

UF College of Veterinary Medicine Research and PHI ZETA Celebration

April 13 & 14, 2017

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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. Ilaria Capua, DVM, PhD



Dr. Ilaria Capua, DVM, PhD, comes to the University of Florida after spending more than three years as a member of Italian Parliament. She was asked to run for election by Mario Monti, a former EU Commissioner who was serving as Italian Prime Minister at the time. She has come to UF to lead the One Health Center of Excellence, a UF center that aims to tackle international health challenges through interdisciplinarity. Dr. Capua ironically describes herself as a PMO — a “politically modified organism.” As a scientist who has been enriched by exposure to the political arena, she now has a broader grasp of the complexity of the science/policy interface.

Before joining Italian Parliament Dr. Capua was the director of the Department of Comparative Biomedical Sciences at the Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, in Padova, Italy. The department hosts the National FAO/OIE Reference Laboratory for Avian Influenza (AI) and Newcastle disease (ND) and the OIE Collaborating Centre for Diseases at the Animal/Human Interface, providing diagnostic assistance at both a national and international level and performing cutting edge research on influenza viruses and viral zoonoses. The group collaborates with major research institutes worldwide and has developed international collaborative projects aiming to capitalize on investments for capacity building in developing countries.

Dr. Capua rose to the international stage in 1999 due to her efforts to quell the 1999-2000 avian influenza outbreak, then the largest outbreak of avian flu ever recorded. Dr Capua’s team developed novel diagnostic and intervention strategies, including the “DIVA” (Differentiating Vaccinated from Infected Animals) strategy, the first ever to combat avian flu by vaccination. This strategy eradicated LPAI and enabled the continuation of trade in poultry commodities. “DIVA” is now included in EU legislation as one of the tools to combat avian influenza infections in poultry.

In 2006 she ignited an international debate on sharing genetic information from panzootic H5N1 virus strains across disciplines, garnering international attention from media outlets such as the Wall Street Journal and The New York Times. Her advocacy of increased openness was endorsed by OIE, FAO and later by the WHO, and resulted in resolutions supporting greater transparency on genetic data of emerging pathogens.. The sharing of virus sequences to allow for better understanding of animal and human infections has now become a core part of pandemic preparedness plans.

In 2007 she was among the winners of the Scientific American 50 award and in 2008 she was included among Seed’s Revolutionary Minds series for her leadership in science policy for promoting the sharing of information at an international level. She received the most prestigious award in veterinary medicine, the Penn World Leadership in Animal Health award in 2011 and the R.F. Gordon Memorial Medal in 2012. In the same year the Italian President of the Republic, Giorgio Napolitano, bestowed upon her the rank of Knight (Grande Ufficiale della Repubblica Italiana).

As the new director of One Health, Dr. Capua plans to expand the outlook of the center beyond the boundaries of biomedical disciplines. “The One Health concept in the biomedical disciplines is already up and running,” she said. “The veterinary, medical and agricultural communities are already working together, and this collaboration should be fostered and enhanced because there is much more to do.” However, she believes that the time is ripe to expand the One Health vision to other disciplines, and she intends to use her breadth of vision, acquired during her years serving as a member of Parliament, to facilitate a wider interaction between experts in diverse fields. “UF is the ideal place to move this project forward, given the enormous wealth in diversity and expertise on campus. I am convinced that completely novel ideas will come to light that will lead to new approaches for addressing health challenges across the globe.”

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Symposium Schedule

April 13

Banfield Room, SAH 340

3:00pm-4:00pm Poster Set-up

4:00pm-6:00pm Poster Presentations & Light Refreshments

April 14

Banfield Room, SAH 340

10:45am-11:00am Dean's Welcome

11:00am-12:00pm Best In Show Graduate Student Research Competition

11:20pm-1:00pm Lunch in the Courtyard (David's Real Pitt BBQ)

1:00pm-1:05pm Zoetis Award for Research Excellence Presented by Dr. Harvey Crumm

1:05pm-1:45pm Keynote Speaker- **Dr. Ilaria Capua, DVM, PhD**

1:45pm- 2:00pm **Break**

2:00pm-3:00pm Top Dog DVM Research Competition

3:00pm-3:20pm Break with Snacks

3:20pm-4:00pm Awards

Poster #, Name, Title, Abstract #

1. Christopher Alling, "Duration of systemic humoral immunity in beef bulls following therapeutic vaccination against *Tritrichomonas foetus*" (abstract 1)
2. Aysem Bengel, "The pharmacokinetics of ponazuril in Red footed tortoises, *Chelonoidis carbonaria*" (abstract 2)
3. Madison Berger, "Acoustoelastographic Evaluation of the Equine Deep Digital Flexor Tendon" (abstract 3)
4. Kaylee Brown, "The precursor hormone pregnenolone increases sex steroid production in the fathead minnow (*Pimephales promelas*) ovary without modulating enzyme or receptor expression" (abstract 4)
5. Carly Dworkin, "Impact of Heat Treatment on *Dirofilaria immitis* Antigen Detection in Shelter Dogs" (abstract 5)
6. Logan Elmore, "Comparison of fetal crown rump lengths in multiple breeds of Florida beef cattle via ultrasound diagnostics" (abstract 6)
7. Nicole Furst, "Cardiovascular effects of epinephrine on Alligator mississippiensis recovering from isoflurane anesthesia" (abstract 7)
8. Jennifer Groover, "Clinicopathologic Characterization of Canine Leptospirosis in Florida: 2007-2016" (abstract 8)
9. Valentina Henao, "Vascular afferents innervating lumbosacral veins have distinct immunohistochemical phenotypes in Dil-traced DRG neurons in the rat" (abstract 9)
10. Sarah Lewis, "Retrospective Comparison of Antebrachiometacarpal Arthrodesis to Pancarpal Arthrodesis in Dogs" (abstract 10)

Poster #, Name, Title, Abstract #

11. Shelby Loos, "Diagnostic Performance of Serum Amyloid A, Protein Fractions determined by Protein Electrophoresis, Iron, and Ferritin for the Diagnosis of Inflammatory Disease in Wild Stranded Bottlenose Dolphins (*Tursiops truncatus*)"

12. Heather Morissey, "Effect of positioning on length of ventral abdomen: relevance to abdominal closure" (abstract 11)

13. Heather Morissey, "Relationship between echotextural and histomorphometric characteristics of stallion testes" (abstract 12)

14. Bryce Miller, "Discrepancy of Plasma Iron Concentrations with Tissue Iron Stores in Chronically Debilitated Loggerhead Sea Turtles (*Caretta caretta*) with Inflammatory Disease"

15. Kelly Mills, "Utility of ATP-Bioluminescence Measured by a Hand-Held Luminometer as a Tool for Monitoring Hygiene of Milk Replacer Feeding Systems for Dairy Calves" (abstract 13)

16. Jacquelyn Mariano, "Pregnancy Associated Glycoprotein Levels and Embryonic Mortality in Florida Beef Cattle" (abstract 14)

17. Andrea Nall, "Every neuron has a silver lining: a novel silver labeling technique for electrophysiological recordings" (abstract 15)

18. Riley Shugg, "The Effect of Cyclic Axial Loading on Fixation Tension in Single Ring Circular External Skeletal Fixator Constructs" (abstract 16)

19. William Smith, "Survey of endoparasite infection loads in exotic cervid and bovid species in Florida" (abstract 17)

20. Alexa Stephen, "The effects of natural and synthetic retinoids on feline squamous cell carcinoma cell lines" (abstract 18)

21. Amy Thibault, "Three Dimensional Morphometric Analysis of Femoral and Tibial Alignment in Dogs with Medial Patellar Luxation" (abstract 19)

22. Haley Violetta, "Validation of Health Assessment Criteria for *Diadema antillarum*" (abstract 20)

Poster #, Name, Title, Abstract #

Graduate Students:

23. Alexandra Burne, "Broad host range of *Mycoplasma agassizii*: Exposure and infection in Chelonian species" (abstract 21)
- 24.. Maite De Maria, "Is the Herbicide Glyphosate Functioning as a Nephrotoxicant in Fish and Manatees?" (abstract 22)
25. David Dreier, "Utilizing In Vitro Assays To Predict Vitellogenin Induction In Male Fish" (abstract 23)
26. Nick Musselwhite, "Adaptive Mechanisms Governing Tracheobronchial Cough: In Vivo Experiments and Model Simulations" (abstract 24)
27. Andrew Nelson, "Establishment of a Regulatory Microenvironment by *Lactococcus* Expressing Colonization Factor Antigen I (CFA/I) Fimbriae Ameliorates Type 1 Diabetes (T1D) in Non-Obese Diabetic (NOD) Mice" (abstract 25)
28. Dhani Prakoso, "Evaluation of Viral Enrichment Methods for West Nile Virus RNA Extraction from Equine Central Nervous System Tissue" (abstract 26)
29. Massimiliano Tagliamonte, "Malaria in Haiti: A Genomic Approach to Its Epidemiology and Biology" (abstract 27)
30. Marissa Valentine- King, "Investigation of Antimicrobial Resistance in *Ureaplasma* species and *Mycoplasma hominis* Isolates from Urine Cultures in College-aged Females" (abstract 28)
31. Anderson Veronese, "Estrous Characteristics of Heifers with differing Genotype for Daughter Pregnancy Rate and Heifer Conception Rate" (abstract 29)
32. Maria von Chamier, "Prenatal nicotine exposure alters maternal and fetal inflammatory response to infection" (abstract 30)

Post Doc:

33. Whitney Burda, "Comparative evaluation of RpoS⁺ and RpoS⁻ RASV strains" (abstract 31)
34. Manjunatha Nanjappa, "Role of epigenetic regulator enhancer of zeste homolog 2 (EZH2) in uterine and mammary gland development and function in mice" (abstract 32)
35. Tabitha Shen, "Frequency Dependent Inhibitory Mechanisms Controlling the Laryngeal Adductor Response (LAR)" (abstract 33)
36. Arun Wanchoo, "An innovative method for rapid detection of spot urinary oxalate in goats with urolithiasis." (abstract 34)

Poster #, Name, Title, Abstract #

Interns:

37. Michelle Hasiuk, "Perioperative and Long-term Outcomes Following Plate Stabilization to Address Spontaneous Luxation of the Long Digital Extensor Tendon of Origin in Two Dogs " (abstract 35)
38. Cabral Keriann, "Does Do Not Resuscitate (DNR) always mean DNR? Exploring DNR orders in veterinary medicine" (abstract 36)
39. Jennifer Martinez, "Acute Kidney Injury in Dogs with Pit Viper Envenomation" (abstract 37)
40. Tessa Meland, " Anti-Convulsant use patterns for suspect idiopathic epileptic dogs among board-certified emergency and neurology specialists" (abstract 38)

Residents:

41. Rachael Davy, " The Effect Of Catheter Location And Flush Volume On The Time For Contrast To Reach The Heart In A Porcine Model Of A Cardiac Arrest" (abstract 39)
42. William Fox-Alvarez, " Temporary Percutaneous T-fastener Gastropexy and Continuous Decompressive Gastrostomy in Dogs with Experimentally Induced Gastric Dilatation" (abstract 40)
43. Kayla Harding, " Receptor Tyrosine Kinase Expression in Spontaneously Occurring Canine Adrenal Tumors" (abstract 41)

Fellows:

44. Jo Anne Au Yong, "Appendicular fracture management in wild Florida panthers (*Puma concolor coryi*): Six Cases (2000 - 2014)" (abstract 42)
45. Jo Anne Au Yong, " Clinical and radiographic outcomes of cortical allograft demineralized bone fibers for fracture management in dogs" (abstract 43)

Biological Scientist:

46. Shilpa Sanapala, " Comparative evaluation of Protective Efficacy of Salmonella Vaccines derived from UK-1 and 14028" (abstract 44)

Faculty:

47. Rowan Milner, " Immune platforms to monitor GD3 based osteosarcoma vaccine given concurrently with a carboplatin chemotherapy protocol and surgery." (abstract 45)
48. Allison O’Kell, " Two-Stage Model To Study Idiopathic Calcium Oxalate Stone Formation (abstract 46)
49. Shifeng Wang, " Triple-sugar regulated Salmonella Vaccines protect against Clostridium perfringens-induced necrotic enteritis in broiler chickens" (abstract 47)

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



Ana Bascunan

Small Animal Clinical Sciences

"In-Vivo Three-Dimensional Stifle Kinematics In Goats With Anterior Cruciate Ligament Deficiency" (abstract 48)



L. Cody Smith

Physiological Sciences

"Investigating estrogen receptors as targets of pro-fibrogenic signaling in the lung." Dr. Tara Sabo-Attwood and Dr. Nancy Denslow, advisors (abstract 49)



Anje G. Bauck

Large Animal Clinical Sciences

"Effects of lidocaine on inflammation in equine intestine subjected to manipulation and ischemia" Dr. David Freeman, advisor (abstract 50)



Jessica Jacob

Infectious Disease & Pathology

"Phylogenomic Characterization of a Novel Sea Otter Poxvirus" Dr. Thomas Waltzek, Advisor (abstract 51)

Top Dog DVM Competition

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



Geof Zann

Class of 2017

“The Effects of Tibial Plateau Leveling Osteotomy on Patellofemoral Kinematics in Dogs: An In Vivo Study.” (abstract 52)



Rachel Corlett

Class of 2018

“Survey of Angiostrongylus cantonensis in Northwestern Florida” Dr. Bobbie Conner, advisor (abstract 53)



Roasalind (Lindy) Kopp

Class of 2019

“Evaluation of New Sternal Bone Marrow Aspiration Techniques to Increase Initial Stem Cell Yield and Reduce Time for Culture Expansion of a Therapeutic Dose” Dr. McCarrel, project advisor, Dr. Whitley, advisor (abstract 54)



Federico Cunha

Class of 2020

“Droplet digital PCR quantification of uterine bacteria associated with metritis in lactating dairy cows.” Dr. Klibs Galvão, Advisor (Abstract 55)

Abstract 1

Duration of systemic humoral immunity in beef bulls following therapeutic vaccination against *Tritrichomonas foetus*

Christopher Allinga, D. Owen Raea, Xiaojie Maa, Laura Neumann, Raoul Boughton, Elizabeth Steelec, John Yelvington, Hermant K. Naikared, Heather Stockdale Waldene, John Crews

The preputial epithelium of the bull serves as the reservoir for the venereal parasite *Tritrichomonas foetus*, which is innocuous to the bull but may cause vaginitis, pyometra, early embryonic death, and abortion in cows. Systemic humoral immunity is not instigated by natural infection in the bull but seems to play a useful role in preventing infection following immunization. The utility of therapeutic vaccination has been advocated in previous studies, but anecdotal reports suggest that this practice does not clear infections and may additionally confound diagnostic testing by reducing parasite burdens below detectable limits using standard culture-based and PCR techniques. The objective of this study was to characterize the systemic humoral immune response to vaccination in *T. foetus*-infected bulls over a period of four months using an indirect ELISA protocol and to compare the dynamics of this response to culture and PCR results to establish the existence of a relationship (or lack thereof) between immunization and infection status. A study population of 4- to 6-year-old beef bulls ($n = 20$) was divided equally into a treatment group and a control group. The treatment group was administered two doses of commercially prepared whole cell killed vaccine 2 weeks apart while the control group received injections of vaccine diluent. Blood samples were collected at the time of each injection and at four monthly intervals thereafter for measurement of IgG1 and IgG2 γ antibody subisotype response via the indirect ELISA. Preputial smegma samples were collected at the four monthly intervals following vaccination for diagnosis of infection via InPouch™ culture, Modified Diamond's Medium (MDM) culture, and PCR. The ELISA results indicated a statistically significant humoral response for both IgG isotypes from the 2nd week of study through the 14th week, after which time IgG1 absorbance remained significantly increased ($p = 0.043$) relative to the control group in the 18th week. By contrast, IgG2 concentrations fell below statistical significance in the 18th week ($p = 0.53$). However, despite this augmented immunity, no statistically significant decrease in infection rate was detected in the treatment group for any of the diagnostic methods tested. We propose that the secondary immune response stimulated by the labeled two-dose vaccination schedule is insufficient to promote effective pathogen neutralization and that a third dose may be necessary to achieve the desired therapeutic effect. This indicates that there may likely be a role of various modes of *T. foetus* immune system evasion in the poor clinical response to therapeutic vaccination.

Abstract 2

The pharmacokinetics of ponazuril in Red footed tortoises, *Chelonoidis carbonaria*

Shemi L. Benghe (1), M. Tobias Heinrichs (2), Sarah E. Crevasse (1), Behrang Mahjoub (2), Charles A. Peloquin, Pharm D, FCCP (2,3), James F.X. Wellehan Jr., DVM, PhD, DACZM, DACVM, DECZM(1)

Abstract

Coccidial disease is significant in tortoises; Testudines intranuclear coccidiosis (TINC), caused by an unnamed coccidia, causes high mortality in diverse species of infected tortoises. There is a lack of information on anticoccidial drugs in tortoises. The drug ponazuril has demonstrated efficacy in treating mammals infected with similar coccidial disease. Previous empirical use of ponazuril in TINC cases suggests that it may have the potential for therapy. We predicted that the oral administration of 20 mg /kg of ponazuril would result in blood concentrations found to be effective for anticoccidial therapy in mammals. We measured ponazuril in tortoise plasma and determined pharmacokinetic parameters using noncompartmental analyses. Tortoises showed prolonged oral absorption, and despite sampling for 168 hours (1 week), we were unable to determine the terminal elimination rate constant and half-life. Additional studies are needed to fully characterize ponazuril pharmacokinetics in red-footed tortoises. The optimal dose for treating TINC remains to be determined.

Abstract 3

Acoustoelastographic Analysis of the Equine Deep Digital Flexor Tendon

Madison L. Berger*; Adam Biedrzycki, DVM

Objective

To determine biomechanical characteristics of the normal equine deep digital flexor tendon using a sonographic technique called acoustoelastography.

Animals

Eight clinically normal adult horses.

Procedures

Each horse was sedated and then evaluated sonographically. Ultrasound videos of deep digital flexor tendons transitioning from non-weight bearing to weight bearing at two sites on all four limbs were taken. An analytical software was then used to evaluate the strain of the tendons via Acoustoelastography. Two-way ANOVA with limb (fore vs hind) and location (proximal vs distal) as factors was performed. $P < 0.05$ was considered significant.

Results

For maximum strain, there was a significant difference between proximal and distal regions ($p < 0.001$) and no significant difference between hind and fore limbs ($p = 0.72$). For maximum echo gradient, there was no significant difference between proximal and distal regions ($p = 0.46$) or between hind and fore limbs ($p = 0.28$). For maximum pathology index, no significant difference between proximal and distal regions ($p = 0.24$) or between hind and fore limbs ($p = 0.52$).

Conclusions and Clinical Relevance

Results suggest that maximum strain values acquired from acoustoelastography analysis show similarities between fore and hind limbs and significant differences between proximal and distal regions. These results may be useful when utilizing this technology for rehabilitating deep digital flexor tendon injuries, but further acoustoelastography analysis of injured tendons is necessary.

Abstract 4

The precursor hormone pregnenolone increases sex steroid production in the fathead minnow (*Pimephales promelas*) ovary without modulating enzyme or receptor expression

Kaylee A. Brown¹, Christopher J. Martyniuk¹.

¹Center for Environmental and Human Toxicology and the Department of Physiological Sciences, UF Genetics Institute, College of Veterinary Medicine, University of Florida, Gainesville, Florida, 32611, USA

Steroid hormones regulate the development and maturation of oocytes in vertebrates, including teleost fishes. Pregnenolone is a hormone synthesized from cholesterol in the steroid biosynthesis pathway and is the major precursor for progestogens, mineralocorticoids, androgens and estrogens among other steroids. However, little is known as to the role of this hormone in teleost reproduction. The objectives of this study were to assess the effects of pregnenolone treatment on testosterone (T) and 17 β -estradiol (E2) production from the teleost ovary and to determine whether this hormone modulates the expression of transcripts related to steroidogenesis and hormone receptor signaling in this tissue. Ovary explants from individuals were incubated for 12 hours with either control media or one of three concentrations of pregnenolone (10⁻⁶ M, 10⁻⁸M, and 10⁻¹⁰M) which spanned physiological relevance. The highest concentration of pregnenolone (10⁻⁶ M) significantly increased T and E2 production from the ovary, yet there was no effect on the expression levels of transcripts that included those in the steroid pathway (*cyp17*, *cyp19a*, *cyp19b*, *hsd11b2*, *hsd17b*, and steroidogenic acute regulatory protein or *star*) or steroid hormone receptors (androgen receptor or estrogen receptors *esr1*, *esr2a*, *esr2b*). Interestingly, while T and E2 production was increased at the highest dose of pregnenolone, there was no change in the E2/T ratio in terms of overall production, suggesting that the ovary may act to maintain an overall steroid balance, rather than to regulate individual steroids. Moreover, as mRNA levels did not significantly change following pregnenolone treatments compared to controls, we propose that the early modulation of the steroid pathway by steroid does not involve *de novo* transcription of converting enzymes. Thus, pre-existing proteins are more likely involved in the rapid increase in T and E2. Lastly, Spearman correlation analysis revealed that pregnenolone shifted well documented relationships between transcript levels and steroid production. For example, previous studies show that *cyp19a* is positively and strongly correlated to E2 production in the fathead minnow ovary, however endogenous pregnenolone treatments negated this close relationship. This was also true of the negative relationship between androgen receptor expression and testosterone production. This study sheds light on the steroid regulation in the ovary, which can be perturbed by environmental contaminants and endocrine disruptors. As such, continued evaluation of the mechanistic processes underlying steroid production in the ovary is warranted with endogenous hormone treatments.

Abstract 5

IMPACT OF HEAT TREATMENT ON REGIONAL PREVALENCE OF DIROFILARIA IMMITIS ANTIGEN DETECTION IN SHELTER DOGS

BA DiGangi,¹ C Dworkin,¹ J Stull,² J O'Quin,² M Elser,² AE Marsh,² L Groshong,³ W Wolfson,⁴ B Duhon,⁴ K Broaddus,⁵ EN Gingrich,⁶ E Swiniarski,⁷ EA Berliner⁸

¹University of Florida, Gainesville, FL, USA

²The Ohio State University, Columbus, OH, USA

³The Humane Society of Boulder Valley, Boulder, CO, USA

⁴Louisiana State University, Baton Rouge, LA, USA

⁵Austin Humane Society, Austin, TX, USA

⁶Larimer Humane Society, Fort Collins, CO, USA

⁷Pet Orphans of Southern California, Van Nuys, CA, USA

⁸Cornell University, Ithaca, NY, USA.

The diagnosis and management of canine heartworm disease is an area of growing importance to shelter veterinarians nationwide. Although the accuracy of commercially available antigen test kits has been widely studied, recent reports have renewed interest in antigen blocking as a causative factor for false negative test results. The objectives of this study were to determine the prevalence of false negative antigen test results due to the presence of antigen blocking in dogs entering shelters in 3 different regions of the country and to identify historical and clinical risk factors for such results.

Blood samples were collected from adult dogs upon admission to shelters in 3 regions of the USA. Serum samples were evaluated for *Dirofilaria immitis* antigen using a commercially available point-of-care ELISA; negative samples underwent a heat treatment protocol and repeat antigen testing. Whole blood samples were submitted to a diagnostic laboratory for modified Knott's testing to identify and quantify the presence of microfilariae. Historical and clinical findings were analyzed in light of diagnostic test results.

A total of 616 samples were analyzed. Prevalence of positive antigen test results (prior to heat treatment) and frequency of false negative results due to antigen blocking (i.e., samples negative prior to heat treatment and positive after heat treatment) are summarized in Table 1. Multivariable exact logistic regression demonstrated greater odds of antigen blocking (i.e., false negative test results) when microfilaria were detected via modified Knott's testing (OR = 32.30, p-value = 0.013) and in dogs known to have received a heartworm preventive prior to sample collection (OR = 3.81, p-value = 0.016). Geographical region of origin was not identified as a risk factor for antigen blocking.

Table 1. Prevalence of positive and false negative antigen test results.

Region	Number Positive (%)	95% CI	Number False Negative (%)	95% CI
All	45 (7.3)	5.4, 9.7	29 (5.2)	3.5, 7.4
North	3 (1.6)	0.3, 4.5	6 (3.2)	1.2, 6.8
South	38 (15.9)	11.5, 21.2	14 (7.3)	4.0, 12.0
West	4 (2.2)	0.6, 5.4	9 (5.0)	2.3, 9.3

Study findings identified specific clinical and historical risk factors for false negative antigen test results secondary to antigen blocking of non-heat treated samples. Heat treatment of serum samples should be considered to improve diagnostic test accuracy, particularly in dogs with circulating microfilaria and those known to have received a heartworm preventive prior to antigen testing.

Abstract 6

Comparison of Fetal Crown Rump Lengths in Multiple Breeds of Florida Beef Cattle Via Ultrasound Diagnostic

Logan Elmore and Dr. Owen Rae

The beef cattle industry relies heavily on a thriving herd of weaned calves each year. With a host of variables, it is difficult to determine what can cause a cow to abort or lose a pregnancy and fail to wean a calf. The purpose of this paper is to evaluate/compare fetal crown rump (FeCR) lengths via transrectal ultrasound to days pregnant (DPg) across different breeds prominent in the Florida beef cattle industry. Measuring FeCR length provides a fairly accurate indicator of pregnancy development and, as hypothesized in this study, can be used to monitor a potentially unsuccessful pregnancy based on the fetal size at measurement in early gestation. FeCR was also compared across the six Angus x Brahman breed crosses to determine if a size difference in developing fetuses by breed was observed. Results were displayed in scatter plots, showing that a distinct size pattern throughout each breed is evident, based on the first 60 days of pregnancy. In regards to predicting future embryonic loss based on developing FeCR lengths, it is impossible to make an assumption due to having only one embryonic death during the time frame studied.

Logan Elmore and Dr. Owen Rae

Abstract 7

Cardiovascular effects of epinephrine on *Alligator mississippiensis* recovering from isoflurane anesthesia

Nicole Furst, Bonnie Gatson, Simon Swift, Jim Wellehan

Departments of Large and Small Animal Clinical Sciences

Abstract: Crocodylians are commonly anesthetized using inhalant agents during procedures to minimize risk to patients and personnel. However, crocodylians often times are slow to recover from inhalant anesthesia, which delays return to their enclosures and increases costs to clients. Previous studies indicate that intramuscular epinephrine significantly reduces recovery time from inhalant anesthesia in American alligators (*Alligator mississippiensis*). It is conceivable that cardiovascular right to left shunting, which occurs naturally during underwater submersion, plays a role in delaying anesthetic recovery. However, it is unknown how volatile anesthetics and epinephrine affect the complex cardiovascular anatomy and shunting patterns of the crocodylian heart. In this blinded cross-over study, six American alligators underwent isoflurane anesthesia for ninety minutes. After isoflurane was discontinued, epinephrine (0.1mg/kg) or equal volume of saline was administered intramuscularly. Echocardiography was performed before anesthetic induction, during maintenance of anesthesia, and immediately following administration of treatment. Physiologic parameters (heart rate and respiratory rate), concentration of airway gases, and recovery parameters were recorded every five minutes during anesthetic recovery. Description of shunting patterns observed by echocardiography during different stages of anesthesia and recovery were described. Also, the time from discontinuing isoflurane to spontaneous ventilation, return of palpebral and withdrawal reflexes, and spontaneous movement was compared between treatment groups.

Research Grant: Association of Reptilian and Amphibian Veterinarians, University of Florida

Student Support: Merial Veterinary Scholars Program

Abstract 8

Clinicopathologic Characterization of Canine Leptospirosis in Florida: 2007-2016

Authors: Groover, Jennifer; Londoño, Leonel

Abstract:

The objective of this study is to characterize the demographics, exposure risks, clinicopathologic features, treatments, outcomes, and environmental conditions for dogs diagnosed with leptospirosis in Florida. Medical records of twenty-five dogs diagnosed with leptospirosis at a referral hospital between December 2007 and July 2016 were reviewed. Data was also gathered from the National Oceanic and Atmospheric Administration of the United States Department of Commerce to determine the average precipitation and temperature for Florida each month at the time of diagnosis. The study did not find a correlation between precipitation and temperature with the number of leptospirosis cases submitted. *L. grippityphosa* was the most common serovar and most likely to cause the worst degree of renal and hepatic involvement. The overall prognosis for these patients was usually good although 40% required hemodialysis for acute kidney injury (AKI). Further research is warranted to determine if the increasing number of leptospirosis patients seen may be secondary to increased frequency of testing and awareness or higher prevalence.

Abstract 9

Vascular afferents innervating lumbosacral veins have distinct immunohistochemical phenotypes in DiI-traced DRG neurons in the rat.

Emphasis in Neurology (more applicable to humans)

Abstract:

Henao, V., Nguyen, H.D., Dugan, V.P., Cooper, B.Y., Johnson, R.D.

Input from vascular sensory neurons has been implicated in painful conditions including migraine headache and deep tissue pain. Our previous work on DRG neurons used a combined in vitro electrophysiology and immunohistochemical phenotyping approach utilizing target-specific tracing from the periphery (skin, muscle, viscera, mucocutaneous, etc.) in the rat lumbosacral region. We have found distinct target-specific phenotypic patterns, and in the present report, extend this investigation to the vasculature.

Under aseptic procedures in adult male Sprague-Dawley rats, the proximal segment of the left lateral tail vein was surgically isolated (1.5-2 cm), a glass microsphere luminal plug placed at the cranial end, and the vein sutured closed at the caudal end. The closed-end venous luminal space was filled with a fluorescent DiI-paste delivered through a 26-gauge catheter. After 13 days of tracer transport time, the animals were euthanized and perfused transcardially with 4% paraformaldehyde. The left and right L5-S2 DRGs were dissected free, post-fixed overnight and cryoprotected. The Di-I injection site was examined postmortem to verify that no dye leaked into non-vascular structures. Serial cryosections at 14 μm were thaw-mounted on alternating slides. Nucleated DiI-positive DRG neurons were directly visualized via multi-label fluorescence microscopy, digitally imaged with Zeiss optics, and measured with morphometric software. Using our previously published techniques (e.g. Petruska et al, 2000), slides were immunohistochemically processed for various cellular markers including CGRP, SubP, Neurofilament-M (NF-M), IB4, TRPV1, TRPV2.

Di-I positive cells neurons (n=929) traced from the proximal tail vein were found almost exclusively in the ipsilateral L6 and S1 DRGs (some in S2) and exhibited small to medium cell diameters (96% between 15-45 μm). Only 19% were NF-M positive but had a significantly greater cell diameter ($41.5 \pm 1.1 \mu\text{m}$) compared to NF-M negative cells ($31.2 \pm 0.5 \mu\text{m}$; $p < .01$). The latter were mostly IB4 negative. While only 21% were typical peptidergic cells containing both CGRP and SubP, those containing only one peptide were CGRP+ (92%). Combined with our finding that one third of the total Di-I cells contained CGRP, our data support the known importance of CGRP in vascular control. Consistent with our in vitro patch clamp data from capsaicin-sensitive and -insensitive Di-I traced vascular DRG neurons (type 8, type 19 and type 20; Cooper et al., 2014), a portion of the vascular afferent population were positive for TRPV1 and TRPV2 with most CGRP+ cells co-labeled with the capsaicin-insensitive TRPV2 receptor (63%).

Abstract 10

Retrospective Comparison of Antebrachiometacarpal Arthrodesis to Pancarpal Arthrodesis in Dogs

Lewis ST1, Lewis DD1, Cross AR2, Radasch RM3; 1University of Florida, Gainesville, FL; 2 Georgia Veterinary Specialists, Atlanta, GA; 3Dallas Veterinary Surgical Center, Dallas, TX

This study evaluated the indications, technical aspects, complications, and clinical outcomes associated with antebrachiometacarpal arthrodeses ABMCA and compare results to a cohort group which underwent pancarpal arthrodesis (PCA). Medical records, including radiographs, of dogs that underwent ABMCA and PCA were reviewed. Data was analyzed using a t-test: $p < 0.05$ was considered significant.

Four dogs had ABMCAs performed; eight dogs (10 PCAs) comprised the cohort group. Disease processes necessitating ABMCA included chronically infected fracture-luxation of the carpus (1) and severe carpal flexure contracture (3). Carpal bone excision was necessitated to allow the manus to be stabilized in a functional position in the dogs with contracture. Disease processes necessitating PCA included traumatic hyperextension injuries (7), antebrachial deformity (2), and osteoarthritis (1). All arthrodeses were grafted and stabilized with dorsal plates. Postoperative frontal and sagittal plane angulation was not a significant difference between groups. The incidence of complications was significantly greater ($p=0.02$) for ABMCAs (4/4 vs 5/10). The implants stabilizing all 4 ABMCAs and 5 PCAs had to be removed due to infection ($p=0.003$). All ABMCAs, while only 7 PCAs obtained complete osseous union ($p=0.04$). There were no significant differences in time to early osseous union between arthrodesis groups. All ABMCA dogs obtained acceptable function, while 2 PCA dogs had poor function necessitating revision arthrodesis.

While ABMCA provided acceptable long-term function, the procedure was fraught with a higher complication rate (100%) than PCA (50%). ABMCAs, however, obtained consistent osseous union and the high incidence of complications may reflect unique, challenging pathologies necessitating ABMCA.

Abstract 11

Effect of positioning on length of ventral abdomen: relevance to abdominal closure

H. A. Morrissey, D.J. Schaeffer and D. E. Freeman

Department of Large Animal Clinical Sciences, University of Florida, College of Veterinary Medicine, Gainesville, Department of Large Animal Clinical Sciences, 2015 SW 16th Avenue, Gainesville, FL, USA (Morrissey and Freeman) and University of Illinois, College of Veterinary Medicine, Urbana, IL, USA (Schaeffer).

Telephone No: (904) 446-5928

Fax Number: (352) 392-8289

Email: heathermorrissey@ufl.edu

Approximately 30% of horses develop incisional complications after colic surgery. Our longterm goal is to determine if suture tension increases as a horse is transferred from dorsal to lateral recumbency. If so, this increased tension could strangulate the linea alba and predispose to infection along the suture line. This study was designed to provide preliminary data to support a more complete study on the effects of body position on suture tension.

The hypothesis of this study states that the ventral abdomen is shorter from cranial to caudal when the horse is in dorsal recumbency than when it is converted to lateral recumbency or standing.

Data from 20 adult horses of body weight ≥ 400 kg that had colic surgery included the length of the ventral abdomen from umbilicus to xiphoid after the abdominal closure in dorsal recumbency, again in lateral recumbency in the recovery stall, and in the standing position. Differences were analyzed by Spearman correlations with $P < 0.05$ significant.

The length of the ventral abdomen increased by 18% from dorsal recumbency to lateral recumbency (20 horses) and by 16% from dorsal recumbency to standing (10 horses). These changes represented significant increases in length ($P < 0.05$).

Based on these findings, the ventral midline incision is closed when the abdomen is at its shortest length. Therefore, continuous suture patterns could become tighter and thereby reduce blood flow to the incision edges after surgery. The common practice of maximally tightening abdominal wall sutures could exacerbate this problem.

Abstract 12

HISTOMORPHOMETRIC CHARACTERICS OF STALLION TESTES

Heather Morrissey, Malgorzata Pozor, Valeria Albanese, Natalie Khouzam, Alexis Derberprey
Department of Large Animal Clinical Sciences, University of Florida, College of Veterinary Medicine,
Gainesville, FL, USA

Computer based analysis of testicular echotexture from ultrasonographic images has been used in humans and other species. Accurate diagnosis of testicular disorders often requires invasive techniques, such as testicular biopsies. The goal of this study was to investigate the relationship between testicular echotextural and histomorphometric parameters in stallion testes.

A total of eighty testes were evaluated; fifty-four scrotal testes, 10 retained inguinal testes, and 16 retained abdominal testes. The scrotal testes were divided based on age of castration into colts (<1 year of age), young stallions (1-5 years of age), and mature stallions (>5 years of age). Using a testicular ultrasonographic training model of our own design, cross sectional and longitudinal ultrasonographic images were obtained for each testis. Numerical pixel value (NPV) and pixel standard deviation (PSD) were determined for each image. Three tissue samples per testis were sampled for histologic evaluation. Histomorphometric parameters of the seminiferous tubules were taken using Advanced SPOT 5.2 microscope imaging software.

Differences between groups for all parameters were analyzed using one-way ANOVA and Tukey's test. The Pearson product-correlation procedure was used to calculate correlations between echotextural and histological parameters.

The scrotal testes from the young and mature stallions had the highest echotextural and histomorphometric parameters. Our data demonstrated a significant correlation between NPV, PSD, and histomorphometric parameters from the three groups of scrotal testes. However, there was no correlation between these parameters when only scrotal testes from young and mature stallions were included in analysis. There was no correlation between NPV and the histomorphometric parameters in retained abdominal testes.

The positive correlation of pixel intensity and heterogeneity in stallions during the first year of life is likely reflective of seminiferous tubule development. This tool does not seem to reflect the histomorphometric composition of testicular parenchyma in stallions after puberty. Further investigation of computer based analysis of testicular echotexture is warranted.

Abstract 13

Utility of ATP-Bioluminescence Measured by a Hand-Held Luminometer as a Tool for Monitoring Hygiene of Milk Replacer Feeding Systems for Dairy Calves

Kelly Mills, Fiona Maunsell , G. Arthur Donovan

Bioluminescence uses the concept that all living organisms produce ATP so the presence of ATP is a sign of living organisms. Bioluminescence uses the enzyme luciferase which reacts with ATP and creates a photon of light, which is then measured. Bench-top ATP-bioluminescence instruments are used in the food industry to test foods, including raw and ultra heat treated milk, for microbial contamination.. Recently, hand held portable units (luminometers) have become available for field testing of foods and water for microbial contamination. The luminometer could offer significant advantages over standard bacterial culture as results are available immediately. A rapid field test to evaluate bacterial contamination of milk replacer would be helpful in monitoring hygiene of dairy calf feeding systems. The goal of this project was to investigate the hypothesis that the hand held luminometer would measure calf milk replacer systems hygiene rapidly and reliably. Water and milk replacer were tested with increasing levels of bacteria. The luminometer was able to detect bacteria at levels of 10^6 colony forming units (cfu) and higher in the milk replacer. In spiked water, the luminometer was able to generally detect contamination at $<10^3$ cfu. These findings indicate that for milk-replacer systems, there is limited ability of the system to detect bacterial contamination. The differences noted between milk replacer and water likely is due to background fluorescence. Use of the luminometer for determination of milk replacer system hygiene is not recommended.

Abstract 14

Pregnancy Associated Glycoprotein Levels and Embryonic Mortality in Florida Beef Cattle

Jacquelyn Mariano and Dr. Owen Rae

This study examined circulating serum pregnancy associated glycoproteins (PAGs) as a potential predictor of embryonic mortality (EM) in beef cattle. The main objective of the study was to identify the relationship between PAG concentrations at day 30 of gestation and EM in beef cattle between day 30 and day 60 of gestation. A secondary objective was to identify a PAG value below which would likely predict EM. It was hypothesized that cattle with lower circulating PAG concentrations at day 30 of gestation would be more likely to experience EM than cattle with higher circulating concentrations. All of the animals received pregnancy examinations at 30 and 60 days after breeding following a timed artificial insemination protocol. Pregnancy was diagnosed by the presence of a fetal heartbeat via ultrasonography during these checks. EM was said to occur if the animal was diagnosed pregnant during the 30 day check, but not pregnant (open) during the 60 day check. Only animals diagnosed pregnant during the 30 day check were included in the analysis (Pregnancy maintained, n = 224; EM, n = 6). Animals that experienced EM had significantly lower PAG values at day 30 of gestation (1.65 ± 0.14 ng/mL; mean \pm SEM) than animals that maintained their pregnancy until day 60 of gestation (3.12 ± 0.05 ng/mL; mean \pm SEM). The study was not able to identify a definitive PAG level below which EM would likely occur; however, a logistic regression curve was used to confirm an increasing probability of EM as PAG values fall below 2 ng/mL at day 30 of gestation. In conclusion, low circulating PAG concentrations at day 30 of gestation display a clear relationship with EM in beef cattle, but further studies with larger sample sizes are needed to definitively identify a PAG value below which would predict EM.

Abstract 15

A Novel Silver Labeling Technique to Anatomically Identify Cardio-Respiratory Network Neurons in the Brainstem of the In Situ Rodent Preparation

Nall AC, Howard CA, Denson HB, Baekey DM

Cardio-respiratory coupling (CRC) is governed by a complex network of neurons within the ventrolateral brainstem distributing inspiratory and expiratory modulated signals to airway musculature and autonomic control circuitry. While some functional and anatomic components of this network have been identified, specific anatomic locations and neurophysiological connections between neural ensembles of this circuitry remain undefined. Importantly, the physiological coordination of these automatic and autonomic systems has significant health implications and improved knowledge of their interactions may lead to better treatment of associated disease states such as sleep apnea and hypertension. Research in this area has applied a variety of animal preparations and numerous histologic staining procedures to map respiratory neural infrastructure with electrophysiological recording. Individually these methodologies produce relevant findings regarding single neurons, but the coupling of multiunit electrophysiological recordings with discreet neural populations will provide researchers a powerful new technique to determine cardio-respiratory circuitry. While there are several techniques to perform juxtacellular labeling from single glass pipette electrodes, the practice of silver-plating tungsten metal microelectrodes is unique. To accommodate this deposition approach, we have introduced a novel silver staining technique and incorporated it with our custom-built multielectrode array, creating an integrated approach toward phenotyping the cardio-respiratory brainstem. Incipient results suggest that this method is effective in visualizing recorded brainstem neurons.

Abstract 16

THE EFFECT OF CYCLIC AXIAL LOADING ON FIXATION WIRE TENSION IN SINGLE RING CIRCULAR EXTERNAL SKELETAL FIXATOR CONSTRUCTS

Riley A Shugg
Daniel D Lewis, DVM, Diplomate ACVS
Nicole J Mohajer
Mathew C Banks
Scott A Banks, PhD

Introduction

The objective of this study was to evaluate the effects of repetitive cyclic axial loading on residual wire tension in single ring circular fixator constructs. We hypothesized that there would be a significant decrease in wire tension in constructs subjected to cyclically loaded and that the majority of the drop in tension would occur during the initial loading cycles.

Methods and Materials

Five single 84 mm ring constructs with two 1.6 mm olive fixation wires were subjected to cyclical axial load of 200 N over 22,000 cycles. Wire strain values were recorded and analyzed to derive the mean percent of tension lost in the fixation wires over time.

Results

The mean decrease in strain was 22.6% in wire 1 and 7.5% in wire 2 after 22,000 cycles of loading. The majority of the decrease in wire tension occurred during the first 3,500 cycles (2 hours) of loading. Mean displacement at maximum load was 19% more after 22,000 cycles than the displacement recorded during initial cycle of loading.

Discussion Conclusion

This reduction in wire tension was ascribed to slippage of the wires in the fixation bolts which secure the wires to the ring. An equation was developed, based on our data, that would predict the potential decrease in wire tension (percentage) in a circular construct over time.

Acknowledgements

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Abstract 17

Survey of Endoparasite Infection Loads in Exotic Cervid and Bovid species in Florida

William Smith and Heather S Walden

Parasite species known to infect exotic cervid and bovid species is a topic not well studied in Florida. This study involved collecting fecal samples from a ranch in Quincy, Florida. Species collected from included: Père David's (*Elaphurus davidianus*), Axis (*Axis Axis*), Fallow (*Dama dama*), and Sitka deer (*Odocoileus hemionus sitkensis*), North American Elk (*Cervus canadensis*), Nilgai (*Boselaphus tragocamelus*), Blackbuck (*Antilope cervicapra*), Scimitar Oryx (*Oryx dammah*), Gemsbok (*Oryx gazella*), Hawaiian sheep, and Jacob's sheep (*Ovis spp.*). Fecal samples were examined for endoparasites using centrifugal flotation and sedimentation techniques. Out of 69 fecal samples collected, 38 were found to be positive for some type of endoparasite. Parasitic species identified in the study included *Trichostrongyle spp.*, *Eimeria spp.*, *Trichuris spp.*, *Strongyloides spp.*, and unknown nematode larvae. All of these parasites, with the exception of *Strongyloides spp.*, were identified in another study conducted on White-tailed deer on the same property indicating that cross-transmission is a possibility. Confounding variables include low sample numbers and a tri-annual deworming practice that could have impacted the number of parasites in each species.

Abstract 18

The effects of natural and synthetic retinoids on feline squamous cell carcinoma cell lines

Alexa A. Stephen, Marc E. Salute, Carlos H. Souza

Squamous cell carcinoma is the most common oral malignancy in cats. The existing therapies include combinations of surgery, radiation, and chemotherapy, which often fail to control this locally invasive tumor and can be associated with significant morbidity. Retinoids, derivatives of vitamin A, have been shown to inhibit cell proliferation by terminal differentiation and apoptosis. The current study evaluated the effects of two retinoids, all-trans retinoic acid (ATRA) and fenretinide, on feline squamous cell carcinoma cell lines (SCCF1 and SCCF2). In addition, we evaluated if these cell lines produced vascular endothelial growth factor and tumor-promoting cytokines (IL-1, IL-6, IL-8, MCP-1). Fenretinide induced apoptosis in clinically achievable concentrations. ATRA induced apoptosis in only one cell line; however, this concentration cannot likely be achieved in vivo. Both SCCF1 and SCCF2 cell lines produced IL-8. ATRA had no effect on IL-8 production. Fenretinide seems to have altered IL-8 production in a time and concentration dependent manner.

Abstract 19

Morphometric Analysis of Femoral and Tibial Alignment in Dogs with Medial Patellar Luxation

Thibault AE; Kim SE1; Tinga S1; Petazzoni M2, Banks SA3

(1) Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, (2) Clinica Veterinaria Milano Sud, Milan, Italy, (3) Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL

Hindlimb abnormalities of the femur and tibia have been associated with the presence of medial patellar luxation (MPL) in dogs. Cause and effect relationships between these deformities and the presence of MPL have not been well investigated. We utilized morphometric analysis through 3D reconstructions of the femur and tibia to quantify 3D alignment of the femur and tibia in each plane for dogs with and without MPL. Ten small breed dogs with MPL and 8 small breed dogs unaffected with MPL were recruited. All dogs underwent orthopedic examinations and hindlimb CT scans. Volumetric 3D reconstructions of the femurs and tibias were created from CT scans. Coordinate systems were assigned to the proximal and distal regions of the femur and tibia based on local anatomical landmarks. Degrees of torsion, varus/valgus, and angulation were measured by calculating the angle formed between the coordinate systems created. Subtle morphometric differences in femoral and tibial alignment were identified between normal dogs and dogs with MPL. Dogs with MPL had a mean of 4 degrees more external rotation of the distal tibia (or internal rotation of the proximal tibia), 3 degrees more valgus angulation of the tibia, 6 degrees more external rotation of the intertrochanteric axis, and 6 degrees less femoral procurvatum.

Abstract 20

Validation of Health Assessment Criteria for *Diadema antillarum*

Haley M. Violetta^{1*}+, Ruth Francis-Floyd¹, Roy P.E. Yanong², and Jan H. Landsberg³

¹University of Florida, Department of Large Animal Clinical Sciences, College of Veterinary Medicine Gainesville, Florida, 32610, USA; ²Tropical Aquaculture Laboratory, Fisheries and Aquatic Sciences Program, Institute of Food and Agricultural Sciences, University of Florida, Ruskin, Florida, 33570, USA; ³Florida Fish and Wildlife Conservation Commission, Fish and Wildlife Research Institute, St. Petersburg, Florida, 33701, USA

Abstract

The long-spined sea urchin (*Diadema antillarum*) is a keystone species in coral reef ecosystems, and is considered an important factor in the planning and development of coral reef restoration.^{1,2} A mass mortality event of *D. antillarum* in the 1980's left population levels at unrecoverable densities required for this species to maintain its primary ecological role.^{3,4,5} As a component of coral reef restoration efforts a stock enhancement program is being developed to supplement reduced endemic populations of *D. antillarum*.⁶ Health assessment criteria have been developed to qualify cultured long-spined sea urchins for release into the wild.⁷ These assessment criteria have not yet been utilized in practice, and their validation is needed to determine whether they are appropriate for designating cultured *D. antillarum* healthy for release. In this study, the health assessment protocol was applied to animals comprising two different data sets: 1) wild and 2) captive. The first was collected during a sampling of wild-caught individuals and retrospectively analyzed and the second was collected from captive animals at three Florida facilities. The wild-caught sea urchin data comprised 189 individuals divided into six batches to be spatiotemporally assessed. The captive sea urchin data collected from 50 individuals was also comprised of six batches that were delineated by animals in the same facility and system. The health assessment protocol as defined in the "Diagnostic Manual for Health Assessment of *Diadema antillarum*" was applied to each batch of animals, and the overall percentage of individuals qualifying for release was calculated.⁷ It was expected that all batches assessed would have 90% of the individuals qualify for release, and ten out of the 12 batches assessed fell into this category. Two batches had 50–89% of individuals qualify for release. None of the batches had $\leq 49\%$ of individuals qualify for release. These results support use of the health assessment criteria as both reasonable and effective for qualifying cultured *D. antillarum* for release. During results collection, a relationship between increased holding time post-capture and decreased health assessment scoring was noted in data from wild-caught animals in the retrospective analysis. This information may have relevance to handling and husbandry considerations and will be considered for further investigation.

Acknowledgements

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Abstract 21

BROAD HOST RANGE OF MYCOPLASMA AGASSIZII: EXPOSURE AND INFECTION IN CHELONIAN SPECIES

Alexandra M. Burne, Lori D. Wendland, Daniel R. Brown, Paul A. Klein, and Mary B. Brown

Mycoplasma agassizii is a primary etiologic agent of a well-characterized upper respiratory tract disease (URTD) in free-ranging desert and gopher tortoises in the U.S. Although narrow host specificity traditionally has been considered a feature of mycoplasmal species, clinical signs compatible with mycoplasmal URTD have been observed in a number of chelonian species. In order to assess the host range of this pathogen, we screened 2,773 sera from 31 species of turtles and tortoises in the Chelidae (N=1), Chelydridae (N=1), Emydidae (N=6) and Testudinidae (N=23) families for the presence of antibody to the pathogen. In addition, nasal lavage/swab samples (N=460) were tested by PCR and culture. Clinical isolates obtained from 10 different tortoise species representing 8 genera in Testudinidae were confirmed to be *M. agassizii* by sequencing of the 16S rRNA gene. Our results demonstrate the ability of *M. agassizii* to colonize a wide range of tortoise species within the Testudinidae family, supporting the need to include this pathogen as a differential diagnosis in diverse chelonian species presenting with URTD, and also to consider risks for pathogen transmission across multiple species when establishing husbandry and breeding protocols.

Abstract 22

IS THE HERBICIDE GLYPHOSATE FUNCTIONING AS A NEPHROTOXICANT IN FISH AND MANATEES?

Maite De María¹, Viet Dang¹, Kevin Kroll¹, Mike Walsh² and Nancy Denslow¹

¹ Department of Physiological Science, University of Florida

² Aquatic Animal Health Program, University of Florida

Glyphosate or its commercial versions Rodeo[®] and Roundup[®], is an herbicide widely used in Florida, for sugarcane, citrus, tomato farming or sprayed directly in lakes to control vegetation. It is supposed to rapidly break down diminishing its toxicity, although, previous studies have linked glyphosate exposure to reproductive consequences in fish and kidney alterations in rats. Moreover, farmers working with herbicides have unusual chronic kidney disease. Fish and manatees swim in waters that receive runoff from agricultural areas where this herbicide is directly sprayed. To date there is sparse research addressing molecular changes from oxidative damage in kidney of exposed animals. Exosomes are small vesicles that are released from kidney into the urine and blood and may contain markers of oxidative damage. The first two objectives of this study are to assess glyphosate/Rodeo[®] injury to largemouth bass and to characterize exosomes isolated from urine and blood. We will expose largemouth bass to two concentrations of glyphosate and two of Rodeo[®] (equivalent to the chemical amount of glyphosate) to assess nephrotoxicity and develop a biomarker in both blood and urine. We will analyze alterations in gene expression in the kidney by RNA sequence of glyphosate and rodeo-exposed fish (equivalent doses) compared to control. Gene expression changes will be correlated with kidney histopathology. Both lipid and protein biomarkers will be assessed in exosomes that relate to kidney function, for example KIM-1, NGAL and beta-microglobulin have previously been linked to kidney disease. Our third objective is to assess glyphosate/Rodeo[®] injury to manatees, through analysis of blood and urine exosomes. We expect the biomarker levels will correlate with the concentration of glyphosate or its breakdown product in manatee blood and urine. The results of this study will contribute to understanding kidney disease of manatees and sensitivity of other stressors (red tide) which have important consequences for manatee populations.

Abstract 23

Utilizing In Vitro Assays To Predict Vitellogenin Induction In Male Fish

David A. Dreier, Nancy D. Denslow, Christopher J. Martyniuk

Center for Environmental & Human Toxicology, Department of Physiological Sciences, College of Veterinary Medicine, University of Florida

Vitellogenin induction in male fish has long been used a biomarker for estrogenic chemicals. In the Endocrine Disruptor Screening Program (EDSP), this unique response has been used to identify estrogenic chemicals with the Fish Short-Term Reproduction Assay (FSTRA). In recent years, in vitro assays have also provided valuable information for endocrine responses. In ToxCast, there are several assays for estrogen receptor (ER) activity, and there is also an integrated ER AUC (area under the curve) model to identify estrogenic chemicals. As ER agonism is a molecular initiating event in several adverse outcome pathways (AOPs), it is important to understand whether these assays provide a suitable alternative for in vivo testing. Thus, the primary objective of this work was to validate the use of ToxCast data to predict vitellogenin induction in male fish. A comprehensive review was performed for the FSTRA, where LOEC values for male vitellogenin induction were extracted from 50 publications for 10 known estrogen receptor agonists. These data were correlated with results from 16 ToxCast assays and the integrated assay model. In this comparison, the TOX21_ERa_BLA_Agonist_ratio assay showed the strongest correlation ($\rho = 0.857$, $p = 0.014$, $n = 7$), while the ACEA_T47D_80hr_Positive assay had the weakest correlation ($\rho = 0.588$, $p = 0.074$, $n = 10$). When the integrated ER AUC model was evaluated, there was comparable correlation ($\rho = -0.721$, $p = 0.019$, $n = 10$) to most of the assays. These results indicate that in vitro assays can be used to identify estrogenic chemicals and predict thresholds of toxicological concern for vitellogenesis in male fish. Such assays will be important to reduce animal use and move toward quantitative AOPs.

Abstract 24

Adaptive Mechanisms Governing Tracheobronchial Cough: In Vivo Experiments and Model Simulations

M Nicholas Musselwhite¹, Teresa Pitts², Kendall F Morris³, Tabitha Y Shen¹, Paul W Davenport¹ and Donald C Bolser¹

¹Dept. of Physiological Sciences, Univ. of Florida, Gainesville, FL

²Kentucky Spinal Cord Injury Research Center, University of Louisville

³Dept. of Molecular Pharmacology & Physiology, Univ. of South Florida, Tampa, FL

Repetitive coughing can be suppressed in a dose-dependent manner in response to administration of cough suppressant drugs in anesthetized cats. Cough suppression by these drugs manifests as a reduction in cough number and expiratory motor drive. Cough phase durations and inspiratory motor drive are unaffected by these drugs in the cat. The mechanism by which antitussive drugs exert these effects is unknown. We speculated that depression of synaptic excitability at the first central synapse of cough receptor afferents could contribute to this effect. To test this hypothesis, we conducted simulations using a well-established computational model of the peripheral afferents and the central circuit that is proposed to account for cough and breathing. Modulation of the conductance of the first central synapse and then the firing probability of cough receptors predicted inverse relationships between these parameters, and cough number and expiratory motor drive. However, these simulations also predicted that reductions in the magnitudes of these parameters would result in longer cough cycle times, with a greater impact of synaptic conductance on this relationship. These predictions are not consistent with the known action of antitussive drugs on the cough motor pattern. Similarities to the results of these simulations were observed between coordinated in vivo experiments in anesthetized spontaneously breathing cats. A repetitive cough protocol was conducted in which naive animals were challenged with a sequence of trials in which the intrathoracic trachea was mechanically stimulated to elicit coughing. Cough number and expiratory motor drive decreased by approximately 50% over the course of 15-20 consecutive trials. Total cough cycle duration increased by 46% ($p < 0.02$) accounting for the decreased number of coughs during the protocol. Significant prolongations of cough TI, E1, and E2 were also observed. The data support: (i) significant adaptive processes of naive animals to a sequential cough stimulation protocol, (ii) a different phenotype of this adaptive process than that associated with cough suppressants, (iii) potential mechanisms for this adaptive process that could include reduced excitation of both peripheral afferents and their first synapse with a larger contribution of the central component to the adaptation.

Abstract 25

Establishment of a Regulatory Microenvironment by Lactococcus Expressing Colonization Factor Antigen I (CFA/I) Fimbriae Ameliorates Type 1 Diabetes (T1D) in Non-Obese Diabetic (NOD) Mice

Andrew S. Nelson, Massimo Maddaloni, Carol Hoffman, Jeffrey R. Abbott, David W. Pascual
Dept. Infect. Dis. & Path., Univ. of Florida, Gainesville, FL

Oral treatment with the adhesin from enterotoxigenic *E. coli*, CFA/I fimbriae protect in murine models of multiple sclerosis and rheumatoid arthritis. Although CFA/I fimbriae initial mode of action is in a bystander or in an antigen (Ag)-independent fashion, protection was found to be ultimately dependent upon the induction and/or activation of auto-Ag-specific regulatory T cells (Tregs). Our recent findings show that oral dosing with a *L. lactis* vector expressing CFA/I fimbriae (LL-CFA/I) reduces incidence of T1D in NOD mice by 45% with a concomitant 8-fold increase in their splenic Foxp3+CD25+ Tregs. However, little is known about how protection transitions from bystander suppression to Ag-specific Tregs. We hypothesized that LL-CFA/I stimulates dendritic cells (DCs) to establish a regulatory microenvironment since DCs play an integral role in fate decisions for T cells becoming inflammatory or tolerogenic. In this study, 4 wk-old NOD mice were orally dosed with LL-CFA/I and treated every 2 wks; control groups were given *L. lactis* vector or PBS. At 11 wks of age, frequency of insulinitis was reduced by more than half, and insulin-specific T cells were reduced in the pancreatic lymph nodes (PaLNs; $P < 0.05$). To discern how this was mediated, groups of mice were examined at 3, 7, 14, and 21 days post-treatment for changes in DC and T cell phenotypes. As early as 3 days post-treatment, DCs exhibited significantly less expression of costimulatory molecules, CD40 and CD86, and stably maintained this phenotype for at least 7 wks. Splenic DCs showed reduced IL-6 production. These data show that orally dosing with LL-CFA/I ameliorates T1D in NOD mice by establishing a regulatory microenvironment via DCs. Work is supported by AI121745.

Abstract 26

Evaluation of Viral Enrichment Methods for West Nile Virus RNA Extraction from Equine Central Nervous System Tissue

D Prakoso¹, TB Waltzek¹, AF Barbet¹, MJ Dark¹, M Salemi², JJ Liu¹, N Wenzlow¹, GH Delcambre³, MT Long¹

¹Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, US

²Department of Pathology, Immunology, and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, Florida, US

³Department of Biomedical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, Colorado, US

Abstract

Physical virus enrichment is thought to increase sensitivity and enhance detection of viral diversity, but the use of these techniques are limited for many mammalian tissues. We hypothesized that elimination of host brain RNA will enhance sensitivity of viral detection and render undetectable virus available to sequence. Specifically, since West Nile virus (WNV) causes grave clinical signs in the face of low to undetectable virus, the aim of this research was to develop repeatable methods for detecting and generating WNV sequences from horses in which virus was either not detected or detected in limited quantity. Twenty-one normal horse brains were collected and small pieces of brain from each were inoculated with WNV NY99 strain (low passage to maintain diversity). Each sample underwent eight different RNA extraction protocols. The protocols utilized combinations of low-speed centrifugation, syringe filtration, and nuclease treatment. Quantitatively, WNV viral and host RNAs were analyzed using real-time PCR targeting WNV Envelope (e) protein and equine G3PDH, respectively. Then, deep sequencing targeting overlapping fragments of the whole e protein was performed. Real-time PCR results showed that the more enrichment applied to a sample, the less viral and host RNA was obtained. DNA sequencing results showed no significant difference in sequence total variation between each RNA extraction protocol. A method utilizing combination of direct RNA extraction followed by host RNA depletion was most suitable for samples of horse brain infected with WNV. The study highlights the importance of viral enrichment and extraction method selection to the sample type being tested.

Abstract 27

Malaria in Haiti: A Genomic Approach to Its Epidemiology and Biology

Tagliamonte, M.S.1; Yowell, C. A.1; El-Badry, M.A.2; Okech, B.A.2; Salemi, M. 3;
Dame, J.B.1

1 Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida

2 Department of Environmental and Global Health, College of Public Health and Health Professions, University of Florida

3 UF Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida

Malaria in the Caribbean has been eliminated from all islands except Hispaniola. In Haiti, located in the western half of the island, malaria is endemic and becomes epidemic in the rainy seasons. The malaria parasite (*Plasmodium falciparum*) was introduced into Hispaniola through the slave trade, and during this 300 year period ending in early 1800s, ~11 million Africans were brought to the Caribbean. Hispaniola received a large fraction of this total, and a substantial percentage of these individuals carried the parasite, since malaria is holoendemic in West Africa. We hypothesize that in establishing transmission through its new vector (*Anopheles albimanus*), the parasite population underwent bottlenecks and was subjected to powerful selection pressures. Knowing historically when the parasite arrived on the island and the comparative isolation of this parasite population on Hispaniola, represents a unique opportunity to examine the evolution of *P. falciparum*. We have undertaken a detailed study of the genetics of this parasite population utilizing whole genome sequencing. Principal component and phylogenetic analyses based upon single nucleotide polymorphisms (SNPs) indicate that the Haitian parasites have an ancestral relationship with parasites from Africa, and are clearly distinguishable from those found in South America. Loci under selection represent mutations allowing *P. falciparum* to adapt to the novel vector and environmental conditions on Hispaniola. Mutations of biomedical relevance, such those conferring drug resistance or potentially altering future vaccine efficacies, plus those suited to tracking the movement of the Haitian parasite to other countries, provide information useful for tailoring elimination plans.

Abstract 28

INVESTIGATION OF ANTIMICROBIAL RESISTANCE IN UREAPLASMA SPECIES AND MYCOPLASMA HOMINIS ISOLATES FROM URINE CULTURES IN COLLEGE-AGED FEMALES

Marissa A. Valentine-King, Mary B. Brown

Abstract

Urinary tract infections (UTIs) affect nearly 20% of women aged 15-29 and account for an estimated \$3.5 billion in US costs. Antibiotic resistance (ABR) prolongs UTI treatment, and resistance profiles vary regionally and serve as important considerations in treatment guidelines. Regional studies in the US have identified tetracycline resistance in over a third of *Ureaplasma* spp. isolates, but no studies have evaluated ABR levels in college-aged women with first-time UTI. This study determined the minimum inhibitory concentrations (MICs) of 73 urinary *Ureaplasma* spp. isolates and 10 *Mycoplasma hominis* isolates from college-aged women with first time-UTI against a panel of antibiotics, and evaluated the genetic mechanisms of resistance. Among the *Ureaplasma* spp. isolates, two isolates were resistant: one to levofloxacin and one to tetracycline. All *M. hominis* isolates were sensitive. For the *Ureaplasma* spp. isolates, MIC₉₀s were highest against gentamicin (32ug/ml) and lowest against doxycycline (0.25ug/ml). PCR amplification identified tetM present in the tetracycline resistant isolate, and a S83W mutation within the quinolone resistant isolate. A previous study identified a clinical isolate with a S83W mutation, but was unable evaluate its susceptibility to quinolones. In conclusion, low antibiotic resistance was found in this population of women and phenotypic evidence was provided to support that a S83W mutation confers levofloxacin resistance.

Abstract 29

Estrous Characteristics of Heifers with differing Genotype for Daughter Pregnancy Rate and Heifer Conception Rate

Anderson Veronese^{1*}, Odinei Marques¹, Rafael Moreira¹, Francisco Peñagaricano², Ricardo C. Chebel^{1,2}. ¹Department of Large Animal Clinical Sciences & ²Department of Animal Sciences, University of Florida, Gainesville, USA

Genetic selection of dairy animals until the early 2000s focused on productive traits, which may have compromised reproductive performance. Since the mid 2000s, genetic selection of dairy breeds has included reproductive traits. The hypothesis was that genotype for daughter pregnancy rate (GDPR) and heifer conception rate (GHCR) are associated with estrous characteristics. Objectives were to evaluate the association among GDPR and GHCR with estrous characteristics. Holstein heifers (n = 1,019) were enrolled at 11 months of age and were fitted with a collar that determined estrus according activity and rumination changes. Using the DataFlow² software, estrous characteristics (onset, duration, activity peak, rumination nadir, and heat index) of spontaneous (SPE) and PGF₂ α induced (PIE) estruses were recorded. Continuous variables were analyzed by ANOVA. Binary variables were analyzed by logistic regression. Hazard of estrus was analyzed by the Cox proportional hazard ratio. Duration of SPE tended to increase according to GDPR (P=0.08), but it decreased according to GHCR (P<0.01). Rumination nadir at SPE decreased according to GDPR (P=0.03), but it increased according to GHCR (P=0.05). GDPR tended (P=0.09) to be associated with likelihood of activity peak > 80 of SPE, whereas GHCR was not (P=0.22) associated with this outcome. There was a tendency for GDPR (P=0.06) to be positively and GHCR (P=0.08) to be negatively associated with heat index > 80 of SPE. Hazard of PIE increased (P=0.03) according to GDPR. There was no association among GDPR (P=0.19) and GHCR (P=0.15) and duration of PIE. Rumination nadir at PIE decreased according to GDPR (P=0.01), but it was not associated with GHCR (P=0.26). The interaction between GDPR and GHCR was (P=0.05) associated with activity peak > 80 of PIE. There was no association among GDPR (P=0.51) and GHCR (P=0.99) and heat index > 80 of PIE. Genomic breeding value for DPR was positively associated with SPE characteristics. Conversely, the associations of GDPR and GHCR with PIE were not clear. Further experiments that elucidate physiological differences of dairy animals with differing GDPR and GHCR are needed.

Abstract 30

Prenatal Nicotine Exposure Alters Maternal and Fetal Inflammatory Response to Infection

Maria von Chamier, Leticia Reyes, Linda Hayward, Mary B. Brown

We investigated the interaction between prenatal nicotine exposure and intrauterine infection using established rat models. Beginning at gestation day (GD) 6, dams were continuously infused with either saline or 6 mg/kg/day nicotine (Nic). At GD 14, dams received either sterile broth or 10⁵ CFU *Mycoplasma pulmonis* (MP), resulting in 4 treatment groups: Control (4 dams, 33 fetal units); MP only (5 dams, 55 fetal units); Nic only (5 dams, 61 fetal units) and Nic + MP (7 dams, 82 fetal units). At GD 18, nicotine exposure significantly increased ($P \leq 0.02$) the percentage of amniotic fluids and fetuses infected by MP but did not impact colonization rates of maternal sites. Nicotine exposure significantly reduced the numbers of MP in the placenta required for high microbial loads ($\geq 10^4$ CCU) in the amniotic fluid ($P < 0.01$). Fetal inflammatory response lesions were most extensive in the Nic only and Nic + MP groups ($P < 0.0001$). Control and MP only placentas were IL10-dominant, consistent with an M2/Th2 environment. Placentas exposed to nicotine shifted to a neutral environment, with equivalent levels of IFNG and IL10. Both IL6 and TNF levels in amniotic fluid were highly elevated when both nicotine and infection were present. Our study suggests that prenatal exposure to nicotine increases the risk for intrauterine infection, lowers the infectious dose required to breach the placental barrier and infect the amniotic fluid and fetus, and alters the pathology and inflammatory profile associated with maternal and fetal sites.

Abstract 31

COMPARATIVE EVALUATION OF RPOS+ AND RPOS- RASV STRAINS

Whittney N. Burda, Katie Brenneman, Amanda Gonzales, Roy Curtiss III

The vast majority of live attenuated typhoid vaccines are constructed from the *Salmonella enterica* serovar Typhi strain, Ty2, which is devoid of the alternative sigma factor, RpoS. RpoS is a specialized sigma that plays an important role in the general stress response of a number of Gram-negative organisms, including *Salmonella*. Previous studies have demonstrated that this sigma factor is necessary for the survival following exposure to acid, hydrogen peroxide, and nutrient limiting conditions and during starvation. In addition, studies have shown that RpoS is important in colonization and survival within the host. Our recent clinical trial in human volunteers indicated that an RpoS+ derivative of Ty2 was superior in providing protection from disease than its RpoS- counterpart. Therefore, we hypothesize that a functional RpoS allele is necessary for developing an effective live attenuated vaccine. We converted 4 clinically relevant candidate typhoid vaccine strains derived from Ty2 (CVD908-htrA, Ty800, and 9639(pYA4088)) and the licensed live Typhoid vaccine Ty21a (also derived from Ty2) to RpoS+ and compared their ability to withstand certain environmental stresses that may be encountered within the host to those of the RpoS- parent strains. The results of our study indicate that those strains that contain a functional RpoS were better able to survive following stress, and that they would be ideal for further development of effective recombinant attenuated *Salmonella* vaccines.

Abstract 32

Role of epigenetic regulator enhancer of zeste homolog 2 (EZH2) in uterine and mammary gland development and function in mice

Manjunatha K. Nanjappa¹, Ana M. Mesa^{1,2}, Theresa I. Medrano¹, Ellis R. Levin^{3,4}, Paul S. Cooke¹
Departments of ¹Physiological Sciences and ²Animal Sciences, University of Florida, Gainesville, FL. ³Division of Endocrinology, Department of Medicine, University of California, Irvine, CA.
⁴Department of Veterans Affairs Medical Center, Long Beach, CA.

Introduction Polycomb repressive complex 2 (PRC2) is a histone methyltransferase that represses gene transcription by trimethylation of histone 3 at lysine 27. EZH2, a rate-limiting catalytic subunit of PRC2, is critical for embryo development and tissue differentiation, and may mediate epigenetic effects of early estrogen exposure. This study investigated the role of EZH2 in uterine and mammary development and function.

Methods Since global EZH2 knockout is lethal, EZH2 conditional knockout mice (EZH2cKO) were generated using cre-lox technology. Mice expressing one copy of cre recombinase under the transcriptional control of the progesterone receptor (Pgr) promoter, and two copies of floxed EZH2 (PgrCre/+, EZH2floxed/floxed), should have deletion of EZH2 in Pgr-expressing tissues. At postnatal day 60 (PND60), uterus and mammary glands were compared in EZH2cKO and control (i.e., EZH2floxed/floxed) mice. To investigate EZH2's role in uterine estrogen signaling, adult control and EZH2cKO mice were ovariectomized and were given oil or 1 ng/g bw 17 β -estradiol (E2). Uteri were weighed 24h later and immunostained for MKI67, a cell proliferation marker; labeling indices (LI; labeled/total cells) were determined in luminal epithelium (LE). In addition, fertility was evaluated in EZH2cKO females.

Results Uterine immunostaining for EZH2cKO confirmed EZH2 deletion in both luminal and glandular epithelium. However, some stromal cells were EZH2-positive. At PND60, EZH2cKO mice uteri and mammary glands showed increased weights and reduced terminal end bud numbers, respectively, compared to controls. Treatment of control and EZH2cKO mice with E2 increased LE proliferation compared to vehicle-treated control and EZH2cKO mice, respectively. However, E2 produced greater LE proliferation in EZH2cKO mice compared to controls. Interestingly, vehicle-treated EZH2cKO mice showed increased uterine weights, LE and glandular epithelial proliferation and number of glands per uterine cross section. The EZH2cKO female mice (n=10/10) were sub-fertile, with reduced litter size and reproductive problems such as dystocia (n=4/10) and cystic endometrial hyperplasia (n=1/10).

Conclusions Loss of EZH2 results in sub-fertility, reduced mammary growth, and alteration of uterine epithelial proliferation and E2 responsiveness. Thus, EZH2 is required for normal uterine and mammary development and function in the mouse, and may mediate uterine epigenetic effects.

Abstract 33

Frequency Dependent Inhibitory Mechanisms Controlling the Laryngeal Adductor Response (LAR)

Tabitha Y. Shen¹, Christian Gestreau², M. Nicholas Musselwhite¹, Melanie J. Rose¹, Teresa E. Pitts³, Kendall F. Morris⁴, Bruce G. Lindsey⁴, Paul W. Davenport¹, Donald C. Bolser¹

¹University of Florida, Gainesville, FL, ²Physiologie et Neurophysiologie, Aix-Marseille Université, Marseille, France, ³University of Louisville, Louisville, KY, ⁴University of South Florida, Tampa, FL.

Electrical stimulation of the superior laryngeal nerve (SLN) has been used to induce behaviors with airway protective components, such as the LAR. The LAR has both short (R1) and long latency (R2) peaks in response to stimulation, but the neuronal pathways that produce and modulate the LAR are not well understood. It has been reported that there exists a frequency-dependent suppression of R2 for SLN stimulation frequencies above 2.5 Hz. The R1 can follow frequencies of electrical stimulation of the SLN approaching 200 Hz, suggesting a high fidelity of brainstem neuronal pathways, but to our knowledge there is no report on whether R1 exhibits frequency-dependent depression. We hypothesized that the R1 would exhibit frequency-dependent depression consistent with the existence of modulatory short latency inhibitory mechanisms. To test this hypothesis, we monitored EMG activity from the thyroarytenoid (TA) muscle and electrically stimulated the SLN unilaterally with a range of frequencies between 1 and 40 Hz. All frequencies employed induced both repetitive swallowing and LAR. The R1 peak of the LAR was time-locked to each stimulus pulse (peak R1 latencies were 13.7 ± 0.5 ms (5-10 Hz) and 14.2 ± 0.6 ms (35-40 Hz)), and the R2 peak was not observed. Lower frequency stimulations (5-10 Hz) of the SLN elicited significantly higher LAR amplitudes (52.8 ± 2.9 % max) than higher frequency stimulations (35-40 Hz; 36.8 ± 3.2 % max). In other experiments, analysis of neuronal responses recorded in the ventral respiratory column revealed evidence of stimulus-induced synchrony as well as disruption of concurrent functional relationships among respiratory-modulated neurons. These observations suggest that more complex regulatory mechanisms exist in addition to frequency-dependent depression of the LAR. Supported by NIH HL 103415, HL 111215, HL109025.

Abstract 34

An innovative method for rapid detection of spot urinary oxalate in goats with urolithiasis

Arun Wanchoo PhD¹, Demia de Tonnerre BVSc², Elizabeth Nelson, BVSc², Jim Burrow², Samuel Tao¹, Robert J. MacKay, BVSc, PhD, DACVIM², PhD Sarah M. Reuss VMD², Cuong Q. Nguyen PhD^{1,3,4}

¹Department of Infectious Diseases and Pathology, ²Department of Large Animal Clinical Sciences, College of Veterinary Medicine, ³Department of Oral Biology, ⁴Center for Orphaned Autoimmune Diseases, College of Dentistry, University of Florida, Gainesville FL

Oxalic acid is a natural and abundant by-product of metabolism. It is also a highly oxidized organic compound with powerful chelating activity that, in high concentrations, can cause death in both animals and humans due to its corrosive effects. Since mammalian species cannot break down oxalic acid, it must be excreted via urination. High levels of oxalic acid in urine, referred to as hyperoxaluria, have been found to correlate with a number of human diseases, especially urolithiasis or kidney stone disease (KSD), a disease also prevalent in goats. Current methods of measuring oxalate are highly technical, cumbersome, and time-consuming process. Often clinics are forced to utilize expensive diagnostic laboratories to measure urine oxalate. Therefore, in this study we designed an innovative technique called Kidney Stone ALERT to measure urinary oxalate. Our results indicate that oxalate can be determined within one minute using our patented device. More importantly, we determined that goats with urolithiasis exhibited higher levels of oxalate than healthy goats. This proof-of-concept study supports the future application of the device in determining on-the-spot oxalate levels and bring kidney stone prevention to point-of-care practice. Further work will be needed to optimize the Kidney Stone ALERT device to increase resolution and accuracy so that the device can be read visually.

Abstract 35

TITLE: Perioperative and Long-term Outcomes Following Plate Stabilization to Address Spontaneous Luxation of the Long Digital Extensor Tendon of Origin in Two Dogs

Michelle MM Hasiuk, DVM, Kevin A Drygas, DVM, DACVS, Daniel D Lewis, DVM, DACVS

INTRODUCTION: The long digital extensor (LDE) muscle originates in the extensor fossa on the lateral femoral condyle and the tendon is maintained in the sulcus extensorius on the proximal tibia by a restraining band of fibrous tissue. Spontaneous luxation of the LDE tendon of origin is an uncommon cause of pelvic limb lameness in dogs. This report describes the clinical abnormalities, surgical management utilizing plate stabilization and functional outcomes of two dogs ascribed to spontaneous luxation of the LDE tendon of origin.

MATERIALS & METHODS: Reduction of the luxation LDE tendon of origin was maintained using plate stabilization. Perioperative (surgery-12 weeks) follow-up was conducted with examination and radiographs. Long-term follow-up (>12 months) consisted of examination, force plate analysis, radiographs, goniometry and limb circumference measurements.

RESULTS: Luxation of the LDE tendon of origin was palpable in both dogs and confirmed intraoperatively; the retinacular fibrous band could not be identified. Groove sulcoplasty was performed. A plate was contoured to bridge the sulcus. Both dogs had mild lameness 2 weeks post-operatively, but the luxation resolved. Lameness and recurrence of luxation was not present 4, 8 and 12 weeks post-operatively or on long-term follow-up for dog #1.

CONCLUSIONS: Reduction of the LDE tendon of origin using plate stabilization is an effective technique. No recurrence of luxation or lameness occurred. Clinical outcome was considered excellent in both dogs with direct long-term documentation of acceptable limb function in dog #1.

ACKNOWLEDGMENTS: Supported by discretionary funds from the University of Florida Collaborative Orthopaedics and Biomechanics Laboratory.

Abstract 36

Does Do Not Resuscitate (DNR) always mean DNR? Exploring DNR orders in veterinary medicine
1Keriann Cabral DVM, 1Gareth J Buckley MA VetMB, 2Elizabeth A Rozanski DVM, 3Howard J Cabral PhD

1 - University of Florida College of Veterinary Medicine, Department of Small Animal Clinical Sciences

2 - Tufts University Cummings School of Veterinary Medicine, Department of Small Animal Clinical Sciences

3 - Boston University School of Public Health, Department of Biostatistics

At the time of submission, Dr Cabral's affiliation is University of Florida College of Veterinary Medicine

Objective:

To investigate the interpretation and implementation of DNR orders in veterinary medicine.

Design:

A survey consisting of respondent characteristics, followed by case examples where interpretation of the DNR order was questionable were distributed. These included examples where a patient with pre-existing DNR suffered an unexpected cardiopulmonary arrest and one case where a terminally ill dog's owner requested CPR.

Sample:

733 individuals, including 533 veterinarians, 66 veterinary technicians, 134 veterinary students responded.

Procedures:

A link to an online survey was distributed via email to variety of veterinary listserves, Data were evaluated using the chi-square or Fisher's exact test.

Results:

Significant differences were found based on gender, occupation, years of experience, and location. Men, veterinarians that have been in practice longer, and respondents who participate in multiple CPR events per year were more likely to perform CPR in the face of a pre-existing DNR order. Respondents within North America were more likely to provide CPR, compared to those outside. The majority of respondents would override an existing DNR order in the case of an in-hospital iatrogenic cardiac arrest and an unexpected arrest during sedation but would provide CPR when requested by the owner, even if judged futile. Codes are not routinely suspended when undergoing anesthesia or regularly re-evaluated during hospitalization.

Conclusions and Clinical Relevance:

This study supports that there is potential for confusion surrounding DNR in veterinary patients. The definition of "DNR" is not universal, and may result in different interpretations. The profession would benefit from more consistency regarding this topic.

Abstract 37

ACUTE KIDNEY INJURY IN DOGS WITH PIT VIPER ENVENOMATION: 50 CASES (2008-2017)

Martinez J, Schaer M, Londoño L.

Objective To investigate the incidence of acute kidney injury (AKI) in dogs diagnosed with pit-viper envenomation and the association of AKI with length of hospitalization, antivenom dose received and survival.

Design: Retrospective study.

Animals: 50 dogs with pit-viper envenomation and diagnosis of AKI developed during their time of hospitalization.

Procedures: The medical records of dogs receiving crotalid antivenom at the University of Florida from January 2008 to January 2017 and at UF Pet Emergency Treatment Services from July 2012 to January 2017 were reviewed. Dogs were included for analysis if they received at least 1 vial of crotalid antivenom, had at least 2 creatinine measurements performed within 48 hours over the course of hospitalization and if they were hospitalized for more than 24 hours. Dogs were excluded if they had a history of CKD, or received NSAIDs or other nephrotoxic drugs prior to presentation. The diagnosis of AKI was based on an absolute creatinine increase of > 0.3 mg/dL from baseline within 48 hours, or based on creatinine measured at presentation according with the International Renal Interest Society (IRIS) AKI grading criteria.

Results: A total of 57 dogs met the inclusion criteria, 1 patient was excluded due to a suspected history of CKD and 6 were excluded due to NSAID administration prior to presentation. Sixteen dogs developed AKI (32%). Patients with AKI received a significantly higher dose of antivenom, 9.1 vials versus 4.2 in the non-AKI group ($P = 0.017$). A moderate correlation was identified between the number of vials of antivenom received and initial creatinine measured ($r = 0.392$, $P = 0.015$), IRIS AKI staging ($r = 0.588$, $P = < 0.001$), and the length of hospitalization ($r = 0.537$, $P = < 0.001$). No significant association was identified between the development of AKI and the length of hospitalization, dogs with AKI had a mean hospital stay of 2.8 days versus 2.3 in the non-AKI group ($P = 0.605$). Twelve dogs died or were euthanized (24 %) and 11/12 (92%) were in the AKI group. Based on calculation of odds ratio, dogs that developed AKI were 182 times more likely to be euthanized ($P < 0.001$); 30 times more likely to die due to progression of the envenomation ($P = 0.003$) and 93 times less likely to be discharged from hospital ($P = < 0.001$).

Conclusions: AKI has not been previously recognized following pit-viper envenomations in dogs. This study suggests that development of AKI following pit-viper envenomation carries increased risk of mortality and is significantly associated with increased dose of antivenom administration.

Abstract 38

ANTI-CONVULSANT USE PATTERNS FOR SUSPECT IDIOPATHIC EPILEPTIC DOGS AMONG BOARD-CERTIFIED EMERGENCY AND NEUROLOGY SPECIALISTS

Meland T, Carrera-Justiz S, Buckley GJ

Abstract.

Epileptic seizures in the dog are one of the most common neurological disorders presented to veterinary neurologists and criticalists. If idiopathic epilepsy is suspected, antiepileptic drug (AED) therapy is often initiated. The goal of AED therapy is to provide an appropriate balance between seizure control and side effects of the drug for the individual patient. There are multiple AED options available and, ultimately, the choice of treatment depends on clinician, efficacy, safety, and price. The goal of this study was to document AED use patterns for suspect idiopathic epileptic dogs among board-certified emergency and neurology specialists. A survey inquiring about AED use for dogs newly diagnosed with idiopathic epilepsy was distributed to diplomates on the databases of the American College of Veterinary Emergency and Critical Care and the American College of Veterinary Internal Medicine (Neurology). Results were obtained from 300 completed responses by 128 board-certified neurologists and 172 board-certified criticalists. Case volume was slightly different between groups, with 36% of neurologists and 18% of criticalists managing >50 cases of first time canine seizure patients per year. The initial AED of choice was similar between groups with 66% of neurologists and 64% of criticalists using phenobarbital. Whereas 16% neurologists and 26% criticalists used levetiracetam, only 15% neurologists and 9% of criticalists used zonisamide as their initial AED. Bromide was used by 1% or less by both groups. Academic vs. Private Practice residency training did not appear to affect the clinician's initial choice of AED. While most responders gave a loading dose of phenobarbital, roughly one fourth of neurologists did not load the drug versus only 6% of criticalists. Total loading dose was similar between responders (16 mg/kg) but varied in administration protocol. Approximately half of neurologists gave a higher initial dose of levetiracetam, where nearly one third of criticalists did not give a higher initial dose. Almost all the responders who gave a higher initial dose of levetiracetam used 60 mg/kg IV once. Understanding the common practices in the use of anti-epileptic drugs may promote future discussions regarding the best practices in the management of canine idiopathic epilepsy.

Abstract 39

THE EFFECT OF CATHETER LOCATION AND FLUSH VOLUME ON THE TIME FOR CONTRAST TO REACH THE HEART IN A PORCINE MODEL OF CARDIAC ARREST

Davy R, Buckley GJ, Shih A

Introduction: Drug delivery during CPR may be impaired by slow delivery of medications from the periphery. Central venous catheters result in rapid delivery of drugs to their site of action but are challenging to place quickly. Large flush volumes may enhance delivery from a peripheral catheter to the central circulation.

Objective: To compare four different catheter locations, jugular, cephalic, saphenous, and intraosseous, and high versus low flush volumes on time for contrast to reach the heart using a porcine model of cardiac arrest.

Methods: The study was approved by the University of Florida Institutional Animal Care and Use Committee. Four pigs were heparinised then euthanised with pentobarbital. The cephalic, saphenous, and jugular vein were catheterised and an IO catheter placed in the humerus. CPR was initiated in dorsal recumbency with a mechanical compression device at 120 compressions/minute. 4ml iohexol contrast was injected into the jugular catheter followed by 4ml of saline (low flush). Fluoroscopy was used to visualize the contrast entering the central circulation and the time recorded from injection to the time the contrast first appeared in the central circulation. This was repeated in each of the remaining catheters. The process was then repeated for all catheters, this time with 20mls of saline (high flush). Each measurement was taken in duplicate.

Results: There was no significant difference between central venous and intraosseous injections on time to reach the heart using either a low flush volume (7.49 seconds v 9 seconds), or high flush volume (6.4 seconds versus 6.6 seconds), ($p=0.6$). There was no significant difference between the central venous injections and cephalic injections with a low flush volume (7.49 versus 17.7 seconds, $p=0.053$) or high flush volume (6.49 seconds versus 11.6 seconds, $p=0.07$). Saphenous injection resulted in very slow and inconsistent delivery of contrast to the heart, irrespective of flush volume ($>60s$, $p=0.001$).

Conclusions: Catheters in the cranial body, whether jugular, cephalic, or humeral IO result in efficient delivery of contrast to the heart. The volume of flush delivered after contrast is not statistically significant for these sites. Use of the saphenous site during CPR is not recommended.

Abstract 40

DESCRIPTION OF A NOVEL TECHNIQUE FOR GASTRIC DECOMPRESSION IN DOGS USING AN IATROGENIC GASTRIC DILATATION MODEL: TEMPORARY PERCUTANEOUS GASTROPEXY AND CONTINUOUS DECOMPRESSIVE GASTROSTOMY, PILOT STUDY AND EARLY CLINICAL USE

W. Alexander Fox-Alvarez, DVM1; J. Brad Case, MS, DVM, DACVS1; Kirsten L. Cooke, DVM, DACVIM1; Fernando L. Garcia-Pereira, DVM, MS, DACVAA1; Gareth J. Buckley, MA, VetMB, MRCVS, DACVECC1, Eric Monnet DVM, PhD, DACVS, DECVS2, Beau Toskich, MD, VIR3

1Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL

2Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, CO

3Department of Radiology- Vascular Interventional Radiology, College of Medicine, University of Florida, Gainesville, FL

Abstract:

Introduction: Gastric dilatation and volvulus (GDV) is a life-threatening medical and surgical emergency brought on by massive gastric distention which obstructs critical abdominal vasculature, leading to hypovolemic shock, myocardial ischemia, cardiac arrhythmias, electrolyte imbalance, and gastric necrosis. Rapid, effective gastric decompression is critical in the treatment of dogs with GDV. Currently used methods for gastric decompression have a temporary effect and do not provide sustained decompression.

The objective of this study was to describe and evaluate a novel, percutaneous, continuous gastric decompression technique using a temporary T-fastener gastropexy and self-retaining, decompression catheter. Six male dogs were anesthetized and their gastric lumen insufflated endoscopically using room air until tympany was present. Three T-fasteners were placed percutaneously into the gastric lumen via the right-lateral abdomen, caudal to the 13th rib, lateral to the rectus abdominis. In the center of the T-fasteners, a 5fr locking pigtail catheter was placed into the gastric lumen, then attached to a device to measure outflow and intra-gastric pressure. The stomach was insufflated to 23mmHg and allowed to passively drain from the catheter until intraluminal pressure reached 5mmHg for a total of three cycles. After removal, the gastrostomy site was evaluated endoscopically and laparoscopically. Patients were hospitalized for 72 hours.

Results: Mean catheter placement time was 3.3 ± 0.47 minutes. The mean time to reach $\geq 50\%$ reduction in intra-gastric pressure and ≤ 6 mmHg was 2.1 ± 1.3 and 8.4 ± 5.1 minutes, respectively. After catheter removal, no evidence of gas or fluid leakage at the catheter site was visible laparoscopically or endoscopically. All dogs were clinically normal at 72 hour post-operative.

Conclusion: The described technique can be performed rapidly, and provides continuous gastric decompression with no evidence of post-operative leakage in normal dogs. This technique may be an effective method for sustained gastric decompression in clinical GDV patients but clinical investigation is required.

Abstract 41

EXPRESSION OF MULTIPLE RECEPTOR TYROSINE KINASES IN SPONTANEOUSLY OCCURRING CANINE ADRENAL TUMORS AND CORRELATION WITH CLINICAL OUTCOME
Expression of Multiple Receptor Tyrosine Kinases in Spontaneously Occurring Canine Adrenal Tumors

Authors:

Kayla Harding, DVM; Carlos Henrique de Mello Souza, DVM, MS; Sarah Boston, DVM, DVSc; Amandine Lejeune, DVM; Elizabeth Whitley, DVM, MS, PhD; Anna Szivek, DVM; Jeffrey Abbott, DVM, PhD; Rowan Milner, DVM, BVSc Hons, MMedVet, PhD.

Affiliation:

University of Florida. Gainesville, Florida.

Abstract:

The most common canine primary adrenal gland tumors are adrenal-cortical adenomas, adrenal-cortical carcinomas, and pheochromocytomas. Receptor Tyrosine Kinase Inhibitors (RTKIs) have anecdotally been reported to be efficacious for the adjuvant treatment of adrenal tumors in dogs. Toseranib phosphate is an RTKI that works in part via inhibition of multiple receptor tyrosine kinases (RTKs) including C-KIT, VEGFR, PDGFR- β and Flt-3. The objective of this study was to analyze canine adrenal tumors for the presence of these receptors and explore a theoretical basis for the adjuvant use of toceranib. Secondly, we attempted to correlate RTK expression with clinical tumor characteristics and outcome. Forty-three spontaneously occurring canine adrenal tumors were removed and analyzed for intensity, distribution and localization of C-KIT, VEGFR, PDGFR- β and Flt-3 using immunohistochemistry. Tumor RTK expression was compared to sections of non-neoplastic and normal adrenal tissue. Medical records were retrospectively reviewed to identify prognostic factors and to explore correlations between RTK expression and tumor aggressiveness and patient survival. Overall RTK expression was moderate to high in cortical tumors and was generally low in pheochromocytomas. C-KIT expression was completely absent in all pheochromocytomas. RTK staining characteristics did not correlate with clinical tumor characteristics such as size or invasiveness or survival for any tumor type. Identified clinical prognostic factors included: maximum tumor diameter, age, and performing a concurrent invasive surgical procedure at the time of adrenalectomy. The median survival time for all dogs undergoing adrenalectomy and surviving at least 14 days post-operatively was 559 days. The examined receptor staining characteristics revealed moderate to high cellular staining for various RTKs targeted by toceranib in adrenal cortical tumors. The findings suggest that toceranib may be efficacious for adrenal-cortical adenomas and adenocarcinomas. Though RTK expression was not prognostic or predictive of tumor behavior, several clinical prognostic factors were identified which may help predict outcome for dogs undergoing adrenalectomy in the future.

Purpose:

The most common canine primary adrenal gland tumors are adrenal-cortical carcinomas, adrenal-cortical adenomas, and pheochromocytomas. Receptor Tyrosine Kinase Inhibitors (RTKIs) have anecdotally been reported to be efficacious for the adjuvant treatment of adrenal tumors in dogs. Toseranib phosphate is an RTKI that has been licensed for use in dogs and works via inhibition of multiple receptor tyrosine kinases (RTKs). The objective of this study was to analyze canine adrenal tumors for the presence of various RTKs including C-KIT, VEGFR, PDGFR- and Flt-3 to explore a theoretical basis for the adjuvant use of RTKIs. Secondly, we attempted to correlate RTK expression with clinical tumor characteristics and outcome.

Methods:

Forty three spontaneously occurring canine adrenal tumors were removed and analyzed for intensity, distribution and localization of C-KIT, VEGFR, PDGFR- and Flt-3 using immunohistochemistry. Medical records were retrospectively reviewed to identify prognostic factors and to explore correlations between RTK expression and tumor aggressiveness and patient survival.

Results:

RTK expression was not significantly different between adrenal tumors and non-neoplastic adrenal tissue. C-KIT expression was completely absent in all pheochromocytomas. RTK staining characteristics did not correlate with clinical tumor characteristics or survival except a significant positive correlation that was found between increased Flt-3 staining intensity and adrenal-cortical tumor size. Identified prognostic factors included: maximum tumor diameter, age at time of surgery, and concurrent invasive surgical procedure at the time of adrenalectomy.

Conclusions:

Receptor staining characteristics examined in this study do not provide support for the use of RTKIs in the adjuvant treatment of adrenal tumors in dogs, however, further prospective studies are required to confirm these findings as all mechanisms of RTKI action were not explored. Though RTK expression was not prognostic or predictive of tumor behavior, several prognostic factors were identified indicating that increasing tumor size, age, and concurrent invasive surgical procedures at the time of adrenalectomy are predictive of decreased survival.

Abstract 42

SURGICAL MANAGEMENT OF APPENDICULAR LONG-BONE FRACTURES IN FREE-RANGING FLORIDA PANTHERS (*PUMA CONCOLOR CORYI*): SIX CASES (2000 – 2014)

Jo Anne Au Yong, B.V.Sc., Daniel D. Lewis, D.V.M., Dipl. A.C.V.S., Scott B. Citino, D.V.M., Dipl. A.C.Z.M., Mark W. Cunningham, D.V.M., M.S., Dipl. A.C.V.P.M., Alan R. Cross, D.V.M., Dipl. A.C.V.S., James P. Farese, D.V.M., Dipl. A.C.V.S., and Luisito S. Pablo, D.V.M., Dipl. A.C.V.A.

From the University of Florida, College of Veterinary Medicine, 2015 SW 16th Ave., Gainesville, Florida 32608, USA (Au Yong, Lewis, Cross, Farese, Pablo); White Oak Conservation Center, 581705 White Oak Rd, Yulee, Florida 32089, USA (Citino); Florida Fish and Wildlife Conservation Commission, 1105 SW Williston Rd, Gainesville, Florida 32601, USA (Cunningham). Present address (Cross): BluePearl Veterinary Partners, 1071 Howell Mill Rd NW, Atlanta, Georgia 30318, USA; (Farese): North Bay Veterinary Surgical Services, 121 Laurel Grove Ave, Kentfield, California 94904, USA. Correspondence should be directed to Dr. Lewis (LewisDa@ufl.edu)

The clinical outcomes of six free-ranging Florida panthers (*Puma concolor coryi*) which underwent surgical stabilization of appendicular long-bone fractures were evaluated. These Florida panthers presented to the University of Florida between 2000-2014 for diaphyseal appendicular long-bone fracture (three femoral fractures, one tibial and fibular fractures, and two radial and ulnar fractures) stabilization. Estimated age of the panthers ranged from 0.5 - 4.5 years and weights ranged from 22 - 65 kg. Causes of injuries were vehicular collision (n = 4) and capture-related (n = 2). All panthers underwent open reduction and fracture stabilization. Fixation failure necessitated three subsequent surgeries in one panther. Five panthers survived the immediate post-operative period and all of these panthers' fractures obtained radiographic union (range: 8 - 36 [mean: 22] weeks). The five surviving panthers underwent convalescence for 7 - 14 months' duration at White Oak Conservation Center before being released back into the wild; however, one panther was killed when hit by a car three days after release. The remaining four panthers were tracked for up to 106 months in the wild and successfully integrated back into the native population. Surgical stabilization of appendicular long bone fractures in free-ranging Florida panthers can be successful, but must take into account the stress that a large undomesticated felid will place on the stabilized limb during convalescence as well as the difficulties involved in rehabilitating a wild panther in captivity.

Abstract 43

CLINICAL AND RADIOGRAPHIC OUTCOMES OF CORTICAL ALLOGRAFT DEMINERALIZED BONE FIBERS FOR FRACTURE MANAGEMENT IN DOGS

Jo Anne Au Yong BVSc; Stanley E. Kim BVSc MS Dipl. ACVS, Sarah Townsend BVSc, Daniel D. Lewis DVM Dipl. ACVS

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL

INTRODUCTION:

This prospective clinical case series evaluated the efficacy of Demineralized Bone Fibers (DBF™) (Vet Fiber Matrix™ K-9, TheraCell-Vet, Inc., Littleton, MA), a novel form of allogenic demineralized bone matrix, in promoting osseous union in dogs.

MATERIALS & METHODS:

Dogs with high potential for delayed- or non-union received DBF™ at the time of fracture repair or corrective osteotomy. Dogs were required to return for monthly rechecks including repeat radiographs until radiographic union was documented.

RESULTS:

DBF™ was used in 20 dogs but only 12 met our inclusion criteria: three dogs were euthanized for reasons unrelated to the study and five dogs did not return for follow-up evaluations. Two dogs had bilateral radial and ulnar fractures. DBF™ was used in 14 sites involving the radius/ulna (n = 8), tibia/fibula (n = 3), femur (n = 2), mandible (n = 1). There were seven acute fractures, three fracture revisions, and four angular limb deformities.

Twelve cases achieved radiographic union by 1 month (n = 3), 2 months (n = 7), and 4 months (n = 2) post-operatively. One site had delayed union, healing at 6 months, while the other case experienced a non-union. Mean and median time to radiographic union was 2.4 months and 2 months, respectively.

DISCUSSION/CONCLUSION:

Results are encouraging, although limited by the small sample size. The graft was convenient to use and had excellent handling characteristics. No adverse events were attributable to the use of DBF™.

ACKNOWLEDGEMENT:

Drs. Kim and Lewis are members of the scientific review board for TheraCell-VET, Inc., which manufactures and distributes DBF™

Abstract 44

Comparative Evaluation of Protective Immunity Induced by Salmonella

Vaccines Derived from UK-1 and 14028s Strains

Shilpa Sanapala, Leandra Mosca and Roy Curtiss III.

The initial virulence and invasiveness of a bacterial strain seem to be important in leading to a maximally efficacious attenuated live vaccine to prevent infection. Here we show that χ 9909, derived from UK-1 χ 3761, the most virulent Salmonella Typhimurium strain known, is very effective in protecting mice against lethal UK-1 and 14028 challenge. As opposed to this, 14028-derived vaccine χ 12359 offered suboptimal levels of protection, with survival percentages that were significantly low against higher U-1 challenge doses.

Abstract 45

Title: Immune platforms to monitor GD3 based osteosarcoma vaccine given concurrently with a carboplatin chemotherapy protocol and surgery.

Authors: Milner RJ a, Sahay B a, Hutchison S a, Pei S a, Cascio M b, Bowles K, Sayour E b, Whitley E a, Lejeune A a, Boston S a, Souza CH a, Salute M a.

Abstract

Introduction. The disialyl gangliosides GD2/GD3 have been implicated in the enhancement of malignancy in a number of human and animal cancers and as a tumor antigen target for immunotherapy. We reported on the coexpression of GD2/GD3 in four canine OSA cell which confirmed the expression of GD2/GD3. In a prospective trial we propose to vaccinate dogs with naturally occurring OSA receiving standard of care to improve survival and to investigate the expression profiles of GD2/GD3 and immune response from tumor aspirates and blood samples overtime. Data from the research will be used to determine the appropriate timing for chemo-immunotherapy.

Methods. Dogs will be entered into the study only if they meet the following inclusion criteria; have a diagnosis of osteosarcoma, confirmed by histopathology or cytology and have no other life threatening diseases. The study will accrue 40 cases, 20 will receive the vaccine plus standard of care and 20 dogs will receive only the standard of care (amputation and intent to treat with 6 doses of carboplatin. The dogs will then be vaccinated according to a predetermined protocol during chemotherapy and staged. The median survival, immune response and GD2/GD3 expression profile of the vaccine group will be compared to dogs receiving standard of care alone using flow cytometric platforms to monitor changes in immune cells (CD5, CD21, CD4, CD8, CD14, CD11b, MHCII, Foxp3, and Tetramer). Flow cytometry platforms and RNAscope arrays have also been developed for check points of immunity PD1, PDL-1 etc.

Results. Currently fifteen dogs have enrolled into the study and have received standard of care and vaccination. Preliminary expression profiles of immune cells compared to normal dogs show changes consistent with carboplatin myelosuppression and changes in immune response. High expression of Foxp3+/Cd4+ cells were found in dogs on presentation to the trial and on receiving chemotherapy, a significant decrease in Foxp3+/Cd4+ cells occurred.

Conclusions Anticipated results from the study will be used to adjust the vaccine according to the immune monitoring and expression of GD2/GD3 profiles and immune profiles.

Acknowledgement: The study is funded by a grant from the American Kennel club and The UF CVM.

Abstract 46

TWO-STAGE MODEL TO STUDY IDIOPATHIC CALCIUM OXALATE STONE FORMATION

Allison O'Kell, Archana Lovett, Benjamin Canales, Laurie Gower, Saeed Khan

Idiopathic calcium oxalate (CaOx) kidney stones grow attached to Randall's plaques (RPs), which are calcium phosphate (CaP) deposits on renal papillary surfaces. Our goal is to develop a model system consisting of 1) CaP mineralized biomimetic RPs (BRPs) formed in-vitro using classical mineralization or the polymer-induced liquid precursor (PILP) process, followed by 2) CaOx overgrowth into a stone in-vivo on BRPs implanted as foreign bodies into the urinary bladders of hyperoxaluric male rats. BRPs were developed by mineralizing decellularized porcine kidney tissue (DPK) with CaP in the presence or absence of 50 µg/ml of polyaspartic acid (PA) or osteopontin (OPN). Foreign bodies were surgically implanted into the bladders of adult male rats in the following groups: non-mineralized DPK (n=8), classical mineralization (without PA or OPN (n=8)), PILP mineralization with PA (n=8), or with OPN (n=8). Half of the rats in each group were given regular water and half were given water with 0.75% ethylene glycol (EG rats) to induce hyperoxaluria. Urine was collected at days 7 and 21 for determination of pH, microscopy, and oxalate excretion. Rats were sacrificed after 4 weeks and the foreign bodies analyzed via scanning electron microscopy and x-ray diffraction. Results showed that DPK mineralized via the PILP process in vitro showed features resembling native plaques, such as concentric spherules and collagen fibrils with intrafibrillar mineral. EG rats had higher urinary oxalate excretion and lower urine pH than rats given regular water, and formed CaOx crystals. Bladder foreign bodies from rats given regular water were mineralized with magnesium phosphate or CaP, and those given EG water were mineralized with CaOx. Both CaOx monohydrate and dihydrate crystals were detected on foreign bodies mineralized with PA in EG rats, while mostly CaOx monohydrate was detected in the other EG rat groups. In conclusion, mineralization through PILP process led to the production of BRPs. When exposed to hyperoxaluria, BRPs became covered with CaOx crystals, morphologically similar to human CaOx kidney stones. Further studies to determine the repeatability of these findings and investigate the utility of BRPs for use in therapeutic models of CaOx stone treatment and prevention are indicated.

Abstract 47

Triple-sugar regulated Salmonella Vaccines protect against Clostridium perfringens-induced necrotic enteritis in broiler chickens.

Shifeng Wang,¹ Thomas J. Bigger,² Bob Nordgren,² Charles L. Hofacre,³ Roy Curtiss III^{1,2}

¹ College of Veterinary Medicine, University of Florida, Gainesville, Florida, 32611, USA

² Curtiss Healthcare Inc., Alachua, Florida, 32615, USA

³ College of Veterinary Medicine, University of Georgia, Athens, Georgia, 30602, USA

Gram-positive Clostridium perfringens type A, the causative agent of necrotic enteritis (NE), gained more attention in the broiler industry due to governmental restrictions on the use of growth-promoting antibiotics in poultry feed. To date, there is only one commercial NE vaccine available, based on the C. perfringens alpha toxin. Our previous work has proved that regulated delayed lysis Salmonella vaccines delivering a plasmid pYA5112 encoding an operon fusion encoding the synthesis of the C-terminal adhesive part of alpha toxin and a GST-NetB toxin fusion were able to elicit significant protective immunity in broilers against both mild and severe C. perfringens challenges. We recently improved our S. Typhimurium antigen delivery vaccine strain by integrating a rhamnose regulated O-antigen synthesis gene enabling a three-sugar regulation system for virulence and antigen-synthesis traits. The new system confers on the vaccine strain a super safe profile and much improved induction of immune responses. The strain with triple-sugar regulation systems delivering pYA5112-encoded antigens protected chickens in a field setting at levels observed for antibiotic-treated chickens. Feed conversion and growth performance were also superior. These studies made use of a severe C. perfringens challenge with lesion formation and mortality enhanced by pre-exposure to Eimeria maxima oocysts. The vaccine achieved similar effectiveness through three different immunization routes, oral, spray and in the drinking water. The vaccine has the great potential for application in commercial hatcher and broiler-rearing conditions. Further improvement of this vaccine is ongoing.

Abstract 48

In-Vivo Three-Dimensional Stifle Kinematics In Goats With Anterior Cruciate Ligament Deficiency

Ana Bascuñán, DVM

Stanley E Kim, BVSc, MS, Diplomate ACVS

Daniel D Lewis, DVM, Diplomate ACVS

Adam Biedrzycki, BSc, BVSc, PhD, Diplomate ACVS

Scott A Banks, PhD

College of Veterinary Medicine, University of Florida, Gainesville, FL

Introduction:

Goats are commonly used for translational ACL research. Reported deleterious effects of ACL transection in this species are inconsistent; thus the limitations of this model are poorly defined. Quantification of in vivo, 3D kinematics of ACL-deficient caprine stifles will improve insight regarding the value of caprine ACL research.

Materials and Methods:

Fluoroscopic imaging of six goats' stifles and force plating was performed before and at 2 weeks and 3 months after unilateral, arthroscopic ACL transection. Three-dimensional bone models were superimposed over fluoroscopic images. Relative alignment in 6 degrees of freedom was calculated and kinematics were compared as a percentage of gait cycle. Repeated measures ANOVA was used to compare mean kinematic parameters of the right stifle. $p < 0.05$ was considered significant.

Results:

A significant reduction in peak vertical force occurred in the operated limb at 2 weeks post-ACL transection. Axial rotation and abduction/adduction were not significantly different from baseline. Flexion angle was significantly decreased 3 months post-operatively compared to baseline. Anterior tibial translation was significantly increased during stance phase at 2 weeks and 3 months compared to baseline, with mean increases of 2.73 ± 0.78 mm and 4.52 ± 0.67 mm, respectively.

Discussion/Conclusion:

The magnitude of anterior tibial translation following ACL transection in this caprine model is similar to previous reports in humans. Lameness resolved by 3 months despite significant persistent kinematic abnormalities. The results of this study suggest that the caprine model is suitable for translational research of ACL biomechanics, as the kinematic consequences of ACL transection are representative of what has been observed with ACL deficiency in humans.

Abstract 49

INVESTIGATING ESTROGEN RECEPTORS AS TARGETS OF PRO-FIBROGENIC SIGNALING IN THE LUNG

L. Cody Smith^{1,2}, Santiago Moreno², Dale Porter³, Tara Sabo-Attwood^{2,4}

¹Department of Physiological Sciences, University of Florida, Gainesville, FL, ²Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL, ³National Institute for Occupational Safety and Health, Morgantown, WV, ⁴Department of Environmental and Global Health, University of Florida, Gainesville, FL

Pulmonary fibrosis is an irreversible condition that if progressive can lead to respiratory failure. Pulmonary fibrosis can have unknown etiology or be associated with exposure to airborne particulates like asbestos and potentially emerging particles such as carbon nanotubes (CNTs). The contribution of the pro-fibrogenic cytokine, transforming growth factor beta1 (TGF- β 1), has been well studied but sex-specific trends in the incidence and prevalence of disease suggest a role for sex hormones and their receptors. The goal of this research was to examine a role for estrogen (E2) in CNT-induced fibrogenic signaling in vivo using a whole-body inhalation mouse model and in vitro using human bronchial epithelial cells (BEAS-2Bs). Estrogen receptor alpha (ESR1) mRNA expression was reduced in lung tissue of mice exposed to 10 mg/m³ CNTs for 2, 4 ($p < 0.05$), 8, and 12 ($p < 0.05$) days and in BEAS-2Bs exposed to 0.2 ($p < 0.05$), 2 ($p < 0.05$), and 20 μ g/mL CNTs ($p < 0.05$) for 48-hr. As expected, TGF- β 1 was induced by exposure to CNTs in mouse bronchoalveolar lavage fluid and interestingly, pre-exposure in vitro to a TGF- β 1 receptor inhibitor blocked CNT-induced reduction of ESR1. To identify targets of TGF- β 1-E2 signaling, RNA-Seq was performed in BEAS-2Bs exposed to E2 in the presence and absence of TGF- β 1. Gene set enrichment analysis revealed that both E2 and TGF- β 1 caused statistically significant enrichment of genes involved in extracellular matrix turnover, but many of the targets were down-regulated by E2 and up-regulated by TGF- β 1. These results highlight ESR1 as a target for TGF- β 1-dependent pro-fibrogenic signaling induced by CNTs and identify opposing actions of E2 and TGF- β 1 in processes central to pulmonary fibrosis.

Abstract 50

Effects of lidocaine on inflammation in equine intestine subjected to manipulation and ischemia

Anje G. Bauck DVM, Astrid Grosche DVM, PhD, Alison J. Morton DVM, A. Sarah Graham DVM, Thomas W. Vickroy PhD, David E. Freeman MVB, PhD

Objective - To examine effects of a continuous rate infusion (CRI) of lidocaine on transmural neutrophil infiltration in equine intestine subjected to manipulation and ischemia.

Animals - Fourteen healthy adult horses.

Procedures - In anesthetized horses, mild ischemia was induced in segments of jejunum and large colon and a one-meter segment of jejunum was manipulated by massaging its wall 10 times. Horses randomly received lidocaine (n=7) or saline (n=7) throughout anesthesia. Biopsies were taken to assess tissue injury, neutrophil influx, cyclooxygenase (COX) expression and hypoxia-inducible factor-alpha (HIF-alpha) for up to 4 hours after manipulation and ischemia. Transepithelial resistance (TER) and mannitol fluxes were measured in Ussing chambers in manipulated and control tissues. ANOVA was used to compare data with P<0.05 significant.

Results - Ischemia, reperfusion and manipulation of the small and large intestine caused a measurable global inflammatory response in control, ischemic and manipulated tissues at 4 hours as demonstrated by a significant influx of neutrophils in the seromuscular layer (P<0.05). There was also superficial mucosal injury. Lidocaine did not consistently decrease neutrophil infiltration in ischemic, manipulated, or 4-hour control tissues. Lidocaine also did not affect other measures of inflammation, including histomorphometric measurements, COX and HIF-alpha expression, TER and mannitol fluxes..

Conclusions - Lidocaine did not consistently affect neutrophil infiltration. Findings of this study raise the concern that the widespread use of lidocaine in horses after colic surgery might not be justified.

Abstract 51

PHYLOGENOMIC CHARACTERIZATION OF A NOVEL SEA OTTER POXVIRUS

Jessica M. Jacob,¹ Kuttichantran Subramaniam,¹ James F. X. Wellehan Jr.,¹ Ole Nielsen,² Pamela A. Tuomi,³ Thomas B. Waltzek¹

¹University of Florida, College of Veterinary Medicine, Gainesville, Florida, 32611, USA

²Department of Fisheries and Oceans Canada, Winnipeg, Manitoba, R3T2N6, Canada

³Alaska SeaLife Center, Seward, Alaska, 99664, USA

Abstract

Family Poxviridae are large, double-stranded DNA viruses that replicate in the cytoplasm of the host cells. The subfamily Chordopoxvirinae infects a wide range of vertebrates including marine mammals. A novel poxvirus was discovered in two orphaned sea otter pups that developed small, superficial ulcerated skin lesions during captive care in 2009 and 2011. Histopathologic examination of the skin lesions revealed epithelial hyperplasia with affected cells displaying intracytoplasmic eosinophilic inclusions. Additionally, the epithelial cells in both pups showed different degrees of ballooning degeneration and necrosis. In the northern sea otter, transmission electron microscopy revealed epithelial intracytoplasmic virions. The virions were brick shaped and had dumbbell electron-dense cores. Despite being known for more than a decade, the phylogenetic relationships of marine mammal poxviruses are not well established because of the lack of complete genome sequences. The purpose of the current study was to sequence the entire sea otter poxvirus (SOPV) genome using an Illumina MiSeq Next Generation Sequencer. The approximately 133,000-bp genome is 31.5% G+C, encodes 128 proteins, and has 5,132-bp inverted terminal repeats. Phylogenetic analysis based on 14 core poxvirus genes showed that SOPV is divergent from other known poxviruses. The SOPV genome is the first marine mammal poxvirus to be fully sequenced and is the smallest poxvirus genome known. Sequencing of the SOPV genome is the first step in unraveling the position of a marine mammal poxvirus within the larger Poxviridae tree and provides the necessary sequence to develop future molecular tools for diagnostics and epidemiological studies.

Abstract 52

The effect of tibial plateau leveling osteotomy on patellofemoral kinematics in dogs: An in vivo study

Geoffrey J. Zann, Stanley E. Kim, BVSc, MS, DACVS, Selena Tinga, DVM, Antonio Pozzi, DVM, MS, DACVS, Scott A. Banks, PhD

OBJECTIVE: To quantitatively evaluate the patellofemoral kinematics in clinical subjects with cranial cruciate ligament (CrCL) deficiency treated by tibial plateau leveling osteotomy (TPLO).

SUBJECTS: Ten client-owned dogs with unilateral CrCL insufficiency.

PROCEDURES: Lateral projection fluoroscopy of the stifles during treadmill walking was performed 6 months following TPLO. Computed tomographic scans were also performed to create digital bone models of the patella and femur. These digital models were superimposed over the fluoroscopic images with shape-matching software, and patellofemoral kinematics in the sagittal plane were calculated.

RESULTS: Cranial-caudal translation, proximal-distal translation, and patellar flexion were linearly coupled with femorotibial flexion angle in both normal and TPLO-treated stifles. TPLO treatment was associated with increases in cranial displacement of the patella ($P < 0.05$) when compared to the normal contralateral stifle, at equivalent femorotibial flexion angles between 120 - 142°. Proximal-distal translation and patellar flexion angle were not significantly affected by TPLO surgery at equivalent femorotibial flexion angles.

CONCLUSIONS AND CLINICAL RELEVANCE: In-vivo patellofemoral kinematics in TPLO-treated stifles differ from normal. Our findings indicate small increases in cranial displacement following TPLO. These results may provide further insight into extensor mechanism abnormalities associated with TPLO.

Abstract 53

SURVEY OF ANGIOSTRONGYLUS CANTONENSIS IN NORTHWESTERN FLORIDA

R. Corlett*, J. Slapcinsky†, S.R. Roff, H.S. Walden*

*Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Gainesville, FL 32608. †Florida Museum of Natural History, University of Florida, Gainesville, FL 32611.

ABSTRACT: *Angiostrongylus cantonensis*, the rat lungworm, is a parasitic nematode found in the pulmonary artery of the rat, and the most common cause of eosinophilic meningitis worldwide. It is endemic to Southeast Asia, the Pacific Islands, and the Caribbean. In the United States, this parasite is established in Hawaii and has been reported in Louisiana and Florida. The life cycle requires a gastropod intermediate host and a rat definitive host. Once ingested, infective larvae (L3) migrate to the brain of the rat, then continue migration to the pulmonary artery where they mature. First stage larvae (L1) are then released in feces and ingested by the snail. A range of species serve as incidental hosts, including humans and non-human primates. In the incidental host, the infective L3s migrate to the brain, however maturation to an adult in the pulmonary artery does not occur. Since 2003, *A. cantonensis* has been associated with the death of two non-human primates in South Florida. Preliminary studies have discovered *A. cantonensis* in rats and snails in South and Central Florida. The purpose of this study is to determine if *A. cantonensis* is present in Northwest Florida.

Abstract 54

Evaluation of bone marrow aspiration methods to increase yield and reduce time for therapeutic dose expansion

Rosalind Kopp, Taralyn McCarrel, Kelly Kirkendall-Merritt

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, Florida.

Musculoskeletal injuries in the horse are among the most common applications of adult mesenchymal stem cell (MSC) therapy as well as an active area of research for the benefit of the equine and human patient. One of the major limiting factors for the utility of MSC therapy is the time required for isolation and culture expansion of an adequate dose resulting in delay of MSC injection or cell based surgeries for 3 to 4 weeks. Collection of larger numbers of MSC at the time of aspiration will require fewer cell doublings to achieve therapeutic doses and lessen the current delay in therapy. The objective of this study was to compare three different sternal bone marrow aspiration techniques for the purpose of mesenchymal stem cell isolation and culture expansion to determine which technique results in the greatest MSC yield. Six adult Thoroughbred horses of mixed gender will have bone marrow collected using two different techniques from the fifth sternebra at 1-month intervals. The number of MSC collected at the time of bone marrow aspiration and the time to achieve a therapeutic dose of cells will be compared between groups to determine the optimum technique for bone marrow collection from the sternum. We hypothesize that bone marrow aspiration needle type and technique can be optimized to increase the initial yield of MSC, thereby reducing culture time and passage number to achieve clinically relevant doses of cells. We propose that a new multi-site aspiration technique using a fenestrated bone marrow biopsy needle will be clinically feasible and result in greater stem cell numbers in fewer passages in comparison to single site aspiration with fenestrated bone marrow biopsy needles.

Keywords: horse, regenerative, mesenchymal stem cell, cell passaging, culture expansion.

None

Merial Veterinary Research Scholars Program

Abstract 55

Droplet digital PCR quantification of uterine bacteria associated with metritis in lactating dairy cows.

Federico Cunha, Soo Jin Jeon, Anthony F. Barbet, Kwang C. Jeong, Carlos A. Risco, and Klíbs N. Galvão

Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL

Metritis in lactating dairy cows is widely considered to arise from mixed bacterial infections, primarily by pathogenic gram negative facultative and obligate anaerobes.

The objective of this study was to quantify the number of potentially pathogenic uterine bacteria that have been associated with metritis and fever in lactating dairy cows. Based on relative abundance data of recent metagenomic studies we hypothesized that *F. necrophorum*, *B. heparinolyticus*, *B. pyogenes*, *P. levii*, and *H. ovis* will be present in higher numbers in the uterus of metritic cows than in healthy cows, and that metritic cows will not have a significantly higher quantity of *E. coli*, *P. Melaninogenica*, and *T. pyogenes* compared to healthy cows.

Droplet digital PCR, a system that works by partitioning PCR samples into ~1nL individual reactions and estimating copy number based on the ratio of positive to negative reactions, was used for bacterial quantification. Raw data were log₁₀ transformed before analysis. Cows in the Metritis no fever (MNoFever) and Metritis with fever (MFever) had a mean overall bacterial count one order of magnitude greater than the mean bacterial count of the Healthy group (7.83 ± 0.22 vs. 7.51 ± 0.21 vs. 6.75 ± 0.21 ; $P < 0.05$). This higher bacterial count is in accordance with the fact that metritis is caused by mixed bacterial infection. *F. necrophorum* (6.19 ± 0.37 vs. 6.53 ± 0.36 vs. 3.8 ± 0.35 ; $P < 0.001$), *B. pyogenes* (5.33 ± 0.41 vs. 5.89 ± 0.41 vs. 3.77 ± 0.39 ; $P < 0.02$), and *P. levii* (5.95 ± 0.43 vs. 5.78 ± 0.43 vs. 4.25 ± 0.41 ; $P < 0.04$) were for higher MNoFever and MFever cows than for Healthy cows. *E. coli* and *T. pyogenes* abundance was similar ($P > 0.15$) between groups. *E. coli* and *T. pyogenes*, bacteria generally considered to have substantial impact on uterine health, were present at a relatively low amount in comparison to other species (~4.0). These findings suggest *E. coli* and *T. pyogenes* are not significant components in the mixed infection of the uterus at 6 ± 3 DPP. MFever cows had a significantly higher copy number of *P. melaninogenica* than healthy cows (4.10 ± 0.23 vs. 3.01 ± 0.23 ; $P = 0.006$); however, the biological significance of this finding is unclear due to the low abundance of this bacterium. *F. necrophorum*, *B. pyogenes*, and *P. levii* may be important factors in the etiology of metritis. Fever associated with metritis is not dependent on bacterial load but is more likely dependent on host response.

Chapters

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

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.

