UF College of Veterinary Medicine Research and PHI ZETA Celebration

Abstract Book

April 13-14, 2023

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FOR ANIMALS. FOR HEALTH. FOR YOU.

Vision

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

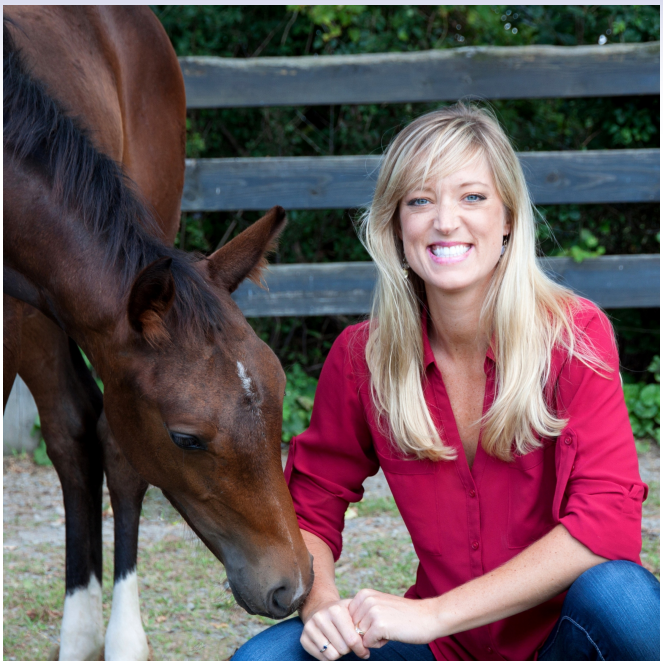
Mission

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 real-world 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.

Keynote Speaker- **Dr. Sally Anne DeNotta, DVM, PhD, DACVIM**



Sally DeNotta is board-certified by the American College of Veterinary Internal Medicine and is a member of the clinical faculty at the University of Florida College of Veterinary Medicine. Originally from the rural Oregon coast, she received her DVM from Oregon State University and spent time in private practice in Oregon and Colorado before completing an internal medicine residency and PhD at Cornell University. She joined the UF faculty in 2018, where her clinical interests include infectious disease and clinical neurology. When not in the hospital, she is the UF equine veterinary extension specialist, serving as the liaison between the College of Veterinary Medicine and the Florida horse industry.

Summary of talk:
"Three right turns on the road ahead: My pathway to academia and clinical research"

Life is curvy. Plans we make for our future evolve and change over the course of one's career, and maintaining an open mind for opportunities to grow and learn can lead us down the ideal path - often to places we never expected. Take a walk down the meandering path of an equine veterinarian determined to remain in practice and avoid research at all costs…ultimately leading to a rewarding career in academia and clinical research.

Dr. Sally Anne DeNotta
Clinical Assistant Professor
Large Animal Internal Medicine
UF Equine Veterinary Extension Specialist UF College of Veterinary Medicine

Symposium Schedule

April 13 ***UF IFAS Straughn Center***

2:00pm-4:00pm	Poster Set-up
4:00pm-6:00pm	Poster Presentations & Reception

April 14 ***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- 12:20pm	Lunch
12:20pm-1:20pm	Keynote Presentation - Dr. Sally DeNotta DVM, PhD, DACVIM
1:20pm-2:20pm	Awards

April 13; Posters - UF IFAS STRAUGHN CENTER

Poster #, Name, Title, Poster

1. Ashley Clarke. Mutations in a novel transcriptional regulator of *Francisella* virulence, AraC, results in resistance to Tolfenpyrad

2. Alexis Irvin. An interactome of the *Francisella* type VI secretion system reveals novel protein-protein interactions

3. Jenna Annalise Yungmann. Surface Modification to Increase the Tumor Targeting Ability of *Salmonella*

4. Melanie Zamora. Multi-locus variable tandem repeat analysis (MLVA) of *Mycoplasma bovis* to determine allelic profiles of past and current isolates

5. Cole D. English. Molecular and behavioral responses to the antineoplastic ifosfamide in zebrafish (Danio rerio) embryos and larvae

6. Ciara Saccente. Transcriptome and behavioral investigation of atorvastatin exposure in developing zebrafish (*Danio rerio*)

7. Keith Y. Gandy. Pilot evaluation of the effects of positive end-expiratory pressure and inspiratory pause on dead space in mechanically ventilated dogs

8. Jessica Steen. A single-cohort retrospective analysis of factors associated with morbidity and mortality in 193 anesthetized domestic goats

9. Lisette M. Coll-Roman. Reproductive complications and survival after parturition in hospitalized goats: a multi-center study

10. Alexis E. Diaz. Dynamic pneumatic compression therapy can be safely applied following maximal exercise but does not improve systemic lactate clearance in fit Thoroughbred horses

11. Elizabeth Handberg. MRI findings associated with clinical presentation and histopathology in a Mexican redknee tarantula (*Brachypelma hamorii*)

12. Nirali Pathak. Optimizing Gene Editing in Canine Cells Using CRISPR/Cas9 for Pyruvate Dehydrogenase Kinase 4

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

14. Alexis M. Kidd. Validation of a Primary Thyroid Feline Cell Line and Treatment with Methimazole

15. Melissa P. de Carvalho. Current mortality rate and main characteristics of dogs diagnosed with sepsis at the University of Florida Small Animal Hospital: preliminary results of a retrospective study

16. Alyssa R. Berman. Correlation between Urine Anion Gap and Urine Ammonia-Creatinine Ratio in Healthy and Kidney Disease Cats

17. Savannah K. Crowdis. Comparison of exposure of the radius afforded by the craniomedial and craniolateral surgical approaches

18. Rachel Naghi. Acellular fish skin may be used for immediate wound coverage following wide surgical tumor excision in dogs: A prospective clinical trial

19. Cameron B. Seger. Pantarsal arthrodesis stabilized with circular external skeletal fixators in 8 dogs (2010-2022)

20. Kelly Deabold. Inter- and Intra-observer Reliability of Thoracic Limb Circumference Measurement Methods in Healthy Dogs

21. Oscar Alas. Radiographic characterization and establishment of a grading system for tibial avulsion fractures in dogs

22. Eric T. Alexopoulos. Efficacy and safety of topical mydriatic agents in bearded dragons (*Pogona vitticeps*)

23. Bryan Chiang. Evaluation of Intra-observer and Interobserver Agreements to Determine Reliability of 8 Segmental Reflexes in 101 Dogs

24. Rachel P. Lee. Radiographic Features of Cardiogenic Pulmonary Edema in Dogs with Dilated Cardiomyopathy

25. Lauren T. Porter. Selective osmotic shock for the isolation of feline pancreatic Islets of Langerhans

26. Veronique Etienne. Antibody Response to Mosquito Salivary Proteins as a Marker for Exposure

27. Chaitanya Gottapu. Identification of vaccine candidate antigens of *Campylobacter hepaticus* using phage display technology

28. Roshen Neelawala. An avian pathogenic *E. coli* vector system for delivering heterologous vaccine antigens in poultry

29. Nimra Khalid. Discovering Virulence Inhibitors In Highly Pathogenic Bacteria

30. Pacharapong Khrongsee. Engineering of Parasitic Bacteriophages to Combat against *Burkholderia pseudomallei*

31. Dorothea Megarani. Interlaboratory Reproducibility of a TaqMan RT-qPCR Assay for Detection of Tilapia Lake Virus

32. Pumin Sintara. Development of *Burkholderia pseudomallei* Detection Assay Using Bacteriophage Tail Protein-Based Approach

33. David Kiiza. Cost-benefit analysis of an enhanced vaccination program to control brucellosis in (zero-grazing) cattle in Rwanda

34. An-Chi Cheng. Emerging Viral Pathogens in Farmed White-tailed Deer (*Odocoileus virginianus*) in Florida

35. An-Chi Cheng. Trueperella pyogenes, a Lethal Pathogen for Farmed White-Tailed Deer (*Odocoileus virginianus*) in Florida

36. Maria B. Ugarte Marin. Prevalence of anatomical defects and pathological conditions involving the cervical os in lactating dairy cows evaluated using a digital vaginoscope

37. Yasir Khan. Mitigating the antimicrobial resistance along the poultry production: A systematic review

38. Ameer Megahed. Effects of injectable trace minerals on the inflammatory cytokines response to vaccination in dairy calves

39. Steven Beck. Structural remodeling of the basolateral amygdala (BLA) in Ovalbumin (OVA)-induced allergic asthmatic mice: Preliminary Study Findings

40. Kaylie Anne Costa. Reefs vs. Red Tide: Investigating the sublethal impacts of Karenia brevis on South Florida reef-building coral species

41. Mallory J. Llewellyn. Nonlethal detection of PFAS bioaccumulation and biomagnification within fishes in a Great Lakes Urban-Dominated Watershed

42. Qaim Mehdi. Species-specific accumulation and geographic variability of per -and polyfluoroalkyl substances (PFAS) in marine apex predators: Concern for exposure to longer-chain PFAS?

43. Gabriella L. Robilotto. Potential Pathophysiological Role of Angiotensin Receptor Expression in the Urinary Bladder

44. Sierra Chanutin. Comparison of two methods to blind-end small intestine for jejunocecostomy in horses

45. Tomas Gonzalez. Comparison of reproductive performance of dairy cattle following a multivalent modified live post-partum vaccine protocol or a multivalent killed pre-partum vaccine protocol

46. Oscar Alejandro Ojeda-Rojas. Economic Consequences of Clinical Endometritis in Dairy Herds

47. Twisha Jani. Investigation of the in vitro effects of cannabidiol, cannabidiolic acid, and the terpenes β-caryophyllene in lymphocytes harvested from atopic and healthy dogs

48. Logan M. Scheuermann. Clinical Efficacy of Virtual Surgical Planning and 3D-Printed, Patient-Specific Reduction Guides to Facilitate Alignment of Diaphyseal Tibial Fractures Stabilized via Minimally Invasive Plate Osteosynthesis in Dogs: A Historic Case Matched Control Study

49. Jacob Morris. Visual Field of the Albino Rat, Common Ferret, and Northern Tree Shrew

50. Lekshmi K. Edison. High Cross-reactivity between Receptor Binding Domains of Feline Coronavirus Serotypes 1 and 2 with SARS-CoV-2: Its Impact on Diagnostics and Pan-CoV Vaccine Development

51. Courtney Valerio. Into the Matrix: Assessing the Use of Alternative Matrices with Barbiturate Screening Tests

52. Pedro H. O. Viadanna. Characterization of Bluetongue virus serotype 1 strains isolated from farmed white-tailed deer (Odocoileus virginianus) in Florida, USA

53. Lifeng Xiong. Group 3 innate lymphoid cell pyroptosis represents a host defense mechanism against Salmonella infection

54. Wilson P. Carvalho Filho. Influence of different horse transport distances on biochemical and metabolic markers and hematological cells

55. Kalene Jasso. Modulation of the Brain-Derived Neurotropic Factor (BDNF) pathway increases mucin production in human epithelial airway cells: *Preliminary Study Findings*

56. MD Absullah. Targeting Ferroptosis as Novel Therapeutics for Human and Canine Osteosarcoma

57. Donghee Lee. Genome-wide CRISPR screening for identification of a cross-species oncogenic mechanism in osteosarcoma

58. XaioXaio J. Lederer. Comparison of the Efficacy of Cranial vs. Medial Minimally Invasive Plate Osteosynthesis Applications for the Stabilization of Simulated Radius and Ulna Fractures

59. Apichai Tuanyok. One Health and a retrospective study of laboratory – based melioidosis surveillance in Songkhla and Phatthalung Provinces of southern Thailand, 2014 – 2020.

60. Jennifer Kyu. The Role of Zinc in Innate Lymphoid Cells

61. Kristi L. Jones. Optimization of viable qPCR method to differentiate live and dead bacteria in blood

62. Amany M. Salama. Neurorestorative potential of Some Nutraceuticals in Neurotoxicity Induced by Aluminum oxide in Albino rats

63. Karen C. Scott. Leg length and water depth affect the increase in stride length with speed in dogs exercising on a dry or underwater treadmill

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.

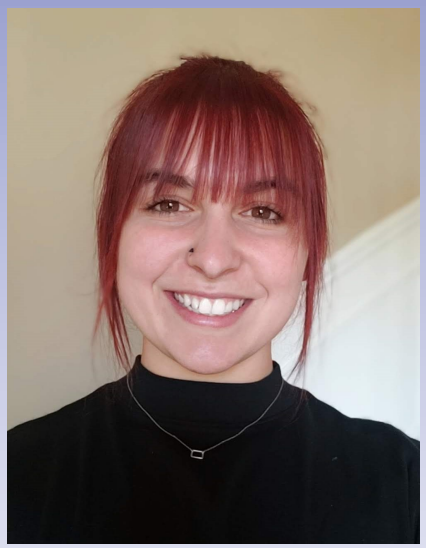
The Top Dog DVM Presentation Competition emphasizes communication, promotes fellowship, and inspires friendly competition among the College of Veterinary Medicine's DVM students.

Please Meet This Year's Top Dog DVM Finalists

Tasha Desiderio

Class of 2024

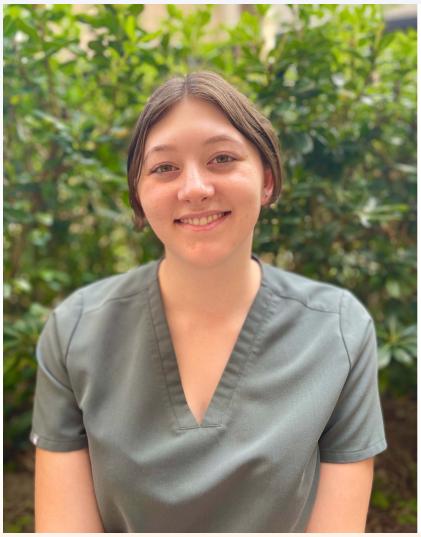
Phylogenetic Analysis of a Novel Atadenovirus from an Aldabra Tortoise (*Aldabrachelys gigantea*) Suggests Lower Barriers to Host Jumping from Squamates to Tortoises
(Abstract 1)



Hannah Quail

Class of 2025

Phylogenomic characterization of ranavirus isolated from wild smallmouth bass (*Micropterus dolomieu*)
(Abstract 2)



Rachel Breitenbach

Class of 2026

The importance of auditory, olfactory, and visual cues for insect foraging in owl monkeys (*Aotus nancymaae*)
(Abstract 3)

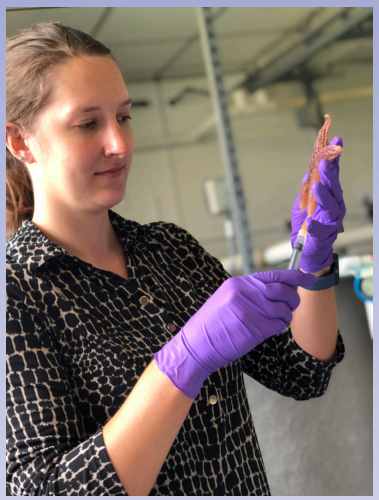
Best in Show

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.

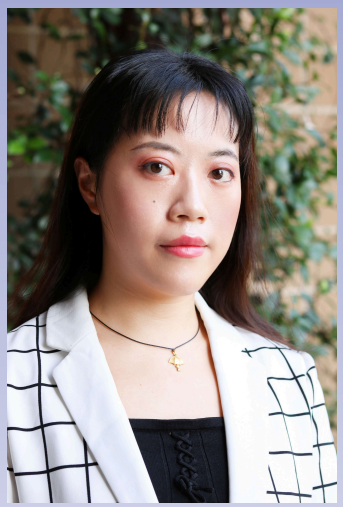
The Best in Show Presentation Competition underscores VGSA's core mission of fostering an environment which encourages excellence in graduate research, scientific ethics, and the exchange of ideas. Additionally, it emphasizes communication, promotes fellowship, and inspires friendly competition among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

Please Meet This Year's Best in Show Finalists



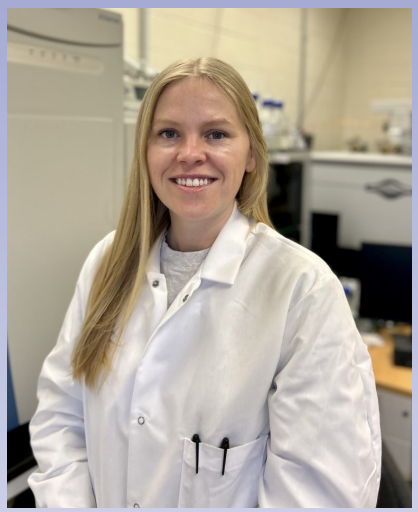
Sarah Wahltinez
CDPM

Coelomic Fluid Changes of Common Sea Stars (Asterias rubens) to Hypoxic Environmental Conditions
(Abstract 4)



Yiran Shen
IDI

Evidence of a Sjögren's disease-like phenotype following COVID-19
(Abstract 5)



Emily Griffin
PS

Aquatic vegetation, an understudied depot for PFAS
(Abstract 6)

Segundo Casaro
LACS

Unraveling the immune and metabolic changes associated with metritis in dairy cows
(Abstract 7)

Lydia Sixta
SACS

Detection of Tumor-Associated Bacterial DNA (Microbiome) Within Canine Melanoma and Osteosarcoma Using the Bacterial 16S Ribosomal RNA Gene
(Abstract 8)

Poster 1

Mutations in a novel transcriptional regulator of *Francisella* virulence, AraC, results in resistance to Tolfenpyrad

Ashley Clarke*, Isabelle Llabona*, Alexis Irvin, Danielle Hulvey M.S., Aria Eshraghi Ph.D

Francisella are highly infectious Gram-negative bacterial pathogens capable of being transmitted from animals to humans. Due to their pathogenicity, ease of transmission, and potential for major public health impact, Francisella are classified as high-priority pathogens by the CDC. The development of Francisella as a bioweapon by multiple countries has spurred interest in the discovery of novel antibiotics that target this and other bacterial pathogens. To identify drugs that inhibit Francisella growth, we screened a library of 1,000 compounds with F. novicida, a surrogate for human virulent species Francisella. Among the most potent inhibitors of F. novicida growth is Tolfenpyrad, a drug previously characterized as an insecticide. To find the molecular targets of Tolfenpyrad, we selected Tolfenpyrad-resistant F. novicida strains and sequenced their genomic DNA. Comparing the genomes of these mutants to wild type revealed point mutations in two genes: araC, an uncharacterized transcriptional regulator, and nuoM, a component of the respiratory electron transport chain. Expression of wild type nuoM did not complement resistance to Tolfenpyrad, making interpretation of the requirement for nuoM unclear. Remarkably, we found that mutating three amino acid residues in araC resulted in resistance to Tolfenpyrad, and expression of araC in these mutants' complemented sensitivity to wild type levels. Interestingly, these point mutants of araC displayed attenuated growth in interferon-gamma-stimulated macrophages but not in untreated macrophages, suggesting that AraC may control evasion of the host immune response in vivo. This research has identified a novel antimicrobial drug, the bacterial pathways it targets, and how one of these targets controls Francisella virulence.

*Contributed equally

Poster 2

An interactome of the *Francisella* type VI secretion system reveals novel protein-protein interactions

Alexis Irvin, Danielle Hulvey MS, Timothy Hamerly PhD, Rhoel R. Dinglasan PhD MPH MPhil, and Aria Eshraghi PhD

Department of Infectious Diseases and Immunology, University of Florida, Gainesville, FL

Francisella tularensis is a causative agent of tularemia, a lethal disease transmitted through aerosolization and arthropod vectors. Owing to it remarkable pathogenicity and ease of transmission, this infectious pathogen is highly concerning to the counter bioterrorism community. Francisella encode a type VI secretion system (T6SS) on a genomic locus termed the Francisella pathogenicity island (FPI). This T6SS injects toxic proteins directly into host cells to facilitate intracellular bacterial growth and virulence. The Francisella T6SS is distinct from other bacterial secretion systems because it is composed of unique proteins that are absent in other bacterial genera. To characterize this system, we set out to define the protein-protein interactions (PPIs) of the Francisella T6SS apparatus. We engineered strains of Francisella novicida to encode epitope fusions with each of the FPI-encoded genes and validated that these strains retain apparatus activity by maintaining their ability to infect cultured macrophages. For the strains that retained T6SS activity, we immunoprecipitated the FPI-encoded proteins, performed quantitative mass spectrometry, and constructed an interactome to map the PPIs of the Francisella T6SS. To determine if the interaction of two of these proteins are required for T6SS activity, we constructed point mutations in a protein that comprises the T6SS sheath, IglA. These mutations abrogated interaction with the second component of the sheath, IglB, and this blocked T6SS activity and Francisella pathogenesis. These results have provided us with amino acid-level details of T6SS sheath assembly and the interactome we have generated will drive future studies on T6SS PPIs that can serve as future drug targets to block Francisella pathogenesis.

Poster 3

Surface Modification to Increase the Tumor Targeting Ability of *Salmonella*

Jenna Annalise Yungmann, Shifeng Wang, Roy Curtiss III

Department of Infectious Diseases and Microbiology, College of Veterinary Medicine, University of Florida

As a gram-negative bacterium, Salmonella has been proven to be an effective immunotherapy agent that induces tumor regression against cancers through multiple mechanisms in mice. Despite this conducted research, clinical trials showed that the targeting efficiency of the live bacterium needs to be increased. A strain that displays an OmpA fused with PLZ4 peptide on the surface of Salmonella was developed to increase the targeting ability of bladder cancer cells. This research focuses on further surface modification and mutations to potentially increase Salmonella tumor targeting via tissue culture cell assay. The OmpA porin is located on the outer membrane of the surface of Salmonella to assist with peptidoglycan linking and is covered by LPS, a molecule composed of lipid A, an inner core, an outer core, and an O-antigen. While the deletion of lipid A is lethal, reducing the LPS-O antigen and cores has the potential to strengthen the exposure of OmpA to the cell. Thus, this research compares the ompA3Ωplz4 strain with various core and O-antigen mutations to test for increased efficiency. The mutations introduced into the Salmonella ompA3Ωplz4 strain included ΔwaaL, ΔwaaC, and ΔwaaG and were compared to the parent strain. The attachment to and invasion of human carcinoma urinary bladder cells (5637), mouse bladder tumor cells with different genetic complexities (BBN963), and urothelial carcinoma bladder fibroblast cells (MB49) in culture were also observed to further determine the tumor targeting efficiency. As a result, some mutations and surface modifications resulted in a more significant effect on cell attachment and invasion than others and an increase in tumor targeting efficiency was seen. This research is significant to increasing the effectiveness of immunotherapy agents in tumor regression and future animal studies will be performed.

Poster 4

Multi-locus variable tandem repeat analysis (MLVA) of *Mycoplasma bovis* to determine allelic profiles of past and current isolates

Melanie Zamora B.S., Alexandra Burne MPH, Mary Brown Ph.D

Department of Infectious Disease & Immunology, University of Florida, College of Veterinary Medicine, Gainesville FL 32608

Mycoplasma bovis was first isolated in the United States over 60 years ago. With global trade of cattle/cattle products M. bovis had spread to multiple countries within 10 years of its initial isolation. Clinical signs associated with infection are pneumonia, mastitis and arthritis. Transmission is typically respiratory, however transmission through milk has been widely demonstrated. While vaccines are available, there is a lack of data surrounding their efficacy and protectiveness. Mycoplasmas lack a cell wall and are naturally resistance to beta-lactam antibiotics. Treatment options are becoming more limited with increasing levels of antibiotic resistance being reported. As M. bovis can persist readily in populations large-scale euthanasia is often required to rid farms of epizootic outbreaks. Thus, there is a substantial detrimental economic impact when it comes to M. bovis infected herds. M. bovis prevalence on domestic farms can range from 0.8-30% where environmental factors compound infection outcome. Our lab has a library of M. bovis isolates from U.S. farms across a 35-year period. In this study we aimed to look at allelic profiles of M. bovis using multi-locus variable tandem repeat analysis (MLVA) to compare isolates from 1987 to our most recent isolates (2017-2023) to look for any allele cycling. Future work will involve analysis of U.S. isolates from the early 2000's, as well as isolates from an underrepresented country (Bosnia and Herzegovina).

Poster 5

Molecular and behavioral responses to the antineoplastic ifosfamide in zebrafish (Danio rerio) embryos and larvae

Cole D. English, Kira J. Kazi, 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

Alkylating antineoplastic agents are employed in chemotherapeutic treatment of various cancers. Although the presence of ifosfamide, one such alkylating antineoplastic, in aquatic ecosystems is well established, the ecotoxicological risks of the chemical have not yet been fully investigated. Hence, the lack of toxicity data for ifosfamide necessitates a need to demarcate its potential negative impacts on endemic aquatic species potentially exposed through wastewater and hospital effluent. The objectives of this study were to (1) Characterize sub-lethal teratogenic, cardiotoxic, and mitochondrial toxicity potential of ifosfamide exposure; (2) Elucidate mechanisms of toxicity with RNA-seq; (3) Determine if ifosfamide exposure altered locomotor and anxiogenic behaviors in larval fish. Survival rate, hatch rate, and deformity incidence were not different amongst treatments following exposure levels up to 1000 μg/L ifosfamide. Tachycardia was noted in early-stage embryos treated with ifosphamide. Reactive oxygen species were not induced in 7 dpf larvae up to 1000 μg/L ifosfamide, but there was reduction in non-mitochondrial respiration with exposure. RNA-seq reveled 231 and 93 transcripts differentially expressed in larvae exposed to 1 μg/L and 100 μg/L ifosfamide respectively. RNA-seq also revealed induction of several gene networks related to vascular resistance, cardiovascular response, and heart rate. Hyperactivity in larval zebrafish was noted at environmentally relevant concentrations (0.1-1.0 μg/L) of ifosfamide, and there was no change in behaviors associated with anxiety. RNA-seq revealed increased expression of dopamine networks in larval fish, which may be related to hyperactivity. This study improves ecological risk assessment of antineoplastics by elucidating molecular and physiological mechanisms related to ifosfamide toxicity, and more broadly, the alkylating agents.

Poster 6

Transcriptome and behavioral investigation of atorvastatin exposure in developing zebrafish (*Danio rerio*)

Ciara Saccente1, Emma Ivantsova1, Aster Pringle1, Christopher L. Souders II1, Christopher J. Martyniuk1,2

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,

Atorvastatin is a statin pharmaceutical that is frequently prescribed to treat high cholesterol and triglyceride levels. This pharmaceutical has been deposited into aquatic environments at low ng/L levels due to incomplete wastewater treatment and once in ecosystems, can exert off target effects in aquatic wildlife. The objectives of this study are to determine the developmental effects of atorvastatin in zebrafish by measuring the expression of transcripts in larval fish, as well as indicators of oxidative stress and locomotor activity of exposed zebrafish. We exposed zebrafish at 6 hours post fertilization to one treatment of either embryo rerio media (control), 100 ng/L atorvastatin (low) or 1 µg/L atorvastatin (high) for 7 days. RNA-seq revealed that there were 2359 differentially expressed transcripts in the low concentration and 1991 in the high concentration treatments ($p.adj<0.05$). KEGG pathway enrichment revealed 100 ng/L atorvastatin altered genes related to fatty acid metabolism, fatty acid biosynthesis, ECM-receptor interaction, and ABC transporters. Conversely, 1 µg/L atorvastatin affected transcripts associated with adrenergic signaling in cardiomyocytes, mitophagy, melanogenesis, and protein processing in the endoplasmic reticulum. Atorvastatin also induced hyperactivity in larval fish at environmental levels. Such endpoints inform on the toxicity of the pharmaceutical to gain a better understanding of its potential risk to aquatic wildlife.

Poster 7

Pilot evaluation of the effects of positive end-expiratory pressure and inspiratory pause on dead space in mechanically ventilated dogs

Keith Y Gandy1 BS, Ignacio Sández-Cordero2 DVM, Ludovica Chiavaccini1 DVM, PhD, MS, DACVAA, Diego A Portela1 DVM. PhD, DACVAA

1 Department of Comparative, Diagnostic, and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA
2 Department of Anesthesiology and Pain Management, Hospital Veterinario Vetsia-AniCura, Leganés, Madrid, Spain

Animals undergoing general anesthesia develop atelectasis, which impairs gas exchange. Positive end-inspiratory pressure (PEEP) is commonly used to prevent atelectasis; however, PEEP could lead to airway and alveolar overdistention, increasing anatomical (VDaw) and physiological dead space (VDphys), which worsens prognosis in ventilated human patients. The present observational study evaluated the effects of PEEP and end-inspiratory pause (EIP) on VDaw and VDphys in anesthetized dogs using volumetric capnography. Volumetric capnograms were recorded at baseline (0 cmH2O PEEP), after adding 5 cmH2O PEEP (PEEP_5), and finally after adding a 30% EIP (PEEP+EIP) in seven anesthetized, dorsally recumbent dogs ventilated with a tidal volume (VT) of 15 mL/kg. Repeated measurements were analyzed using generalized mixed model effects. The median anatomical dead space and VDphys slightly increased with PEEP_5 ($p < 0.01$) and then returned to values not significantly different from the baseline with PEEP+EIP. The median alveolar tidal volume (Vtalv) decreased slightly with PEEP_5 and increased slightly over baseline with PEEP+EIP; however, this difference was not statistically significant ($p = 0.14$). No significant differences were observed in alveolar dead space and CO2 elimination (VCO2) per breath. In conclusion, the addition of PEEP increases VDphys by increasing VDaw; however, the addition of EIP returns dead space values near the baseline, overcoming one of the side effects associated with PEEP. Adding EIP can have beneficial clinical effects in dogs with lung diseases requiring mechanical ventilation.

Poster 8

A single-cohort retrospective analysis of factors associated with morbidity and mortality in 193 anesthetized domestic goats

Jessica Steen, Marta Romano DVM, MS, PhD, Dipl. ACVAA, Diego A. Portela DVM, PhD, Dipl. ACVAA, Alanna N. Johnson DVM, Dipl. ACVAA, Sarah Shippy DVM, MPH, MS, and Ludovica Chiavaccini DVM, DES, MS, Dipl. ACVAA

Department of Comparative, Diagnostic, and Population Medicine, University of Florida, College of Veterinary Medicine, Gainesville, Florida, 32610 USA

Morbidity and mortality are main concerns during general anesthesia, particularly in species where evidence-based knowledge is scarce, such as goats. This retrospective, single-cohort, observational study aimed to define morbidity and mortality in goats undergoing general anesthesia at a large animal teaching hospital. Data were collected from 218 medical records on 193 goats undergoing general anesthesia between January 2017 and December 2021. Demographic data, anesthetic management, recovery period and perianesthetic complications were recorded. Perianesthetic death was defined as anesthesia-related or anesthesia-contributory death occurring within 72 h after recovery. Records of euthanized goats were reviewed to ascertain the cause of euthanasia. Each explanatory variable was investigated by univariable penalized maximum likelihood logistic regression, followed by multivariable analysis. Statistical significance was set at $p < 0.05$. Perianesthetic mortality was 7.34%. Multivariable analysis showed that gastrointestinal surgeries (OR 19.17, SE 12.99, 95% CI 5.08-72.33, $p < 0.01$) and requirement for perianesthetic norepinephrine infusion (OR 10.85, SE 8.82, 95% CI 2.21- 53.33, $p < 0.01$) were associated with increased mortality. Maintaining other variables equal, the use of perianesthetic ketamine infusion was associated with decreased mortality (OR 0.09, SE 0.09, 95% CI 0.01 – 0.73, $p = 0.02$). Anesthesia-related or anesthesia-contributory complications included hypothermia (52.36%), bradycardia (38.07%), hypotension (35.32%), hypoxemia (14.77%), regurgitation/aspiration (7.34%), azotemia/ acute renal failure (4.59%), myopathies/neuropathies (4.13%) and fever of unknown origin (2.75%). In this population, gastrointestinal surgeries and the requirement for perianesthetic norepinephrine infusion were associated with increased mortality in goats undergoing general anesthesia. Ketamine infusion may have a protective effect.

Poster 9

Reproductive complications and survival after parturition in hospitalized goats: a multi-center study

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Despite the growing popularity of goats as production animals and pets, there is very little information available regarding the incidence of periparturient reproductive complications in goats. The objective of this study was to describe the incidence of reproductive conditions in pregnant does admitted to referral hospitals. A multi-center cross-sectional study including 9 veterinary teaching hospitals was conducted, with data collected by questionnaire completed by the admitting clinician. Descriptive statistics were used to report complications, and survival between groups was compared using Chi-square. A total of 184 does were included in the study. One-hundred forty-seven (80%) does had dystocia, 108 (59%) underwent C-section, and 28 (15%) were diagnosed with pregnancy toxemia. Periparturient complications included retained fetal membranes (n = 35, 19%), vaginal/perineal trauma (n = 28, 15%), uterine tears (n = 24, 13%), metritis (n = 21, 11%), uterine/vaginal hemorrhage (n = 7, 4%), and uterine prolapse (n = 1, 0.5%). Does with uterine tears were less likely to survive to discharge than does without uterine tears (29% survival vs 86% survival, $P < 0.001$). Does with uterine/vaginal hemorrhage (43% survival vs 86% survival, $P = 0.002$) or metritis (62% survival vs 87% survival, $P = 0.003$) were less likely to survive to discharge than those without these conditions. This study demonstrated a high incidence of reproductive complications in periparturient does admitted to referral hospitals. Periparturient reproductive complications were associated with non-survival to hospital discharge.

Poster 10

Dynamic pneumatic compression therapy can be safely applied following maximal exercise but does not improve systemic lactate clearance in fit Thoroughbred horses

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Dynamic pneumatic compression therapy (DCT) has had limited investigation for post-exercise recovery in people. There are no publications investigating use of DCT for athletic recovery of horses. This study aims to assess whether the EQ Press DCT would improve systemic lactate clearance in horses following maximal exercise and following standard training 48 hours later. Thoroughbred horses (n=6, 3 mares, 3 geldings) were enrolled in a randomized crossover design with 2 weeks between trials. Each horse underwent an exercise test to exhaustion followed by routine cooldown (control) or routine cooldown and application of EQ Press at 30 minutes and 4 hours post-exercise. Horses were confined to a stall until 48-hour post-test standard training. Blood lactate, heart rate, temperature, and blood pH and time for parameters to return to normal were used to assure horses had met criteria for maximal exercise as well as to assess the effect of EQ Press treatment compared to control. All horses tolerated forelimb DCT and 5/6 horses tolerated hindlimb DCT. Pre- and post-test parameters confirmed that all horses met criteria for maximal exercise. No significant differences between EQ Press and control were observed, except that EQ Press treated horses showed an increased time for blood pH to return to normal ($p=0.01$). The current study findings show that EQ Press DCT was well tolerated but did not improve lactate clearance following maximal exercise in this small population of horses. Future investigation on horses undergoing different exercise programs and assessment using different recovery parameters may be warranted.

Poster 11

MRI findings associated with clinical presentation and histopathology in a Mexican redknee tarantula (*Brachypelma hamorii*)

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A 15-year-old, 9 g, female Mexican redknee tarantula (Brachypelma hamorii) presented to the North Carolina State University College of Veterinary Medicine with a history of anorexia and weight loss. Physical examination revealed an inability to extend the left chelicera, which was suspected to prevent the tarantula from prehending live prey. Magnetic resonance imaging revealed enlargement and T2 hyperintensity of the left chelicera. The patient was provided with supplemental feedings and rehydration until it was found deceased more than 8 months after the initial presentation. A postmortem examination was performed, and histopathology showed an area of chronic inflammation at the level of the left chelicera. Magnetic resonance imaging can be used as an antemortem diagnostic tool to identify lesions in tarantulas.

Poster 12

Optimizing Gene Editing in Canine Cells Using CRISPR/Cas9 for Pyruvate Dehydrogenase Kinase 4

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Dilated cardiomyopathy in canines is associated with a 16-base pair deletion in the intron of the pyruvate dehydrogenase kinase 4 (PDK4) gene. This can lead to impaired cardiac function. The study objective was to determine the efficacy of homology-independent targeted insertion (HITI) gene editing in canine cells. We hypothesized that clustered regularly interspaced short palindromic repeats (CRISPR) and its associated protein-9 (Cas9) would effectively genetically edit PDK4. Guide RNAs (sgRNA) were designed to target the mutant region and enable replacement of the deleted region using the HITI approach. Several sgRNAs were tested with in vitro cleavage reactions, followed by candidate sgRNAs nucleofection into canine cells. Cleavage efficiency was assessed by sequencing and Tracking of Indels by Decomposition (TIDE) and Interference of CRISPR Edits (ICE) analysis. Four sgRNAs showed evidence of cutting in vitro of the desired DNA templates. Post-nucleofection TIDE and ICE analysis showed different results for total editing efficiency of up to 9.5% with SaCas9-61for and KKH-71rev. However, we were not able to detect editing efficiency in sgRNA designed to cut only in presence of the mutation, KKH-123for and KKH-Drop. This study investigates a novel approach to gene editing in canine cells is expected to treat genetic diseases in canines.

Poster 13

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.

Poster 14

Validation of a Primary Thyroid Feline Cell Line and Treatment with Methimazole

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Hyperthyroidism is the most common endocrine disease in cats, with approximately 10% of cats over 10 years of age affected. It can lead to widespread systemic effects such as cardiomyopathy and hypertension and may be fatal if left untreated. In vitro assays are useful for assessing the efficacy of new potential compounds for treating hyperthyroidism. The objective of this study was to validate primary cat thyrocyte cell cultures for in vitro studies and to test the effects of anti-thyroid methimazole (MMI). Thyroid tissue was excised from 10 cat cadavers to develop primary feline thyrocyte cultures. The purity of cell cultures was verified by conducting histology and immunohistochemistry. Immunohistochemistry showed positive reactivity for thyroid peroxidase and thyroid stimulating hormone receptor, confirming purity of the thyrocytes. Thyrocytes were exposed to 20 ng/mL and 2,000 ng/mL MMI, with the former mimicking a realistic therapeutic dosage. The mRNA steady state levels of transcripts related to thyroid hormone production and transport were measured in thyrocytes after a 72-hour treatment. MMI did not alter the expression levels of receptors (thra, thrb), enzymes (dio1, deiodinases), nor transporters. Thyroid hormones (T3 and T4) were also measured in the media using a competitive ELISA. T3 was below detection limits and efforts are currently focused on improving detection. Intriguingly, T4 levels did not show any difference among groups with MMI treatment, suggesting that the therapeutic effects of MMI may require additional feedback mechanisms in vivo. Data demonstrates that thyrocytes can be isolated and cultured to study treatments related to hyperthyroidism.

Poster 15

Current mortality rate and main characteristics of dogs diagnosed with sepsis at the University of Florida Small Animal Hospital: preliminary results of a retrospective study

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Sepsis, defined as a life-threatening organ dysfunction due to dysregulated host response to infection, is a medical emergency due to patient fast decompensation and elevated mortality rates. Pre-clinical and clinical studies over the last years were not able to find a specific medication to treat sepsis, however, guideline-based human hospitals' routines culminated in a significant reduction in patients' mortality rate (approximately 40% nowadays). In dogs, sepsis is a common cause of emergency admittance and ICU deaths. Similarly, there is no specific medication to treat dogs with sepsis and its treatment is also centered on human guidelines, Thus, due to the sparse veterinary literature evaluating the mortality rate in dogs with sepsis and the main factors related to it, this study aimed to evaluate the local mortality rate and main factor (s) of non-survival. The database is composed of 104 cases from 2016 to 2021, all of which were dogs presented to The University of Florida, Emergency Department. Of 104 cases, 34 patients survived (27.2%) and 70 did not (72.8%). Of the non-survivors, 55 dogs died during treatment, whose 38/55 were euthanized (69%). Besides, 15 dogs were euthanized at the diagnosis of sepsis. Considering only deceased patients, the age average was 8 years old, hospitalization 2.9 days, and financial cost of $1837.91. The majority of the deceased patients were obtunded on presentation or were euthanized. This study demonstrates that the mortality rate for sepsis in dogs is still elevated. Financial limitations and poor prognosis were the main factors in patients' outcomes.

Poster 16

Correlation between Urine Anion Gap and Urine Ammonia-Creatinine Ratio in Healthy and Kidney Disease Cats

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Ammonium excretion decreases as kidney function declines in several species including cats, and may have predictive or prognostic significance related to chronic kidney disease (CKD). Urine ammonia measurement is not readily available in clinical practice, so urine anion gap (UAG) has been proposed as a surrogate test. The primary aim was to determine degree of correlation between urine ammonia to creatinine ratio (UACR) and UAG in healthy cats and CKD cats. A secondary aim was to evaluate if there was a significant difference between the UAG of healthy and CKD cats. Previously collected urine samples from client-owned cats assessed to be healthy (n=59) or to have stable CKD (n=17) were thawed and processed. Urine electrolytes were measured using a commercial chemistry analyzer. UAG was calculated as sodium + potassium - chloride. Urine ammonia and creatinine were previously measured using commercially available enzymatic assays and used to calculate UACR. Coefficient of determination (square of Pearson correlation coefficient) between UAG and UACR was calculated for both groups. Unpaired Student's T-test was used to compare mean UAG of healthy versus CKD cats ($p < 0.05$). UAG had a very weak correlation with UACR in healthy ($R^2 = 0.18$) and CKD ($R^2 = 0.07$) cats. There was a significant difference between UAG in healthy and CKD cats ($p< 0.002$). This calculation for UAG cannot be used as a substitute for UACR in cats. The clinical significance of the difference between healthy and CKD cats remains unknown.

Poster 17

Comparison of exposure of the radius afforded by the craniomedial and craniolateral surgical approaches.

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The surgical approach selected in canine antebrachial fractures is likely influenced by the location and configuration of the fracture, implant availability, associated soft tissue trauma, and surgeon training and experience. The aim of this descriptive cadaveric study was to quantitatively compare the circumferential exposure afforded by an open surgical craniomedial approach (CMA) and craniolateral approach (CLA) to the radius, without and with the release of the abductor pollicis longus (APL) muscle. We hypothesized that the CLA would provide greater diaphyseal and epiphyseal exposure, and the CMA would offer more metaphyseal exposure. Standard craniomedial and craniolateral approaches were performed on 12 randomly assigned paired cadaveric forelimbs, with subsequent release (CMA) or elevation (CLA) of the APL muscle. Digital images were uploaded, and the surface area and percent area of exposure was calculated in 10% longitudinal increments. The CLA afforded greater exposure to the radial diaphysis (30-70% longitudinal radial segments mean: 82.8% CM; 90.3% CL) and epiphysis (90-100% longitudinal radial segments mean: 6.2% CM; 40.8% CL). Distal radial metaphyseal plate placement (70-90% longitudinal radial segments) can be more readily accomplished using the CMA (mean: 64.2% CM; 52.2 CL). Distal exposure (80-100% longitudinal radial segments) can be effectively improved using either approach by transecting the tendon of insertion (CMA) or by releasing the origin (CLA) of the APL muscle (mean increased exposure: 86.3% CM; 62.1% CL). This information presents surgeons, particularly less experienced surgeons, with an objective means of deciding which approach might be more useful when addressing fractures at specific locations.

Poster 18

Acellular fish skin may be used for immediate wound coverage following wide surgical tumor excision in dogs: A prospective clinical trial

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To prospectively evaluate the clinical outcomes using acellular fish skin grafts (FSGs) for the management of complete wound healing by secondary intention after wide surgical excision of skin tumors in dogs. 5 dogs undergoing wide surgical excision of distal extremity skin tumors. FSGs were applied to surgical wound beds following wide excision of the tumor. Bandages were changed weekly, and additional grafts placed when integration of the previous graft was complete. The wounds were assessed for: dimensions, health (color), time to complete epithelialization, complications, and tumor recurrence. All masses were excised with 2 cm lateral margins and one facial plane deep to the tumor. Surgical wounds had a mean area of 29.9 ± 16.9 cm2. The number of FSG applications ranged from 4 to 9 (median 5). Complete epithelialization occurred within 7-9 weeks for uncomplicated wounds, and 12-15 weeks for complicated wounds that sustained self-trauma. There were no adverse events related to the use of FSGs. Tumor diagnoses included 3 mast cell tumors and 2 soft tissue sarcomas. Three of the 5 tumors had incomplete histologic margins. Local recurrence was not seen over the follow-up period of 239 to 856.Wide surgical excision of distal extremity skin tumors, followed by repeated application of acellular FSGs, resulted in complete healing of all wounds with no adverse events. This treatment method does not require advanced reconstructive surgical skills and may be useful for the management of skin tumors on the distal extremities.

Pantarsal arthrodesis stabilized with circular external skeletal fixators in 8 dogs (2010-2022)

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The aim of this study was to report the surgical technique, complications, and outcomes of 8 dogs that underwent pantarsal arthrodeses stabilized using circular external skeletal fixators for the treatment of uni or multilevel tarsal instability. Six of the 8 dogs had an excellent return to function following arthrodesis, however; 4 of the 8 dogs developed complications. Two of these 4 dogs had at least 1 major complication and represented the 2 dogs that did not return to full function with an excellent outcome. One dog failed to obtain union of the arthrodesis site and required secondary surgical stabilization. The second dog had persistent lameness following fixator removal. Minor complications occurred in 2 of the 8 dogs and resolved with construct removal. Fixator removal was performed at a median time of 11.5 weeks (IQR 10.3 to 12.8 weeks). Tarsi were stabilized with a median angle of extension of 125.8 (IQR 120.7 to 129.9). Circular constructs can be used successfully to stabilize pantarsal arthrodeses in dogs with tarsal instability. These constructs require continued construct care but obviate the need for supplemental post-operative coaptation. This technique may be considered as an alternative to internal plate fixation avoiding implant-associated complications, such as screw loosening, implant failure, and implant-associated infection.

Inter- and Intra-observer Reliability of Thoracic Limb Circumference Measurement Methods in Healthy Dogs

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Limb circumference, as an indirect measure of muscle mass, is an inexpensive, practical objective outcome measure commonly used for the pelvic limb in canine rehabilitation. Currently, there is no standardized circumference measurement protocol for the canine thoracic limb. The primary objectives of this prospective, blinded study are to (1) compare the inter- and intra-observer reliability of thoracic limb circumference measurement methods in healthy dogs, and (2) determine the most reliable thoracic limb positioning and location on the thoracic limb for performing circumferential measurements. Thoracic limbs of ten healthy dogs (20 limbs) were measured. Triplicate measurements were performed at mid-brachium (50%), distal brachium (70%), and of the proximal antebrachium (25%). Intra-class correlation coefficients (ICC) with a 95% confidence interval were used for data analysis with a $p < 0.05$ being significant. All measures had significantly good to excellent intra- (ICC 0.836 – 0.994, $p < 0.001$) and inter-observer reliability (ICC 0.834 – 0.996, $p < 0.001$), with the most reliable method at 25% antebrachial length in either a neutral or extended elbow position. Measurements of the thoracic limb were most reliable at 25% antebrachial length with the elbow either in a neutral or extended position. Further research is needed on dogs of different conformations and with common disease conditions to ensure these findings also apply to patients with known orthopedic conditions of the thoracic limbs.

Poster 21

Radiographic characterization and establishment of a grading system for tibial avulsion fractures in dogs

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Proximal tibial apophyseal avulsion fractures are the most common avulsion fracture in dogs. One previously proposed classification system for proximal tibial apophyseal fractures in dogs is similar to the Watson-Jones classification system in humans. This canine study however did not include fracture types described by Ryu et al. and Ogden et al. in humans. The authors propose a new classification system of proximal tibial apophyseal fractures in dogs. This is a retrospective and observational study. Radiographic and CT images of dogs admitted to a veterinary teaching hospital were assessed twice in a randomized order two weeks apart by a first-year diagnostic imaging resident and a DACVR. The fracture type, subject age, sex, neuter status, body weight, limb of fracture, comminution, and presence of concurrent fibular fracture were recorded. The intra- and interobserver agreement for fracture classification was calculated using the Cohen's kappa. The fracture type distribution was: type 1 (33/49), type 2 (1/49), type 3 (0/49), type 4 (2/49), and type 5 (13/49). Dogs that had a concurrent fibular fracture had a type 4 or type 5 tibial avulsion fracture ($p < 0.01$). The intra-observer agreement for Observer 1 (kappa= 0.80) and Observer 2 (kappa= 0.88) was strong. The inter-observer agreement was moderate (kappa= 0.71-0.72). This study describes two new tibial apophyseal fracture conformations in dogs (type 4 and 5). The use of the proposed classification system is recommended in the description of tibial apophyseal avulsion fractures in dogs, as it might assist surgeons in patient management and surgical planning.

Poster 22

Efficacy and safety of topical mydriatic agents in bearded dragons (*Pogona vitticeps*).

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The inland bearded dragon captive pet population has grown dramatically since being first imported to the United States in the early 1980's. Their combination of anatomical and personality traits has made them one of the most popular companion reptile species. Bearded dragons, in comparison to mammalian species, possess striated muscle in their iris and ciliary process, meaning parasympatholytic medications should not result in mydriasis. As in other species, mydriasis is important for thorough evaluation. Topical administration of rocuronium bromide, an aminosteroid neuromuscular blocking agent, has recently been shown to be an effective mydriatic agent in loggerhead turtles and several avian species, who also possess striated iris musculature. The goal of this study was to investigate the efficacy and safety of topically applied neuromuscular blocking agents, as well as a traditional mammalian mydriatic agent, atropine. Six privately owned bearded dragons were included in an initial pilot phase, followed by a 3-phase crossover portion. In the pilot phase, 3 randomly selected bearded dragons were treated with either rocuronium, atropine, or rocuronium combined with phenylephrine, to determine the lowest effective dose. During the crossover phases each bearded dragon was treated with each of the medications, with a minimum 1 week washout period between medications. Photo documentation was utilized in each phase to facilitate measurement of pupil size. Pre-medication administration pupil size, as well as serial post-medication administration pupil size were measured and compared. Results are currently still under review.

Poster 23

Evaluation of Intra-observer and Interobserver Agreements to Determine Reliability of 8 Segmental Reflexes in 101 Dogs

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There is a lack of literature that support the popular claim that the patellar reflex and the withdrawal (flexor) reflex are the only reliable segmental reflexes in the normal dog. The objective of the study reported was to identify the segmental reflexes that are most reliably present in dogs by estimating the intra-observer agreement of eight segmental reflexes in the right limbs versus left limbs by three veterinary clinicians and the interobserver agreement of the responses to the selected reflexes. This is a prospective, descriptive study. A segmental reflex was defined as reliable in the present study if it had an observed agreement of greater than or equal to 96% and a Kappa value greater than or equal to 0.61 in both right and left limbs. The intra-observer agreement was highest for the withdrawal reflex in the thoracic limbs, patellar reflex, cranial tibial reflex, and the withdrawal reflex in the pelvic limbs. The interobserver agreement was perfect or near perfect for the withdrawal reflex in the thoracic limbs, patellar reflex, cranial tibial reflex, and withdrawal reflex in the pelvic limbs. In animals without evidence of severe orthopedic disease that inhibits normal range of motion in their limbs, myelopathy, or neuromuscular disease, the patellar and the withdrawal reflexes were found to be reliably present and should be included in the neurologic exam of dogs.

Poster 24

Radiographic Features of Cardiogenic Pulmonary Edema in Dogs with Dilated Cardiomyopathy

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Dilated cardiomyopathy (DCM) is one of the most common causes of acquired cardiac disorder in dogs, second only to myxomatous mitral valve disease. DCM primarily affects large-breed dogs and can present with left-sided congestive heart failure (CHF), as supported by the presence of cardiogenic pulmonary edema on thoracic radiographs. This study aimed to describe the radiographic features of cardiogenic pulmonary edema in dogs with DCM. A total of 52 dogs with left-sided CHF due to DCM met the inclusion criteria. Thoracic radiographs were retrospectively reviewed by two radiologists using a survey to assess the distribution, severity and characteristics of the pulmonary pattern, as well as to identify the presence of pleural effusion and degree of cardiomegaly. Results of the study suggest that the predominant pulmonary pattern differs from myxomatous mitral valve disease, characterized by a mild to moderate (59.6% and 32.7%, respectively), unstructured interstitial (71%), diffuse (92%), and predominantly ventrally distributed (53.8%) pulmonary pattern. A subset of dogs (32.7%) had diffusely distributed linear soft tissue opacities throughout all lung lobes considered to represent pulmonary vascular congestion, bronchial cuffing and/or thickening of the bronchi. The presence of lobar pulmonary venous enlargement was an expected finding in a large number of dogs associated with left-sided CHF. A little over half (57%) of the dogs had pleural fissure lines, possibly representing scant to mild pleural effusion. Recognition of these radiographic findings to support a diagnosis of left-sided CHF is important to guide medical recommendations in dogs with DCM.

Poster 25

Selective osmotic shock for the isolation of feline pancreatic Islets of Langerhans

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Background:
Pancreatic islet isolation is critical for studying islet physiology and transplantation. Traditional protocols use collagenases, causing injury to islets. In cats, collagenase protocols fail to produce functional islets, as demonstrated by glucose-stimulated insulin secretion (GSIS). Since islet cells express GLUT-2 they may adapt to hyperosmolar glucose solutions, enabling islet isolation by selective destruction of exocrine tissue.

Objective:
To compare islet yield, purity, morphology, and GSIS between four protocols for islet isolation using selective osmotic shock (SOS)

Animals:
Pancreata were obtained from cats in which necropsy or pancreatectomy was performed for reasons unrelated to this study.

Methods:
Pancreatic tissue was mechanically disrupted and incubated in hyperosmolar RPMI solution with either 300 mmol/L (protocol A and B) or 600 mmol/L glucose (protocol C and D) for 20 (A and C) and 40 minutes (A and D). Islets were cultured for 24-48 hours. Using light microscopy, islets were quantified and assessed for purity and morphology. GSIS was measured by incubating approximately 120 islets from each protocol for 60 minutes in low (2.8mM) and high (28mM) glucose concentrations. GSIS was calculated as [insulin] at 28mM glucose/[insulin] at 2.8mM glucose. Data are presented as median and range.

Results:
Islet yield was moderate (713 islet/g, [407-2261]) and morphology excellent across protocols. Protocol C resulted in the highest GSIS stimulation index (2.2 [1.0-3.6], $p = 0.05$).

Conclusions:
SOS resulted in isolation of functional feline islets and will allow in vitro studies. Optimization to improve purity and yield are required before use in clinical islet transplantation.

Poster 26

Antibody Response to Mosquito Salivary Proteins as a Marker for Exposure

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Arboviruses (arthropod-borne viruses) are responsible for a massive global burden of disease in humans. Aedes spp. mosquitoes spread the majority of human mosquito-borne diseases (MBDs). Furthermore, many humans also suffer from hypersensitivity/allergy to mosquitos, which can be debilitating in some. More efficient mosquito surveillance is needed that is not as cumbersome and costly as traditional methods and can accurately examine bite exposure (and possible arbovirus exposure). The quantitative measuring exposure to mosquito bites, via technologies such as ELISA, has been demonstrated as a promising alternative to trap-based surveillance. Using PRISMA guidelines, a systematic review and pooled analysis were performed to assess the efficacy of detection of human antibody response to mosquito salivary proteins (MSP) as presented in the literature. A total of 1353 studies were screened by two reviewers; 99 articles were included in the qualitative synthesis. The pooled analysis included 20 papers that met our inclusion criteria, provided individual level human IgG response to MSP via ELISA, and explicitly stated how OD scores were reported. We assessed how subject age, Aedes spp. mosquito, antigen type, collection season, population level of mosquito exposure, Köppen-Geiger climate, and OD reporting method impact OD values in separate univariate analyses as well as a multivariate analysis. We found that OD values correlated positively with antigen complexity and population level of mosquito exposure. While there is considerable variation between studies (ICC=0.12), using human IgG holds promise in complimenting more traditional mosquito surveillance methods as a proxy for individual and population exposure to Aedes spp. mosquitoes.

Poster 27

Identification of vaccine candidate antigens of Campylobacter hepaticus using phage display technology

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Introduction: Spotty liver disease (SLD) caused by C. hepaticus is an emerging cause of morbidity, mortality, and loss of production in commercial laying hens. Currently, the commercial layer industry relies on C. hepaticus autogenous killed vaccines. However, the slow growth of C. hepaticus, the detrimental effects of the high-volume use of killed vaccines on pullet performance, and the cost of production prohibit the use of killed vaccines. Therefore, a live-vectored vaccine expressing protective C. hepaticus antigen/s is a logical approach to control SLD.
Material and methods: The phage display reverse vaccinology was used to select candidate vaccine antigens. First, the antibodies against killed whole-cell C. hepaticus were raised in specific pathogen-free female Leghorn chickens. Then, the protective antigen epitopes were identified using Ph.D.-12 phage display peptide library kit from the New England Biolabs and hyperimmune serum raised against C. hepaticus. Finally, DNA sequencing, Basic Local Alignment Search Tool, and subcellular localization prediction identification were used to prioritize the identified epitopes (mimotopes).
Results and conclusion: We discovered 16 mimotopes matched with 13 different proteins of C. hepaticus as potential vaccine candidates. Some of these proteins have already been tested as potential vaccine candidates against other Campylobacter species, such as Campylobacter jejuni and Campylobacter coli. These epitopes' immunological and protective abilities have to be confirmed through in vivo and in vitro research.

Poster 28

An avian pathogenic E. coli vector system for delivering heterologous vaccine antigens in poultry

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Poultry constitutes the most important protein source for human consumption worldwide. One of the biggest threats to the poultry industry are infectious diseases, which are primarily controlled by antimicrobial and antiparasitic drugs, vaccines, and biosecurity practices. Due to the increasing consumer demand for "organically-raised" or "antibiotic-free" poultry, vaccination has been one of the most common approaches to control economically significant diseases. However, the current volume of vaccines has negatively affected the industry due to many reasons, including rising vaccine production and administration costs and poor growth performances associated with some vaccines. Avian pathogenic Escherichia coli (APEC), a major pathogen causing severe illness in poultry, affects all bird types and ages. In this study, we constructed two attenuated strains of avian pathogenic E. coli lacking the asd gene (Δasd single mutant and ΔasdΔaroA double mutant), which is involved in synthesizing diaminopimelic acid (DAP), to deliver heterologous vaccine antigens via an asd+ expression plasmid. Because the Δasd mutants will have an obligate requirement for DAP, they will not survive in the host without the asd+ plasmid. This system provides a balanced lethal combination to deliver antigens obviating the need for an antibiotic-selective marker in the live vaccine. This vaccine vector strain is expected to deliver vaccine antigens of other pathogens while providing protection against colibacillosis. In vivo experiments are currently underway to demonstrate the safety, immunogenicity, and efficacy of the APEC mutants expressing a novel Salmonella vaccine antigen to protect commercial egg-type chickens against colibacillosis and Salmonella colonization.

Poster 29

Discovering Virulence Inhibitors In Highly Pathogenic Bacteria

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Francisella tularensis is a deadly bacterium that causes a zoonotic disease called tularemia. Due to its low infectious dose and potent virulence, it is a potential agent of bio-terrorism. The chief virulence factor driving pathogenicity in these bacteria is a toxin secretion apparatus called the type VI secretion system (T6SS). An essential component of the T6SS apparatus is a contractile sheath that is composed of two proteins, IglA and IglB. In this study, we will discover novel therapeutics to block the secretion nanomachinary and attenuate virulence. To this end, we have developed a high-throughput method to detect the interaction of IglA with IglB by using a split Renilla luciferase assay. As a proof of principle, we screened a library of one thousand drugs composed of natural and synthetic compounds. Two of these compounds block sheath assembly, secretion of toxins, and bacterial virulence. Since these drugs block virulence but do not affect bacterial viability, they represent a new class of antibiotic that has the promising property of avoiding microbial resistance.

Poster 30

Engineering of Parasitic Bacteriophages to Combat against *Burkholderia pseudomallei*

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Burkholderia pseudomallei is a Gram-negative bacterium residing in soil and fresh water and is the causative agent of melioidosis in both humans and animals in the tropics. Melioidosis is considered untreatable in animals due to the development of antibiotic resistance and the high cost of effective antibiotic treatments for up to 20 weeks, as well as the difficulty of decontaminating infected areas. As a result, bacteriophage (phage) therapy is being investigated as a possible treatment for this disease. Herein, we studied lysogenic phages, the bacterial parasites that incorporate their genome into the host chromosome. We used bioinformatic tools to track the short direct repeat sequences at the 3'end of the recombination event in order to identify prophage regions in B. pseudomallei genomes obtained from the GenBank database. This method uncovered over 200 functional prophage regions in 135 B. pseudomallei genomes. Ten recombination hotspots on B. pseudomallei chromosomes were discovered, with many of them linked to tRNA gene sequences. Transcriptomic analysis revealed that phage integrase genes were highly expressed during normal bacterial growth, whereas other prophage genes were not or only minimally expressed. This suggests that phage integrases may inhibit the phage during normal bacterial growth. Subsequently, deletion mutagenesis was used to characterize a phage integrase gene, BP1026B_I3636, in the lysogenic strain Bp82, which resulted in the lytic phage phenotype. We believe that modifying the integrase gene of a phage could result in a phage phenotype shift from lysogenic to lytic, which could be used in future phage therapy applications.

Poster 31

Interlaboratory Reproducibility of a TaqMan RT-qPCR Assay for Detection of Tilapia Lake Virus

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Tilapia is the second most important aquaculture species globally and a primary source of protein in many developing countries. Although tilapia are known for rapid growth and general hardiness, they are susceptible to common finfish pathogens such as viruses, bacteria, fungi, water molds, and parasites when reared intensively. Tilapia lake virus (TiLV) is the causative agent of an emerging viral disease associated with high morbidity and mortality in cultured tilapia worldwide. Since the first outbreak in 2012 in Ecuador, TiLV has spread globally, causing variable mortality in all ages of tilapia species in Asia, Africa, and the Americas. Although diagnostic assays to detect TiLV (or exposure to TiLV) have been developed, these assays have not been fully validated. The University of Florida Wildlife and Aquatic Disease Veterinary Laboratory has developed and partially validated (analytic and diagnostic performance) a TaqMan RT-qPCR assay to detect TiLV. In the current study, the reproducibility of the TiLV TaqMan RT-qPCR assay was evaluated through a collaborative effort involving six laboratories. All participating laboratories received a standard operating protocol for the RT-qPCR assay and a blinded test panel consisting of 31 positive and 19 negative RNA samples. Seventeen positive RNA samples were extracted from striped snakehead (SSN-1; E11 clone) cell culture supernatant (n=14) and fish tissues (n=3) infected with the same TiLV isolate. Seven positive RNA samples were generated from a single in vitro transcription event by preparing 7 aliquots of 106 copies in vitro standards from a single 107 copies tube; each was then diluted separately down to 104 copies. Furthermore, each of these 7 samples was duplicated within the panel (two vials aliquoted from the same tube) for a total of 14 samples. Nineteen negative RNA samples were extracted from fish tissues unexposed to TiLV. Performance measures, including variation between- and within-laboratory, were evaluated for cell culture supernatant and tissue RNA extracts. For the RNA samples generated by in vitro transcription, variations between-laboratory and within-laboratory were evaluated, including the variation within-vial and between-vial for the latter. All laboratories reliably detected both positive and negative samples, except for one laboratory reporting a negative sample as a suspect. For cell culture supernatant and tissue RNA extracts, the estimated standard deviation (SD) of mean Ct values between-laboratory was nearly double (0.39) that of the within-laboratory (0.20). However, the magnitude of this variation is relatively small, with both SDs being less than a full cycle threshold. The estimated SD of mean Ct values between-laboratory was 0.70 for in vitro transcript samples. For within-laboratory, the estimated SD of mean Ct values between-vial was nearly double (1.24) that of the within-vial (0.65); note that the former is an artifact of the sample preparation rather than the testing process. Thus, standard deviations reflecting the testing process all fell below a single cycle threshold for each sample type. This interlaboratory validation trial provided data to support the reproducibility (stage 3) of the post-extraction component of the TiLV TaqMan RT-qPCR assay as outlined by the World Organisation for Animal Health (OIE) for diagnostic assay validation.

Poster 32

Development of Burkholderia pseudomallei Detection Assay Using Bacteriophage Tail Protein-Based Approach

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Burkholderia pseudomallei is a Gram-negative, facultative intracellular bacteria and a highly pathogenic causative agent of melioidosis, a potentially fatal disease. Since melioidosis can be fatal within 24 - 48 hours, a precise and prompt diagnosis is critical to ensure that patients receive the appropriate therapy. However, melioidosis diagnostic approaches, such as culture, PCR, and antibody response measurement, are insufficiently sensitive or rapid. We aim to develop a rapid bacteriophage protein-based detection of B. pseudomallei. Phages recognize and enter their hosts using receptors that have great specificity and binding efficiency, making them appropriate for detection and comparable to monoclonal antibody-based techniques. We believe that tail proteins from Burkholderia phages, such as Phi-Bp82.1 tail fiber protein, PhiPK23 tail fiber protein GpH, and PhiBt-TXDOH tail fiber protein, are specific to B. pseudomallei. We aim to clone these proteins into E. coli using pET-21b or pET-DUET1 plasmids. Plasmid sequences will be validated by Sanger sequencing before being transformed, expressed and purified using immobilized metal affinity chromatography. We have successfully expressed tail fiber protein of Phi-Bp82.1 and now we are going to clone the others. We try to identify an appropriate candidate protein that may be used to develop a diagnostic application, such as fluorescence-based detection, latex agglutination, and lateral flow assay. While monoclonal antibody-based detection is difficult to generate and has certain cross-reactivity concerns that may lead to misidentification, these phage proteins are highly specific, robust, and easier to produce. This method could be used in conjunction with the gold standard B. pseudomallei diagnosis.

Poster 33

Cost-benefit analysis of an enhanced vaccination program to control brucellosis in (zero-grazing) cattle 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 in humans and livestock include forced unemployment in infected people, cost of treatment in humans, reduced livestock-related household income and access to milk, and interruption of trade in animals and animal products. In a recent systematic review, vaccination alone was cost-effective to control brucellosis in livestock populations (Kiiza et al., 2023). In Rwanda and other African countries, the cost-benefit of implementing mass vaccination strategies to control brucellosis in livestock is not known. Cost-benefit analyses can help policymakers decide whether allocation of resources is economically beneficial to cover the costs of brucellosis control interventions in populations. The objective of this study is to estimate the net present value (NPV) and benefit-cost ratio (BCR) from adopting an enhanced vaccination program for brucellosis control in zero-grazing cattle in Rwanda. The estimated prevalence of brucellosis in zero-grazing cattle is 5% in a population of 650,000. The current vaccination program includes vaccination of about 15% of all female calves using the Brucella abortus strain RB51 vaccine. An enhanced vaccination program would target 100% of all female calves. In a preliminary analysis (using a deterministic benefit-cost model), the NPV was negative and the BCR was below one; an indication that the enhanced vaccination program is not economically justifiable (when animal health benefits only are taken into account). Benefit-cost model structure, parameters, inputs, and equations, as well as the consequences of acting, or failing to act, on policy options of two selected intervention scenarios will be presented at the conference.

Poster 34

Emerging Viral Pathogens in Farmed White-tailed Deer (*Odocoileus virginianus*) in Florida

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White-tailed deer (Odocoileus virginianus) farming is a growing industry in Florida. Viral and bacterial infections are known to be significant sources of mortality in farmed white-tailed deer in Florida, causing important production loss. This study aims to identify the special heterogeneity, possible origins, and transmission routes for two viruses, bovine viral diarrhea virus (BVDV) and deerpox virus, two emerging viral pathogens in the Florida deer farming industry. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the cause of death. From 2016 to date, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. We detected three cases of BVDV1 on one farm and one case of BVDV2 on another farm in Florida white-tailed deer in 2018. The full genome sequence data has shown that BVDV2 from Florida white-tailed deer is highly similar to BVDV2a found in beef cattle in other states in the US. We have found deerpox virus cases throughout Florida since 2017, with the highest mortality cases seen in deer under 12 weeks old. We found that the deerpox virus can be detected through conventional PCR not only from skin scabs and tongue lesions but from most internal organ tissue collected during necropsy. Our results will provide valuable information to improve preventative health measures and clinical management of Florida white-tailed deer, improving herd health and reducing mortality.

Poster 35

Trueperella pyogenes, a Lethal Pathogen for Farmed White-Tailed Deer (Odocoileus virginianus) in Florida

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White-tailed deer (Odocoileus virginianus) farming is a growing industry in Florida. Bacterial and viral infections are significant sources of mortality in farmed white-tailed deer in Florida, causing important production loss. This study aims to identify possible transmission routes, antimicrobial resistant genes, and special heterogeneity of Trueperella pyogenes, one of the most common bacterial pathogens in farmed white-tailed deer in Florida. The University of Florida Cervidae Health Research Initiative (CHeRI) provides a diagnostic service to Florida deer farmers to determine and monitor the cause of death. From 2016 to 2021, participating Florida ranches provided recently deceased farmed white-tailed deer for necropsy or shipped tissues for analysis by the CHeRI diagnostic program. Our data suggested that Escherichia coli and Trueperella pyogenes are the most frequently isolated bacteria in farmed deer. T. pyogenes cases have been increasing since 2018 and showed a much higher prevalence in north Florida. Whole genome sequence of the T. pyogenes isolates is grouped into two clades; one is close to T. pyogenes from pigs and the other from cattle. The high diversity of the T. pyogenes in Florida farmed white-tailed deer indicates the possibility of multiple origins and transmission routes. Likewise, most isolates have numerous antimicrobial resistant genes, such as tetracycline resistance genes, suggesting that antimicrobial resistance could play a significant role in the high mortality of Florida farmed white-tailed deer by T. pyogenes. Our results will provide valuable information to improve preventative health measures and clinical management of Florida white-tailed deer, improve herd health, and reduce mortality.

Poster 36

Prevalence of anatomical defects and pathological conditions involving the cervical os in lactating dairy cows evaluated using a digital vaginoscope

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Involution of the uterus and cervix is critical for reestablishment of reproductive function after calving. Because calving related trauma and persistent inflammation are associated with decreased fertility postpartum, improving identification of pathological conditions afflicting the reproductive tract provides the basis for implementation of therapy or targeted culling decisions early postpartum. Objectives were to characterize the prevalence of anatomical defects and pathological conditions in the cervical os. Lactating dairy cows (n=5,041; primiparous, n=1,601; multiparous, n=3,440) from three farms were examined vaginally using a digital vaginoscope containing a light source and camera connected to a mobile device. Visual assessment of live footage and still images were used to diagnose anatomical defects, evidence of previous trauma, cervical prolapse, and vaginal discharge score (VDS; 0=no discharge, 1=clear mucus or lochia, 2=clear mucus with flecks of pus, 3=mucopurulent with < 50% pus, 4=mucopurulent with ≥ 50% pus, 5=watery/brownish liquid). Metritis prevalence (VDS 5 with ≤ 21 DIM) was numerically greater following evaluation of live footage compared with still pictures (6.3 vs. 2.8%). Prevalence of purulent vaginal discharge (VDS ≥ 3) decreased as DIM progressed (22-35 = 10.0%; 36-50 = 7.8%; 51-70 = 5.5%; 71-100 = 1.3%: 101-150 = 0.6%; >150 = 1.5%). Prevalence of adhesions (0.5%), double cervix (1.7%), masses (1.1%), and scars (0.9%) were small. Conversely, 58.9% of cows were diagnosed with cervical prolapse. Bruising prevalence was 72.4 (0-7 DIM), and 37.3 (8-21 DIM), 12.1% (22-35 DIM), and below 2.7% thereafter. Next steps include evaluation of the association between these findings and reproductive performance.

KEYWORDS: Reproduction; cervix; uterine disease

Poster 37

Mitigating the antimicrobial resistance along the poultry production: A systematic review

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Abstract: Antimicrobial resistance (AMR) is recognized as a significant threat to human, animal and environmental health. Persistent use of antimicrobial drugs (AMU) in livestock and poultry production systems has been implicated as the main driver contributing to development of AMR-enteric pathogens that could potentially be transmitted to humans via food chain. Various antimicrobial classes of medical importance are used in poultry production for disease treatment, control, prevention, and growth promotion. Therefore, the objectives of this systematic review are to [1] determine risk associated with AMU and AMR development in poultry (broiler, layer and turkey) and [2] identify the improved best management practices to mitigate AMR development along the poultry production. Eligibility criteria for this systematic review was developed using PECO/PICO framework and includes poultry (population), AMU or other management practices (exposure or intervention) and prevalence or incidence of AMR-enteric pathogens (outcome). Peer reviewed primary research studies in scientific databases such as Agricola, Embase, PubMed, Scopus and Web of Science will be searched to identify relevant studies. Risk of bias in selected studies will be assessed using standard quality assessment tools. Results of this systematic review will be synthesized and presented narratively using tables and figures according to PRISMA-2020 guidelines.

Poster 38

Effects of injectable trace minerals on the inflammatory cytokines response to vaccination in dairy calves

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Cytokines response post-vaccination is an important driver of both humoral and cellular immunity. Here, we characterize the effects of injectable trace minerals (ITM) on the cytokines response to the modified-live virus (MLV) vaccine containing BVDV, BHV1, PI3V, and BRSV and attenuated-live Mannheimia-Pasteurella (MP) bacterin in dairy calves. Several inflammatory cytokine gene expression (GE) profiles were determined in thirty calves randomly assigned into two groups: 15 calves received two doses of ITM subcutaneously (ITM), and 15 calves received saline (CONT) concurrently with the vaccines. Liver biopsy and blood samples were collected on days 0 (treatment +vaccination), 7, 14, 21 (treatment+vaccination), 28, and 42 for the determination of trace mineral concentrations, GE, flow cytometry markers, and serum antibodies titer. Marked associations were reported between CD80+ and Zinc (rs=0.50; P<0.001) and BHV1-antibody titer (rs=0.60; P<0.001), and between cobalt and IL-1β (rs=0.60; P<0.001), IL-12 (rs=0.50; P<0.001), and IFN-γ (rs=0.4; P<0.01). Hepatic manganese concentration was associated with TLR-7 (rs=0.7; P<0.001) and TNF-α (rs=0.5; P<0.01). Compared to CONT calves, ITM-treated calves showed a reduction in TNF-α, and increments in IFN-α (P=0.03), IFN-γ (P=0.02), and TLR-7 (P=0.04). Compared to baseline level (d0), all cytokines (IFN-α, IFN-β, IFN-γ, CD86, CD80, IL-1β, IL-10, IL-12, TLR-7, TNF- TNF-α) were decreased (P<0.001) on d42, except TLR-3. In conclusion, ITM supplementations likely potentiate the immunogenicity of the bovine respiratory diseases complex (BRDC) vaccine through the regulation of inflammatory cytokines release post-vaccination in dairy calves; however, it is unclear whether these effects contribute to improved protection against BRDC in dairy calves.

Structural remodeling of the basolateral amygdala (BLA) in Ovalbumin (OVA)-induced allergic asthmatic mice: *Preliminary Study Findings*

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Asthma is a chronic inflammatory disorder that causes airway obstruction and airflow limitation. Though previous and current asthma studies focus on the immune system, the involvement of the nervous system, particularly the neural connections that control the airway, has yet to be understood as a driver of asthma. It has been proposed that the amygdala, a central brain region that controls anxiety, is impacted in asthma and may contribute to asthma pathogenesis. For example, rodents exposed to experimental asthma have heightened activity of the amygdala. Given the amygdala's anatomical connections with neurons that control bronchoconstriction, understanding the link between anxiety and asthma may elucidate possible therapeutic interventions. Here, we provide a preliminary understanding of the effects of asthma on structural remodeling in excitatory neurons of the basolateral amygdala (BLA). Male mice were randomly assigned to two treatment groups 1) control mice (saline-sensitized) and 2) asthmatic mice (ovalbumin sensitized). Dendritic spine density was determined using traditional Golgi staining (FD Rapid GolgiStain™ Kit) and microscopy techniques. Our studies suggest that ovalbumin-induced asthma increases dendritic spine density in excitatory neurons of the BLA. Because increased spine density is associated increased amygdala activity, our data suggest that functional remodeling of the amygdala may be a consequence of and/or contribute to asthma pathogenesis. Future studies include expanding findings to female mice and probing molecular expression of pro-plasticity genes associated with functional remodeling of the BLA.

Reefs vs. Red Tide: Investigating the sublethal impacts of Karenia brevis on South Florida reef-building coral species

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Coral reefs cover only 1% of the ocean floor, yet support more life per area than any other marine ecosystem. Due to drastic declines observed in coral reefs in response to changes in climate and other anthropogenic stressors, the survival of these ecosystems hinges on the health and reproductive success of stony corals. Specifically in Florida, Karenia brevis blooms, which have drawn increased public attention in recent decades, have been documented to reach the Florida Keys and therefore represent an expanding threat. The purpose of this project was to investigate the sublethal impacts of K. brevis exposure on three South Florida coral species. Fragments of P. astreoides, A. cervicornis, and S. radians were exposed to environmentally relevant levels of K. brevis for 2-weeks in a controlled aquaria setting. At the end of this exposure, ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (UHPLC-HRMS/MS) workflows were used to perform nontargeted lipidomics and metabolomics analyses to assess physiological changes in the corals in response to red tide exposure as well as highlight possible biological processes and pathways altered. Significant changes to the lipidome and metabolome were observed in all three species following exposure. Furthermore, the multi-omics analysis highlighted important pathways for further study including oxidative stress, energy metabolism, immune impacts, and nitrogen fixation/photosynthesis of symbionts. This is the first study to conduct an exposure of stony corals to K. brevis using an environmentally relevant timeline; therefore, these results are invaluable to inform future coral reef restoration and management decisions.

Nonlethal detection of PFAS bioaccumulation and biomagnification within fishes in a Great Lakes Urban-Dominated Watershed

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PFAS are contaminants of emerging concern spilling into global ecosystems. Properties that make them desirable for manufacturing also prevent them from degrading. Two compounds, PFOS and PFOA, have been phased out of use due to evidence of biomagnification and findings of adverse animal and human health outcomes. This phasing out has led to replacement analogs of equal or greater concern. Despite studies linking PFAS to adverse health outcomes, there is a lack of international regulations of PFAS as a hazardous material. The Great Lakes Huron to Erie corridor (HEC) is an EPA Area of Concern, drinking water source for ~4 million people, hub of >30% of Michigan's fishing effort, and home to vital habitats. For the past two years, we have analyzed the concentration of 40 PFAS from muscle biopsy and serum samples of sport fish along the HEC to assess PFAS bioaccumulation and potential human-health implications related to consumption. Unlike previous studies, native fish were sampled using non-lethal methods. Three species of fish were used to represent low, intermediate, and high trophic levels: Sander vitreus, Perca flavescens and Neogobius melanostomus. Eight PFAS congeners were detected in muscle and fifteen in serum, including the detection of five novel PFAS in Great Lakes fish. PFOS was identified in 100% of muscle and serum pools across all species and is biomagnifying in the studied food webs. We have used this data to inform follow-up studies in the laboratory zebrafish model and are evaluating behavioral, developmental, sexual, reproductive, transcriptomic, and transgenerational effects.

Poster 42

Species-specific accumulation and geographic variability of per -and polyfluoroalkyl substances (PFAS) in marine apex predators: Concern for exposure to longer-chain PFAS?

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Per- and polyfluoroalkyl substances (PFAS) are persistent and bioaccumulative chemicals found ubiquitously in various environmental matrixes over the past several decades. Data is scarce on the occurrence and bioaccumulation of PFAS in apex predators and the processes by which these chemicals integrate into food webs is still not well understood. Marine apex predators, such as many marine mammals and sharks, act as drivers of the ecosystems and are considered sentinels of environmental and ecosystem health. Here, we report the current accumulation profile of PFAS in the blood of marine mammals and sharks collected from two different geographical regions (Korean coastal waters and the Southeast coast of the United States). The results showed a similar pattern of PFAS in both marine mammals and sharks, with longer-chain carboxylates (PFCAs) and perfluorooctane sulfonate (PFOS) being the most frequently detected compounds. The highest concentrations of ∑PFAS were found in the spotted seal (Phoca largha; 122.23 ng/L), followed by the beaked whale (Ziphius cavirostris; 121.76 ng/L) from the Korean coast, while the fin whale (Balaenoptera physalus) showed the lowest concentration of (0.3 ng/L), due to its lower trophic level. Among the shark samples studied, the bonnethead shark (Sphyrna tiburo) showed the highest concentration of ∑PFAS (57.80 ng/L) probably due to its unique omnivore feeding behavior. Regardless of its continued removal globally from production and use, PFOS was still the most dominant compound in all species due to its strong bioaccumulation potential. The study suggests that marine mammals showing relatively higher concentrations of PFAS, as compared to sharks, might be due to the metabolic variations between the two groups

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Potential Pathophysiological Role of Angiotensin Receptor Expression in the Urinary Bladder

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Several inflammatory diseases, including interstitial cystitis/bladder pain syndrome (IC/BPS), present increased mast cell infiltration, oxidative stress, and fibrosis, like those that involve renin-angiotensin signaling. While we know the renin-angiotensin system is well-studied in various organ systems, including the lungs, kidneys, and heart, there is a lack of information regarding the urinary bladder. Thus, the initial goal of this work is to characterize angiotensin receptor type 1a (AT1aR) in the bladder. Our long-standing objective is to investigate the potential pathophysiological role of angiotensin receptor signaling in the urinary bladder. We utilized a transgenic mouse model that expresses tdTomato under the AT1aR promotor. We utilized the chemotherapeutic drug, cyclophosphamide (CYP), which is well-documented to induce acute bladder cystitis, to study the changes in AT1aR expression under inflammatory conditions. We found that AT1aR is expressed in various bladder cell types. We observed AT1aR co-expression with multiple urothelial and myofibroblast markers. Preliminary analysis indicates the mean fluorescence intensity and total area percentage (in pixel units) were significantly greater in CYP-injected animals, indicating an increase in the AT1aR-expressing cells post-bladder inflammation. The data suggest that there is angiotensin receptor expression occurring in the bladder. With minimal treatment options for bladder diseases, such as IC/BPS, improving the understanding of the pathophysiology of both normal and disease bladder state cellular signaling will aid in the development of new effective therapies. Numerous FDA-approved therapeutic drugs target the renin-angiotensin mechanism that could be targets of interest for bladder diseases if angiotensin signaling plays a role in the disease.

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Comparison of two methods to blind-end small intestine for jejunocecostomy in horses

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The ileum is reportedly involved in 44% of small intestinal strangulating colic, necessitating bypass by jejunocecostomy (JC). JC is reported to adversely affect outcomes when compared with alternative anastomotic techniques.
Jejunocecostomy can be performed in a side-to-side manner, using a stapling device or hand-sewn technique. A critical step in the side-to-side JC is blind-ending the small intestine to bypass the ileum. We hypothesized the stapled blind-end would be larger and induce greater inflammation compared to the hand-sewn. Additionally, the blind-end construct time and short-term outcome would be similar between groups.
Twelve healthy adult horses underwent exploratory celiotomy with hand-sewn side-to-side JC performed using a Parker-Kerr (6-horses) or stapled (6-horses) technique. At surgery peritoneal fluid and tissue samples were collected as controls and dimensions of the blind-ends obtained. Postoperative monitoring included physical examination and serial hematology. Horses were euthanatized 7-days postoperatively. The peritoneal cavity was examined, peritoneal fluid collected for cytology, and tissue samples collected for histology.
Post-operative colic occurred in three horses and post-operative reflux occurred in one horse. No association with procedure was found. The intraluminal projection of the jejunal blind-end was significantly larger in the stapled group at surgery. The Parker-Kerr construction was significantly faster than the stapled method (mean difference of 7.3 minutes). Both groups demonstrated similar inflammatory response on hematologic and peritoneal fluid analysis. Preliminary histopathology suggests greater inflammation in the hand-sewn closure. This study demonstrates side-to-side anastomosis is well tolerated in healthy horses. Both the hand-sewn and staple techniques are acceptable methods for blind-end formation.

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Comparison of reproductive performance of dairy cattle following a multivalent modified live post-partum vaccine protocol or a multivalent killed pre-partum vaccine protocol

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Our objective of this study was to compare pregnancy per artificial insemination (P/AI) following first insemination postpartum, time to pregnancy, and proportion of cows pregnant by 150 days in milk (DIM) between animals that received one of two vaccination strategies; 1) killed multivalent vaccine prepartum (KV) or 2) multivalent modified life vaccine postpartum (MLV). Nulliparous cows with 196 to 202 days carried calf (DCC), primiparous cows with 209 to 217 DCC, and multiparous cows with 215 to 223 DCC were assignat to one of two treatment groups. Cows assigned to the KV group received two doses (28 to 35 days interval) of a killed multivalent vaccine (Vira Shield 6 L5, Elanco, Greenfield, IN) prior to calving according to label directions. Cows assigned to the MLV (Bovis-Shield GOLD FP5 L5, Zoetis, Parsippany, NJ), received a single dose of multivalent modified live vaccine at least 30 days after calving according to label directions. For pregnancy per AI was not statistical difference between groups (P = 0.60) on 30 days pregnancy confirmation (KV= 49.9% vs MLV= 50.6%), neither in the confirmation of pregnancy on 60 days post AI (P = 0.93) (KV= 47.3% vs MLV= 47.4%). There was no (P = 0.29) effect of treatment on pregnancy loss (KV= 4.4% vs MLV= 5.2%). Hazard of pregnancy until 150 DIM have a tendency (P = 0.06) between the groups in the study, 72.8% for the KV group and 70.3%. The use of a killed vaccine in the dry period compared to modified live vaccine in the postpartum period did not impact first breeding outcome at the two farms.

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Economic Consequences of Clinical Endometritis in Dairy Herds

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Cows with clinical endometritis (CE) have several disruptions in their performance, such as reduced pregnancy per artificial insemination, extended calving to conception intervals, increased pregnancy losses, and decreased survival in the herd. The objective of this study was to perform an economic analysis of the consequences of CE in dairy herds. The data set used in this study was composed of 10,956 cows (35.60% primiparous and 64.40% multiparous) collected from 16 dairy herds located in 4 different regions of the US. Vaginal discharge was collected at 28 ± 7 days in milk (DIM) for diagnosis of CE, and CE was characterized as mucopurulent, purulent, or reddish-brownish discharge. 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 at the end of the lactation (i.e. 305 DIM). Expenses were divided into feed cost, reproductive cost, and replacement cost. Continuous and dichotomous variables were analyzed using analysis of variance and logistic regression, respectively. Compared with healthy cows, cows with CE produced 255 kg less milk (10,213 vs. 10,468 kg/lactation), had a lower proportion of cows pregnant at 305 DIM (73.8 vs. 82.1%), and greater culling by 305 DIM (31.4 vs. 23.7%). Consequently, the gross profit for cows with CE was $301 lower than for cows without CE. In conclusion, CE affected the economic efficiency of dairy cows by being associated with decreased milk yield, reproductive efficiency, and survival in the herd.

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Investigation of the in vitro effects of cannabidiol,cannabidiolicacid,and the terpenes β-caryophyllene in lymphocytes harvested from atopic and healthy dogs

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Abstract:Cannabidiol (CBD)hasbeenshownto have anti-inflammatory and antipruritic properties without thepsychoactive effects.Cannabidiol is associated with other substances like terpenes andcannabidiolicacid(CBDA) in commercially available formulations.This study aims to evaluate the anti-inflammatory and cytotoxic properties of CBD, CBDA and β-caryophyllene (BCP) on atopic lymphocytes. Sixhealthy and five atopic dogswereenrolled. Peripheral blood mononuclear cells (PBMC) were harvested andincubated for 24h with different concentrations of CBD, CBDA, and BCP alone and in combination corresponding to a dose of 2 and 8 mg/kg of CBD. Cell viability was assessed by lactate dehydrogenase and ATP assay. Thirteen cytokineswere tested using MILLIPLEX®Canine Cytokine/Chemokine Magnetic Bead Panel (Sigma Aldrich, St. Louis, MO, USA). The results were analyzed with Mann Whitney and Friedman's multiple comparison test followed by a Dunn's test. There was no significant difference in cell viability and cytokine secretion between baseline and any treatments.This is the first study that tested the effect of CBD, CBDA, and BCP at different dosages and concentrations on atopic PBMC. The results show CBD, CBDA and BCP at tested concentrations are safe in harvested PBMC. Additional future studies using higher doses of CBD to achieve clinical benefits are needed. Unfortunately, CBD, CBDA and BCP did not show any anti-inflammatory effect. However, changes in cytokines were noted. This preliminary data may warrant further research to assess a larger population since there were some cytokines with changes noted for CBD, CBDA, and BCP at higher doses.

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Source of funding: Self-funded.

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Clinical Efficacy of Virtual Surgical Planning and 3D-Printed, Patient-Specific Reduction Guides to Facilitate Alignment of Diaphyseal Tibial Fractures Stabilized via Minimally Invasive Plate Osteosynthesis in Dogs: A Historic Case Matched Control Study

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Obtaining acceptable alignment and reduction during MIPO can be challenging and may be facilitated by application of a patient-specific 3D-printed fracture reduction system (PSFRS). The objective of this study was to compare the efficacy of PSFRS to conventional indirect fracture reduction techniques for tibial MIPO applications in dogs. We hypothesized that the PSFRS would result in shorter surgical times and more accurate tibial length and alignment restoration than traditional MIPO applications. Eleven dogs weighing >15 kg with diaphyseal tibial fractures were enrolled to evaluate the efficacy of utilizing a 3D-printed PSFRS to facilitate MIPO (3D-MIPO). An historic case matched control cohort was established of dogs that underwent MIPO stabilization of diaphyseal tibial fractures using conventional indirect reduction techniques (c-MIPO; n=11). Tibial length and alignment, surgery and anesthesia duration, and healing times were compared between reduction groups. Fracture reduction using custom 3D-printed PSFRS reduced surgical duration by a mean of 35 minutes (p=0.025). Median post-operative tibial length and frontal and sagittal alignment were within 4 mm, 3°, and 3°, respectively, of the contralateral tibia in both reduction groups, with no differences between reduction groups (p>0.099). Post-operative complications occurred in 18% of fractures in both reduction groups. Median time to union was 63 and 62 days in the 3D-MIPO and c-MIPO groups, respectively, with no difference between reduction groups (p=0.230). Surgical duration was shorter when using 3D-MIPO than c-MIPO. Accurate restoration of tibial length and alignment was achieved using both 3D-MIPO and c-MIPO.

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Visual Field of the Albino Rat, Common Ferret, and Northern Tree Shrew

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Establishing the extent of the visual field accessible to the retina provides insight into the egocentric perceptual experience of animals. Visual fields were measured using an ophthalmoscopic reflex technique in six northern tree shrews, four common ferrets, and four albino rats. Animals were anesthetized and the midpoint between their eyes was centered inside a spherical space. A rotating arm was manipulated in 10-degree increments around the head. At each increment, a direct ophthalmoscope was used to visualize the limits of the retinal reflex for each eye, the overlap being the extent of the binocular visual field. Maximum mean binocularity was 69° +/- 1.6° in the ferret, 90° +/- 3.1° in the rat, and 53.6° +/- 12.2° in the shrew, located at 10° above, 40° above, and at the horizontal plane, respectively. Binocularity extended 160°, 200°, and 180° in the sagittal plane in the ferret, rat, and shrew, respectively, from at least below the nose to above the head in all animals. Overhead binocularity in these animals enhances anti-predatory vigilance in a natural setting. In describing the visual field, we provide a reference for the representation of the visual space in different cortical regions, many of which represent specific sub regions of the visual field.

High Cross-reactivity between Receptor Binding Domains of Feline Coronavirus Serotypes 1 and 2 with SARS-CoV-2: Its Impact on Diagnostics and Pan-CoV Vaccine Development

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Feline coronavirus (FCoV), when mutated into fatal feline infectious peritonitis virus (FIPV), shows similar pathogenic features to SARS-CoV-2 (SCoV2). Both FCoV and SCoV2 infect cats. The current study was initiated when our specific-pathogen-free, laboratory toms developed unexpectedly high levels of cross-reactive antibodies to the human SCoV2 receptor binding domain (RBD) upon mating with FCoV-positive queens. Multi-sequence alignment analysis of SCoV2 Wuhan RBD and FCoV serotypes 1 and 2 (FCoV1, FCoV2) demonstrated 11.5% amino acid (aa) sequence identity and 31.8% similarity with FCoV1 (12.2% identity and 36.5% similarity with FCoV2). The sera from toms and queens were cross-reacted with SCoV2 and FCoV1 RBDs, FCoV2 spike-2, nucleocapsid, and membrane proteins, but not with FCoV2 RBD. Conversely, the plasma from FCoV2-inoculated cats reacted with FCoV2 and SCoV2 RBDs only. Additionally, group-housed laboratory cats possessed persistent serum cross-reactivity with SCoV2 RBD over 15 months. Such cross-reactivity was also observed in FCoV1-positive pet cats, complicating SCoV2/FCoV/FIPV serological diagnosis. The SCoV2 RBD at a high non-toxic dose and FCoV2 RBD at a 60-400-fold lower dose blocked the in vitro FCoV2 infection, demonstrating their close structural conformations. Remarkably, such cross-reactivity was also detected by the T cells from FCoV1-infected cats. The broad cross-reactivity between human and feline RBDs and the ability to induce pan-CoV-specific T-cell responses provide essential insights into developing a pan-CoV vaccine by combining FCoV1, FCoV2, and SCoV2 RBDs, each in a configuration of trimer RBD-HR (heptad repeats) with highly-conserved, SCoV2 stem helix core (SHC). The addition of conserved pan-CoV cytotoxic-T-lymphocyte epitopes from highly-conserved SCoV2/FCoV proteins (e.g., RNA-dependent RNA-polymerase) to the triple RBD-SHC-HR, as an mRNA or DNA vaccine, may be required to induce sterilizing immunity against pan-CoVs in animals to prevent future zoonotic variants. Such minimalistic pan-CoV vaccine may be essential to decrease adverse effects and to induce sterilizing immunity against SCoV2 in humans.

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Into the Matrix: Assessing the Use of Alternative Matrices with Barbiturate Screening Tests

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The barbiturate drug, Pentobarbital, is a drug commonly used by veterinarians for the euthanasia of domestic animals. During the veterinary forensic autopsy, it is sometimes necessary to screen for barbiturates in order to determine whether or not the animal was chemically euthanized with pentobarbital. The use of a human immunochromatographic test for barbiturates screening utilizing dog or cat urine have been previously validated; however, use of alternative matrices for this purpose has yet to be explored when urine is not available. In this study, heart, liver, and spleen from 20 dogs and heart or skeletal muscle, spleen, and liver from 20 cats with known chemical euthanasia status were screened using a human immunochromatographic barbiturate screening test. Tissues were processed using a combination of a bead homogenizer and centrifugation and also by sonication. There was 100% agreement for all tested samples using the commercial immunochromatographic test using sonication and bead homogenization with the euthanasia status of the dogs and cats. Liver from 10 dogs and 10 cats were sent for confirmation testing for pentobarbital via GC/MS, with 100% agreement with known euthanasia status in cats and 80% agreement with known euthanasia status in dogs. Overall our results support the use of this immunochromatographic test for the screening of postmortem collected alternative animal matrices to assess for the presence of barbiturates, specifically pentobarbital, in cats and dogs as no false negatives were observed. The disagreement between the euthanasia status and GC/MS is hypothesized to be due to a limit of detection issue.

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Characterization of Bluetongue virus serotype 1 strains isolated from farmed white-tailed deer (Odocoileus virginianus) in Florida, USA

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Bluetongue virus (BTV) particularly concerns farmed white-tailed deer (WTD) in Florida, given its severe clinical signs and wide distribution. In this study, we reported the clinical findings, ancillary diagnostics, and complete coding sequence of two new BTV serotype 1 strains in farmed WTD in Florida. A 2-year-old doe (OV1049) in 2019 and a 3-month-old buck fawn (OV1706) in 2022 presented with neurological signs, and the necropsy report revealed lung pneumonic lesions. RNA extract from spleen fragments were screened for BTV, epizootic hemorrhagic disease virus, eastern equine encephalitis virus, and West Nile virus using a multiplex RT-qPCR assay. Both animals were positive for BTV and negative for the other viruses. Next-generation sequencing was performed on cDNA libraries generated from the RNA extracts of virus isolation in Vero E6 cell cultures displaying cytopathic effects. The 5′ end of the coding sequence of segment 1 of sample OV1049 was determined using a 5′ Rapid Amplification for cDNA End (RACE) PCR Kit, followed by Sanger sequencing. Nucleotide identity and phylogenetic analyses supported both isolates as BTV serotype 1. All BTV sequences that showed a high degree of identity to OV1409 and OV1706 strains were classified as either established or reported BTV serotypes in the U.S. Sequence identities, phylogenetic analyses, and recombination analysis indicated BTV-1 strains OV1409 and OV1706 were reassortants. Continued surveillance efforts are needed to determine the prevalence and potential threat of new BTV strains that may pose to domestic and wild ungulates.

Keywords: Bluetongue virus, Orbivirus, Reovirus, White-tailed deer

Group 3 innate lymphoid cell pyroptosis represents a host defense mechanism against Salmonella infection

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Group 3 innate lymphoid cells (ILC3s) produce interleukin (IL)-22 and coordinate with other cells in the gut to mount productive host immunity against bacterial infection. However, the role of ILC3s in Salmonella enterica serovar Typhimurium (S. Typhimurium) infection, which causes foodborne enteritis in humans, remains elusive. Here we show that S. Typhimurium exploits ILC3-produced IL-22 to promote its infection in mice. Specifically, S. Typhimurium secretes flagellin through activation of the TLR5-MyD88-IL-23 signaling pathway in antigen presenting cells (APCs) to selectively enhance IL-22 production by ILC3s, but not T cells. Deletion of ILC3s but not T cells in mice leads to better control of S. Typhimurium infection. We also show that S. Typhimurium can directly invade ILC3s and cause caspase-1-mediated ILC3 pyroptosis independently of flagellin. Genetic ablation of Casp1 in mice leads to increased ILC3 survival and IL-22 production, and enhanced S. Typhimurium infection. Collectively, our data suggest a key host defense mechanism against S. Typhimurium infection via induction of ILC3 death to limit intracellular bacteria and reduce IL-22 production.

Poster 54

Influence of different horse transport distances on biochemical and metabolic markers and hematological cells

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The study evaluated the interference of the transport of horses over distances of 50 and 300 kilometers (km) on biochemical, metabolic and hematological components. Eleven quarter horses, 8.5±1.4 years old, seven geldings, and four mares were used. The study was conducted in the southeast of Brazil, and horses were submitted to two trips in a truck, the first for 50 km and the second 21 days later for 300 km. Blood was collected before the trip, immediately after, six hours, 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, and 144 hours after the end of each trip. The hematocrit (VG), total proteins (TP), platelets (PL), total leukocytes, segmented lymphocytes, monocytes, eosinophils, and basophils were analyzed. Muscle activity was evaluated through creatine kinase (CK) and aspartate aminotransferase (AST) and metabolic activity through glucose (GLU) and lactate (LAC). ANOVA comparisons by Tukey's test and nonparametric by Kruskal-Wallis with Dunn's post hoc were used, with significance at $p<0.05$. No difference was observed in VG, TP, PL, and WBC between distances, but they showed variation between moments, especially neutrophils ($p=0.023$). The GLU did not change between distances but showed depletion immediately after 300 km trip ($p=0.017$). Lactatemia after 50 km compared to 300 km ($p=0.037$), as well as immediately after 50 km ($p=0.001$). The CK did not change, but the AST showed an increase after 6 hours on 300 km compared to 50 km ($p=0.034$). The more extended trip was established as more disturbing, with a variation of muscle enzymes, and justified greater care for horses to ensure animal welfare.

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Modulation of the Brain-Derived Neurotropic Factor (BDNF) pathway increases mucin production in human epithelial airway cells: Preliminary Study Findings

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Asthma is a chronic inflammatory disorder of the airway causing bronchoconstriction and enhanced production of airway mucins. Combined, these features result in airflow limitation and airway obstruction. Though steroidal anti-inflammatories remain the mainstay therapeutic for asthma, there remains a population of patients with steroid resistant asthma. This suggests a gap in our knowledge surrounding the mechanisms responsible for asthma pathophysiology. Brain-derived neurotrophic factor (BDNF) is one candidate molecule that may contribute to asthma pathogenesis either independent of inflammation or as a modulator of inflammation. For example, altered levels of BDNF have been reported in asthma and BDNF polymorphisms have been associated with asthma. Here we tested the hypothesis that BDNF regulates mucin production in airway epithelia. Using qRT-PCR, our preliminary data show that repeated application of recombinant BDNF to human airway epithelia cells increased the mRNA expression of the two major secreted mucins in the human airway, MUC5AC and MUC5B. Protein production as measured with immunofluorescence intensity mimicked the mRNA data. Pharmacologic inhibition of TrkB, the high affinity receptor for BDNF, did not prevent BDNF-mediated increases in mucins, suggesting that other BDNF receptors may be involved. Our data suggest that BDNF may be an overlooked component of asthma pathogenesis that promotes mucus production. Increasing our understanding of this pathway will allow for the development of therapies for refractory asthma.

Targeting Ferroptosis as Novel Therapeutics for Human and Canine Osteosarcoma

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Ferroptosis is an iron dependent cell death accompanying lipid peroxidation. It is morphologically distinct from other types of cell death; ferroptotic cells have an intact nuclear structure but have significantly smaller mitochondrial size, crista, and condensed mitochondrial membrane density. Ferroptosis inducers primarily target to inhibit the system xc− and deplete GSH synthesis -/or directly inhibiting glutathione peroxidase 4 (GPX4), a key regulator of ferroptosis. Recently, it has been shown that an increased intracellular oxidative stress produced by the presence of labile iron promote the susceptibility of cancer cells to ferroptosis as well as inhibit metastatic process. Osteosarcoma is a malignant bone tumor occurring in both humans and dogs. The standard of care for osteosarcoma is primarily surgery with adjuvant and/or neoadjuvant chemotherapy. Yet, identification of a new therapeutic targets is an unmet need for osteosarcoma in both humans and dogs. Aims of this study are to determine the susceptibility of osteosarcoma cells to ferroptosis and to identify novel molecules as key regulators for ferroptosis by multi-species comparative approaches. Our experimental strategy includes the measurement of XCT and GPX4 protein expression along with the determination of IC50 values of ferroptosis inducers in five human (HOS, 143B, Saos-2, MG-63, U2OS) and two canine (COS31 and DOUG) osteosarcoma cell lines. Ongoing work is generating RNA-seq data to establish global gene expression profiles in osteosarcoma cells by treating system xc− inhibitor, Erastin and GPX4 inhibitor, RSL3. By this approach, we will determine therapeutic vulnerabilities of osteosarcoma by targeting ferroptosis.

Keywords: Ferroptosis, system xc−, GPX4, RNA-seq, osteosarcoma, comparative oncology, bioinformatics.

Poster 57

Genome-wide CRISPR screening for identification of a cross-species oncogenic mechanism in osteosarcoma

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Oncogenesis a biological process that cells gain malignant properties through aberrant alterations of intrinsic or extrinsic cellular factors resulting in uncontrolled proliferation and enhanced survival. Genomic profiling via next-generation sequencing enables to identify gene mutations potentially affecting oncogenesis. However, a great number of mutations are possibly stochastic, non-pathogenic, or functionally unknown; even some genes confer oncogenic properties without mutations. The precise oncogenic mechanism still remains unclear, particularly in the context of cellular properties. Recently, we leveraged a whole-genome CRISPR/Cas9-mediated loss-of-function screening to define context-dependent roles in malignant phenotype. Specifically, we used human acute myeloid leukemia (AML) TF-1 cells that can proliferate only in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF). Enriched TF-1 cells in the absence of GM-CSF were selected after infecting a whole-genome CRISPR/Cas9 knockout library. By the screens, genes encoding key enzymes involved in sialic acid biosynthesis such as CMAS, SLC35A1, NANS, and GNE were identified. Further, individual knockout studies revealed that a dysregulated sialylation of cytokine receptors altered intercellular signaling pathways, suggesting a new potential mechanism of how AML cells overcome bone marrow-derived cytokine dependency. In the present study, we utilize CRISPR/Cas9 screening method to establish a novel comparative approach for a better understanding of osteosarcoma biology at a genome-wide level. Our ongoing work is building a canine whole-genome CRISPR/Cas9 knockout library, and we will identify a cross-species oncogenic mechanism and determine therapeutic vulnerabilities in osteosarcoma.

Poster 58

Comparison of the Efficacy of Cranial vs. Medial Minimally Invasive Plate Osteosynthesis Applications for the Stabilization of Simulated Radius and Ulna Fractures

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Minimally invasive plate osteosynthesis (MIPO) is an emerging fracture management modality in small animal orthopedics. Cranial plate application has been consistently advocated when performing radial MIPO applications, however medial application might prove easier, particularly when stabilizing mid-to-distal fractures. We hypothesized both plate applications would yield equivalent fracture alignment, but medial plating would be easier to perform, decreasing surgical times. Cranial or medial radial plating was performed by MIPO on contralateral forelimbs in cadavers, facilitated by a 2-ring fixator. Post-procedural angulation, radial length, procedure times, number of fluoroscopic images obtained, and Likert scores were compared between groups. Ten cadavers were used. Radial length proved to be significantly different (P=0.004) as well as the change in radial length between plating groups, compared to intact radii (P=0.002): medial plating resulted in radial lengthening (1.11 ± 0.54 mm) compared to shortening with cranial plating (-1.06 ± 0.71 mm). The clinical significance of radial lengthening with medial plating is assumed to be negligible. Our results support medial antebrachial MIPO application warrants consideration. The majority of outcome measures were similar between plating groups, despite the surgeon's experience and bias with cranial plating, suggesting fixator-assisted medial radial MIPO has merit and could potentially prove more efficient with increased surgeon experience.

Poster 59

One Health and a retrospective study of laboratory – based melioidosis surveillance in Songkhla and Phatthalung Provinces of southern Thailand, 2014 – 2020

Apichai Tuanyok, Ph.D1., Pacharapong Khrongsee, DVM, MS1., Jedsada Kaewrakmuk, MS2.

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It is well-recognized that melioidosis is endemic in most parts of Thailand. However, the prevalence of melioidosis in humans and animals, and the occurrence of its pathogen, Burkholderia pseudomallei, in natural environment of southern Thailand has not been updated for long time. We used "One Health" approach and multidisciplinary research to investigate epidemiology of melioidosis in southern Thailand. We have been collecting B. pseudomallei isolates from human and animal cases, and soils in two southern provinces, Songkhla and Phatthalung, since January 2014. All culture-confirmed B. pseudomallei isolates from patients admitted to tertiary care hospitals in both provinces were further tested by real-time PCR assays. We have also investigated the presence of B. pseudomallei in soil and water especially in farming areas and a local zoo where animal cases have been reported. We have confirmed at least 473 human melioidosis cases, a one-year long outbreak in a local zoo affected to various animal species, and multiple incidences of melioidosis in animal farms, as well as the presence of B. pseudomallei in soil and water in Songkhla during 2014-2020. The infections were most likely seasonal and associated with rainfall. Genetic analysis using multi-locus sequencing typing (MLST) has indicated that most of recent isolates had same STs with those from the Finkelstein's historic collection from southern Thailand a half century ago. Over seven years of the study, the average annual incidence of human melioidosis was 3.4 per 100,000 population with 40% mortality. We have confirmed that these two provinces of southern Thailand are endemic for melioidosis; even though the incidence rate is much lower than that of the Northeast, the mortality rate is comparable. We believe that implementing the "One Health" approach would provide a current situation of B. pseudomallei infections in humans and animals, as well as its occurrence in the environment in southern Thailand that forms an integral part of regional threat assessment of Thailand and Southeast Asia.

Poster 60

The Role of Zinc in Innate Lymphoid Cells

Jennifer Kyu, BS, Eric Helm, PhD, Lifeng Xiong, PhD, Na Sun, MS, Liang Zhou, MD, PhD

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Zinc is an essential trace element that is important for many biological processes in animals and human. Zinc deficiency is commonly associated with malnutrition, but it can also be attributed to aging, chronic illnesses, and increased consumption of phytates, calcium, and phosphate, which reduce zinc absorption. It is estimated that about 20% of the world's population is at risk of zinc deficiency due to inadequate absorbable zinc in food supplies, making zinc deficiency a widespread concern. In the immune system, zinc has been shown to be crucial for overall immune responses, including T- and B-cell proliferation and function and innate immune activity during infection and inflammation. Innate lymphoid cells (ILCs) are innate counterparts of T cells that are important for shaping and promoting immune responses when exposed to different antigens. However, the role of zinc in ILC development and function remains unknown. Specifically, by developing mouse models of zinc deficiency in ILCs, using genetic and pharmacological approaches, I will examine the significance of zinc in ILC proliferation and function. In addition, using an in vitro cell line (MNK3) that mimics primary ILCs, I will determine the mechanisms of action of zinc in transcriptional control of ILC gene program. Together, through these studies, I hope to gain better understanding of zinc in the immune system.

Optimization of viable qPCR method to differentiate live and dead bacteria in blood

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Determination of bacterial viability usually requires culture; however, a commercially available viability PCR kit (biotium) has reported clinical uses. Herein, we present data showing this kit can be used to differentiate live from heat killed (HK) E. coli in blood. We tested inhibition of whole blood, effectiveness of DNA amplification inhibition, and percent recovery of live cells in mixed samples. BHI was the comparison matrix. This dye inhibits PCR amplification when it is bound to DNA, therefore samples spiked with only HK cells will amplify later than samples spiked with only live cells. This was observed in blood pre-treated with eukaryotic lysis buffer and BHI but not whole blood with direct dye exposure. We concluded whole blood has an inhibitory effect mitigated by eukaryotic lysis prior to dye exposure. Thus, the lysis buffer was used in all subsequent experiments. The dye did not affect live cell DNA amplification. However, 99.96% and 99.99% of HK DNA was removed from blood and BHI, respectively. 55% and 30% live cells were recovered from blood spiked with 50% and 12% live cells. Taken together these data indicate this kit can differentiate live from HK E. coli in blood. However, determination of lower detection limits, optimization of percent recovery of live cells, gram-positive reference strain, and comparison to culture-based methods needs to be evaluated.

Poster 62

Neurorestorative potential of Some Nutraceuticals in Neurotoxicity Induced by Aluminum oxide in Albino rats.

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Aluminum (Al) is a heavy metal that has been linked to neurological dysfunction. This study aimed to evaluate the neurorestorative potential of lecithin (L) and/or gallic acid (GA) in alleviating (Al)-induced neurotoxicity in the central nervous system of rodents.
There were seven experimental treatments: Group (1): served as the control, Groups (2) & (3): received lecithin or gallic acid respectively at a dose of 100 mg/kg body weight (B.wt). Group (4): received Al at a dose of 300 mg/kg B.wt, Group (5): received Al + lecithin, Group (6): received Al + gallic acid and Group (7): received Al + lecithin+ gallic acid. Rats were treated orally for 28 days (5days/week).
Al induced a significant increase in acetylcholinesterase (AChE) activity, along with a significant decrease in dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the brain. Al stimulated apoptosis based on increases of Bax, caspase-3 and the Bax/Bcl-2 ratio with decreased Bcl-2 in several brain regions.
Treatment with lecithin and/or gallic acid mitigated the effect of Al toxicity on the cholinergic, dopaminergic, and apoptotic status. This study suggests that natural products lecithin and gallic acid may be effective in reducing AI-induced neurotoxicity.

Poster 63

Leg length and water depth affect the increase in stride length with speed in dogs exercising on a dry or underwater treadmill

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Underwater treadmills are used for rehabilitation and to facilitate weight loss. While stride length increases linearly with speed in dogs exercising on a dry treadmill, it isn't known how kinematic parameters are affected by leg length and water depth in dogs exercising on an underwater treadmill. Stride length and frequency were measured in nine privately owned small dogs (five dachshunds and four Italian greyhounds) of similar body weight, exercising on a dry treadmill, and in 1inch deep water and elbow depth water in an underwater treadmill, using a 240 frames/s video camera. Dogs walked and trotted at a variety of speeds. Stride length increased linearly with speed under all conditions. The rate of increase (slope of the line) increased with water depth in long-legged dogs, whereas in Dachshunds, the slope increased in any depth of water but was less affected by actual water depth. A difference was still evident when stride and speed were normalized for leg length. Range of motion is important in rehabilitation. This study shows that water depth has less influence when legs are shorter than when they are longer, when the dogs are of similar body weight, which has implications for rehabilitation from injury. These changes are similar to changes in energy requirements reported previously in these dogs, which suggests that the relationship between energy requirements and leg kinematics is similar for dogs exercising in and out of water.

TOP DOG ABSTRACTS

Abstract 1

Phylogenetic Analysis of a Novel Atadenovirus from an Aldabra Tortoise (Aldabrachelys gigantea) Suggests Lower Barriers to Host Jumping from Squamates to Tortoises

Tasha M. Desiderio, Garrett Fraess, DVM, Estelle Rousselet, DVM, PhD, Dipl ACZM, Jane Christman, DVM, Kali Gennette, DVM, Kirsten L Cooke, DVM, DACVIM, Nicole I. Stacy, DVM, DrMedVet, Dipl ACVP, Scott Terrell, DVM, Dipl ACVP, Heather A. Grodi, DVM, Amy B. Alexander, DVM, Dipl ACZM, Sarah E. Crevasse, April L. Childress, and James F. X. Wellehan Jr. DVM, PhD, DACZM, DACVM (Virology, Bacteriology/Mycology)

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Aldabra tortoises (Aldabrachelys gigantea) are an endangered species that live in the Seychelles Islands. An Aldabra tortoise presented in 2015 with chronic, intermittent regurgitation. The patient had recently been transferred from a large mixed-species reptile collection. Gastrointestinal endoscopic biopsies revealed lymphoplasmacytic gastritis and enteritis. Multiple courses of antibiotic therapy over several years resulted in no clinical improvement. Consensus nested pan-adenoviral polymerase chain reaction (PCR) and sequencing of a fecal sample identified a novel adenovirus. Phylogenetic analysis revealed this to be a novel member of the genus Atadenovirus. Adenoviruses have high host fidelity, and their phylogeny often reflects that of their hosts. The genus Atadenovirus appears to be endemic in squamates but has jumped into birds and mammals. When it has done so, it has acquired a high Adenine/Thymine base composition bias (A/T bias). The virus sequence from the Aldabra tortoise did not reveal a high A/T bias, suggesting this was either a longer host relationship or so recent that there had not been time for nucleotide composition change. Phylogenetic analysis shows that while mammal and bird atadenoviruses are distinct clades, tortoise atadenoviruses are admixed within atadenoviruses using toxicoferan squamate hosts, suggesting that barriers to jumping between squamate and tortoise hosts are lower.

Abstract 2

Phylogenomic characterization of ranavirus isolated from wild smallmouth bass (*Micropterus dolomieu*)

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In September 2021, 14 wild smallmouth bass (SMB; Micropterus dolomieu) with skin lesions were collected from Lake Michigan and submitted for diagnosis. All samples tested positive for largemouth bass virus by conventional PCR (LMBV). A homogenized skin sample was inoculated into Epithelioma papulosum cyprini cells and typical ranavirus cytopathic effects were observed after 24 hours. The infected cell culture media was then sequenced using an Illumina MiSeq sequencer. The de novo assembly of paired-end reads using SPAdes v3.15.3 resulted in a 99,354 base pair LMBV genome. Maximum Likelihood (ML) phylogenetic analysis based on the 21 core iridovirus genes supported the LMBV isolated from SMB (21117) as a member of the species Santee-Cooper ranavirus (SCRV). Another ML phylogenetic tree, based on the complete major capsid protein gene (MCP) alignment, grouped isolate 21117 with other LMBV isolates reported from the United States and China, as well as doctor fish virus (DFV) and guppy virus 6 (GV6). In addition, pairwise nucleotide comparison of the MCP gene showed that isolate 21117 is identical to other LMBV reported from the United States and nearly identical to DFV and GV6 (99.2%), as well as LMBV isolates from China (99.1%). Thus, LMBV isolate 21117 represents a different strain within SCRV and is closely related to previously isolated strains from the United States and Asia.

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Abstract 3

The importance of auditory, olfactory, and visual cues for insect foraging in owl monkeys (*Aotus nancymaae*)

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Top Dog Oral Presentation
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Nocturnal mammals have unique sensory adaptations to facilitate foraging at night. Owl monkeys (Aotus spp.) are nocturnal platyrrhines adept at capturing insect prey under low-light conditions. We conducted an experiment to determine which cues (auditory, olfactory, visual) A. nancymaae rely upon when foraging for insects. We scored the behavior of 23 captive owl monkeys during trials in which monkeys were provided sensory boxes with insect cues either present (experimental box) or absent (control box). Each cue was tested alone and in combination with all other cues (multimodal cues). We used generalized linear models to determine which cues elicited the greatest behavioral response. Aotus approached and spent more time near experimental than control boxes. Male owl monkeys were quicker than female partners to approach sensory boxes, suggesting that males may be less neophobic than females. The monkeys exhibited behaviors associated with olfaction and foraging (e.g., sneezing, trilling) during trials with multimodal cues and with only olfactory cues present. When only visual or auditory cues were present, Aotus did not successfully locate insects from afar. After approaching a sensory box, however, they often touched boxes containing visual cues. Aotus may rely on olfactory cues at night to detect a food source from several meters away and then rely on visual cues once they are within arm's reach of the food. Their use of sensory cues during insect foraging differs from nocturnal strepsirrhines, possibly reflecting physiological constraints associated with phylogeny, given that Aotus evolved nocturnality secondarily from a more recent diurnal ancestor.

BEST IN SHOW ABSTRACTS

Abstract 4

Coelomic Fluid Changes of Common Sea Stars (*Asterias rubens*) to Hypoxic Environmental Conditions

Sarah J. Wahltinez, DVM, CertAqV, Nancy Denslow, PhD, Donald C. Behringer, PhD, Alisa L. Newton, VMD, Dipl ACVP, Salvatore Frasca, Jr. VMD, PhD, Dipl ACVP, and Nicole I. Stacy, DVM, DrMedVet, Dipl ACVP

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Common sea stars (Asterias rubens) are keystone species in the North Atlantic Ocean rocky intertidal zone and may be at risk due to expanding coastal hypoxic zones or low dissolved oxygen (DO). Sea star die-offs associated with Sea Star Wasting have been increasing in number of species and individuals affected in recent years, and environmental conditions, such as hypoxia, may be contributing factors. However, little is known about the respiratory gases, acid-base status, and molecular responses of A. rubens to hypoxic conditions. The objective of this study was to use a point-of-care blood gas analyzer (iSTAT®) and proteomic analysis to evaluate the coelomic fluid of A. rubens after a 14-day exposure to hypoxic conditions (mean DO 1.80 mg O2/L, N=15). Compared to the control group (mean DO 9.49 mg O2/L, N=15), sea stars in the hypoxia group had higher pH(TC), lower pCO2(TC), and lower pO2(TC), as well as 24 upregulated and 20 downregulated proteins. The upregulated protein network had significantly increased protein-protein interactions and enriched Gene Ontology molecular functions associated with inflammation. While there were no significant protein-protein interactions in the downregulated protein network, there was a functional enrichment of ubiquitin homologues in the network; since ubiquitin-like proteins defend against the harmful effects of hypoxia in vertebrate animals, downregulation of ubiquitin homologues suggests less protection against effects from hypoxia in sea stars. These analyses indicate that sea stars undergo changes in their respiratory gases and acid-base status in addition to eliciting a pro-inflammatory response to hypoxic conditions.

Evidence of a Sjögren's disease-like phenotype following COVID-19

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*Authors contributed equally to the study
#Co-correspondence authors.

Abstract
Sjögren's Diseases (SjD) is a chronic and systemic autoimmune disease characterized most notably by the development of dry eyes and dry mouth as the result of the secretory dysfunction of the lacrimal and salivary glands. It can be systemic and affect other organs. In recent years, infectious agents have been shown to be associated with triggering SjD, including cytomegalovirus, coxsackievirus, Epstein-Barr virus (EBV), and lymphoma virus-1 (HTLV-1). Many cases suggest that infections caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may induce an increase in their autoantibodies, triggering multiple autoimmune responses including primary rheumatic diseases and SjD. To investigate the relationship between SARS-CoV-2 infection and SjD, we infected humanized angiotensin-converting enzyme 2 (hACE2) mice with SARS-CoV-2 WA1/2020. Infected mice showed symptoms of dry eye, decreased salivary flow, elevated antinuclear antibodies (ANA) and SJD hallmark anti-SSB/La, as well as lymphocyte infiltration in the lacrimal and salivary glands, and the apparent presence of apoptotic cells. Corroborating with clinical data, we also observed elevated SjD-specific autoantibodies (ANA, anti-SSB/Ro52 and anti-SSA/La) in the sera of COVID-19 patients. Salivary gland biopsies from patients diagnosed with SjD several months after SARS-CoV-2 infection showed lymphocytic infiltration and fibrosis, which are typical clinical signs of SjD. Finally, to determine whether the monoclonal antibodies produced by COVID-19 patients reacted to nuclear antigens, antibodies with greater antiviral binding capacity were tested and showed a stronger response to nuclear antigens. This study provides strong evidence for the role of SARS-CoV-2 in inducing SjD-like phenotypes, which has implications for how COVID-19 patients will be diagnosed and treated effectively.

Abstract 6

Aquatic vegetation, an understudied depot for PFAS

Emily K. Griffin, Lauren M. Hall, Melynda A. Brown, Arielle Taylor-Manges, Trisha Green, Katherine Suchanec, Bradley T. Furman, Victoria M. Congdon, Sara S. Wilson, Todd Z. Osborne, Shawn Martin, Emma A. Schultz, Mackenzie M. Holden, Dylan T. Lukacsa, Justin A. Greenberg, Katherine Y. Deliz Quiñones, Elizabeth Z. Lin, John A. Bowden

Center for Environmental and Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, 32611.

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured chemicals that have been extensively utilized worldwide. We hypothesize that the presence, uptake, and accumulation of PFAS in aquatic vegetation (AV) is dependent upon several factors, such as the physiochemical properties of PFAS and proximity to potential sources. In this study, AV was collected from eight locations in Florida to investigate the PFAS presence and spatiotemporal distribution. PFAS were detected in AV at all sampling locations, with a range from 0 to 61 ng/g sum (∑)PFAS. Individual PFAS and their concentrations varied by sampling location, time, and AV species. A total of 12 PFAS were identified, with the greatest concentrations measured in macroalgae. The highest concentrations, across all AV types, were recorded in the Indian River Lagoon (IRL), a location with a history of elevated PFAS burdens. The present study represents the first investigation of PFAS in naturally existing AV, filling an important gap on PFAS partitioning within the environment, as well as providing insights into exposure pathways for aquatic herbivores. Examining the presence, fate, and transport of these persistent chemicals in Florida's waterways is critical for understanding their effect on environmental, wildlife, and human health.

Abstract 7

Unraveling the immune and metabolic changes associated with metritis in dairy cows

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Objective was to unravel the peripartum immune and metabolic changes associated with metritis in Holstein cows. Holstein cows (n=128) had blood collected at -14, 0, 3, and 7 days relative to parturition (DRP). Flow cytometry was used to evaluate blood leukocytes' counts, proportions, and activation. Total cells, live cells (LiveDeadTM-), single cells, monocytes (CD172α+/CD14+), polymorphonuclears (PMN; CD172α+/CD14-/SSChigh), B-cells (CD21+/MHCII+), CD4+ T-cells (CD4+), CD8+ T-cells (CD8+), and γδ T-cells (γδTCR+) were evaluated. CD62L and CD11b were used as markers of cell activation. Major histocompatibility complex class II was used as a marker of antigen presentation in monocytes. A MILLIPLEX® Bovine Cytokine/Chemokine 08-plex kit was used to evaluate plasma concentrations of interferon-γ, Interleukin (IL)-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, and tumor necrosis factor-α. Prepartum body weight and body weight change were evaluated, and plasma fatty acids were measured at -14 and 0 DRP. Data were analyzed by ANOVA for repeated measures. Cows that developed metritis had greater prepartum body weight, body weight loss, and greater fatty acid concentrations at calving. We propose that greater prepartum adiposity in cows that developed metritis leads to persistent systemic inflammation, which was demonstrated by greater B-cell activation, greater pro-inflammatory cytokine concentration, and greater cell damage. Postpartum, we observed a dysregulated immune response, with greater PMN activation and extravasation but lesser monocytes and CD4+ T-cells activation and extravasation, which suggests postpartum immune tolerance. This dysfunctional immune response with overall systemic inflammation followed by immune tolerance may lead to failure to prevent bacterial infection, and development of metritis.

Keywords: inflammation, uterine health, lipolysis, cytokines, transition period

Abstract 8

Detection of Tumor-Associated Bacterial DNA (Microbiome) Within Canine Melanoma and Osteosarcoma Using the Bacterial 16S Ribosomal RNA Gene

Lydia Sixta, Rowan J. Milner, Keijiro Shiomitsu, Sandra Bechtel, Michael Dark, Bikash Sahay, Ian Hawkins, Ann M. Chan, Jessy Castellanos, Fahong Yu, Alberto Riva, Oscar Hernandez

The microbiome in the tumor microenvironment has been implicated in altering the host's immune response and playing a role in cancer progression. Osteosarcoma (OSA) and melanoma are deadly cancers of both humans and canines with a high metastatic potential. Novel immunotherapy treatments for both diseases are at the forefront of cancer therapy research. Recent investigations regarding the tumor microenvironment demonstrate that bacteria can affect a patient's response to cancer therapy. The objectives of this study were to extract, identify, quantify and compare bacterial DNA within canine melanoma and osteosarcoma formalin-fixed paraffin embedded (FFPE) samples via Next Generation Sequencing (NGS) using the V3-V4 regions of the bacterial ribosomal 16S gene. Despite the low biomass of intratumoral bacteria, bacterial DNA was successfully extracted from the 20 primary tumor and lymph node samples, along with the 20 matched paraffin-only samples. Immunohistochemistry (IHC) using antibodies against lipopolysaccharide (LPS) and lipoteichoic acid (LTA) demonstrated the presence of intracellular staining in many of the tumor samples. The alpha and beta diversity of the tumor samples were statistically different from the non-tumor paraffin controls (P value = 0.0186, P value = 0.001). Furthermore, select genera were significantly different among tumor types. In conclusion, canine osteosarcoma and melanoma have tumor-associated bacteria. The findings from this study parallel those of a recent largescale study investigating the microbiome in seven human cancers. A better understanding of the role of the tumor microenvironment may guide development of new therapeutic options for both human and canine cancer patients.

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 Univeristy, 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

PHI ZETA

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.