# **UF College of Veterinary Medicine Research and PHI ZETA Celebration**

# Abstract Book April 18-19, 2024

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#### Vision

Our products, services and people will be the most valued by animal health customers around the world.

#### Mission

We build on a six-decade history and singular focus on animal health to bring customers quality products, services and a commitment to their businesses.

### **Our History**

Becoming Zoetis is only the latest milestone in our long history of providing realworld solutions that matter to our customers. Veterinarians and livestock producers alike have known and trusted our brands and people as Pfizer Animal Health for more than 60 years.

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# **Symposium Schedule**

## April 18 UF IFAS Straughn Center

2:00pm-4:00pm Poster Set-up

**4:00pm-6:00pm** Poster Presentations & Reception

### April 19 UF IFAS Straughn Center

**9:00am-9:05am** Welcome

9:05am-9:15am VGSA Presentation

9:15am-10:00am Top Dog DVM Research Competition

**10:00am-10:10am** Break

**10:15am-11:30am** Best In Show Graduate Student Research Competition

11:30am- 12:30pm Lunch

12:30pm-1:30pm Keynote Presentation - Dr. Charles Vite, DVM, PhD, DACVIM

**1:30pm-2:30pm** Awards

### Keynote Speaker- Dr. Charles Vite, DVM, PhD, DACVIM



Dr. Charles Vite is an ACVIM board-certified veterinary neurologist. He obtained his DVM at Purdue University and obtained his Ph.D. in Cell and Molecular Biology at the University of Pennsylvania. He performed the first study on gene therapy of the CNS in large animal (canine or feline) models of human disease and managed the National Referral Center for Animal Models of Human Genetic Disease for 10 years (RCAM; NIH OD P40-10939; PI: Vite). The RCAM consists of breeding colonies of more than 40 models of human genetic diseases, many of which are lysosomal storage diseases with nervous system involvement. The focus of his laboratory is to improve the characterization and treatment of hereditary neurodegenerative diseases by studying naturally-occurring large animal models of human diseases. His laboratory develops and evaluates experimental therapies including gene therapy, cell-based therapy, and pharmacotherapy in canine and feline models, and develops and validates ante-mortem biochemical and magnetic resonance markers of disease severity and progression. He joined UF as Neurology Professor and Chair of SACS in 2023.

Summary of talk: "Developing therapies for neurodegenerative diseases of cats and dogs"

This talk will discuss the development of therapies for Niemann Pick Type C and Krabbe disease which affect young children, dogs, and cats.

Dr. Charles Vite, DVM, Ph.D., DACVIM
Department Chair, Small Animal Clinical Sciences
Professor, Neurology
UF College of Veterinary Medicine



### Mission

To foster an environment which encourages excellence in graduate research, scientific ethics, and exchange of ideas.

To increase communication and promote fellowship among College of Veterinary Medicine graduate students and faculty.

To provide graduate students with a forum in which to present academic and professional opportunities.



#### Poster #, Name, Title, Poster #

- 1. Makaylah McCray. Serpentovirus Screening in Free-Ranging Snakes Native to Southeastern United States
- 2. Lex Bleckley. Self-destructing Edwardsiella vaccine vector system to prevent Aeromonas hydrophila and Edwardsiella piscicida infection in catfish
- 3. Kelsey Konopka. Development of a specific polymerase chain reaction assay to detect a novel Mycoplasma species isolated from Bearded Dragons
- 4. Hailey Mangio. Characterization of Sugar-Regulated O-antigen Mutations on Salmonella Resistance
- 5. Lev Avidan. Neurotoxicity assessment of the herbicide pethoxamid in zebrafish embryos/larvae
- 6. Nikita David. Behavior and neurotoxicity assessment in zebrafish (*Danio rerio*) embryos and larvae exposed to perfluorooctanesulfonamide
- 7. Nia Dufeal. Passive Clearing of Dorsal Root Ganglia for Holistic Imaging of Bladder Sensory Neurons
- 8. Cole English. Assessing efficacy of antioxidants docosahexaenoic acid (DHA) and vitamin E in X-chromosome deletion (Xq27.3q28)
- 9. Mark Kreychman. A comparative review of the toxicity mechanisms of perfluorohexanoic acid (PFHxA) and perfluorohexanesulphonic acid (PFHxS) in fish
- 10. Victoria Lopez-Scarim. Evidence for neurotoxicity and oxidative stress in zebrafish embryos/larvae treated with HFPO-DA ammonium salt (GenX)
- 11. Madeline McCoy. Investigation of the Effect of Low Dose Perfluorotetradecanoic Acid (PFTeDA) Exposure on Gut Microbiome Diversity in Adult Zebrafish (*Danio rerio*)
- 12. Allison Wetmore. Does brevetoxin exposure cause dysbiosis in economically important pinfish (*Lagodon rhomboides*)
- 13. Abhith Kasala. Investigation: Quantifying the size and concentration dependent effects of polystyrene and polyethylene microplastics on *Danio rerio* (Zebrafish) embryos via embryotoxicity assays (morphology, HR, qPCR, VMR) for modeling the translational impacts on humans and identifying biomarkers through machine learning algorithms (AI)

- 14. Nicole Anclade. Fully Automated Deep Learning Modeling for Prediction of Pain in Domestic Goats: Preliminary Results
- 15. Sarah Florentine. Evaluating Facial Behaviors as a Measure of Stress in Equine Assisted Interventions
- 16. Tori Argenti. A rodent model of chronic kidney disease of unknown etiology (CKDu)
- 17. Sasha Spada-O'Neill. CRISPR-Mediated Gene Editing in Canine Cells for Pyruvate Dehydrogenase Kinase 4 to Treat Dilated Cardiomyopathy
- 18. Edward Daly. Cooled feline intestine does not differ from fresh intestine for enterotomy leak pressure testing or for gross wall thickness measurement
- 19. Madison Hurley. In vitro effects of mitragynine and doxorubicin on canine hemangiosarcoma cells
- 20. Kian Martin. Experiences using a 1.7 mm locking bone plate system for stabilization of appendicular fractures in dogs and cats
- 21. Chanel Shum. Machine Learning-Assisted Canine Lymphoma Classification
- 22. Sierra Chanutin. Comparison of two techniques to blind end jejunum and ileum for side-to-side cutting-thread jejunocecostomy in horse
- 23. Amber Lum. Comparison of thyroid hormones in Steller sea lions (*Eumetopias jubatus*) with and without thyroid disease
- 24. Tomas Gonzalez. Effect of treatment with GnRH 7 days after artificial insemination (AI) on interval to reinsemination and pregnancy per AI (P/AI) in lactating dairy cows
- 25. Kaitlyn Upton. Effect of Single Doses of Formalin on Dissolved Oxygen Concentrations in Reverse Osmosis Filtered Water and Degassed Well Water at Warmwater Aquaculture Temperatures
- 26. Camille Andrews. Use of liposomal bupivacaine in cats undergoing gastrointestinal surgery is associated with use of less full mu opioids postoperatively
- 27. Ashton Berger. Computed tomography imaging features are predictive of survival time for canine adrenal tumors

- 28. Emma Davey. Diaphragmotomy Allows for Improved Surgical Access of Canine Liver Hilus: A Cadaveric Feasibility Study
- 29. Corey Fisher. The Effect of Enteral Nutrition on Length of Hospitalization in Dogs with Severe Acute Kidney Injury: A Retrospective Study of 37 Cases (2011-2023)
- 30. Seth Locker. Computed tomographic characteristics of confirmed and presumed noncutaneous pythiosis in 25 dogs
- 31. Irene Lopez. Toggle pin technique for stabilization of ventral coxofemoral luxation in 9 dogs
- 32. Steven Magidenko. Significance of Pneumorrhachis Detected by Whole-body Computed Tomography Angiography in Dogs without Trauma
- 33. Kristin Reichert. Fresh and Refrigerated Cadaveric Preparations are Ideal for Sentinel Lymph Node Mapping
- 34. Logan Scheuermann. In Silico Comparison of Two Kirschner Wire Arrangements for Stabilization of Femoral Capital Physeal Fractures
- 35. Tomer Shua-Haim. Computed tomographic features of double aortic arch in six dogs
- 36. Anett Szczepanek. A prospective case-control study of appendicular osteosarcoma patients treated with course fractionated radiation and standard of care with or without GD3-based liposomal vaccine
- 37. Ria Watko. Computed Tomographic and Magnetic Resonance Imaging Features of Benign and Malignant Aortic Body Paragangliomas in 44 Dogs
- 38. Ahmad Ali. Prevalence of and risk factors for antimicrobial-resistant fecal bacteria in beef cattle in cow-calf operations: a systematic review
- 39. Rithu Chandran. Effect of Phytochemicals in Mitigation of Horizontal Gene Transfer of Plasmids Harboring Antimicrobial Resistance Genes between Foodborne MDR Salmonella and Commensal E. coli
- 40. Maiara Goncalves Ramos. Chromogenic In situ Hybridization for the Identification of Oomycetes in Formalin-Fixed and Paraffin-Embedded Specimens
- 41. Roshen Neelawala. Assessment of a Novel Poultry Salmonella Vaccine Candidate: Efficacy, Immunogenicity, and Future Prospects

- 42. Rita Grace Sacharia. Multi-state SARS-CoV-2 Surveillance in Bats in the Southeastern and Northeastern United States at the Human-Bat and Animal-Bat Interface
- 43. Ayusha Shrestha. Prevalence, Diversity, and Biological Characterization of Bat Alphacoronaviruses in the United States
- 44. Nimra Khalid. Structure-activity relationship studies reveal potent antibiotic activity of tolfenpyrad derivatives that target *Francisella*
- 45. Dorothea Megarani. One-Pot RT-LAMP CRISPR/Cas12b Platform for Rapid Detection of Tilapia Lake Virus
- 46. Nicole Nazario Maldonado. A novel murine model to assess the role of inflammation and coagulation during the Malaria-associated respiratory distress syndrome
- 47. Austin Surphlis. Characterizing the resistome of farmed white-tailed deer (*Odocoileus virginianus*) in Florida, USA
- 48. Jason Thornton. Multisystemic disease and septicemia caused by Burkholderia cepacia complex in an American Quarter Horse (*Equus caballus*)
- 49. Yasir Khan. Transcriptomic profiling of antimicrobial resistant Salmonella Heidelberg in the presence of commensal Escherichia coli
- 50. David Kiiza. An enhanced vaccination program to control brucellosis in cattle is cost-effective in pastoral, semi-intensive, and zero-grazing farm systems in Rwanda
- 51. Ana Beatriz Montevecchio Bernardino. Effects of heat abatement during the preweaning on post-weaning and first lactation performances
- 52. Elizabeth Moyer. Comparison of end-to-side versus side-to-side jejunocecostomy in healthy horses
- 53. Oscar (Alejandro) Ojeda Rojas. Individual and Combined Effects of Metritis and Clinical Endometritis on Dairy Cow Profitability
- 54. Alireza Rahmani Shahraki. A Case of Severe Abomasal Sand Impaction in a Whitetailed Deer (*Odocoileus Virginianus*) in Florida.
- 55. Maria Belen Ugarte Marin. Reproductive performance and herd removal of lactating dairy cows with anatomical defects in the cranial vagina and cervical ostium

- 56. Monalisa Sousa Dias Lima. Surveillance of Bovine Leukemia Virus Prevalence in Florida Dairy Herds
- 57. Hannah Anderson. Validation of an Intra-Bladder Optogenetic Device in TRPV1-Ai40 Mice
- 58. Qaim Mehdi. Species-specific profiles of per- and polyfluoroalkyl substances (PFAS) in small coastal sharks along the South Atlantic Bight of the United States
- 59. Shane Priester. Mapping of Neural Network Responses to Urothelial Stimulation
- 60. GabbieRobilotto. Investigating Angiotensin Type 1 Receptor in Experimental Autoimmune Cystitis
- 61. Keita Kitagawa. Longitudinal Assessment of QT Apex in Dogs Undergoing Doxorubicin Therapy.
- 62. Parisa Mazdarani. Radiographic Evaluation of Tibial Torsion Following Tibial Plateau Leveling Osteotomy
- 63. Abigail Ortiz. Effect of food on mitragynine pharmacokinetics after oral dose of encapsulated kratom administered to female beagle dogs
- 64. Raiane Moura. Vatinoxan improves sedation quality without improving tissue (muscle) oxygen saturation in privately-owned adult dogs undergoing intradermal testing
- 65. Ashish Shukla. Exploring human placental explant culture as a reliable model for cell death study in placenta malaria
- 66. Firoj Alom. Urothelial signaling can initiate a local bladder contraction
- 67. Eliana Valle. Behavioral response of zebrafish embryos to the insecticide chlorpyrifos associated with perfluorohexanoic acid (PFHxA)
- 68. Jie Zhou. Effects of repeated pesticides and heat exposure on the development of Chronic Kidney Disease of Unknown Etiology (CKDu)
- 69. Md Abdullah. Metabolic Vulnerabilities in Osteosarcoma Through Iron-Mediated Ferroptosis Modulated by the NRF2/GPX4 Axis
- 70. Kelly Deabold. Clinical and Translational Research at The University of Florida College of Veterinary Medicine

- 71. Donghee Lee. The role of oncogenic PIK3CA mutation in canine hemangiosarcoma
- 72. Rong Li. Proximity Labeling with Molecular Signal Sensing of Oncogenic PIK3CA
- 73. Elodie Huguet. Radiographic Pulmonary Vasculature Dimensions in Drug Naive and Medically Managed Dogs with Left Heart Failure from Chronic Mitral Valve Insufficiency
- 74. Daniel Verdugo. The use of postmortem formalin fixed tissues for barbiturate screen in dogs and cats
- 75. Nicole Adams. Francisella secretes PdpE through the type VI secretion system to inhibit host cell death
- 76. Govindaraj Anumanthan. A recombinant probiotic protects mice against Influenza A infection-induced immunopathology
- 77. Ananta Arukha. *Lactococcus lactis* Delivery of Surface Layer Protein A Protects Mice from Colitis by Re-Setting Host Immune Repertoire
- 78. Trevor Perez. Design of a Three-Dimensional Finite Element Analysis Model of Equine Traumatic Brain Injury Following Trauma
- 79. Amany Sultan. Elucidating metabolic effects of dietary GenX in Nile Tilapia liver
- 80. Karen Scott. Heart rate of military-type dogs when resting, and when walking and trotting on 0% and 10% incline

# Top Dog DVM Presentation Competition: Mission Statement

SCAVMA in conjunction with the College of Veterinary Medicine proudly announce, the Top Dog DVM Presentation Competition. This competition will showcase the outstanding DVM student research projects within the four classes of the College of Veterinary Medicine. Emphasis will be placed on each class' representative's ability to clearly and effectively convey their research project's overall aims, methods, and conclusions to both lay and scientific audiences; their ability to handle questions relating to their project; and their presentation outline and appearance.

The Top Dog DVM Presentation Competition emphasizes communication, promotes fellowship, and inspires friendly competition among the College of Veterinary Medicine's DVM students.

# Please Meet This Year's Top Dog DVM Finalists



Fallon Guild

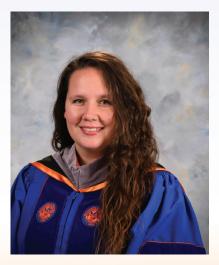
Class of 2027

The Potential Role of the Glandular
Microbiome of Owl Monkeys (Aotus spp.) in
Chemical Communication
(Abstract 1)



Edward Daly
Class of 2025
Use of serum osmolality to identify heart disease stage in dogs and relationship to the degree of mathematical chloride correction.

(Abstract 2)



Bryana Smith
Class of 2024

Modified stapled side-to-side jejunocecostomy
(Abstract 3)

#### **Best in Show**

# Best in Show Presentation Competition: Mission Statement

The VGSA in conjunction with the College of Veterinary Medicine proudly announce, the Best in Show Presentation Competition. The Best in Show will showcase the outstanding graduate student research projects within the four departments of the College of Veterinary Medicine. Emphasis will be placed on each department representative's ability to clearly and effectively convey their research project's overall aims, methods, and conclusions to both lay and scientific audiences; their ability to handle questions relating to their project; and their presentation outline and appearance.

The Best in Show Presentation Competition underscores VGSA's core mission of fostering an environment which encourages excellence in graduate research, scientific ethics, and the exchange of ideas. Additionally, it emphasizes communication, promotes fellowship, and inspires friendly competition among the College of Veterinary Medicine's graduate students and faculty.

### Please Meet This Year's Best in Show Finalists



Chaitanya Gottapu

CDPM

Developing a Vaccine against spotty liver disease in commercial layers

(Abstract 4)



Alexandra Burne

The Invertebrate Galleria mellonella Differentiates Virulence Potential Between Species and Strains of Mycoplasma agassizii and Mycoplasma testudineum.

(Abstract 5)



Kaylie Costa
PS

Sublethal Impacts of Per- and Polyfluoroalkyl Substance (PFAS) Exposure on Acropora tenuis Larvae

(Abstract 6)



Segundo Casaro

LACS

Blood metabolomics and impacted cellular mechanisms during transition into lactation in dairy cows that develop metritis

(Abstract 7)



Victoria Cicchirillo
SACS

Immune cell profile expression analysis of formalin fixed paraffin embedded (FFPE) melanoma and osteosarcoma samples using Nanostring's nCounter assay

(Abstract 8)

#### Serpentovirus Screening in Free-Ranging Snakes Native to Southeastern United States

Makaylah McCray1, Steven B. Tillis1, Corinna Hazelrig2, Ellen Haynes2, Christopher Cleveland2, Nicole M. Nemeth 2,3, Robert J. Ossiboff1

- 1 Department of Comparative, Diagnostic & Population Medicine, University of Florida, College of Veterinary Medicine, Gainesville, Florida USA
- 2 Southeastern Cooperative Wildlife Disease Study, University of Georgia, College of Veterinary Medicine, Athens, Georgia USA
- 3 Department of Pathology, University of Georgia, College of Veterinary Medicine, Athens, Georgia USA

Serpentoviruses (Order *Nidovirales*, Family *Serpentoviridae*) are single-stranded, positive-sense RNA viruses that are associated with respiratory disease primarily in captive reptiles. However, recent studies have identified divergent serpentoviruses in free-ranging snakes, including invasive pythons and native snakes in southern Florida. To better understand the prevalence of serpentoviruses in snakes native to regions of Florida, Georgia, and South Carolina, choanal swab samples were collected from wild snakes and screened for serpentoviruses using reverse-transcription PCR and sequencing. Screening of 232 swabs, including 14 recaptures, representing 26 native species did not detect serpentoviruses in the wild snakes. The screened samples represent only a portion of snakes to be sampled during this project, and additional sampling is ongoing. The final results of this study will provide important information regarding serpentovirus diversity and prevalence in the southeastern United States.

# Self-destructing Edwardsiella vaccine vector system to prevent Aeromonas hydrophila and Edwardsiella piscicida infection in catfish

Lex Bleckley, Roy Curtiss III, Banikalyan Swain

1University of Florida, Department of Infectious Diseases & Immunology, College of Veterinary Medicine, Gainesville, Florida 32608

We engineered regulated delayed lysis plasmid vectors encoding Aeromonas hydrophila protective antigens specified by codon-optimized sequences to enable and enhance synthesis and delivery by recombinant attenuated Edwardsiella vaccine (RAEV-Ah) strains. This vaccine construct is sensitive to all antibiotics and designed to exhibit regulated delayed attenuation. Programmed self-destructing features enable them to efficiently colonize host lymphoid tissues and allow release of bacterial cell contents after lysis. None of the bacterial vaccine cells are able to survive and thus exhibit complete biological containment. RAEV with the regulated delayed antigen synthesis system (RDAS) phenotype enhance immune responses by reducing the adverse effects of high-level antigen synthesis. LD50 of A. hydrophila and E. piscicida strains were determined in zebrafish and catfish. RAEV strains with regulated delayed lysis, regulated delayed attenuation and regulated delayed antigen synthesis phenotype are attenuated in vivo and display increased LD50 values. Plasmid pG8R8517, which encodes the A. hydrophila antigen TonB was introduced into lysis RAEV strain x16018 with genotype ΔasdA10 ΔPfur170::TT araC ParaBAD fur ΔPmurA180::TT araC ParaBAD murA. Zebrafish immunized with x16018(pG8R8517) showed 60% higher survival compared to control groups. We will fully evaluate immunogenicity of optimized RAEV-Ah strains and their ability to induce protective immunity against A. hydrophila and E. piscicida challenge in channel catfish. Ultimately, we develop a new mucosal antigen delivery RAEV system for aquaculture that will prevent important infectious diseases and therefore increase the sustainability and profitability of the finfish aquaculture industry in the US.

# Development of a specific polymerase chain reaction assay to detect a novel *Mycoplasma* species isolated from Bearded Dragons

Kelsey Konopka1, Alexandra Burne1, Javier Nevarez2, Tithipong Plangsangmas2, Dina Michaels1, Mary Brown1

1Infectious Disease and Immunology, College of Veterinary Medicine, University of Florida

Mycoplasmas are important pathogens of a wide variety of host species. Reptilian hosts are of particular concern as many species are listed as threatened or endangered. Disease is generally chronic and clinically silent; however, there are species capable of eliciting fulminant disease (*Mycoplasma* alligatoris). In 2023, an acute death of an individual animal within a bearded dragon research colony occurred at Louisiana State University. Necropsied tissue of the individual was sent to the University of Florida Mycoplasma testing lab where a Mycoplasma was identified via culture and PCR. 16S rRNA sequencing indicated 99.86% homology to a previously reported undescribed *Mycoplasma*. Further screening of the colony was conducted monthly for 3-months. While >70% of samples were negative by direct PCR, we successfully isolated colonies in >90% of the samples over the course of testing. Culture methods thus far have proven to be the most reliable diagnostic method; however, cultivation can be costly and time consuming. To address these challenges, we first developed a primer set specific for this Mycoplasma based on the screening of three housekeeping genes, dnaK, uvrA, and recA. We then determined the limit of detection via qPCR using both 16S and our housekeeping primer sets. Type strain reptilian mycoplasmas were used as control species, including M. testudineum, M. agassizii, M. alligatoris, M. crocodyli, and a novel corn snake Mycoplasma, M. floridensis, to ensure specificity. Through these methods we hope to provide an efficient and rapid diagnostic test for the screening of bearded dragon populations.

#### Characterization of Sugar-Regulated O-antigen Mutations on Salmonella Resistance

Hailey Mangio, Shifeng Wang, Ashley Pitzl, Roy Curtiss III

Department of Infectious Diseases and Immunology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, 32610 USA

The O-antigen is a polysaccharidic component of lipopolysaccharide (LPS), located on the outer membrane of Gram-negative bacteria. It is a critical surface structure that influences bacterial pathogenesis and contributes to resistance to serum, particularly to heat-sensitive complement components. The O-antigen ligase enzyme, waaL, is involved in the biosynthesis and attachment of Oantigen to the core of LPS, and the pmi gene is required to synthesize the O-antigen side chain. In this project, Salmonella strains with waaL gene expression regulated by the sugars arabinose and rhamnose and with pmi mutations requiring mannose for O-antigen side chain synthesis were generated. Singlesugar and double-sugar regulated strains were compared by analyzing motility, serum resistance, and acid tolerance. Motility was assessed by measuring swimming zones on motility test plates. Serum resistance was measured by conducting colony-forming unit counts after 2-hour incubations of bacteria in normal human serum (NHS) and heat-inactivated human serum (IHS). Additionally, 48-hour growth curves based on strains' optical density in 10% NHS and IHS were evaluated. Acid tolerance was also tested by measuring 48-hour bacterial growth curves in E-mediums with varying pH. Mutationmediated variations in O-antigen synthesis were found to influence differences in motility, serum resistance, and acid tolerance among the strains tested. This study contributes to current understandings of the impact of surface modifications on Salmonella pathogenesis and informs directions for recombinant attenuated Salmonella vaccine development. Subsequent research could test additional measures such as bile resistance and examine effects of the mutations on Salmonella virulence in animal models.

#### Neurotoxicity assessment of the herbicide pethoxamid in zebrafish embryos/larvae

Lev Avidan, Cole D. English, Eliana Valle, Isaac Konig, Emma Ivantsova, Christopher L. Souders II, Christopher J. Martyniuk

Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Florida, 32603 USA

Pethoxamid, a member of the chloroacetamide herbicide family, is a recently approved chemical for pre-or post-emergence weed control. While pethoxamid has not been considered by the EPA to have a high risk of groundwater contamination or bioaccumulation in species, pethoxamid is a Group 15 herbicide — potentially inhibiting cell division. Furthermore, sub-lethal toxicity data for aquatic organisms are lacking in current literature. To address this knowledge gap, we exposed zebrafish embryos/larvae to pethoxamid over a 7-day period post-fertilization and measured several toxicological endpoints related to oxidative stress and neurotoxicity. Continuous pethoxamid exposure did not affect survival, hatch success, nor did it cause deformity in embryos/larvae for 7 days up to concentrations of 1000µg/L. Exposure to pethoxamid was not measured to affect basal respiration nor ATP-linked respiration of embryos, but pethoxamid was observed to low non-mitochondrial respiration in embryos. Following exposure to pethoxamid, we noted a significant increase in both levels of apoptosis and of reactive oxygen species (ROS). Levels of ROS were associated with genes sod1, sod2, catalase. To assess neurotoxicity potential, we measured several transcripts (including ache, elav3, gfap, mbp, ) implicated in neural processes in the central nervous system. Locomotor activity of larval zebrafish was affected with pethoxamid exposure, as hyperactivity was observed at concentrations below 1 μg/L; hypoactivity was noted at exposures to 10 and 100 µg/L of pethoxamid. These data contribute to ecological and toxicological risk assessments for chloroacetamide-based herbicide, representing the first reporting sublethal toxicity data for this new herbicide.

# Behavior and neurotoxicity assessment in zebrafish (*Danio rerio*) embryos and larvae exposed to perfluorooctanesulfonamide

Nikita R. David, Cole D. English, Lev Avidan, Amany Sultan, Eliana Valle, Mark Kreychman, Christopher J. Martyniuk

Department of Physiological Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Florida, 32603 USA

Perfluorinated chemicals, also known as "forever chemicals" due to their low ability to break down in the environment, are a group of synthetic chemicals used in a variety of consumer products. Perfluorooctanesulfonamide (PFOSA) has been utilized in the production of packaging material, textile material, and surface protectants. PFOSA has been found in ecosystems around the world, including in the tissues of aquatic predators and fish, but there are few studies detailing its ecotoxicological consequences. The objectives of this study were to 1) determine the neurotoxic toxicity potential of sublethal PFOSA exposure and 2) observe effects of PFOSA on the behavior of zebrafish larvae. The survival rate, hatch rate, and presence of deformities were not different in treatment groups following exposure levels up to  $100~\mu g/L$  PFOSA, suggesting low acute toxicity. Fish exposed to  $100~\mu g/L$  PFOSA experienced hypoactivity, indicating a change in optomotor response. Multiple genes were also studied from RNA-seq, and the expression of elav3 and gfap were altered. This study improves understanding of PFOSA toxicity and its ecological consequences, and on a broader scale, perfluorinated compounds.

#### Passive Clearing of Dorsal Root Ganglia for Holistic Imaging of Bladder Sensory Neurons

Nia Dufeal1, Gabbie Robilotto1, and Aaron Mickle PhD1,2,3

1Department of Physiological Sciences, 2Department of Neuroscience, 3J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, Florida, 32610 USA

Dorsal root ganglia (DRGs) house the cell bodies of sensory neurons that communicate between the bladder and the spinal cord. Increased sensory neuron activity can indicate abnormal conditions within either organ system, including infections and inflammation. Analyzing DRGs under these conditions is important in understanding disease pathways and organ physiology. Current DRG analysis requires serial sectioning, in which tissue is sampled in a time- and labor-intensive manner. This method often results in lost sections, severely limiting the scope of what is observable on a larger scale during organ reconstruction. CLARITY (Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel), a technique utilized to analyze the three-dimensional tissue morphology within transparent samples, aims to expand upon current techniques for microscopic analysis of biological tissues. In the context of DRG communication with the bladder, CLARITY provides the ability to identify neurons transduced by viruses injected peripherally and visualize innervations stemming to the bladder. To clear tissue, the mPACT (modified Passive Clearing Technique) method will be utilized to achieve transparency of DRGs in a more reduced clearance time than traditional PACT and psPACT (process-separate PACT) methods. Unlike other processes, mPACT uses an 8% SDS-based clearing solution and 0.5% α-thioglycerol for the most effective tissue clearing. Rodent tissue samples are expected to clear in about 17 days, becoming nearly transparent. Upon successful completion of tissue clearing, the transparent tissues will then be available for holistic imaging without altering organ configuration, reducing analysis time and improving accuracy.

Assessing efficacy of antioxidants docosahexaenoic acid (DHA) and vitamin E in X-chromosome deletion (Xq27.3q28)

Cole D. English1, Christopher L. Souders II1, Christopher J. Martyniuk1,3\*

1Center for Environmental and Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida, 32611, USA 2UF Genetics Institute, Interdisciplinary Program in Biomedical Sciences Neuroscience

The human X-chromosome contains over 1400 genes and more than 150 million base pairs, of which approximately 95% have been determined. Duplications, mutations, or deletions in the X-chromosome are associated with several diseases and rare conditions associated with reproduction, growth, musculature, cognition, and neural development which compromises lifelong healthy learning and living. The major deletion in the X-chromosome between loci Xq27-q28 can result in the loss of over 200 genes, and includes small molecule signaling proteins (heat shock transcription factors, ubiquitin, Rho GTPase activating protein 4), neuropeptide-related (arginine vasopressin receptor 2), muscle (myotubularin related protein 1), reproduction-related (pregnancy up-regulated non-ubiquitous CaM kinase, sperm protein associated with the nucleus, X-linked, family member A1), neurotransmitterrelated (gamma-aminobutyric acid subunits alpha3, epsilon, theta), miRNAs, and others. Several genes associated with mitochondrial function and ATP production in this chromosomal region are impacted by the deletion. These genes include ATP binding cassette subfamily D member 1, ATPase H+ transporting accessory protein 1, ATPase phospholipid transporting 11C, ATPase plasma membrane Ca2+ transporting 3, and ATP synthase subunit O (mitochondrial), all of which are located within Xq27-Xq28. Loss of these genes can therefore have detrimental consequences to cell bioenergetics and ATP production. Here, we profile neuro-progenitor cells harboring X-chromosome deletion Xq27.3q28 for their metabolic function and transcriptomic character. Given enhanced production of antioxidants and ROS-scavenging enzymes with DHA, vitamin C, or vitamin E administration, the overarching hypothesis was that antioxidants would improve metabolic capacity of dopaminergic neurons containing the X-chromosome Xq27-q28 deletion. Results to date will be discussed.

# A comparative review of the toxicity mechanisms of perfluorohexanoic acid (PFHxA) and perfluorohexanesulphonic acid (PFHxS) in fish

Mark Kreychman1,2, Emma Ivantsova1,2, Austin Lu3, Joseph H. Bisesi Jr. 1,4, Christopher J. Martyniuk1,2, 5  $^{\star}$ 

1Center for Environmental and Human Toxicology, University of Florida, Gainesville, Florida, 32611 Industrial and consumer goods contain various perfluoroalkyl substances (PFAS), notably perfluorohexanoic acid (PFHxA) and perfluorohexanesulphonic acid (PFHxS), drawing increased scrutiny for their potential aquatic toxicity. This review compares PFHxA and PFHxS levels in aquatic environments and fish tissues, alongside toxicity mechanisms encompassing morphological, endocrine, metabolic, and behavioral endpoints. A computational assessment was also performed to identify putative mechanisms of toxicity and to characterize exposure biomarkers. PFHxA and PFHxS residues have been detected in marine and freshwater fish tissues, reaching concentrations of up to 37.5 ng/g PFHxA and 1290 ng/g PFHxS, but persistence in water and tissue degradation necessitates further investigation. Both oxidative stress and endocrine disruption have been reported. Based on evidence for endocrine disruption, we modelled interactions of estrogen and androgen receptors of several fish species with. Molecular docking revealed a stronger affinity of PFHxS over PFHxA with fish estrogen and androgen receptors and that estrogen and androgen receptors of fathead minnows, zebrafish, Atlantic salmon, and largemouth bass show comparable binding affinities for each chemical except for salmon Esr2b which shows lower affinity for PFHxA relative to Esr2a. While mechanistic data is lacking in fish, computational assessments revealed that PFHxA can perturb the endocrine system, nervous system, impacting kidney and liver weight. Proteins associated with PFHxA/PFHxS exposure in fish include those involved in lipid and glucose regulation, reproductive proteins like KISS metastasis suppressor, and immune system proteins like RAG1 and RAG2, serving as potential exposure biomarkers. Taken together, we synthesize current knowledge regarding the environmental fate and ecotoxicology of PFHxA/PFHxS in fish.

Evidence for neurotoxicity and oxidative stress in zebrafish embryos/larvae treated with HFPO-DA ammonium salt (GenX)

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"GenX" [ammonium perfluoro (2-methyl-3-oxahexanoate] was developed as a replacement chemical for toxic perfluorinated compounds to be used in product manufacturing. Here, we assessed developmental, mitochondrial, and behavioral toxicity endpoints in zebrafish embryos/larvae exposed to GenX. GenX exerted low toxicity to zebrafish embryos/larvae up to 20 mg/L. GenX did not affect mitochondrial oxidative phosphorylation nor ATP levels. ROS levels were reduced in larvae fish exposed to 10 and 100  $\mu$ g/L, indicative of an antioxidant defense; however, ROS levels were elevated in fish exposed to 1000  $\mu$ g/L. Increased expression of cox1 and sod2 in GenX exposed 7-day larvae was noted. GenX (0.1 or 1  $\mu$ g/L) altered transcripts associated with neurotoxicity (elavl3, gfap, gap43, manf, and tubb). Locomotor activity of larvae was reduced by 100  $\mu$ g/L GenX, but only in light periods. Perturbations of anxiety-related behaviors in larvae were not observed with GenX exposure. These data inform risk assessments for long-lived perfluorinated chemicals of concern.

Investigation of the Effect of Low Dose Perfluorotetradecanoic Acid (PFTeDA) Exposure on Gastrointestinal Microbiome Diversity in Adult Zebrafish (*Danio rerio*)

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Perfluorinated compounds (PFCs) are a class of manufactured chemicals used as lubricants, protectants, pesticides, detergents, and flame retardants. PFCs have been widely recognized to produce detrimental physiological and behavioral impacts in humans and animals, such as developmental toxicity, neurotoxicity, immunotoxicity, and endocrine disruption. Perfluorotetradecanoic Acid (PFTeDA) is a long-chain PFC prevalent within aquatic environments. Past studies have shown disruption of gene regulation for mitochondrial transcription, induction of oxidative stress, and endocrine disruption. The gastrointestinal (gut) commensal microbiome is a diverse microbial niche that is heavily integrated into host homeostatic function. Dysbiosis in the gut microbiome can lead to decreased enteric infection resistance and increased host stress. This study aims to investigate the effect of environmentally relevant doses of PFTeDA toxicity on the gut microbiome the zebrafish (Danio rerio). Bacterial DNA from fecal samples will be isolated and sequenced using Illumina next generation sequencing to assess microbial abundance and diversity from PFTeDA exposed zebrafish. It is expected that experimental groups will have a decreased diversity, due to harmful effects of PFTeDA toxicity, such as oxidative stress. These data are important because dysbiosis can lead to opportunistic growth of pathogenic bacteria which may synergistically decrease individual health. This research connects and contributes to the growing fields of PFC toxicity and microbiome physiological research.

# Does brevetoxin exposure cause dysbiosis in economically important pinfish (*Lagodon rhomboides*)?

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Florida's red tide is caused by the overgrowth of Karenia brevis, a dinoflagellate that produces brevetoxin, a neurotoxin. Brevetoxins can accumulate through trophic cascades resulting in mortality of fish, marine mammals, and other coastal species. The impact of brevetoxins on gastrointestinal function and microbiome has not been investigated. Our objective is to understand how brevetoxin exposure may alter the natural gastrointestinal microbiome in pinfish (Lagodon rhomboides). We hypothesize that dietary brevetoxin exposure will result in gastrointestinal dysbiosis which may lead to reduced nutrient absorption and overall health of the animal. To test this, a 7-day dietary dose experiment was conducted on pinfish in 3 treatment groups (control = no PbTx-3, low = 0.18 µg PbTx-3/g food, and high = 1.8 μg PbTx-3/g food). The experiment was replicated. Bacterial DNA was isolated from dissected tissues and PCR amplified for Illumina next-generation sequencing. Microbiome richness and diversity in the gut will be assessed. We expect the results to show that there will be a negative relationship between brevetoxin concentration and abundance, and diversity of bacteria species present in the intestines and feces of the pinfish. This research is significant because brevetoxins are not only toxic but may also disrupt nutrient absorption and allow for pathogenic bacteria to increase. These data will contribute to the understanding the negative impacts of red tide events on Florida fisheries and ecosystems.

#### Global Microplastic Pollution - The Emerging Health Crisis

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**Investigation:** Quantifying the size and concentration dependent effects of polystyrene and polyethylene microplastics on *Danio rerio* (Zebrafish) embryos via embryotoxicity assays (morphology, HR, qPCR, VMR) for modeling the translational impacts on humans and identifying biomarkers through machine learning algorithms (AI).

Remediation: A Novel low-cost water purification method for microplastic extraction incorporating *Moringa oleifera* seed powder as a natural coagulant.

The escalating scale of "Global Plastic Pollution" is leading to the omnipresence of Microplastics (MPs), negatively impacting the environment, world's water quality, aquatic organisms, and human health in trophic transfer. Annually, >400 million tons of plastic waste enter landfills and oceans due to its non-biodegradable nature. Plastics depolymerize through physical/chemical processes fragmenting their long carbonaceous chains, producing microplastics, ≤5 mm. Polyethylene (PET) and polystyrene (PS) are the most commonly used plastic amounting to >125 million tons produced annually. Studies show that MPs are endocrine disruptors, carcinogens, diabetogens, obesogens, neurotoxins, and genotoxins. Stage 1 is a comprehensive novel investigation to bridge the knowledge gaps in the size/ concentration-dependent effects of PS and PET-MPs on Danio rerio embryos as a model organism for human comparison. Embryos were exposed to environmentally relevant sizes 4, 10, and 15µm PS-MPs, and 100µm PET-MPs at low/high concentrations for 6 days-post-fertilization. Embryotoxicological effects were assessed via morphology, HR, VMR assays (behavior), and qPCR (gene expression). 3 AI models were developed to identify zebrafish deformity image recognition (convolutional neural network), predict molecular binding of chemicals in microplastics with zebrafish and human enzymes to analyze inhibitory effects (Deep Learning), and identify biomarkers for diseases and cancers using gene expression levels from experimental data (Deep Learning) for effectively analyzing large datasets/discovering new patterns. Stage 2 is a novel remediation method for extracting MPs in water sources utilizing *Moringa oleifera* seed powder as a natural coagulant in a scalable, sustainable, and low-cost water purification prototype for mitigating the global water microplastic pollution crisis.

# Fully Automated Deep Learning Modeling for Prediction of Pain in Domestic Goats: Preliminary Results

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Acknowledging the need to alleviate stress and pain in livestock drove interest in developing precise pain assessment techniques. Facial expressions are vital in nonverbal communication, conveying emotional information and regulating peer interactions. Despite its success in various species, facial expression recognition poses drawbacks such as experience, training, costs, and bias. In contrast, Automated Pain Recognition (APR), driven by Artificial Intelligence, utilizes mathematical algorithms to detect pain-related traits, overcoming challenges inherent in traditional scoring systems. In this preliminary study, we established the architecture and hyperparameters for a custom Artificial Neural Network (ANN) designed to discern pain facial features in goats. Two networks were trained and externally validated: one based on a custom convolutional base with five Conv2D layers and five MaxPooling2D layers and the other based on a pre-trained standard convolutional base (VGG16) with fine-tuning of the upper layer. Both networks used the same densely connected classifier placed on the convolutional base. The training dataset comprised 11,778 images, with 1,308 validation images, sourced from two-minute high-definition footage featuring eight client-owned adult goats of diverse breeds, sexes, and ages. Pain definition and classification were based on the Unesp-Botucatu Goat Acute Pain Scale. Both models demonstrated acceptable performance, achieving over 90% accuracy in discriminating pain in validation. However, the VGG16 model with fine-tuning consistently outperformed, reaching an accuracy of 98.4%. These preliminary findings suggest the potential of the deep learning approach in goat pain detection. Further studies are warranted to evaluate the model across various facial morphometric features and pain intensities.

#### Evaluating Facial Behaviors as a Measure of Stress in Equine Assisted Interventions

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The horse is a remarkably expressive animal, possessing seventeen unique facial action units that can be directed to generate an expansive repertoire of facial behaviors. These behaviors serve as a physical form of communication within the herd and can be used to convey extensive information about the horse and its environment. Decoding the context of these facial behaviors has become a recent focus within the field of equine behavioral research, and the existence of numerous facial ethograms offers a rich foundation for examination of the equine emotional state. The facial behaviors of pain in horses are some of the most well-described, with a variety of pain scales developed as metrics for their analysis. The Horse Grimace Scale (HGS) is a unique pain scale that numerically scores muscular tension in the face; as facial muscle tightening has been linked with equine stress, it has a promising secondary application for stress analysis. Equine stress is a notable concern in Equine Assisted Interventions (EAIs), programs that explore the human-horse relationship for therapeutic benefit. While the emotional impacts of EAIs on human participants are overwhelmingly positive, those on their equine partners remain unclear. This study used the HGS to evaluate stress levels in four horses during an eight-week EAI program for combat veterans with post-traumatic stress disorder (PTSD), a psychological disorder potentially linked to higher stress levels in EAI horses. Mean HGS scores across the eight-week program and individual sessions were found to differ insignificantly between the EAI and control groups of horses.

#### A rodent model of chronic kidney disease of unknown etiology (CKDu)

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This study aims to investigate the role of heat stress in chronic kidney disease of unknown etiology (CKDu) and to identify urinary biomarkers that may allow for early detection strategies. We placed rats into a temperature-controlled environmental chamber for 4 hours per day over 6 weeks. An internal remote temperature sensing probe collected real-time core temperature data. Additionally, we collected urine samples for analysis with multiplex assays to identify unique biomarkers, and we collected weekly body weight measurements. There was a statistically significant difference between the mean core body temperatures of the experimental and control rats. The most frequent core temperature experienced by the experimental rats was approximately 1.5° Celsius higher than that of the control rats. Osteopontin (OPN) and cystatin-C (CysC) levels showed the most significant differences between the experimental and control groups. Finally, there was a statistically significant difference between the mean body weights of the groups, with the experimental group experiencing an overall negative trend in body weight. The findings suggest chronic increases in core body temperature may play a role in CKDu progression. Furthermore, OPN and CysC may be candidates for acute kidney damage screening, allowing for early detection of the tubular injury that manifests as CKDu.

# CRISPR-Mediated Gene Editing in Canine Cells for Pyruvate Dehydrogenase Kinase 4 to Treat Dilated Cardiomyopathy

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Cardiovascular disease is a leading cause of mortality in canines, with one of the most common forms being dilated cardiomyopathy (DCM). In the Doberman Pinscher, DCM is linked to two known genetic mutations. One is a 16 base pair deletion located at the donor splice site of an intron in the pyruvate dehydrogenase kinase 4 (PDK4) gene which affects metabolic capacity of cardiomyocyte cells. The objective of this study was to determine the efficacy of homology-independent targeted insertion (HITI), a CRISPR/Cas9 gene editing strategy, to correct the PDK4 mutation. We designed single guide RNA (sgRNA) using two approaches: 1) dropping out the exon adjacent to the mutation and 2) targeting and cutting the unique mutant sequence to enable integration of the deleted region using a HITI approach. We tested five sgRNAs in vitro using ribonucleoprotein (RNP) based cleavage assay. Four sgRNA candidates showed evidence of cutting *in vitro* in the homozygous DNA template. We then carried out transfection using RNPs in vivo. Cleavage efficiency was assessed by Sanger sequencing and computational analysis. For the two sgRNA candidates designed to exon drop, postnucleofection analysis showed up to 100% editing efficiency specific to the exon deletion. However, post-nucleofection analyses detected a range of 0-5.4% editing efficiency for sgRNAs designed to cut the mutant sequence for integration, indicating the need for further optimization in vivo for these candidates. With further investigation, the CRISPR/Cas9 HITI approach may be used to edit the PDK4 mutation, advancing potential for genetic therapies in veterinary medicine.

Cooled feline intestine does not differ from fresh intestine for enterotomy leak pressure testing or for gross wall thickness measurement

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**Objective:** To report gross anatomical gastrointestinal measurements and compare enterotomy leak pressures between fresh and cooled feline cadavers.

Animals: Fresh feline cadavers (n=20).

**Procedures:** Jejunal segments (8 centimeters) were harvested the same day as euthanasia. From each cadaver, one segment was randomly assigned to control (C), fresh enterotomy (FE), and cooled enterotomy (CE) groups. Enterotomy construction and leak testing were performed within 12 hours of euthanasia for C and FE groups and after 17-29 hours of cooling for the CE group. Initial leak pressure (ILP) and maximum intraluminal pressure (MIP) were compared. Gastrointestinal wall thickness and intraluminal diameter were measured on harvested applicable gastrointestinal divisions at up to 3 time points: day 1 fresh, day 2 cooled, and day 3 cooled.

**Results:** Mean  $\pm$  SD ILP for C, FE, and CE constructs was  $600 \pm 0.0$ ,  $200.3 \pm 114.7$ , and  $131.3 \pm 92.6$  mmHg, respectively. C ILP was higher (P<0.001) than FE and CE ILP. ILP (P=0.11) and MIP (P=0.21) were not different between FE and CE constructs. Wall thickness (mm, millimeters) was not different between duodenum day 1 fresh and day 2 cooled groups (P=0.18) and between any jejunum day groups (P=0.86). Intraluminal diameter (mean  $\pm$  SD) for duodenum, jejunum, and ileum was  $5.7 \pm 0.7$ ,  $5.8 \pm 0.8$ , and  $7.2 \pm 2.2$  mm, respectively.

Clinical Relevance: Cadaveric feline intestine cooled for up to 29 hours may be used for determining intestinal leak pressures.

#### In vitro effects of mitragynine and doxorubicin on canine hemangiosarcoma cells

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Visceral hemangiosarcoma in dogs is a highly malignant neoplasm of endothelial origin that exhibits an aggressive biological behavior. While surgery is the mainstay of treatment for this disease, it has minimal impact on survival times. Therefore, various chemotherapy protocols have been investigated. Currently, doxorubicin is a commonly used chemotherapy protocol but has a high rate of adverse effects. Mitragynine is an indole-based alkaloid and the most abundant active alkaloid in the Southeast Asian plant Mitragyna speciosa. Mitragynine displays a high affinity to opioid receptors and has been used for medicinal and recreational purposes in both eastern and western countries. Mitragynine has also demonstrated concentration-dependent cytotoxicity and profound antiproliferative efficacy towards various human cancer cell lines. To assess the in vitro effects of mitragynine and mitragynine combined with doxorubicin on cells harvested from an immortalized canine hemangiosarcoma cell line, cell viability assays were performed at 24, 48, and 72 hours. Exposure of cells to the combination of mitragynine and doxorubicin resulted in greater cytotoxicity than either mitragynine or doxorubicin alone. Furthermore, cytotoxicity was seen at doses lower than standard intravenous doxorubicin used clinically. These findings provide support for a clinical trial investigating the use of combination therapies for treatment of canine hemangiosarcoma.

Experiences using the Fixin 1.7 micro-locking bone plate system for stabilization and fixation of appendicular fractures in both the canine and feline patient; A clinical and radiographic retrospective assessment

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**Objective:** To retrospectively evaluate the stabilization of appendicular fractures using a 1.7 mm bone plating system.

**Methods:** A review of medical records of dogs and cats with fractures stabilized with the Fixin 1.7 mm locking bone plate system from 2019 to 2023 was performed. Fractures were stabilized using both minimally invasive plate osteosynthesis (MIPO) and open reduction and internal fixation (ORIF) methods. Data pertaining to the signalment, nature of the fracture, implants, evidence of fracture healing, and limb function were recorded. The outcomes, including complications and explants, were determined from clinical and radiographic follow-up exams. The function of the affected limb was evaluated between 30 days and 270 days.

**Results:** One hundred and two of 133 fractures met the inclusion criteria. Fractures were excluded due to lack of follow-up radiographs, prophylactic plating, or the use of non-1.7 mm additional plates. Of the 102 fractures that were included, 37 were repaired via MIPO and 65 were repaired via ORIF. Complete healing occurred in 29/37 (78%) using MIPO and 48/65 (74%) with ORIF, combined successful outcome was 77/102 (75%). Follow-up evaluations were performed between 30 days and 270 days post-operatively. Complications occurring in 7/37 (19%) fractures when done via MIPO and 8/65 (12%) fractures when done via ORIF, with an overall complication rate of 15/102 (15%).

**Conclusion:** Despite having a moderate complication rate, the Fixin 1.7mm micro-locking bone plate system appears to be an acceptable implant for the stabilization and fixation of traumatic appendicular fractures in the dog and cat.

# Machine Learning-Assisted Canine Lymphoma Classification

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Our study harnesses the power of machine learning, specifically Convolutional Neural Networks (CNN), to classify lymphomas in dogs. We sourced a total of 2,668 images: 1,110 from B-cell lymphomas across 84 patients, 520 from T-cell lymphomas across 41 patients, and 1,038 from reactive lymphoid hyperplasia across 77 patients. To standardize our dataset, we center-cropped each image to a uniform size of 600 x 800 pixels and enhanced our dataset through vertical and horizontal image flipping for data augmentation. Leveraging a transfer learning approach, we employed the pre-trained ResNet-50 CNN for efficient training, modifying it for a tri-level classification task: distinguishing benign lymphocytes and T- and B-cell malignancies. Over 15 epochs, our model achieved a training accuracy, peaking at 93.7% in the 14th epoch and slightly dropping to 92.9% in the 15th epoch. Our model's performance achieved an overall accuracy of 85.9%, along with a precision of 89.8%, a recall rate of 85.9%, and an F1 score of 86.7%. Confusion matrix analysis revealed notable insights: while our model excelled in differentiating between reactive lymphoid hyperplasia and B-cell malignancies (98.3% accuracy), it demonstrated lower accuracy in distinguishing between B-cell and T-cell lymphomas (86.4%) and between reactive lymphoid hyperplasia and T-cell lymphoma (91.5%). Future steps involve expanding our methodology by incorporating other pre-trained CNN models like VGG-16 and AlexNet for comparative analysis with our initial ResNet-50 results. Additionally, we plan to employ Grad-CAM software to further interpret and visualize the distinctive features within the images that drive the classification decisions.

Comparison of two techniques to blind end jejunum and ileum for side-to-side cutting-thread jejunocecostomy in horses

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**Background:** Jejunocecostomy (JC) is used to bypass diseased ileum, but methods to blind end small intestine for side-to-side (SS) JC have not been compared. Available evidence suggests that JC performs better when blind end closures are not required.

**Objectives:** To compare handsewn and stapled blind end closures of the ileum and jejunum in side-to-side JC and to assess the cutting thread method for JC in healthy horses.

**Methods:** Cutting thread JC with a Parker-Kerr (PK; 6 horses) or oversewn stapled technique (OS; 6 horses) was used for jejunal and ileal blind ends. Postoperative monitoring included physical and hematological examinations and SAA. At necropsy 7 days postoperatively, measurements were repeated, peritoneal fluid was analyzed, and tissue samples collected.

**Results:** Two PK horses and one OS horse had mild postoperative colic and another PK horse was euthanized because of an anastomotic impaction. Necropsy demonstrated that colic in PK horses was caused by intussusception of the jejunal blind end into the cecum. Postoperative peripheral neutrophil counts and SAA were similar between groups but SAA increased (P=0.03) in combined groups. The OS jejunal blind ends were significantly larger (P=0.004) than the PK blind ends and were slower to complete (P=0.02). The OS blind ends were inflamed between the staples and inverting suture, and the PK closure was inflamed along cut edges. Peritoneal fluid protein was significantly elevated (P<0.001) at necropsy.

**Conclusion:** Both blind end constructions for JC are acceptable. The cutting thread method produces an effective anastomosis with low risk of complications.

Ethical animal research: Approved by University of Florida's Institutional Animal Care and Use Committee. Source of funding: Ardin and Claudia Sims Colic Research Foundation.

# Comparison of Thyroid Hormones in Steller Sea Lions (Eumetopias jubatus) With and Without Thyroid Disease

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An evaluation of morbidity and mortality in Steller sea lions (Eumetopias jubatus; SSLs) under professional care in North America identified a high prevalence of thyroid disease in aged animals. While studies have investigated effects of age, season, nutrition, stressors, and pollutants on thyroid hormones in pinnipeds, data from individuals with thyroid lesions are lacking. The objective of this study was to compare triiodothyronine (TT3), total thyroxine (TT4), free thyroxine (fT4), and thyroid stimulating hormone (TSH) in SSLs with histologically normal and abnormal thyroids and assess whether these assays aid in diagnosis of thyroid disease. Histopathological reports of adult SSLs under professional care in the United States with available banked serum were reviewed. Serum from SSLs with normal (n=2) and abnormal (n=6) thyroid histology, and from clinically unaffected live individuals (n=2), was analyzed via radioimmunoassay and chemiluminescent immunoassay undergoing validation for use in SSLs. Thyroid lesions included follicular cysts (n=2), adenoma with thyroglossal duct or follicular cysts (n=2), medullary or follicular C-cell carcinoma (n=1), and neuroendocrine carcinoma with follicular cysts (n=1). Values were compared between groups using Welch's t-test (TT3 p=0.71, TT4 p=0.34, fT4 p=0.20, TSH p=0.64); the lack of statistically significant differences suggests this species may present similarly to canines which are frequently euthyroid. However, further investigation with a larger sample size and uniform thyroid lesions is warranted. These data expand the current understanding of thyroid disease in SSLs and underscore the need for additional diagnostics such as cervical palpation and ultrasound in aged animals to screen for underlying abnormalities.

# Effect of treatment with GnRH 7 days after artificial insemination (AI) on interval to reinsemination and pregnancy per AI (P/AI) in lactating dairy cows

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Dairy cows with short reinsemination intervals have reduced fertility. Objectives of this ongoing study were to evaluate inter-estrus interval and pregnancy outcomes in lactating dairy cows treated with GnRH 7 days after AI. Daily cohorts were blocked by parity and AI number on d 7 after AI (d 0) and randomly assigned to one of two treatments: 1) GnRH7 (n=1,076) cows were treated with 172  $\mu$ g of gonadorelin 7 days after a previous AI; 2) Control (n=1,087) cows were not treated with GnRH. Ovaries from a subgroup of cows (GnRH7, n=139; Control, n=143) were scanned on d 7 and 14 to determine ovulation. Collar-mounted activity and rumination monitoring devices were used for evaluation of estrus behavior. Logistic regression and Cox-proportional hazard models were used for analyses of binary and time-dependent variables. Treatment with GnRH increased (P<0.001) the proportion of cows that ovulated between d 7 and 14 (GnRH7=79.1 vs. Control=1.4%). However, treatment did not affect (P $\geq$ 0.36) P/AI on d 67 (GnRH=43.5 vs. Control=45.4%) or pregnancy loss between d 33 and 67 after previous AI (GnRH=5.2 vs. Control=6.1%). Treatment with GnRH did not affect (P=0.94) the hazard of reinsemination (AHR=1.01, 95%CI=0.86-1.18). Finally, treatment with GnRH did not affect (P=0.31) P/AI on d 33 after the AI post treatment (GnRH7=47.0 vs. Control=43.8%).

# Effect of Single Doses of Formalin on Dissolved Oxygen Concentrations in Reverse Osmosis Filtered Water and Degassed Well Water at Warmwater Aquaculture Temperatures

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Formalin, a 37-40% solution of formaldehyde in water, is an FDA-approved treatment for control of external protozoa and monogeneans on fish. It is commonly used as a prolonged bath treatment at 15-25 mg/L or as a short-term bath treatment of 30-60 minutes at up to 250 mg/L. Formalin treatment has been correlated with the depletion of dissolved oxygen (DO), and a well-known adage is that every 5 mg/L of formalin causes a 1 mg/L reduction in DO. The objective of this study was to determine if there is a decrease in DO in reverse osmosis filtered water or degassed well water without aquatic organisms at 21°C or 28°C treated with a single dose of 25 mg/L or 250 mg/L of formalin. Each treatment, control (CL), 25 mg/L formalin treatment (25F), and 250 mg/L treatment (250F), was performed in triplicate for both water types and temperatures. All tanks began at 100% oxygen saturation or greater. DO decreased in all tanks throughout the study with a slightly larger reduction in DO in 25F and 250F compared to CL. This difference was often statistically significant but not clinically relevant. Including both water types and temperatures, the average decrease in DO was 0.211 mg/L over 72 hours for 25F and 0.108 mg/L over 6 hours for 250F. These findings suggest that a reaction between formalin and water does not cause a clinically relevant decrease in DO. The relationship between DO and formalin is likely multi-factorial and warrants further investigation.

Use of liposomal bupivacaine in cats undergoing gastrointestinal surgery is associated with use of less full mu opioids postoperatively

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This study analyzes the association with the administration of liposomal bupivacaine (Nocita) with postoperative opioid use in cats undergoing gastrointestinal surgery. Additionally, to analyze the administration of antiemetics postoperatively in this study population to qualify the degree of nausea related to opioid administration. Records of 187 cats undergoing gastrointestinal surgery at the Matthew J. Ryan Veterinary Hospital at the University of Pennsylvania and the University of Florida Small Animal hospital were analyzed for Nocita administration at the time of closure, postoperative injectable opioid use, postoperative injectable antiemetic use, and length of hospitalization. In cats that received only full mu opioids, those that were administered Nocita at the time of closure, received significantly less full mu opioids postoperatively. Significantly more cats that received Nocita were managed with full mu + partial mu opioids postoperatively than any other opioid profile. Cats that received Nocita received less ondansetron in hospital compared to cats that did not receive Nocita. There was no significant difference in the length of hospitalization between cats that did and did not receive Nocita. This study suggests that in cats requiring analgesia postoperatively, the use of Nocita at closure reduces the use of full mu opioids postoperatively. Additionally, more patients who received Nocita were de-escalated from full mu agonists to partial mu agonists during their hospital stay, suggesting that less analgesia was required throughout their hospitalization. Lastly, less ondansetron, a typical second-line anti-nausea medication after gastrointestinal surgery, was used in cats who received Nocita, which may suggest less postoperative nausea.

# Computed tomography imaging features are predictive of survival time for canine adrenal tumors

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The assessment of canine adrenal tumors is influenced by diagnostic imaging modalities such as computed tomography (CT); however, data regarding prognostic indicators on CT imaging is currently lacking. To identify CT features associated with or predictive of worse survival for canine adrenal tumors, medical records were reviewed for dogs undergoing adrenalectomy that had a CT scan performed prior to surgery. Features including mass dimensions, shape, margin definition, neovascularization, and vascular invasion were extracted from preoperative CT imaging and were interrogated alongside histopathology and clinical data using Kaplan Meier and Cox proportional hazards survival analysis. In total, 230 tumors across 204 dogs were evaluated with an overall survival time of 671 days. Dogs with bilateral adrenal tumors had worse overall survival (n=26, MST=567d) than dogs with unilateral tumors (n=178, MST=685d). Those that had tumors with poorly defined margins (n=40, MST=605d) had worse overall survival than those with clear margins (n=164, MST=799d). Overall survival was also worse with renal vessel invasion (n=12, MST=201d) versus without (n=191, MST=697d). When comparing dogs based on disease laterality, mineralization was significantly associated with worse survival in left-sided tumors (uHR=2.084, p=0.025), but not right-sided tumors (uHR=0.882, p=0.662). Conversely, having a larger normalized tumor size was associated with worse survival in right-sided tumors (uHR=1.941, p=0.028) but not in left-sided tumors (uHR=1.335, p=0.374). Tumor shape and neovascularization were not significantly associated with prognosis when used in univariate models as predictors for survival.

# Diaphragmotomy Allows for Improved Surgical Access of Canine Liver Hilus: A Cadaveric Feasibility Study

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Hilar liver lobectomy has been associated with increased perioperative and post-operative complications compared to non-hilar liver lobectomy, possibly because of the increased amount of dissection required for hilar tumors and the associated increased risk of hemorrhage. To avoid excessive traction of friable and neoplastic liver tissue, techniques that do not rely on excessive manipulation of the hepatic parenchyma are needed. Diaphragmotomy has previously been reported as a technique for improving hepatobiliary access and visualization and was reported to be both a simple and successful technique. This descriptive study quantifies the amount of hilar liver retraction achieved with and without diaphragmotomy in canine cadavers. Fiducial radiopaque markers were placed on the hepatic coronary ligament and diaphragm. Fluoroscopic images were taken for each cadaver pre-retraction, at retraction, and at retraction post-diaphragmotomy. To assess effect of landmark choice on robustness of hepatic craniocaudal displacement measurements, two reference point methods were used. Method 1 was the cranial edge of the closest rib caudal to the liver. Method 2 was a fixed point on an esophageal measurement catheter. Linear displacement for both methods at the three stages of retraction was estimated by linear repeated measures mixed models. Retraction alone resulted in approximately 3 cm mean linear field increase, and diaphragmotomy coupled with retraction increased linear displacement by 6 to 7 cm. Measurements based on esophageal catheter landmarks consistently exceeded rib-based landmarks by 1 to 3 cm. Diaphragmotomy coupled with manual retraction permits substantially more caudal displacement of the liver hilus than retraction alone.

The Effect of Enteral Nutrition on Length of Hospitalization in Dogs with Severe Acute Kidney Injury: A Retrospective Study of 37 Cases (2011-2023)

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Enteral nutrition (EN) has been previously reported to have benefits in various critical illnesses in dogs such as parvovirus, pancreatitis, and septic peritonitis, including decreased mortality, improved gut barrier function, earlier clinical improvement, and decreased time to discharge. However, the effect of enteral nutrition on dogs with acute kidney injury is not known. This retrospective study from 2011 to 2023 evaluates the effect of EN via nasogastric or esophageal feeding tube in 37 dogs with severe acute kidney injury (AKI) that were hospitalized for greater than 48 hours. Severe AKI was defined as International Renal Interest Society AKI Grade  $\geq 4$  [i.e., increase in serum creatinine to  $\geq 5.1$ mg/dL within 48 hours and decreased urine production (i.e., oliguria: <1mL/kg/h or anuria: no urine production for >6h)]. Main outcomes of interest include length of stay in hospital (LOS) and changes in several key variables throughout hospitalization (body weight, serum values of blood urea nitrogen, creatinine, albumin, potassium, and peripheral blood cell counts). No significant difference in LOS in dogs that received EN via tube feeding when compared to dogs that did not. Concurrent disease(s) and dialysis treatment were associated with longer LOS. Progressive decrease in body weight, body condition score, and albumin were common regardless of nutritional interventions. Throughout hospitalization, no significant differences in trends were detected for BUN, creatinine, phosphorous, potassium, and peripheral blood cell counts. These results demonstrate a lack of benefits with the current EN practices and the necessity of considering other nutritional approaches in dogs with AKI.

# Computed tomographic characteristics of confirmed and presumed noncutaneous pythiosis in 25 dogs

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Pythium insidiosum is an aquatic oomycete that causes granulomatous infection in dogs, most commonly cutaneous and gastrointestinal. Ultrasonographic characteristics of gastrointestinal pythiosis have been described; however, CT features of pythiosis have not been published. The purpose of this retrospective, multicenter, descriptive study is to describe CT characteristics of noncutaneous canine pythiosis. The following CT parameters were recorded: lesion anatomic location, number, shape, margination, size, attenuation pre- and postcontrast, enhancement pattern, lymph nodes affected, other lesions identified, and presence of peritoneal effusion or steatitis. Descriptive statistics demonstrating the frequency of lesion appearances were performed. Twenty-five dogs with noncutaneous pythiosis lesions that underwent CT were included; 19 had primarily gastrointestinal infections, four primarily arterial infections, one intrathoracic and intra-abdominal infection, and one primary pulmonary infection. In dogs with primary gastrointestinal infection, lesions were most common at the ileocolic junction and were most frequently focal, well-defined, moderate to marked circumferential wall thickening that was homogeneous and smoothly marginated precontrast, with moderate heterogeneous contrast enhancement. Most dogs had involvement of multiple gastrointestinal regions. Of four dogs with primary arterial involvement, three had large aneurysmal dilatations of the cranial mesenteric artery with severe mural thickening. All dogs had regional lymphadenopathy, which was variable but generally mild. Nine dogs had peritoneal effusion; six dogs had steatitis. CT features of pythiosis can overlap with neoplasia, but pythiosis should be considered as a differential, especially in young dogs. Findings supported using CT as an adjunct imaging test for increasing clinical suspicion of noncutaneous pythiosis.

# Toggle pin technique for stabilization of ventral coxofemoral luxation in 9 dogs.

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The coxofemoral joint is the most commonly luxated articulation in dogs and cats resulting in up to 90% of all joint luxations. Most frequently, the femoral head is luxated in a craniodorsal direction; however ventral dislocation can occur. Although multiple surgical techniques have been described for coxofemoral stabilization, no ideal technique has been established yet for ventral coxofemoral luxations. In 1953, Knowles et al., described the toggle pin technique for craniodorsal coxofemoral luxations. This technique replaces the round ligament of the femoral head with a prosthetic device until the periarticular fibrous tissue is developed, and the coxofemoral joint becomes stable. This case series described nine dogs who successfully underwent surgery with the toggle pin technique for ventral coxofemoral luxation. Major complications were not observed in any of the patients, and satisfactory one-month post-operative radiographs with non-implant complications were achieved in all patients. Although the toggle pin technique does not seem to be biomechanically superior to other techniques, most surgeons are familiar with this technique. To the authors' knowledge, this is the largest cases series using the toggle pin technique in dogs with ventral coxofemoral luxation. The results of this case series support that the toggle pin technique can be effectively used to repair ventral hip luxation in dogs.

# Significance of Pneumorrhachis Detected by Whole-body Computed Tomography Angiography in Dogs without Trauma

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Pneumorrhachis (PR) is a rare condition in human and veterinary medicine, defined as gas within the spinal canal. Iatrogenic causes are the most common source of nontraumatic PR reported in humans. PR has been anecdotally recognized by the authors in dogs on CT. Our study aims to identify the cause, prevalence, and distribution of PR and intraforaminal gas in dogs undergoing CT, and identify any immediate post-CT complications.

The medical records of dogs who underwent CT of the thorax, abdomen, and pelvis were retrospectively reviewed. Patients were excluded if they presented with a history of trauma, neurologic deficits, recent surgery, or epidural injection. PR and intraforaminal gas were identified as present or absent and quantified subjectively. Patient positioning, intravenous catheter location, and complications in the 24 hours following CT were recorded.

50/263 (19%) dogs with PR met the inclusion criteria. All dogs with PR had CT performed in sternal recumbency. Catheters were evenly distributed in laterality of placement, yet PR was predominantly right-sided (74%). The volume of gas identified was mild (87%) or moderate (13%). An increase in the amount of PR in post-contrast images was documented in 13 cases (20%). Intraforaminal gas was identified in 16.3% of dogs and 88.4% had right-sided intraforaminal gas. No dogs developed neurologic deficits 24-hours post-CT.

The incidence of PR and intraforaminal gas in this study was significantly higher than previously documented. Intravascular right-sided gas is proposed to be secondary to the ipsilateral location of the azygous vein and of no clinical significance.

# Fresh and Refrigerated Cadaveric Preparations are Ideal for Sentinel Lymph Node Mapping

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The objective of this study is to compare sentinel lymph node (SLN) mapping of peripheral lymph nodes between canine cadaveric specimens that were prepared using one of five techniques: frozenthawed, frozen-thawed-heated, refrigerated, refrigerated-heated or fresh. Six adult mixed breed canine cadavers were used for this study. Sentinel lymph node mapping was performed on all cadavers with injection of indocyanine green (ICG) at standardized locations for mapping of the superficial cervical, superficial inguinal and popliteal lymph nodes. Lymph nodes were dissected out to assess fluorescence. The lymphatic tracts were apparent in all cadaveric preparations. Fluorescence was confirmed within the lymph nodes at all sites in the fresh and refrigerated cadavers, regardless of whether heat was applied. Frozen cadavers had inconsistent florescence of the superficial cervical and superficial inguinal lymph nodes, but successful fluorescence of the popliteal lymph node, regardless of heat application or duration of thaw. Although heating of the refrigerated and frozen-thawed cadavers increased the rate of uptake and migration of ICG through the lymphatics tracts, there was no influence on lymph node fluorescence. Results of this study indicate that both fresh and refrigerated cadavers are ideal for use in sentinel lymph node mapping of the superficial cervical, popliteal, and superficial inguinal lymph nodes. Inconsistent fluorescence of the lymph nodes in frozen-thawed cadavers was primarily noted in the lymph nodes closely associated with the axial skeleton (superficial cervical and superficial inguinal), but the popliteal lymph node had consistent successful fluorescence.

# In Silico Comparison of Two Kirschner Wire Arrangements for Stabilization of Femoral Capital Physeal Fractures

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Proximal femoral physeal fractures are commonly stabilized with multiple, parallel Kirschner wires; however, Kirschner wire penetration of the articular surface can result in substantial coxofemoral pathology. The objective of this study was to compare the epiphyseal purchase afforded by a linear or triangular pattern of Kirschner wires for stabilizing femoral capital physeal fractures. Our hypothesis was that Kirschner wires placed in a triangular pattern would result in more epiphyseal purchase. Computed tomography scans of the pelvic limbs of sixteen skeletally immature dogs were obtained. Virtual femoral models were created. For each dog, the left or right femur was randomly selected for analysis. Separate linear and triangular arrangements of three virtual 1.6 mm Kirschner wires were centered on the thickest area of the proximal epiphysis. The length of each Kirschner wire within the proximal femoral epiphysis was measured. Kirschner wire placement in triangular and linear patterns resulted in a mean epiphyseal purchase for each pin of 8.4 mm and 8.0 mm, respectively. Overall pin purchase was greater in the triangular pattern than the linear pattern for 88% dogs (p = 0.005). The mean difference in overall pin purchase was 1.34 mm (p < 0.001). Use of a linear or triangular pattern of Kirschner wires for proximal femoral epiphyseal fracture stabilization resulted in a greater implant purchase within the epiphysis. The triangular pin pattern may reduce the risk of intra-articular implant placement by more closely centering the Kirschner wires within the thickest region of epiphyseal bone.

### Computed Tomographic Features of Double Aortic Arch in Six Dogs

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Double aortic arch (DAA) is a rare, congenital anomaly in small animals, resulting in a complete vascular ring encircling the esophagus and trachea, and subsequent compression of these organs. Few studies have reported utilizing computed tomography angiography (CTA) for diagnosing DAA in dogs; thus, the imaging features are currently lacking in the literature. The objectives of this retrospective, multicenter, descriptive case series were to report the clinical and CTA characteristics of DAA in surgically treated cases. Medical records and CTA images were reviewed. Six juvenile dogs met the inclusion criteria (median age: 4.2 months; range: 2-5 months). The most common clinical signs included chronic regurgitation (100%), decreased body condition (67%) and coughing (50%). Common CTA features of DAA included a dominant left aortic arch (median diameter: 8.1 mm) and minor right aortic arch (median diameter: 4.3 mm) (83%), an aberrant right subclavian artery arising directly from the right aortic arch (83%), segmental esophageal constriction (100%) and variable degrees of dilation cranial to the heart base, and marked tracheal luminal compression (median %change: -55%) (100%) and leftward curvature of the trachea at the level of the bifurcation of the aortic arches (100%). All dogs underwent successful surgical correction with only minor postoperative complications. Due to the similarity of clinical and imaging characteristics described to that of other forms of vascular ring anomalies (VRA), CTA is vital for the specific diagnosis of DAA in dogs.

A prospective case-control study of appendicular osteosarcoma patients treated with course fractionated radiation and standard of care with or without GD3-based liposomal vaccine

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Osteosarcoma is an aggressive mesenchymal tumor of bone, characterized by osteolytic and proliferative lesions, and comprises 85% of bony tumors. Humans and animals with osteosarcoma have a poor prognosis due to metastasis to the lungs despite treatment. Immunotherapy is an adjuvant treatment option to improve quality and quantity of life with comparative application. A prospective case-control study with a ganglioside (GD3) vaccine was conducted at the University of Florida, with rolling enrolment from 2015 to 2019 with follow-up. The objectives were to evaluate the overall survival time (OST) and progression to pulmonary metastasis (PTM) for patients treated with stereotactic radiation therapy (SRT), chemotherapy, with and without the GD3 vaccine. Seven patients received SRT alone, 12 received SRT with chemotherapy and 15 received all three treatments. Data regarding patient demographics, treatments, and clinical signs were collected. There was no significant difference in OST noted between the groups (SRT alone = 221 days, SRT with chemotherapy = 355 days, SRT with chemotherapy and GD3 = 295 days; P = 0.76). PTM showed significant difference between the groups (SRT alone = 140 days, SRT with chemotherapy = 177 days, SRT with chemotherapy and GD3 = 204 days; P = 0.059), although this may be confounded by the SRT only group, which is not standard of care. A multivariate analysis revealed significance in breed, although groups were small. The lack of OST significance may be due to Zoledronate administration, which can inhibit the immune response, impacting immunogenic stimulation. Further research is needed to evaluate the efficacy of the GD3 vaccine.

# Computed Tomographic and Magnetic Resonance Imaging Features of Benign and Malignant Aortic Body Paragangliomas in 44 Dogs

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Aortic body paragangliomas (ABPGLs) are the most common heart base tumor in dogs; however, descriptions of their CT and MRI findings are lacking. Although generally considered benign, ABPGLs can present as malignant neoplasms with local invasion and metastasis. The objectives of this study were to describe the CT and MRI features and topography of confirmed benign and malignant ABPGLs and identify significant features associated with malignancy. Forty-four dogs with 43 CTs and 3 MRIs were included. Most dogs were males and less than half were brachycephalic breeds. ABPGLs were mostly heterogeneously contrast-enhancing, well-marginated, lobular or ovoid, and frequently cavitated. Local invasion was noted in 11 cases and confirmed metastatic lesions in 13 cases. The most common sites of confirmed metastasis were local lymph nodes, heart, and bones. ABPGLs frequently displaced and compressed regional structures, and less often encircled vasculature. Pericardial and peritoneal effusion were significantly associated with confirmed metastasis, and encircling of vessels, mediastinal effusion, and right-sided congestive heart failure were significantly associated with invasion. Based on ROC analysis, tumor width >4.4 cm and pre-contrast attenuation >49.9 HU had a sensitivity of 91% and 82% and specificity of 52% and 72%, respectively, for vascular/cardiac invasion. MRI features included variably T1 and T2 hyperintense parenchyma and hypointense flow voids attributed to intratumoral vessels. ABPGLs present as lobular or ovoid, heterogeneously contrast-enhancing, often cavitated, middle or combined cranial/middle mediastinal masses with variable mass effect and the potential for local invasion and metastasis.

Prevalence of and risk factors for antimicrobial-resistant fecal bacteria in beef cattle in cow-calf operations: A systematic review

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**Background:** This systematic review aims to identify the prevalence of and risk factors for antimicrobial-resistant (AMR) fecal bacteria in beef cattle in cow-calf operations.

Methods: Inclusion criteria determined for prevalence studies were condition (antimicrobial-resistant fecal bacteria), context (cow-calf operations), and population (beef cattle in cow-calf operations). Inclusion criteria determined for risk factors were population, exposure (antimicrobial use, biosecurity, management practices), and outcome. Peer-reviewed publications were identified in Agricola, Embase, PubMed, and Web of Science databases. The last search for all four databases was performed on May 19, 2023. A critical appraisal of all selected studies was conducted using a standardized critical appraisal checklist for prevalence and cross-sectional studies separately. The results of this systematic review were synthesized narratively.

**Results:** A total of 12 cross-sectional studies conducted between 1997-2020 were included for data extraction, critical appraisal, and synthesis. These studies were conducted in the USA (n = 4), Canada (n = 6), Italy (n = 1) and South Africa (n = 1). In the studies selected, Escherichia coli was the most frequently reported enteric bacteria (n = 9), followed by Salmonella (n = 3), Enterococcus (n = 2), Campylobacter (n = 2), and ESBL/AmpC-producing E. coli (n = 1). The prevalence of AMR fecal bacteria varies between 0.1% to 100% against different antimicrobials. Use of antimicrobials, farm size, body condition score, age, and location of farms were potential risk factors associated with AMR fecal bacteria in beef cattle in cow-calf operations.

**Discussion:** The prevalence of and risk factors associated with AMR fecal bacteria in cow-calf operations are inconsistent due to the heterogeneity in the study populations, study designs, outcome measures, and study risk of bias. However, a high prevalence of AMR fecal bacteria in cow-calf operations could potentially be transferred to the next level of beef production. The results of this systematic review can inform beef producers about their management practices.

# Effect of Phytochemicals in Mitigation of Horizontal Gene Transfer of Plasmids Harboring Antimicrobial Resistance Genes between Foodborne MDR Salmonella and Commensal E. coli

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The presence of antibiotic-resistant (AR) bacteria in animal-derived foods raises a significant public health concern. Despite implementing diverse mitigation strategies and banning the use of antimicrobials as growth promoters in livestock, antibiotic resistance genes (ARGs) persist in the environment. Poultry gut serves as the reservoir for various ARGs, with their persistence facilitated by conjugation-based horizontal gene transfer (HGT) among various bacterial genera. To mitigate the spread and acquisition of ARGs by commensals from AR bacteria, our research focuses on utilizing infeed supplementation-based strategies to address ARGs dissemination in the poultry gut without affecting the intestinal microbiota.

The study aimed to investigate the effect of three generally recognized as safe (GRAS) phytochemicals, namely, cuminaldehyde, zingerone and ferulic acid, in reducing HGT of ARG harboring plasmid between multidrug-resistant (MDR) Salmonella Heidelberg (blaCTX-M1) and commensal bacteria. Sub-inhibitory concentrations (SIC) of phytochemicals in a bacterial broth system co-cultured with MDR S. Heidelberg and commensal E. coli, revealed that only cuminaldehyde exhibited effectiveness in reducing HGT frequency of transconjugant E. coli (p < 0.05). A qPCR analysis evaluating Salmonella conjugal transfer (tra) gene expression revealed downregulation of functional pilus biogenesis genes. To validate phytochemical activity, surface pili visualization and TraA protein semi quantitation studies will be performed using-Atomic Force Microscopy and Liquid Chromatography–Mass Spectrometry (LC-MS), respectively. Further, in vivo chicken experimental trials will analyze the persistence and spread of ARGs in the cecal gut microbiota using metagenomic sequencing and proximity-ligation methods.

# Chromogenic In situ Hybridization for the Identification of Oomycetes in Formalin-Fixed and Paraffin-Embedded Specimens

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Pythium insidiosum, Lagenidium giganteum forma caninum, and Paralagenidium karlingii are oomycetes that cause life-threatening cutaneous and mucocutaneous infections in mammals. These fungus-like organisms exhibit different behavior in tissues. P. karlingii usually has slow growing and infection restricted to the skin, whereas L. giganteum f. caninum and P. insidiosum often promote more severe and invasive lesions. Thus, treatment regimens and responses vary according to the oomycete species resulting in these infections. Conventional diagnosis relies on serology, oomycete culture, and histology combined with panfungal PCR, followed by DNA sequencing, that is required for species-level identification. Formalin-fixed, paraffin-embedded (FFPE) tissue usually results in poor DNA/RNA quality due to formaldehyde reactions occurred during tissue fixation. This degradation affects DNA amplification, resulting in low yield of the DNA product, and interfering with DNA sequencing, increasing turnaround time, labor, and costs. Since *L. giganteum f. caninum* and the novel *P. karlingii* were identified, not much progress has been achieved towards the diagnose approach of oomycosis in FFPE material. Our goal is to implement chromogenic in situ hybridization (CISH) based on AMPIVIEW<sub>TM</sub> RNA probes to identify these 3 common oomycetes in FFPE specimens in a single plex and a multiplex approach. The use of genus/species-specific probes will add another option to improve diagnosis of oomycosis, allowing clinicians to predict lesion progression and select treatment options for these infections in animals more accurately.

# Assessment of a Novel Poultry Salmonella Vaccine Candidate: Efficacy, Immunogenicity, and Future Prospects

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Salmonella is a leading cause of foodborne disease worldwide, ranking among the four primary contributors to diarrheal disease in humans. Poultry eggs and meat have been identified as the most common sources of foodborne salmonellosis in humans. Consequently, the poultry industry is consistently under pressure to implement rigorous measures to reduce Salmonella colonization of poultry and fecal shedding. Currently, the poultry industry relies on vaccination, biosecurity practices, and screening as primary tools for controlling Salmonella. Although vaccines improved pre-harvest control of Salmonella in poultry, they were not as effective as the industry and regulatory authorities desired. Therefore, there is a need for novel Salmonella vaccines that can help poultry producers to achieve their food safety targets.

Our laboratory has previously recognized InvG, a structural protein of the type 3 secretion system of *Salmonella*, as a potential vaccine candidate. This study entails a preliminary assessment of the immunogenicity and efficacy of InvG in commercial layer chickens. Chickens were vaccinated three times intramuscularly with recombinant InvG at the ages of 2, 4, and 6 weeks and then challenged with *Salmonella* Enteritidis orally one-week post-vaccination. The vaccinated chickens elicited a robust serum antibody (IgG) response against InvG. Further, there was a reduction of Salmonella counts in the ceca and spleen of vaccinated chickens compared to the unvaccinated control group. However, the mucosal antibody response (IgA) in the intestinal mucosa was poor. Based on our preliminary data, the next step is to develop a live-attenuated vaccine expressing InvG that would further improve the immune response, including the intestinal IgA response, and confer better protection against Salmonella colonization of poultry and their fecal shedding.

# Multi-state SARS-CoV-2 Surveillance in Bats in the Southeastern and Northeastern United States at the Human-Bat and Animal-Bat Interface

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Emerging bat-borne viruses are at the forefront of global health concerns, as epitomized by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. Although there has been an increase in bat-related virus surveillance in many countries due to bats being recognized as important reservoirs for zoonotic viruses, we know little regarding the viruses that normally circulate in U.S. bat populations, including whether SARS-CoV-2 may have been introduced into native bats during the pandemic and the epidemiological ramifications of such an event.

The overarching goal of this project is to monitor bat species in the southeastern and northeastern U.S. for SARS-CoV-2 infection and determine the risk it may pose if identified in native bat species. Bats are acquired through state health departments (human-bat interface) or wildlife agencies (animal-bat interface) prior to necropsy. Internal tissues are removed from each bat and used for up to four purposes: (i) deep sequencing (RNA-Seq) to detect viral RNA reads via bioinformatics, (ii) virus isolation to propagate virus for biological studies, (iii) formalin-fixed paraffin-embedded tissues for microscopic evaluation (histology) of viral infection, and (iv) development of a tissue repository for longitudinal or retrospective studies.

Surveillance studies of free-ranging bats are an effective way of determining the bat species that may be important in maintaining SARS-CoV-2 in nature and monitoring for the potential emergence of novel strains with altered phenotypes (e.g., distinct antigenicity). Importantly, this study will also identify other viruses naturally occurring within U.S. bat populations which may be of public or veterinary health concern.

# Prevalence, Diversity, and Biological Characterization of Bat Alphacoronaviruses in the United States

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Bats serve as natural reservoirs for a diverse array of coronaviruses, including severe acute respiratory syndrome (SARS)-related coronaviruses. Although an immense amount of research has been conducted on SARS-related coronaviruses (genus Betacoronavirus) owing to their pandemic disease potential, comparatively little is known regarding bat coronaviruses classified in the genus Alphacoronavirus, a lineage related to betacoronaviruses. Interestingly, human alphacoronaviruses such as HCoV-NL63 are known to share phylogenetic relatedness to alphacoronaviruses in bats, suggesting previous cross-species transmission. However, the prevalence and diversity of bat alphacoronaviruses which are endemic to the U.S. remains largely unknown. This study aims to identify bat species in the U.S. that harbor alphacoronaviruses through a comprehensive multi-state surveillance system, followed by biological assays to elucidate the viral life cycle within various hosts. As bat alphacoronaviruses in the U.S. have never been isolated in cell culture, nor do any reverse genetics systems currently exist to create infectious clones of these viruses, they have yet to been studied biologically. To address these deficiencies, we will investigate their host range and relative infectivity using retroviral pseudotypes and reverse genetics. Given the limited surveillance and research on bat alphacoronaviruses in the U.S., this study addresses a significant knowledge gap. Importantly, it lays the groundwork for a comprehensive understanding of the geographical and temporal distribution of bat alphacoronaviruses in the U.S., as well as potential risk factors (if present) that may lead to cross-species transmission to other animals and/or humans, contributing to improved strategies for disease surveillance and potential outbreak management.

# Structure-activity relationship studies reveal potent antibiotic activity of tolfenpyrad derivatives that target *Francisella*

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Francisella tularensis is a highly infectious and deadly intracellular bacterial pathogen that establishes an infection with few as ten viable bacteria. Due to its extreme virulence and potential for weaponization, the identification of new antibiotics against Francisella is essential for global health security. We previously discovered that Tolfenpyrad, a pesticide used worldwide, displays antimicrobial activity that specifically targets Francisella species. To improve safety and potency, we performed structure-activity relationship studies on 262 synthesized analogs based on the Tolfenpyrad scaffold. Though these analogs have diverse chemical structures, none of them exhibits activity toward closely related Gramnegative bacteria. Mutations in two genes that make Francisella resistant to tolfenpyrad also confer resistance to these compounds, indicating that they share a common mechanism of action with Tolfenpyrad. Compared to Tolfenpyrad, 10 compounds display greater than 5-fold better antibiotic activity. We employed stringent conditions to evaluate the toxicity of these compounds to cultured macrophages and found three drugs that demonstrate potent efficacy against Francisella without affecting viability of host cells. These compounds potently inhibit intracellular growth of *Francisella* in immortalized bone marrow-derived macrophages. These compounds not only serve as promising candidates for the development of anti-Francisella therapeutics but also provide valuable tools for uncovering the intricate mechanisms of bacterial pathogenesis. Future discovery of the bacterial target(s) of these drugs will open doors to the development of targeted interventions against this highly virulent pathogen.

# One-Pot RT-LAMP CRISPR/Cas12b Platform for Rapid Detection of Tilapia Lake Virus

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Tilapia lake virus disease (TiLVD) is a viral disease that has been associated with high morbidity and mortality in cultured and wild tilapia worldwide. Although several diagnostic tools have been developed for TiLV detection, they require advanced equipment and time-consuming procedures, making them impractical for laboratories with limited resources or pondside use. Addressing this challenge, we have developed and partially validated an innovative, rapid, and cost-effective one-pot diagnostic assay that combines thermostable Cas12b enzyme with RT-LAMP amplification, targeting a conserved region within segment 4 of the genome. Notably, this assay can be conveniently used, as the incubation process is done at 62°C for 75 minutes, and the results can be observed using an inexpensive portable fluorescence viewer. The TiLV one-pot assay is sensitive and specific, with a limit of detection of 50 RNA viral copies, and no cross-reaction was detected with other fish DNA and RNA viruses. In addition, the assay could also detect 12 TiLV transcripts from other regions despite primer mismatches. The analysis of 98 positive and 94 negative samples previously determined by TaqMan qPCR assay resulted in both diagnostic sensitivity and specificity of 96.9% and 100%, respectively. Additional samples from different populations or field outbreaks will be utilized to validate the diagnostic performance of the assay further. The present study outlines a report on the development and partial validation of a diagnostic assay for TiLV in stages 1 and 2, following the guidelines from WOAH. Finally, this assay can serve as a valuable tool in global surveillance efforts that fish health professionals can easily employ to screen, monitor, and control TiLV disease in tilapia production.

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A novel murine model to assess the role of inflammation and coagulation during the Malariaassociated respiratory distress syndrome

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Malaria is a bloodborne parasitic disease caused by several human infectious Plasmodial species mostly transmitted to humans by a single mosquito bite. Malaria-associated acute respiratory distress syndrome (MA-ARDS) is a complication of severe malaria, characterized by pulmonary edema, inflammation, hemorrhages, and alveolar damage. Seeking to understand the MA-ARDS pathogenesis pathways that lead to pulmonary lesions and vascular leakage, the proposed research aims to develop an experimental mouse model that can help us elucidate the underlying pathogenic mechanisms of lung malaria to determine if sexual dimorphism and pregnancy can lead to more severe pathology. As our first aim, we will establish the role of innate immune cells and parasite byproducts leading to vascular leakage and hemorrhages in an in vivo model lacking expression of tissue factor, the primary initiator of blood coagulation. We hypothesized that altered tissue factor expression would suppress the inflammatory response and endothelial disruption, resulting in reduced vascular leakage in the lung. We will implement a murine model, pregnant and nonpregnant, with genetically modified tissue factor expression. These mice utilize cre recombinase under the control of the Tie2 (or Tek) promoter to delete the tissue factor gene, which has been floxed. We will assess disease progression, measure gene expression of inflammatory targets, and conduct histology to characterize vascular leakage, hemorrhage, and hemozoin accumulation. This study will provide a suitable experimental murine model to better understand what are the mediators and pathogenic pathways of lung malaria, aiming to provide the basis for the development of therapeutic treatments against this severe and life-threatening manifestation of malaria.

# Characterizing The Resistome of Farmed White-Tailed Deer (*Odocoileus virginianus*) in Florida, USA

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Antimicrobial resistance (AMR) constitutes one of the most urgent public health concerns of the 21st century, with many healthcare leaders suggesting that the post-antibiotic era is already here. Antibiotics and antimicrobials are commonly employed in agriculture as well as human and veterinary medicine, thus facilitating the spread of antibiotic resistance genes (ARGs) within humans, companion animals, livestock, wildlife species, and the environment. White-tailed deer (WTD) farming in Florida provides significant benefits to both the economy and the environment; however, the common use of antimicrobial agents in farmed WTD, alongside their proximity to urban, agricultural, and sewage areas, can create a high selective pressure for AMR. Understanding the resistance profiles and patterns in these populations is critical in promoting the health and productivity of these managed cervids, as well as the health of endemic wildlife and the ecosystem where they live. Hence, the purpose of this work is to analyze and characterize the resistome of Florida-farmed WTD, one of the most important game species in North America. Escherichia coli is a suitable candidate to study AMR in WTD due to its status as an indicator strain, its nonpathogenic nature, and its ease of culturability. The E. coli strains were isolated from various tissue samples, including kidney, lung, liver, and heart, during necropsy of the WTD. A NovaSeq high-throughput sequencing system was utilized to determine the E. coli genome. The raw pair-end reads were mapped to known ARGs using the AMR++ v 3.0 pipeline, and the ResistoXplorer tool was implemented for data normalization, analysis, and visualization. Our results suggest that the diversity and variation of ARGs found for each experimental group depends on the resistance level. Higher diversity and variation were observed at lower resistance levels (e.g., group and gene) compared to higher resistance levels (e.g., class or mechanism). Just under 400 unique ARGs were identified, conferring resistance to 16 classes of antibiotics by 31 mechanisms. The three most abundant classes for all samples were beta-lactams, multi-drug resistance, and bacitracin. The current study is the first to analyze and characterize the resistome of farmed WTD in Florida. Further research has been planned to determine the phenotype of the observed ARGs through antimicrobial susceptibility testing.

Multisystemic disease and septicemia caused by *Burkholderia cepacia* complex in an American Quarter Horse (*Equus caballus*)

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The Burkholderia cepacia complex (Bcc) is a heterogeneous group of Gram-negative rod-shaped bacteria from the Burkholderia genus that typically cause opportunistic infections in immunocompromised hosts. Outbreaks of disease often occur in the hospital setting via contaminated medical devices or in patients with chronic respiratory diseases such as cystic fibrosis. Bcc infections are often refractory to antibiotic treatment, and the disease can progress to life-threatening septicemia. A recent increase in case reports in healthy humans and animals suggests Bcc may be an emerging pathogen of concern. An 8-year-old quarter-horse gelding presented to the University of Florida Veterinary Medical Center with the chief complaint of a left retropharyngeal abscess. Clinical examination and radiographs revealed a well-circumscribed 20x10x18 cm round soft tissue mass caudal to the ramus of the mandible with displacement of the guttural pouch. Other exam findings included diffuse interstitial pneumonia, multiple cutaneous ulcers on the dorsal midline, anterior uveitis in the right eye, enlarged mesenteric lymph nodes, and suspected aneurysm of the right renal artery. Due to a declining clinical condition, the animal was humanely euthanized. Post-mortem aerobic culture of the retropharyngeal and mesenteric lymph nodes revealed pure growth of *Burkholderia cepacia*. This finding was confirmed with a fatty acid analysis of the bacterial isolate. This report describes the histomorphologic identification, culture, and characterization of an unusual case of Bcc. This case underscores the potential pathological impact of Bcc in horses, emphasizing the need for increased awareness and understanding of its emergence as a potential pathogen in diverse species.

# Transcriptomic profiling of antimicrobial resistant Salmonella Heidelberg in the presence of commensal Escherichia coli

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Non-typhoidal *Salmonella enterica* serotype Heidelberg is a major foodborne pathogen primarily transmitted to humans through contaminated poultry products. Current control measures emphasize novel approaches to mitigate S. Heidelberg colonization in poultry and contamination of poultry products thereby reducing the transmission to humans. In the recent years, increase in antimicrobial resistant (AMR) non-typhoidal Salmonella, including S. Heidelberg has become an additional public health concern. In light of this, it is important to understand the role of intestinal commensal bacteria in modulating, fitness, virulence and antibiotic resistance of AMR Salmonella. To explore the effect of commensal Escherichia coli on AMR Salmonella and vice versa, we co-cultured poultry strains of commensal E. coli and S. Heidelberg and performed an in-depth analysis of their transcriptomes using high-throughput RNA sequencing. In the context of co-culturing, the study identified 4,890 differentially expressed genes in S. Heidelberg when co-cultured with Escherichia coli. Among these, 192 genes were found to be downregulated and 202 were upregulated, following the filtration of expression data. Interestingly, the genes involved in pathogenicity, virulence, biofilm formation, growth, metal-ion hemostasis, signal transduction, chemotaxis, stress response, and transmembrane transport of xenobiotics were down-regulated one to eighty-six folds in S. Heidelberg. Importantly, a downregulation of one to twelve folds of genes involved in AMR and drug efflux was also noted in Salmonella Heidelberg. These findings highlight that commensal E. coli can potentially reduce the fitness, persistence, and virulence of S. Heidelberg, and can be used to mitigate AMR S. Heidelberg in poultry.

Keywords: Antibiotic resistance, commensal Escherichia coli, Salmonella Heidelberg, fitness, virulence

An enhanced vaccination program to control brucellosis in cattle is cost-effective in pastoral, semiintensive, and zero-grazing farm systems in Rwanda

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Brucellosis in livestock is a disease of paramount importance to animal and human health authorities due to its socio-economic and public health consequences. In livestock, Brucella spp. infection can cause abortion and reduced milk yield. Brucella spp. infection can be transmitted to humans through the consumption of unpasteurized milk or milk products from an infected animal, and by direct contact with infected animals, particularly aborted fetuses. The socio-economic consequences of Brucella spp. infections include forced unemployment and cost of treatment in infected people, reduced livestockrelated household income and access to milk, as well as interruption of trade of animals and animal products. In Rwanda, the estimated prevalence of brucellosis in cattle is 18% (pastoral farm system), 6.5% (semi-intensive), and 4% (zero-grazing). Because of limited funding, the current vaccination program targets about 15% of all female calves in the national herd by using the *Brucella abortus* strain RB51 vaccine. The objective of this study is to estimate the benefit-cost ratio (BCR) from adopting an enhanced vaccination program for brucellosis control in cattle in all three farm systems, where all female calves (100%) are vaccinated. Study results indicate that vaccination is cost effective in cattle in all three farm systems; the BCR were 2.08, 1,54, and 1.15, respectively. My presentation will include highlights of disease transmission models, livestock production models, and financial analyses used, as well as policy options that can be considered by animal health authorities for possible implementation.

# Effects of heat abatement during the pre-weaning on post-weaning and first lactation performances

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Heat stress abatement strategies for pre-weaned dairy calves are seldom evaluated. We conducted an experiment to evaluate the effects of provision of cooling through fans to calves housed under a barn on post-weaning and first lactation performances. Female Holstein calves from a commercial dairy in southern GA were assigned randomly at birth (d 0) to 1 of 2 treatments: individual hutch in a barn with no cooling (SH = 125) vs. individual hutch in a barn with ceiling fans (SHF = 101). All calves were housed in frame-wire hutches placed under the same barn and treatments were applied in 3 alternating sections of the barn. Fans (diameter = 2.1 m) were 4.1 m from the ground and 7.6 m distant from each other (center-to-center). Shade cloths (80%) separated the sections of SH and SHF treatments. Post-weaning, all heifers were managed identically. Body weight (BW) and ADG were recorded at weaning, 5, 7, and 10 months of age. Pregnancy to first AI (P/1AI) and hazard of pregnancy of heifers and hazard of starting the first lactation are reported. Body weight at first calving, P/1AI, hazard of pregnancy, and milk yield are reported for the first lactation. In the current experiment, cooling the environment during the pre-weaning phase through fans negatively impacted the reproductive performance and overall survival of Holstein females.

### Comparison of end-to-side versus side-to-side jejunocecostomy in healthy horses

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Jejunocecostomy (JC) is frequently used in horses, either as an end-to-side (ES) or side-to-side (SS) anastomosis. However, published reports provide contradictory results about the performance of these methods in clinical cases. Previous research has also demonstrated that the larger stoma size achieved with the SS method could reduce postoperative complications. The objective of the study is compare ES JC versus SS JC in healthy horses to and including necropsy at 28 days after surgery. After a brief exploratory celiotomy, either an ES JC (7 horses) or stapled SS JC (7 horses) was completed by random assignment after jejunoileal resection. At surgery, surgical time was recorded, and the outer length of the anastomosis was measured. At euthanasia 28 days postoperatively, the anastomosis was measured and photographed. One horse in the ES group was euthanized at 4 days after surgery because of a jejunal infarction attributed to a technical error. Postoperative colic or reflux did not develop in remaining horses. The stoma produced by SS JC was significantly larger than the ES JC at necropsy (P=0.0009), was significantly faster to complete (P=0.002), and had a shorter total surgery time (P=0.022). However, the ES anastomosis was of satisfactory size and functioned well. Both anastomoses performed well, both were acceptable, and the ES would provide an alternative in the absence of staplers. Specific steps in the ES method were used to maximize stoma size.

## Individual and Combined Effects of Metritis and Clinical Endometritis on Dairy Cow Profitability

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This study aimed to evaluate the individual and combined effects of metritis (MET) and clinical endometritis (CE) on dairy cow profitability. Cows (n = 11,051) were classified as having no uterine disease (NUD), only MET, only CE, or both MET and CE (METCE). Gross profit was calculated as the difference between incomes and expenses. Incomes were calculated from milk production, cow sale value at culling, and residual cow value by 305 DIM. Expenses were divided into feed, reproductive, and replacement costs. Cows in the METCE group produced less milk (P < 0.01) than cows in the NUD group (9,514 vs. 10,109 kg/cow). Compared with NUD (82 %), the proportion of cows pregnant was smaller (P < 0.01) in MET (76 %), CE (75 %), and METCE (67 %). No difference (P > 0.05) was observed between the MET and CE groups. The proportion of cows leaving the herd was greater (P < 0.01) in MET (30 %), CE (31 %), and METCE (38 %) than in NUD (25 %). No difference (P > 0.05) was observed between the MET and CE groups. Finally, gross profit per cow was less (P < 0.01) in MET (\$3,905), CE (\$3,921), and METCE (\$3,627), compared with NUD (\$4,084). No difference (P > 0.05) was observed between the MET and CE groups. Uterine diseases impacted the profitability of dairy cows by being associated with diminished milk yield, reduced reproductive efficiency, and compromised herd survival. The combination of MET and CE led to additive negative effects.

# A Case of Severe Abomasal Sand Impaction in a White-tailed Deer (*Odocoileus Virginianus*) in Florida

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The University of Florida's Cervidae Health Research Initiative (CHeRI) was engaged to conduct a post-mortem examination on a 2-year-old white-tailed doe discovered deceased at a northern Florida white-tailed deer farm. The carcass of the deer displayed slight emaciation and more bloating than anticipated. Upon opening the carcass, clear signs of lung infection were evident, and the rumen was notably enlarged. Additionally, a distinct distension of the abomasum was observed, which, upon further examination, was found to be filled with sand, weighing approximately 5kg. It's not uncommon for white-tailed deer to engage in geophagia (eating soil or sand), which typically does not result in diseases or fatalities. However, in this particular instance, we suspect it was a chronic case that created a physical barrier, hindering nutrient absorption and leading to persistent inflammation of the serosa and mucosal stomach epithelial layer. This condition allowed the colonization of secondary bacteria, ultimately resulting in the death of the animal.

# Reproductive performance and herd removal of lactating dairy cows with anatomical defects in the cranial vagina and cervical ostium

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Female reproductive tract anomalies and pathological conditions have been increasingly studied in human and animal species because of its possible association with fertility. However, studies evaluating such conditions in dairy cows remain sparse. This study aimed to evaluate the association between cervical and vaginal conditions in dairy cows and the odds of receiving artificial insemination (AI), pregnancy, and reproductive culling (DNB), and leaving the herd. Lactating Holstein cows (n=3,618) were evaluated vaginally between 0 and 70 days in milk (DIM). Independently of DIM, 5.6% of cows were diagnosed with anatomical anomalies (adhesion, double cervical ostium, masses, scar tissue, urovagina, vaginal septum). Prevalence of cervical prolapse ranged from 52.3 to 67.7% among DIM categories. Proportion of cows with well-defined cervical folds increased and cervical bruising decreased as lactation progressed. Cows with well-defined folds from 0 to 7 DIM had lower odds (P=0.04) of becoming pregnant by 305 DIM. Cows with urovagina tended to (P=0.07) have lower odds of becoming pregnant and greater odds (P=0.02) of becoming DNB compared with cows without urovagina. The proportion of cows that received AI, became pregnant after first AI, or became pregnant, DNB, or left the herd by 305 DIM were not associated with most visual characteristics evaluated in the present study.

#### Surveillance of Bovine Leukemia Virus Prevalence in Florida Dairy Herds

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**Objective:** Determine the prevalence of bovine leukemia virus (BLV) infection in dairy herds in Florida. **Methods:** This study collected samples continuously over a six-month period from a total of 13 herds, with each herd contributing 40 cows, resulting in a total sample size of 520 (n = 520). Ten cows from each lactation, from the first to those in the fourth or more lactations, were randomly selected for testing. Blood samples were obtained from tail vessels using evacuated tubes without anticoagulants and allowed to clot for one hour, then centrifuged at 3,000 g for 10 minutes to separate the serum and one aliquot was sent to the Iowa Veterinary Diagnostic Laboratory at Iowa State University for BLV (Bovine Leukemia Virus Antibody Test Kit, ELISA, VMRD Inc, Pullman, Wash). Dry cows or cows with 21 days of lactation were excluded from the study as false negatives are more likely in this specific group. The prevalence of BLV was compared between lactations using the Chi-square method from the stats package of R studio (Version 2023.09.1). P-values were adjusted for multiple tests using Bonferroni corrections.

**Results:** The study found an average BLV prevalence of 66.5%, ranging from 36.2% to 83.1%. When assessing parity, BLV prevalence was lower in first lactation (36.2%) compared to second (62.3%), third (84.6%), and fourth or greater lactations (83.1%). BLV prevalence in the second lactation was lower than in the third and fourth or greater lactations. No significant difference in BLV prevalence was observed between the third and fourth lactations.

**Conclusion:** The study concludes that BLV prevalence varies across different lactation stages, with higher prevalence correlating with more lactations

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#### Validation of an Intra-Bladder Optogenetic Device in TRPV1-Ai40 Mice

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Optogenetics is a technique that involves the genetic expression of light-activated proteins to modulate cellular activity. In bladder research, optogenetics is being used to investigate how neuronal activity influences bladder function. However, the need for light penetration of deep tissue in freely moving animals has posed continuous challenges in this field. This work aims to validate small (~35mm), flexible, biocompatible, intra-bladder optogenetic devices wirelessly powered to emit green light (~575 nm) in vivo. We validate these devices using a mouse model that expresses ArchT in TRPV1 lineage neurons (TRPV1-Ai40), which are primarily nociceptive sensory neurons. When ArchT-EGFP becomes expressed upon exposure to green light, it inhibits the neural activity of nociceptive neurons. By implanting this device directly within the bladder, we aim to forego previous challenges of light penetration, isolate optogenetic inhibition of the TRPV1 ion channel, and determine the effects of nociceptor inhibition on pain and voiding behaviors. We hypothesize that activation of ArchT in these neurons will decrease nociception and voiding frequency following bladder inflammation. The devices are validated with behavioral assays, including 20-minute place preference and 2-hour void assays before and after cyclophosphamide injections a week after implantation. Preliminary data (n=9) has shown patterns of improved voiding behavior upon exposure to green light both before and after cyclophosphamide, indicating the functionality of the device. This work demonstrates how utilizing optogenetics to manipulate particular neural functions in bladder physiology can be a powerful tool for further research in pain, physiology, and optogenetics.

### Species-Specific Profiles of Per- and Polyfluoroalkyl Substances (PFAS) in Small Coastal Sharks Along the South Atlantic Bight of the United States

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Per- and polyfluoroalkyl substances (PFAS) have gained widespread commercial use across the globe in various industrial and consumer products, such as textiles, firefighting foams, and surface coating materials. Studies have shown that PFAS exhibit a strong tendency to accumulate within aquatic food webs, primarily due to their high bioaccumulation potential and resistance to degradation. Despite such concerns, their impact on marine predators like sharks remains underexplored. This study aimed to investigate the presence of 34 PFAS in the plasma (n=315) of four small coastal sharks inhabiting the South Atlantic Bight of the United States (U.S). Among the sharks studied, bonnetheads (Sphyrna tiburo) had the highest  $\Sigma$ PFAS concentration (3031 ± 1674 pg g–1 plasma, n = 103), followed by the Atlantic sharpnose shark (Rhizoprionodon terraenovae,  $2407 \pm 969 \text{ pg g} - 1$ , n = 101), blacknose shark (Carcharhinus acronotus,  $1713 \pm 662$  pg g-1, n = 83) and finetooth shark (Carcharhinus isodon,  $1431 \pm$ 891 pg g-1, n = 28). Despite declines in the manufacturing of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the long-chain (C8 – C13) perfluoroalkyl acids (PFAAs) were frequently detected. Furthermore, males exhibited significantly higher ΣPFAS concentrations than females in bonnetheads (p = 0.001037), suggesting possible sex-specific PFAS accumulation or maternal offloading in some species. The results of this study underscore the urgency for more extensive biomonitoring of PFAS in aquatic/marine environments to obtain a comprehensive understanding of the impact and fate of these emerging pollutants on marine fauna.

#### Mapping of neural network responses to urothelial stimulation

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Urothelial cells play an active role in bladder filling, responding to stretching and releasing neurotransmitters to activate sensory neurons. Numerous bladder diseases, including overactive bladder, pain related to a recurrent bladder infection, chemotherapeutic cystitis, and bladder pain syndrome, are suspected to disrupt urothelial sensory signaling, leading to pathological changes to sensory signaling including pain. An exploration of signaling to the central nervous system (CNS) is necessary to understand these complexities. This approach will chart pathways by which afferent pain signals interpret stimuli and characterize interactions between urothelial components of regional visceral pain. In the urothelium, the UPK2-ChR2 line expresses channelrhodopsin 2 (ChR2), a lightactivated cation channel. We used the chemotherapeutic drug, cyclophosphamide (CYP), as an inflammatory agent to induce acute cystitis and to replicate nociceptive conditions. The correlations to the CNS are confirmed by Blood-Oxygen-Level-Dependent (BOLD) signaling via an 11.1T fMRI. Our preliminary results indicate urothelial stimulation evokes a different neural pattern of activation before and after inflammation of the bladder. Pre-treatment groups exhibit a direct pathway in micturitionassociated regions. Mapping of post-treatment groups communicates alternative functional connectivity patterns in response to stimuli under disease conditions, indicating a change in urothelial signaling under cystitis.

#### Investigating Angiotensin Type 1 Receptor in Experimental Autoimmune Cystitis

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Interstitial cystitis/bladder pain syndrome (IC/BPS) is a severe condition with limited treatment options. Research reveals that IC/BPS patients often exhibit heightened bladder inflammation, mast cell activity, fibrosis, and oxidative stress. Interestingly, similar pathological changes occur in other organs, like the lungs, due to increased angiotensin signaling via the type 1 receptor (AT1R). However, the role of angiotensin signaling in bladder function remains unclear. Using the Multi-Disciplinary Approach to the Study of Chronic Pelvic Pain Research Network (MAPP), we analyzed IC/BPS reports and the use of anti-hypertensive drugs in hypertensive patients. The MAPP data revealed that hypertensive patients not taking anti-hypertensive medications reported more severe bladder pain symptoms. In addition to the MAPP data, we used a mouse model of bladder autoimmune cystitis, where we assessed alterations in AT1R expression after disease onset, employing RT-qPCR and histology to measure cell markers, AT1R gene expression, and fibrosis. Furthermore, our mouse studies illustrated AT1R expression alongside various cell markers, suggesting numerous bladder cell types may be involved in angiotensin signaling. Additionally, ex vivo bladder contraction assays confirmed the AT1R functional role. Preliminary RT-qPCR data further confirms AT1R expression in the bladder detrusor muscle and urothelium. In conclusion, our mouse model underscores AT1R's role in bladder contractions. Further research into angiotensin signaling could pave the way for innovative IC/BPS therapies.

#### Longitudinal Assessment of QT Apex in Dogs Undergoing Doxorubicin Therapy

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Doxorubicin-induced cardiotoxicity (DOX-IC) is an important dose-limiting factor. Electrocardiography (ECG) is the least invasive way widely used to assess DOX-IC. In human medicine, the prolongation of the QT apex (QTa: interval between the Q wave and the peak of the T wave) is a sensitive marker for the early detection of DOX-IC. In this study, we evaluated the longitudinal change in QTa during DOX therapy in dogs with various cancers. We retrospectively reviewed the medical records. The dogs included in the study had a confirmed malignant neoplasm, underwent DOX as a monotherapy at least 4 doses, and had at least 4 ECG analyses longer than 30 seconds. All ECGs included in the study were recorded prior to the DOX administration. The QTa was blindly evaluated in lead II and/or lead III, measured QTa five times, and then averaged. Subsequently, the linear mixed model was employed to analyze the QTa data. In the results, a total of 45 dogs matched our inclusion criteria. Among them, 39/45 received 5 doses and 26/45 received 6 doses. There were no significant differences between the QTa values, and the dose of DOX administered; however, as opposed to human literature, the QTa value tended to decrease over the course of DOX. The size of dogs (i.e., 15 > kg vs ≤15kg) was not significantly associated with QTa. Nevertheless, QTa showed less fluctuation in dogs weighing >15kg, compared to those weighing ≤ 15kg. In summary, DOX administration doesn't affect QTa in the studied population.

#### Radiographic Evaluation of Tibial Torsion Following Tibial Plateau Leveling Osteotomy

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The objective of this study is to assess the accuracy of radiography for detecting tibial torsion following tibial plateau leveling osteotomy (TPLO) using standard postoperative radiographs. Seven normal hind limbs from medium-large sized dog cadavers were utilized. Preoperative plain radiography included both craniocaudal and mediolateral views for each limb, following guidelines for TPLO planning. Subsequently, a TPLO was performed on each limb. A custom-designed 3D-printed guide was used to stabilize the osteotomy without inducing torsion (0 degrees), while allowing for internal and external torsion of 5 and 10 degrees. Postoperative radiographs were acquired with the tibia in 5 and 10 degrees of internal and external torsion. Radiopaque pins and the osteotomy sites were covered, then the radiographs were randomly arranged alongside their respective plain radiographs. Three small animal surgeons independently assessed the radiographs to determine the presence and degree of tibial torsion. For the 7 tibias without torsion, 3 to 4 sets were correctly identified as not having any torsion. Internal tibial torsion was correctly identified in 2 to 5 out of 14 radiographs, and external tibial torsion was correctly identified in 0 to 3 out of 14 radiographs. Most (11 to 21) tibias were deemed as having no torsion;12 to 20 were deemed as having internal tibial torsion, and only 2 to 7 were deemed as having external torsion. Internal and external tibial torsion of up to 10 degrees following TPLO is difficult to identify on plain radiography.

# Effect of food on mitragynine pharmacokinetics after oral dose of encapsulated kratom administered to female beagle dogs

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The objective of this study is to evaluate the oral pharmacokinetics of an encapsulated kratom formulation in healthy beagle dogs. Dogs were randomly assigned into two groups (4 dogs in each group): one group received encapsulated kratom with a meal and the other on an empty stomach. The plasma concentrations of all kratom alkaloids were measured using ultra-performance liquid chromatography coupled with a tandem mass spectrometer and the pharmacokinetic properties were analyzed using non-compartmental analysis. Following oral administration, peak plasma concentrations (Cmax) of the major kratom alkaloid, mitragynine, in the fasted group was  $55.22 \pm 6.10$  ng/ml and was observed at  $1.25 \pm 0.25$  h (Tmax). In contrast, the peak plasma of mitragynine in the fed group was  $25.55 \pm 6.68$  ng/ml and occurred at  $7.50 \pm 2.06$ . Oral bioavailability of mitragynine for the fasted group was 67.9% and 45.8% for the fed group. Administration of kratom capsules was well tolerated, with no adverse effects observed Rapid absorption and greater plasma concentrations were achieved when kratom capsules were administered on an empty stomach. Based on this study, greater pharmacologic potential should be expected when encapsulated kratom is administered to fasted animals. Administration of kratom with food proved to be pharmacologically inconsistent.

Vatinoxan improves sedation quality without improving tissue (muscle) oxygen saturation in privately-owned adult dogs undergoing intradermal testing

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This double-blinded randomized cross-over study investigated the impact of co-administration of vatinoxan hydrochloride (HCl), a peripheral a2-adrenoceptor antagonist, and medetomidine HCl on muscle oxygenation (StO2) in healthy privately-owned dogs undergoing intradermal testing (IDT), and compared the effects to dexmedetomidine HCl injection. After written owner consent, dogs received intramuscular (IM) injections of either dexmedetomidine (0.5 mg/m2, DEX) or medetomidine (1 mg/ m2) and vatinoxan (20 mg/m2) (MVX). Once sedated, intradermal injections of histamine and compound 48/80 were given on the lateral thorax of each dog. The study was repeated with the alternative sedation on the opposite side one week later. After IDT assessment, sedation was reversed with atipamezole (5 mg/m2). Sedation quality, cardiopulmonary parameters, StO2, and rectal temperature were recorded and compared using mixed effect linear models ( $\alpha \le 0.05$ ). MVX achieved optimal sedation faster [median (interquartile range), 10 (8, 10) minutes] compared to DEX [18 (15, 22) minutes; hazard ratio = 7.44, p = 0.013), with higher scores at 10- and 15-minutes post-injection. StO2 was significantly reduced for 30 minutes after injection (p < 0.001), independently of the treatment (p =0.68). Cardiopulmonary variables favored MVX, but MVX's higher heart rate did not correlate with improved StO2. There was no difference in either subjective or objective assessment of the wheal size between sedations (p > 0.05). Atipamezole reversal increased StO2 in both groups (p < 0.05). Both sedation protocols, MVX and DEX, were deemed suitable for IDT in dogs, with mild reductions in StO2 that were not significantly different between treatments.

## Exploring human placental explant culture as a reliable model for cell death study in placenta malaria

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The research on placental explant culture has delved extensively into the dynamics of feto-maternal interaction. The placenta serves as a crucial intermediary for nutrient exchange, ensuring fetal development. Placental malaria introduces significant risks to both maternal and fetal health, with oxidative stress-mediated cell death playing a pivotal role in its pathogenesis. A comprehensive understanding of the mechanisms governing syncytiotrophoblast (STB) cell death in placental malaria is imperative for the development of effective interventions. Our research aims to investigate the mechanisms mitigating STB cell death in placental malaria and to identify potential treatment options. The utilization of human placental explants offers a controlled and physiologically relevant setting to scrutinize the interactions between the placenta and the malaria parasite. In this study, we have developed a technique for cultivating placental explants for up to 10 days, employing a modified culture media with additional supplements. Analysis of human chorionic gonadotropin (hCG) and lactate dehydrogenase (LDH) data revealed ~2 folds increase in hCG levels from day 7, maintaining a favorable range until day 9, as compared to day 0. Conversely, LDH levels decreased ~3-4 folds from day 5 to 9, followed by a modest increase on day 10, suggesting reduced cell death during days 7-9. Furthermore, our investigation into cell death demonstrated that cultivated placental explants respond effectively to positive control treatments for cell death. This finding underscores the importance of our established explants model in unraveling the mechanisms of cell death in placental malaria.

#### Urothelial signaling can initiate a local bladder contraction

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The mechanical stretch of the urothelium leads to the release of signaling molecules which play a sensory role in bladder contraction via neuronal signaling. It is also theorized that these molecules released from the urothelium could act locally to induce a local bladder contraction. Here, we specifically stimulated urothelial cells using optogenetics to evaluate potential local signaling mechanisms which initiate detrusor contraction. We have bred a transgenic mouse model that expresses light-activated cation channels, Channelrhodopsin (ChR2) in urothelial cells. Using an ex-vivo whole bladder preparation, we stimulated the urothelial cells by activating ChR2 with blue light and studied the signaling events which initiated local bladder contraction by applying pharmacological tools such as PPADS, atropine, nifedipine and brefeldin A. The application of the P2X receptor antagonist, PPADS, almost completely inhibited these contractions. Light-evoked contractions were also significantly inhibited when we applied the muscarinic receptor antagonist, atropine. Nifedipine, which blocks calcium entry into the smooth muscle cells, abolished bladder contractions initiated by urothelial cell stimulation. Brefeldin A, which inhibit the release of transmitter by exocytosis showed to significantly inhibit the local contraction induced by urothelial stimulation. Our data suggests that direct urothelial cell stimulation produces local bladder contractions. The local contractions induced by urothelial stimulation involve purinergic and muscarinic signaling pathways. Furthermore, the signaling events initiating local bladder contraction by urothelial stimulation involve calcium entry pathways and releasing transmitters through exocytosis. This work may further our understanding of the signaling mechanisms which play a role in pathophysiological bladder conditions.

# Behavioral response of zebrafish embryos to the insecticide chlorpyrifos associated with perfluorohexanoic acid (PFHxA)

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Perfluorinated compounds and pesticides are pollutants that have attracted attention due to their harmful effects on the environment and on non-target species. Only a fraction of PFAS is monitored and regulated, and the biological effects of many PFAS on aquatic organisms are unknown. Similarly, pesticide toxicity is highly complex and specific. However, in the natural environment, chemicals often present as complex mixtures rather than individual compounds. To date, the toxicity mechanisms of mixtures including both PFAS and pesticides are not yet known. Chlorpyrifos (CPF) is a widely used organophosphate insecticide, and one of the most toxic pesticides for aquatic life, causing adverse effects on non-target organisms. Perfluorohexanoic acid (PFHxA), although showing lower toxicity than chlorpyrifos, may modulate the toxicity of other chemical products existing in the same environmental milieu. The objective of this study was investigate the toxicological response of zebrafish embryos when exposed to chlorpyrifos in the presence of PFHxA. We hypothesize that PFHxA enhances the toxicity of CPF. Embryos were randomized into one of eleven experimental groups (CPF 620 μg/L, 62 μg/L, 6.2 μg/L and 0.62 μg/L or in a mixture of CPF/PFHxA of each concentration of CPF with 10 μg/L of PFHxA or ERM control (embryo-rearing media), DMSO control (0.5%), and 10 μg/L of PFHxA alone). Initial experiments indicate that embryos/larvae did not survive 620 μg/L CPF exposure over 7 days and this group was therefore excluded from subsequent studies. Further experiments will assess the molecular and behavioral responses of fish to CPF with and without co-exposure to PFHxA and these will be discussed.

Effects of repeated pesticides and heat exposure on the development of Chronic Kidney Disease of Unknown Etiology (CKDu)

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Chronic kidney disease of unknown etiology (CKDu) is an urgent health issue with increased mortality among farmworkers across the globe. Multiple factors, including pesticides exposure and a hot and humid climate, have been reported to contribute to the development of CKDu, but the underlying cause(s) of CKDu remains unclear. We established a controlled exposure system in male Sprague-Dawley (SD) rats to model the effects of pesticide (glyphosate and paraquat) and/or heat stress exposure on the development of kidney disease. We gathered urine samples for protein biomarker analysis and exosome assessment, and carried out kidney histopathology using an AI-guided image analysis platform. Further, we investigated urine samples from 34 farmworkers, focusing on existing urinary protein biomarkers and novel exosome-based biomarkers. In our animal model, quantification of urinary protein biomarkers by Luminex assay showed increased KIM-1 and Calbindin levels in rats exposed to pesticides or heat treatments, consistent with their role as universal renal damage biomarkers. Other urinary protein biomarkers were responsive to particular treatments, suggesting sitespecific and treatment-specific renal damage. In our analysis of farmworkers' urine samples, proteins including TFF-3, Cystatin C, and NGAL appeared to have the largest effects in participants with both acute kidney injury and reduced kidney function. Furthermore, our next-generation sequencing (NGS) study of urine exosome indicated miR-30a-5p and miR-10a-5p were elevated in individuals with reduced kidney function. Our present study reveals the similarities and differences in renal damage induced by pesticides and heat exposure, that may ultimately contribute to the early diagnosis of CKDu among farmworkers.

## Metabolic Vulnerabilities in Osteosarcoma Through Iron-Mediated Ferroptosis Modulated by the NRF2/GPX4 Axis

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Osteosarcoma, an aggressive bone malignancy primarily affecting adolescents and young adults, presents challenges in recurrence and chemoresistance despite recent advances. Although ferroptosis, driven by iron-dependent lipid peroxidation, shows promise, the molecular mechanisms of the NRF2/GPX4 axis and its therapeutic potential in osteosarcoma are yet unknown.

In this study, we first determined GPX4 and xCT expression levels, as well as cellular responses to RSL3 (a GPX4 inhibitor) and Erastin (an xCT inhibitor) in seven osteosarcoma cell lines, including five human (HOS, 143B, Saos-2, MG-63, U2OS) and two canine (COS31, DOUG) lines. IC50 values ranged from 0.02 uM to 1.01 uM with RSL3 and 0.35 uM to 1.41 uM with Erastin, correlating with GPX4 and xCT levels. U2OS, the most sensitive, and Saos-2, the least sensitive based on IC50 values, were selected for further investigation. In U2OS cells, RSL3 reduced NRF2 and GPX4, while increasing ferroportin-1 and ferritin. GPX4 knockdown also led to comparable changes in NRF2, ferroportin-1, and ferritin expression, implicating NRF2 in iron metabolism, ferroportin-1 as an iron exporter, and ferritin in intracellular iron storage. Our findings suggest that GPX4-mediated ferroptosis is regulated by iron metabolic pathways to potentially prevent cell death. Additionally, in Saos-2, RSL3 decreased GPX4, but Ferrostatin-1 restored GPX4, NRF2, ferroportin-1, and ferritin levels. Cell death inhibitors, Z-VAD, Necrosatin-1s, and Ferrostatin-1 prevented RSL3-induced cell death in Saos-2, COS31, and DOUG cells, but not in U2OS. These data highlight the importance of intracellular iron in NRF2/GPX4-mediated ferroptosis in osteosarcoma cells.

Furthermore, we generated RNA-seq and LC-MS/MS to identify key ferroptosis-related targets in osteosarcoma, while ongoing assays post-gene modifications (overexpression, knockout) in cells and evaluating metabolic vulnerabilities in xenografts through targeted iron-mediated ferroptosis.

Key words: Ferroptosis, GPX4, Ferroportin-1, NRF2, Metabolomics

#### Clinical and Translational Research at The University of Florida College of Veterinary Medicine

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The University of Florida (UF) College of Veterinary Medicine (CVM) is a full-time teaching hospital with multiple departments actively recruiting patients for clinical trials. The Oncology department collaborates with the doctors at the UF Health Cancer Center (UFHCC) as part of a Comparative Oncology Initiative, which has many ongoing canine and feline trials focusing on immunotherapy. The objectives of this study are to demonstrate a successful example of clinical and translational research at a busy veterinary teaching hospital and highlight a collaborative effort in Comparative Oncology between the University of Florida's Colleges of Medicine and Veterinary Medicine. As of August 2023, there are 60 clinical trials actively recruiting and enrolling patients at the UF CVM. 57% of these trials are interventional studies, while the other 43% are observational studies. The UFHCC Comparative Oncology Initiative has successfully completed one clinical trial focusing on canine gliomas; has 4 clinical trials that are actively recruiting patients, and 6 trials that are opening for enrollment in the near future. These studies focus on osteosarcoma, melanoma, and squamous cell carcinoma. It is anticipated that with continued successful collaborations, more clinical trials will be possible, and new treatment options will become available. Clinical and translational research is an important part of veterinary medicine to further patient care. Due to ongoing collaborative efforts, not only veterinary patients but also human patients will benefit from the research being conducted at the UF CVM.

#### The role of oncogenic PIK3CA mutation in canine hemangiosarcoma

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Hemangiosarcomas (HSA) are common and aggressive sarcomas characterized by disorganized vascular structures in domestic dogs. Previous studies revealed the highly immunogenic subtype of HSA which regulates the tumor microenvironment to potentiate tumor growth and metastasis. Furthermore, canine HSAs have been shown to exhibit activated PI3K/AKT/mTOR pathways, regulating the expression of inflammatory cytokines including IL-8. However, the underlying molecular mechanisms and the therapeutic potential of alpelisib, a PI3K-specific inhibitor, are largely unknown. In this study, we created two different isogenic cell lines (C8 and C35) harboring PIK3CA hotspot mutations from DHSA-1426 HSA cell line. Multi-omics analysis using bulk RNA-seq, single-cell RNAseq, and ATAC-seq revealed that the PIK3CA mutant cells turned on the expression of immune-related genes. Multiplex cytokine assay confirmed the secretion of inflammatory cytokines including IL-6, IL-8, and MCP-1. We also demonstrated the therapeutic potential of alpelisib by inhibiting cytokine secretion and AKT phosphorylation in PIK3CA mutant cells. Subsequently, our finding also demonstrated the potential for therapeutic resistance of alpelisib. Single-cell RNA-seq analysis identified drug-resistant subclones within the C35 clone. Marker gene analysis revealed that the resistant cell clusters upregulated genes associated with RAS-MAPK signaling. Further, the C8 clone exhibited the highest level of alpelisib resistance, comparable to that of wild-type cells. Sequencing and metabolomics analyses illustrated that the C8 clone employs HIF1A-mediated metabolic reprogramming to manifest more malignant phenotypes. Here, we suggest roles of oncogenic PIK3CA mutations in modulating immune tumor microenvironment of HSA. In addition, our results propose alpelisib as a possible approach to treat PI3KCA mutant HSAs.

#### Proximity Labeling with Molecular Signal Sensing of Oncogenic PIK3CA

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PIK3CA mutations are major oncogenic drivers for oncogenesis. Mutant PIK3CA continuously activates the PI3K pathway, transmitting oncogenic signals within the transformed cells. Mapping of proximity proteins in the oncogenic PI3K pathway and identifying mutant signals will signify a broad range of therapeutic applications. We hypothesize that PIK3CA mutant cancer cells establish a unique molecular program networking with distinct proteins, which differs from that of cancer cells with wild-type (WT) PIK3CA. To test this hypothesis, we have developed a novel approach to decode protein-protein interactions of oncogenic PIK3CA and identify key molecular partners.

First, we applied the proximity labeling technique, based on the fusion of a biotin ligase to a targeting protein of interest. The promiscuous biotin ligases, BioID and BioID2, were utilized to integrate WT PIK3CA or gain-of-function hotspot mutations at E545K or H1047R residues with multiple tags. A total of 15 plasmids were constructed in pcDNA3.1 vector. HEK293FT cells expressing proteins were transiently transfected with plasmids and selection with neomycin began post-transfection. Next, we employed a programmable nuclease, the prokaryotic Argonaute (pAgo), assisted by fluorescence resonance energy transfer technology, to detect oncogenic signals derived from mutant PIK3CA in cell-free conditions.

Our technical approach enables temporal and spatial molecular sensing of oncogenic PIK3CA and its functional interactome in living cancer cells. Our ongoing work involves biotinylation and streptavidin pulldown assay performed in cells. Western blotting and MS/LC-MS will be conducted to analyze the diversity of proteins in the oncogenic PI3K pathway. Furthermore, we will detect molecular signals from oncogenic PIK3CA.

# Radiographic Pulmonary Vasculature Dimensions in Drug Naive and Medically Managed Dogs with Left Heart Failure from Chronic Mitral Valve Insufficiency

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Chronic mitral valve insufficiency (CMVI) is the most common acquired cause of cardiovascular overload in dogs. A diagnosis of ensuing left heart failure (LHF) is supported by pulmonary venous enlargement and edema on radiographs. However, the absence of venous enlargement has been anecdotally observed after initiation of therapy in dogs with LHF; therefore, we aimed to compare radiographic pulmonary vessel size in LHF patients with or without chronic +/- acute cardiac pharmaceutical support.

Dogs with an echocardiographic diagnosis of CMVI and radiographic evidence of LHF were retrospectively selected. Objective measurements of the pulmonary vessels were obtained by a radiologist. Dogs were divided into 10 groups if drug naïve (control) or based on the type of chronic +/- acute cardiac pharmaceutics provided. Summary statistics were measured for all data and calculations of pulmonary vessel size ratios.

200 radiographic studies from 163 dogs, prevalently mixed breeds, were reviewed. The mean age was 11.7 years, and weight was 6.9 kg. In all dogs and dogs in the control group, the veins measured larger than the arteries in all lung lobes. Chronically untreated dogs who received furosemide immediately before radiographs had no venous enlargement. All other groups had vascular asymmetry or venous enlargement in at least two lung lobes. All control dogs with reoccurrence of LHF had absence of venous enlargement after initiation of therapy.

Dogs medically managed chronically for CMVI can decompensate into LHF without radiographic pulmonary venous enlargement. Normal vessel size may also be observed in dogs administered furosemide prior to radiographs.

#### The Use of Postmortem Formalin Fixed Tissues for Barbiturate Screen in Dogs and Cats

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Pentobarbital is a drug commonly used by veterinarians for the chemical euthanasia of dogs and cats. When performing an autopsy, tissues are routinely collected in 10% neutral buffered formalin to prevent the decomposition of tissue that will be used for microscopy studies; however, fresh tissue samples for toxicology testing are not routinely collected. In this study, we assessed the use of formalin fixed tissues (heart, spleen, and/or skeletal muscle) as an alternative matrix for the detection of barbiturates in 10 dogs and 9 cats with a known chemical euthanasia status using a human immunochromatographic test for barbiturate screening. Fresh liver sample from each animal was analyzed via gas chromatography-mass spectrometry (GC-MS) to confirm the presence/absence of pentobarbital. There was 100% agreement of the immunochromatographic test results at all time points with the reported euthanasia status and GC-MS results of both dogs and cats. The results of this study support the use of select formalin fixed tissues for barbiturate screening up to six months postmortem if the euthanasia status is in question and no fresh or frozen tissues are available for analysis.

#### Francisella secretes PdpE through the type VI secretion system to inhibit host cell death

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Francisella tularensis is the causative agent of the fatal zoonotic disease tularemia and poses a major threat to global public health. This virulent Gram-negative bacterium is highly infectious, requiring fewer than 10 viable bacteria to establish infection in humans. F. tularensis requires a unique contractile toxin secretion apparatus, known as the type VI secretion system (T6SS), to invade host cells, proliferate, and cause disease. Although the T6SS has been studied for over 15 years, we have yet to characterize the full repertoire of the substrates that it injects into host cells. In this project, we took a comprehensive approach to query the genomic locus in Francisella that encodes the T6SS and discovered that PdpE is a secreted toxin. Western blot analyses revealed that PdpE is secreted in a T6SSdependent fashion, and it interacts with another secreted toxin that is critical for pathogenesis. To determine the role of PdpE in Francisella virulence, we infected cultured murine macrophages with a  $\Delta pdpE$  mutant. Unexpectedly, compared to wild type, the deletion mutant caused increased macrophage toxicity and secretion of IL-1β, and expression of PdpE in this mutant restored these phenotypes. In vivo studies in Galleria mellonella larvae showed more rapid death in those infected with the  $\Delta p dp E$  mutant, indicating greater pathogenicity in the absence of PdpE. Taken together, these data indicate that PdpE is a secreted toxin that controls host cell viability, and this research has provided new insights into host-Francisella interactions.

#### A recombinant probiotic protects mice against Influenza A infection-induced immunopathology

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Seasonal Influenza causes millions of hospitalizations and thousands of deaths in the USA. The conventional preventive measure for influenza A is seasonal vaccination, which has varied protection in different groups of individuals from 40-75%. Antivirals are used in cases diagnosed early. Otherwise, primarily symptomatic treatment has been employed. Here, we propose a recombinant probiotic that prevents Influenza disease by boosting the host's immunity. The surface layer protein (SlpA) of *Lactobacillus acidophilus* expressing *Lactococcus lactis* (R110) has been a proven immunomodulator in the gut under human clinical trials. We found that oral feeding of R110 recruits regulatory T cells and prevents neutrophils, a key mediator for lung inflammation. Additionally, we report heightened antigen-specific CD8 T cells at the later stage of the disease. Further studies are in progress to develop a mechanistic understanding of these events.

# Lactococcus lactis Delivery of Surface Layer Protein A Protects Mice from Colitis by Re-Setting Host Immune Repertoire

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Inflammatory bowel disease (IBD) is characterized by gastrointestinal inflammation comprised of Crohn's disease and ulcerative colitis. Centers for Disease Control and Prevention report that 1.3% of the population of the United States (approximately 3 million people) were affected by the disease in 2015, and the number keeps increasing over time. IBD has a multifactorial etiology, from genetic to environmental factors. Most of the IBD treatments revolve around disease management, by reducing the inflammatory signals. We previously identified the surface layer protein A (SlpA) of Lactobacillus acidophilus that possesses anti-inflammatory properties to mitigate murine colitis. Herein, we expressed SlpA in a clinically relevant, food-grade Lactococcus lactis to further investigate and characterize the protective mechanisms of the actions of SlpA. Oral administration of SlpA-expressing L. lactis (R110) mitigated the symptoms of murine colitis. Oral delivery of R110 resulted in a higher expression of IL-27 by myeloid cells, with a synchronous increase in IL-10 and cMAF in T cells. Consistent with murine studies, human dendritic cells exposed to R110 showed exquisite differential gene regulation, including IL-27 transcription, suggesting a shared mechanism between the two species, hence positioning R110 as potentially effective at treating colitis in humans.

# Design of a Three-Dimensional Finite Element Analysis Model of Equine Traumatic Brain Injury Following Trauma

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TBI is typically a product of harsh whiplash of the head against the ground and often occurs in young or excited horses. The aim of this study is to design a finite element model to study equine brain traumatic injury to contribute to a more accurate understanding of trauma and explore preventative devices. Following common human FEA simulations of brain trauma, a simplified model and an anatomically accurate model were created, consisting of the skull, the CSF, and the brain. Due to the availability of mechanical properties of equine brain anatomy, material properties were assumed to be the same as human anatomy. The simplified head model successfully impacted the rigid body floor with contact stress at the point of impact measured to exceed the yield stress of 120 MPa and approximately 10 mm of deformation of the CSF and brain was observed. At impact, the CSF and brain can be seen to impact the inner bottom surface of the skull and bounce back and subsequently impact the inner top surface of the skull. The simplified model showed successful impact of the bone with resultant deformation of the CSF and brain that follows typical behavior during brain trauma. The anatomical equine head model has shown that a simulation of brain trauma can be produced and used in testing preventative methods. This model will continue to be refined to accurately represent the physics and response during equine brain trauma.

#### Elucidating metabolic effects of dietary GenX in Nile Tilapia liver

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Gen-X is an emerging short chain per- and polyfluoroalkyl substances (PFAS) consisting of six-carbon perfluoroether carboxylates. This chemical is highly soluble in water and can dissociate into the environment as the anionic form. GenX is stable to photolysis, hydrolysis, and biodegradation and is not expected to be removed during drinking water or wastewater treatments. Exposure to GenX can occur through drinking water, dermal and inhalation exposures. This study aimed to assess the effect of 1 ppm and 10 ppm Gen-X fed in the diet to tilapia at the transcript and lipid level. Dietary exposure to GenX altered elongation of very long chain fatty acid enzyme 5 (elovl5), catalase and superoxide dismutase1 expression in the liver, indicating oxidative damage and impacts on lipid metabolism. GenX also altered 56 lipids (p-value < 0.05, FDR < 0.2) categorized as ceramides (3), diacylglycerols (4), fatty acids (8), lysophosphatidylcholine (5), lyso-phosphatidyl-ethanolamines (2), phosphatidylcholines (20), phosphatidylethanolamines (3), phosphoinositides (1), sphingomyelins (6), and triglycerides (4). We conclude that Gen-X has prominent adverse effects on lipid metabolism that should be considered and further studied in aquatic species.

## Heart rate of military-type dogs when resting, and when walking and trotting on 0% and 10% incline

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Heart rate (HR) was measured for 9 military-type dogs while the dogs were resting, walking at 2.5mph, and trotting at 4.5mph, on 0% or 10% incline. Measurements were repeated at least 3 times on separate days, or until %CV was less than 10% for the activity for that dog. Rest was defined as the dog being offleash in the room, free to lay down or move around. Walking and trotting occurred on treadmill in climate-controlled room.

Table 1. Range of heart rate (HR) values of 9 dogs 0% incline 10% incline HR at rest 84-105 bpm HR at 2.5mph (walk) 110-140 bpm 108-165 bpm HR at 4.5mph (trot) 125-176 bpm 155-216 bpm

Percentage increase in HR was larger for trotting between 0% and 10% incline than for walking between the two inclines. Variability was higher the harder the dogs worked. HR was counted for 10 beats at 5, 10, and 15 min of each activity. TeleVet II ECG monitor and SkinTact FS50 electrodes were used without clipping the fur. Electrodes were held in place using Vetrap. Each activity was done for 15 minutes at a time, with an average of 15 min at rest and 30 min on treadmill.

### TOP DOG ABSTRACTS

#### **Abstract 1**

## The Potential Role of the Glandular Microbiome of Owl Monkeys (Aotus spp.) in Chemical Communication

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Chemosignals are the most common form of social communication in animals. This is often done through the release of pheromones created by the individual. The fermentation hypothesis of chemical communication provides an alternative hypothesis for the diversity of chemosignals. In this hypothesis, fermenting bacteria that reside on the scent glands of their host release volatile organic compounds (VOCs) into the air. The diverse microbial community of each host releases a unique profile of VOCs that can be used in olfactory communication. Recent research looking into the subcaudal scent gland of owl monkeys (Aotus spp.) has found that aerobic bacterial diversity may be influenced by their sex, environment, and social group. This paper expands upon this by using cultureindependent techniques to identify aerobic bacteria, anaerobic bacteria, and archaea on the subcaudal scent gland. DNA was extracted from swabs of owl monkey (n = 2 males; 6 females) subcaudal glands and Sanger sequencing was performed on the 16s rRNA gene. The microbiomes of the owl monkeys were compared in relation to their genetics, environment, and species. We identified obligate anaerobic bacteria and archaea that were previously unknown to grow on the subcaudal gland. Fermentative bacteria that commonly grow on the skin and in the intestinal tract were identified that are known to produce VOCs in other primates. Preliminary results indicate that cohabitation of owl monkeys may result in similar microbiomes. This indicates that sharing a similar environment and the transfer of microbes between owl monkeys could be used to show group membership.

Use of serum osmolality to identify heart disease stage in dogs and relationship to the degree of mathematical chloride correction.

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Heart failure-associated hypochloremia can be depletional from diuretics or dilutional from free water retention. Serum osmolality reflects free water but has not been evaluated in dogs with heart disease. In this study, we investigated the relationship of osmolality to heart disease stage and the amount of mathematical correction of serum chloride (a proposed indicator of relative free water status) in dogs. Serum samples from 20 healthy dogs and 57 dogs with myxomatous mitral valve disease (25 Stage B (preclinical), and 32 Stage C/D (congestive heart failure)) were biochemically analyzed, chloride concentrations were mathematically corrected, and osmolality was calculated (cOsm) and measured by freezing point depression (mOsm). Correlations between biochemical variables and osmolality were explored and variables were compared among groups. Bland-Altman analysis was used to assess agreement between osmolality methods. The cOsm was different among groups (P=.003), with Stage B (median 310 mOsm/kg; IQR 306, 316) and C/D dogs (median 312 mOsm/kg; IQR 308, 319) having higher cOsm than healthy dogs (median 305 mOsm/kg; IQR 302, 308). Osmolality methods were moderately correlated (P<.0001, r=0.46) but with proportional bias and poor agreement. The amount of chloride correction was negatively correlated with cOsm (P<.0001, r=-0.78) and mOsm (P=.004, r=-.33). Serum bicarbonate was negatively correlated with chloride (P<.0001, r=-.67). In conclusion, dogs with Stage B and Stage C/D heart disease had higher cOsm than healthy dogs. Osmolality was inversely related to the amount of chloride correction, supporting use for the assessment of relative free water. Calculated and measured osmolality cannot be interchanged.

#### Modified stapled side-to-side jejunocecostomy

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**Background:** Jejunocecostomy (JC) can be required in 9% to 68% of small intestinal resections. A simple method needs to be developed to improve outcomes with JC.

**Objectives:** To evaluate short-term postoperative outcome after a modified stapled side-to-side (MS) JC.

**Methods:** Six healthy adult horses underwent exploratory celiotomy and a MS JC after resection of approximately 1.6 m of distal jejunum and ileum. A 6- to 7-cm transverse incision was made in an elevated cecal pouch between medial and dorsal bands, level with the cecocolic fold. The transected jejunum was aligned perpendicular to the cecal opening and the stapler forks were then inserted distal to proximal (jejunum) and body to apex (cecum). When the stapler was fully inserted along the antimesenteric side of jejunum, midway between and parallel to the cecal bands, it was fired. The remaining open edges were closed in 2 layers and the staple lines oversewn. Horses were managed as for clinical cases and were euthanized at 7 days.

**Results:** The MS JC was successfully completed in all 6 horses. Necropsy dissection demonstrated that this technique produced a triangular stoma with an expanded opening, 6 to 8 cm long on each side, different to the slit-like opening produced by the standard side-to-side JC.

**Conclusions:** The MS JC method is a viable alternative to other types of JC. It is simple, requires one application of staples, eliminates the need for jejunal blind-end closure, and the triangular shape of the stoma may prevent anastomotic impactions.

### **BEST IN SHOW ABSTRACTS**

#### Abstract 4

#### Developing a Vaccine against spotty liver disease in commercial layers

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UF CVM CDPM

Spotty Liver Disease (SLD) caused by Campylobacter hepaticus has been reported in the U.S. as an emerging cause of morbidity and mortality in commercial layer chickens. Antibiotics are being used for treatment, however transferrable plasmids carrying resistance markers have been identified in some strains. Antibiotics are also not a viable option for antibiotic-free table egg production indicating the need for alternative approaches. Currently, the layer industry relies on autogenous killed vaccines. However, the fastidious and slow growth of C. hepaticus, cost, and the impact on the uniformity and body weight gain of pullets imposed by the current volume of killed vaccines used in layer chickens hinder their use. Therefore, our aim is to identify vaccine candidates by using phage display to develop an avian pathogenic E. coli-vectored attenuated live vaccine to provide protection against both C. hepaticus-mediated SLD and colibacillosis.

The Invertebrate Galleria mellonella Differentiates Virulence Potential Between Species and Strains of Mycoplasma agassizii and Mycoplasma testudineum.

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Both Mycoplasma agassizii and Mycoplasma testudineum are etiological agents of upper respiratory tract disease in captive-raised and free-ranging Chelonian species. Both species have been isolated from clinically ill free-ranging and captive tortoises, but *M. agassizii* is associated with more severe clinical signs. While limited experimental infections have confirmed the pathogenicity and examined transmission dynamics, ethical considerations are a factor in continued research regarding hostpathogen interactions due to the federal protection status of many *Chelonian* species. Additionally, there is a lack of commercially available cell lines for temperature-restricted pathogens isolated from reptiles. To address these challenges, we established an alternative model of pathogenicity testing using the invertebrate Galleria mellonella. To assess the efficacy of this model we selected strains of M. agassizii and M. testudineum isolated from desert and gopher tortoises. A dose-response was run using low, medium, and high infection doses. Larval mortality, pupation, and emergence events were collected over 28 days. We found that, independent of strain, *M. agassizii* is more pathogenic in *G.* mellonella larva compared to M. testudineum. Interestingly, we also found that the pathogenicity of M. agassizii strain PS6 and 723 is highly dependent on dose when comparing differences within strains. Overall, *G. mellonella* is a tractable alternative model for assessing differences in virulence potential of *M. agassizii* and *M. testudineum*, and an important tool to understand how different species and strain impact clinical outcomes.

## Sublethal Impacts of Per- and Polyfluoroalkyl Substance (PFAS) Exposure on *Acropora tenuis* Larvae

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Per- and polyfluoroalkyl substances (PFAS) are an expanding group of man-made chemicals with emerging health concerns in humans and other species. The widespread use of PFAS has made them ubiquitous in the environment, with especially high levels in the marine environment. Drastic declines observed in coral reefs in response to changes in climate and other anthropogenic stressors, have hinged the survival of these ecosystems largely on the overall health and reproductive success of stony corals. No published data exists on the impacts of PFAS on stony coral larvae, despite repeated incidence of high PFAS levels in coastal areas near coral reefs worldwide. This presents a major research gap in coral ecotoxicology that requires further study. The purpose of this experiment was to characterize the impact of (PFOS) on Acropora tenuis larvae. Impacts of PFOS on symbiont acquisition of larvae were measured. Additionally, symbiotic and aposymbiotic A. tenuis larvae were exposed for 72 hours to two levels of PFOS and the impact on larval symbiont acquisition and the larval lipidome (utilizing nontargeted LC-MS) were analyzed. Lipids are the main energy source for coral larvae; and therefore, represent a necessary endpoint to assess larval health. A. tenuis larvae exposed to PFOS during symbiont acquisition showed a decreased number of symbionts acquired. Evidence of alterations to the lipidome were found in symbiotic larvae. Future research should focus on understanding possible impacts of PFOS on coral symbionts or on the symbiotic relationship between symbionts and coral larvae.

Blood metabolomics and impacted cellular mechanisms during transition into lactation in dairy cows that develop metritis.

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The objective was to identify metabolites associated with metritis and use them for identification of cellular pathways and mechanisms affected during transition into lactation. Holstein cows (n = 104) had blood collected in the prepartum period, at calving, and at the day of metritis diagnosis. Cows with reddish or brownish, watery, and fetid discharge were diagnosed with metritis (n = 52). Cows with metritis were paired with herdmates without metritis (n = 52) based on days in milk. The metabolome of plasma samples was evaluated using untargeted gas chromatography time-of-flight mass spectrometry. Metabolites with adjusted  $P \le 0.10$  on t-tests were used for identification of cellular processes. Cellular processes with adjusted  $P \le 0.05$  and predicted Z-scores were affected. t-Tests showed that 89, 49, and 168 metabolites differed between cows that developed metritis and cows that did not develop metritis in the prepartum, at calving, and at the day of metritis diagnosis, respectively. Cows that developed metritis had affected cellular processes associated with lower amino acid metabolism in the prepartum period, greater lipolysis, cell death, and oxidative stress at calving and at metritis diagnosis, and greater leukocyte activation at calving, but lower immune cell activation at metritis diagnosis. These metabolomic changes may lead to failure to prevent bacterial infection, and development of metritis.

Immune cell profile expression analysis of formalin fixed paraffin embedded (FFPE) melanoma and osteosarcoma samples using Nanostring's nCounter assay

#### Victoria Cicchirillo

Osteosarcoma is the most common primary bone tumor in canines and melanoma is the most common oral tumor in canines. The host's immune response is an important component of tumor control for both of these cancers and the tumor's immune profile can potentially give prognostic information. The aim of this study was to describe the immune expression profiles amongst these tumor types in tissue and to compare the immune expression to cancer cell lines, normal tissue samples, and metastatic lesions. We performed gene expression analysis using Nanostring nCounter in RNA extracted from formalin fixed paraffin embedded (FFPE) osteosarcoma tissue, osteosarcoma cell line, normal bone, melanoma tissue, matched metastatic lymph nodes, and matched normal lymph node samples. Looking at 48 different genes involved in the immune system, we found the genes related to macrophages and cytotoxic cells were highly expressed in osteosarcoma tissue samples, melanoma tissue samples, and all lymph nodes but were not overly expressed within the osteosarcoma cell lines. We also found high expression of mast cell related genes within the osteosarcoma tissue samples but low in the cell line samples and varied expression within the melanoma tissue samples.

### Chapters

Alpha, Cornell University, 1925 Beta, University of Pennsylvania, 1929 Gamma, Iowa State University, 1931 Delta, The Ohio State University, 1934 Epsilon, Auburn University, 1948 **Zeta**, Michigan State University, 1950 Eta, Texas A&M University, 1950 Theta, Colorado State University, 1950 **Iota**, Washington State University, 1952 Kappa, University of Minnesota, 1952 Lambda, University of California, 1953 Mu, University of Illinois, 1953 Nu, Oklahoma State University, 1958 Xi, University of Georgia, 1959 Omicron, Purdue University, 1962 Pi, University of Missouri, 1965 Rho, Tuskegee University, 1967 Sigma, Kansas State University, 1969 Tau, Louisiana State University, 1977 **Upsilon**, University of Florida, 1979 Phi, University of Tennessee, 1979 Chi, Virginia-Maryland Regional CVM, 1984 Psi, North Carolina State University, 1984 Alpha Alpha, University of Wisconsin, 1987 Alpha Gamma, Oregon State University, 1987 Omega, Mississippi State University, 1988 Alpha Beta, Tufts University, 1991 Alpha Delta, St. George University, 2006 Alpha Epsilon, Western University of Health Sciences, 2006

#### **PHIZETA**

The purpose of Phi Zeta is to promote, acknowledge, and reward scholarship in the profession of veterinary medicine.

#### History

Phi Zeta was originated in 1925 by a group of senior veterinary students in the New York State Veterinary College at Cornell University. of the College, Dr. Veranus A. Moore, the Society was formally organized, and Dean Moore was elected as the first president of the Alpha Chapter.

The Society of Phi Zeta was organized in 1929 at a meeting in Detroit, Michigan, and Dean Moore became the first president of the Society.

Also in 1929, a charter was granted to the School of Veterinary Medicine at the University of Pennsylvania, and the Beta Chapter was established. In 1931, the Executive Committee approved the petition of a group from Iowa State College and the Gamma Chapter was established.

Since then twenty-four chapters have been chartered, bringing the total number of chapters to twenty-seven. Chapters of the Society may be formed at any recognized veterinary medial college or at any other institution of higher learning.

#### Name & Symbol

The organizers of the Society, when seeking a suitable name, sought the help of a learned Greek scholar, Professor George P. Bristol of Cornell University. Professor Bristol suggested a Greek word, which in the Latin form is spelled PHILOZOI and means "love for animals." The abbreviation of Phi Zeta was adopted as the name of the society.

The emblem consists of a pendant formed by the letter Phi superimposed by the letter Zeta. The design was the work of Louis Agassiz Fuertes, the great naturalist and artist.

