# PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter



November 8, 2017

# Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

# PHI ZETA
## EPSILON CHAPTER
### COLLEGE OF VETERINARY MEDICINE
### AUBURN UNIVERSITY

Welcomes you to our

## PHI ZETA RESEARCH DAY FORUM
### November 8, 2017

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

We also want to thank all sponsors for their generous support without which this event would not be possible:

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# PHI ZETA RESEARCH DAY FORUM

## NOVEMBER 8, 2017 – VETERINARY EDUCATION CENTER

## 8:30:      Opening Statement

**Dr. Frank Bartol, Associate Dean for research and graduate program**

## 8:40-12:00 MORNING Presentations - Overton Auditorium

### Graduate Students and Residents – Moderator: Drs. Jeff Hwang and Ya-Xiong Tao

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| 8:40 | Abdul Mohin Sajib | Evaluation of the Cancer-Specific Functionality of Canine Promoters to Expand Precision Medicine Approaches for Canine Tumors |
| 8:52 | Jack Kottwitz | The Implications of Drugs Utilized for Assisted Breeding in Managed Rhinoceros |
| 9:04 | Randolph Winter | Notched QRS complexes in dogs with and without structural cardiac disease: 85 cases |
| 9:16 | Annie Maguire | Image Analysis of Sub-Gross Stains for a Feline Neurodegenerative Disease |
| 9:28 | Humberto Nobre | Ante-mortem and Post-mortem diagnosis of Ovarian Follicular Dysplasia in Florida Beef Herds through Utilization of Ultrasonography |
| 9:40 | Elise Diffie | Ultrasound Elastography as a Measure of Whole-Body Therapy for GM2 Gangliosidoses |
| 9:52 | Ana Velloso Alvarez | A study evaluating the ability of epinephrine to potentiate and prolong lidocaine's ability to attenuate pain when administered for a palmar digital nerve block |
| 10:04 | Erik Johnson | Pharmacokinetics of Approved and Compounded Extended Release Levetiracetam After Single Oral Dose Administration in Cats |
| 10:16 | Amanda Gross | Intravenous delivery of AAV gene therapy in GM1 gangliosidosis |
| 10:28 | Thibaud Kuca | Experimental Infection of Pregnant Pigs with Bovine Viral Diarrhea Virus (BVDV) 1b |

| 10:40 | Leah McGlinchey | Will anesthesia of the median nerve ameliorate lameness caused by pain in the cubital (elbow) joint? |
| 10:52 | Melissa Singletary | Polyethylene glycol coating changes microbial effects of zinc nanoparticles |
| 11:04 | Jenna Bayne | Use of Accelerometers to Evaluate Behavior Changes in Cattle Administered Continuous, Low-dose Endotoxin |
| 11:16 | Samantha Hagerty | Characterizing the Effects of Heat Stress on Olfactory Response Kinetics |
| 11:28 | Gustavo F. Agne | Pharmacokinetic and biochemical profiles after single administration of oral torsemide in healthy horses |
| 11:40 | Gustavo F. Agne | Pharmacodynamic Properties of Oral Torsemide in Healthy Horses |
| 11:52 | Rebecca Legere | Short-Term Pioglitazone in Equids Increases High Molecular Weight Adiponectin Concentrations and Decreases Insulin Response to Oral Sugar |

## 12:05–1:30   POSTER Presentations -VEC Lobby with Refreshment

## 1:30-5:30      AFTERNOON Presentations - Overton Auditorium

### Veterinary Students -- Moderator: Dr. Bruce F. Smith

| 1:30 | Kaleigh Bush | Stability of Compounded Transdermal Meloxicam Gel |
| 1:42 | Michon Martin | Mapping the Mad Dogs |
| 1:54 | Trey McElroy | A Ketogenic Diet Induces Changes in Gene Expression of Hepatic Drug-Metabolizing Enzymes and Drug-Efflux Pumps in Rats |
| 2:06 | Matthew Miller | Localization and Quantification of Cannabinoid Receptors in Canine Tissue |
| 2:18 | C. Niles Phillips | Therapeutic benefit of a turmeric extract in a nutrionally and oxidatively induced rat model of non-alcoholic steatohepatitis |
| 2:30 | Amanda Burke | Evaluation of Arthritic Lesions in Giant Anteater Feet: Pilot Data |
| 2:42 | Jonathan Tubbs | Utilizing serum antibody levels in post vaccinated piglets to evaluate maternal antibody interference and vaccination compliance |
| 2:54 | Victoria Crabtree | Chronic Diseases and the Organ Systems Affected in Free-Ranging Captive Lemurs |

**3:10 -3:20        Break and Snack - VEC Lobby**


**Faculty – Moderator: Dr. Juming Zhong**

3:20   Amanda R. Taylor        Enhancing Veterinary Student Learning with Mulitmedia 3D
                               Models: a Pilot Study

3:40   Tekla Lee-Fowler        High resolution computed tomography evaluation of BV, AV, and
                               BA ratios in T. cati infected cats


# 4:OO:              KEYNOTE LECTURE

**"Animal Health Research at the Agricultural Research Service, United States
Department of Agriculture"**

**Cyril G. Gay, D.V.M., Ph.D**
Senior National Program Leader, Animal Production and Protection
United States Department of Agriculture-Agricultural Research Service


# 5:OO      INDUCTION AND AWARDS ANNOUNCEMENT

**INDUCTION of new Phi Zeta Members
Presentation Awards**

Dr. Juming Zhong, president of Epsilon Chapter
Dr. Eleanor Josephson, Treasurer of Epsilon Chapter

# 5:3O      RECEPTION  - VEC Lobby

# PHI ZETA KEYNOTE SPEAKER AND
# JOY GOODWIN LECTURER

## "Animal Health Research at the Agricultural Research Service, United States Department of Agriculture"

**Cyril G. Gay, D.V.M., Ph.D**
Senior National Program Leader, Animal Production and Protection
United States Department of Agriculture-Agricultural Research Service

Dr. Gay obtained a B.Sc. in Chemistry and a Doctor of Veterinary Medicine from Auburn University, and a Ph.D. in Microbiology from The George Washington University. Dr. Gay has worked in the animal health research field for the last 25 years holding several positions of increasing responsibility in the federal government and the pharmaceutical industry. As Chief, Biotechnology Section, Center for Veterinary Biologics (CVB), United States Department of Agriculture (USDA), Dr. Gay developed the procedures for licensing molecular vaccines that led to the first license for a live recombinant vectored vaccine. In the pharmaceutical industry (SmithKline Beecham Animal Health and Pfizer Animal Health) Dr. Gay led several cross-functional teams that successfully developed and licensed veterinary vaccines for companion animals and livestock. As Director, Global Product Development, Pfizer Inc., Dr. Gay developed strategic and tactical plans that interfaced R&D, clinical development, manufacturing, marketing, and product life-cycle management. Dr. Gay joined Agricultural Research Service (ARS), USDA, in 2002. Dr. Gay currently holds the position of Senior National Program Leader and provides program direction and national coordination for the Department's intramural animal health research program, with focus on eight research laboratories located in Ames, Iowa, East Lansing, Michigan, Clay Center, Nebraska, Athens, Georgia, Orient Point, New York, Beltsville, Maryland, Pullman, Washington, and Manhattan, Kansas. Dr. Gay also provides technical support within the interagency in the implementation of the President's National Strategy for Combatting Antimicrobial Resistant Bacteria (CARB). Dr. Gay leads efforts within USDA for research on alternatives to antibiotics in animal production (www.ars.usda.gov/alternativestoantibiotics/). Dr. Gay was the 2010 recipient of the USDA Secretary's Honors Award for interagency response to the pandemic H1N1 influenza outbreak; the ARS Special Administrator's Award for outstanding and rapid research support for pandemic H1N1; and the USDA Secretary's Honor Award for Heroism and Emergency Response for contributions as a team member to the ARS/APHIS response to the 2013 Chinese H7N9 avian influenza outbreak.

**POSTER PRESENTATION:**

**Faculty/Staff:**

Eric Fish | Comparison of Serum microRNA Between Dogs with and without Canine Mammary Carcinoma by Deep Sequencing and Comparison to Histopathologic Characteristics

Julie Gard | Mineral analysis of Florida beef cattle diagnosed with ovarian follicular dysplasia

**Graduate students and Residents:**

Amanda Brenna | Pharmacokinetics of fenbendazole in canine CSF and plasma: A pilot study

Erfan Chowdhury | Synopsis of Broiler and Broiler Breeder Diseases Diagnosed at Alabama State Diagnostic Facilities, 2016-2017

Austin Conley | Evaluating Cepalexin MIC to Cephalotin in Escherichia coli

Crisanta Cruz-Espindola | High Performance Liquid Chromatography (HPLC) method development for Fenbendazole in canine plasma and Cerebrospinal fluid (CSF)

Henri Alexandre Giblot-Ducray | Prebiotic Prevents Disruption of Gut Barrier Integrity

Karen Ho | Antimicrobial activity of rifampin in Staphylococcus intermedius group in the dog

Anna Huskey | Canine mammary tumor susceptibility: studying purebred pedigrees and whole genomes for inherited risk factors

Rebecca Jones | Phage-GnRH constructs for population control of feral animals: evaluation in cats

Leah McGlinchey | In Vitro Evaluation of the Mechanical and Physical Properties of the Forwarder Knot exposed to Fluid Media Using Large Gauge Suture

Francesca Mowry | Hypothalamic paraventricular nucleus AngII-mediated microglial activation through AT1r-TLR4 crosstalk in neurogenic hypertension

Daniel Newhard | Successful restoration of pelvic limb perfusion following manual balloon thrombolysis of distal aortic thrombosis in a dog

Mirian Silva-Cutini | Central and peripheral mechanisms of antihypertensive effects of probiotic kefir in spontaneously hypertensive rats

Li-Kun Yang | Functions of the DRY motif and intracellular loop 2 of human melanocortin-4 receptor

Ramon Zegpi | Increased Population Homogeneity in ArkDPI Vaccine Precludes Emergence of Subpopulations after Challenge

**Veterinary Students:**

Brooke Alnwick | Toll-like Receptor 4 Signaling in Equine Mesenchymal Stem Cells

Kaitlyn Bello | B-lymphocyte inflammatory response to PEG-fibrinogen microsphere encapsulated endothelial colony forming cells (ECFCs) in equine distal limb wounds

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| Sara Brisson | Medical Students' Attitudes Regarding the Impact of a Therapy Dog Program on Stress Reduction |
| Sofia Castello | In vitro effects of pitavastatin in combination with benzimidazole anthelmintics on canine glioblastoma cell lines |
| Sarah Christie | Evaluating Therapeutic Potential of Carnitinoid Compounds in Models of Mitochondrial Dysfunction |
| Melissa Crepps | Identification of a MircoRNA Expression Profile as a Biomarker for Cancer |
| Sarah Escaro | In Vitro Measurements of Friction Used to Observe the Drying Properties of Intact Equine Articular Cartilage |
| Joseph Fuller | Long-term co-culture of equine synovial membrane and articular cartilage explants as an in vitro model of osteoarthritis |
| Amanda Hill | Identification of a single base deletion in the glycoprotein IIb gene causing Glanzmann Thrombasthenia in a Golden Retriever |
| Chris Johnson | Mission Thyroidectomy |
| Mandy Kaiser | Effect of Heartworm Disease and Heartworm Associated Respiratory Disease (HARD) on the Right Ventricular Papillary Muscle of Cats |
| Caroline Parker | Tracking PEG-Fibrinogen microsphere encapsulated endothelial colony forming cells after injection into equine distal limb wounds |
| Matthew Pate | Effect of Acidosis and Storage Time on Platelet Aggregation in Horses |
| Ashley Pickett | Anesthesia enhances subthreshold critical slowing-down in a stochastic Hodgkin-Huxley neuron model |
| Kimberly Smart | Concentration of rifampin in the sebum and buffy coat of dogs |
| Brett Story | Sheep Natural History: A Study of Tay-Sachs Disease in Jacob Sheep |
| Natasha Taylor | EGFR and AR regulated genes: profiles in novel African American and Caucasian prostate cancer cell lines |

**Undergraduate Students:**

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|---|---|
| Jonathan Dismukes | Expression of the INK4AB/ARF tumor suppressor transcription factor MSK1 in canine breast cancer: Quantification through QrtPCR and correlation with established phenotypes |

# Graduate Student Platform Presentations

## Pharmacodynamic Properties of Oral Torsemide in Healthy Horses

Gustavo F. Agne[1], SeungWoo Jung[1], Anne A. Wooldridge[1], Sue H. Duran[1], William Ravis[2]
[1] Department of Clinical Sciences, Auburn University, AL, USA
[2] Department of Drug Discovery and Development, Auburn University, AL, USA

**Introduction**. Diuretic therapy has been limited to injectable formulations in the horse due to poor absorption of oral furosemide. The purpose of this study was to determine whether oral torsemide produced clinically significant diuresis in healthy horses.

**Methods**. Torsemide was administered orally at 4 mg/kg/day to 6 healthy adult mares for 6 days. A 28 Fr Foley urinary catheter with a urine collection bag was used to measure urine output and urine specific gravity (USG) over 12 hours on days 0 and 6. Blood samples were collected daily for serum biochemical analysis. Non-invasive mean arterial pressure (MAP) was measured daily with a tail cuff. Student's *t*-test with the Bonferroni correction was used to analyze for differences in laboratory parameters between Day 0 (pre) and Day 6 (post).

**Results**. Urine output (median and range) significantly increased with torsemide [pre: 2.2 L, (1.7-4.4 L); post: 11.5 L (8.7-15.4 L); $p = 0.0054$]. USG markedly decreased [pre: 1.046 (1.032-1.049); post: 1.010 (1.008-1.011); $p = 0.002$]. Significant alterations in biochemical parameters included hyponatremia [pre: 137 mmol/L (136-141 mmol/L); post: 129 mmol/L (128-134 mmol/L); $p = 0.001$], hypokalemia [pre: 3.75 mmol/L (3.5-4.1 mmol/L); post: 1.95 mmol/L (1.6-2.4 mmol/L); $p = 0.001$], and elevated creatinine [pre: 1.4 mg/dL (1.3-1.6 mg/dL); post: 1.95 mg/dL (1.8-2.2 mg/dL); $p = 0.006$]. MAP significantly decreased after torsemide [pre: 76.5 mmHg (70-87 mmHg); post: 56 mmHg (48-74 mmHg); $p = 0.02$].

**Conclusions**. Torsemide administered orally induced clinically significant diuresis with mild pre-renal azotemia and electrolytes changes. These results suggest that oral torsemide therapy could help overcome current therapeutic limitations for horses with excessive fluid retention.

# Pharmacokinetic and biochemical profiles after single administration of oral torsemide in healthy horses

Gustavo F. Agne[1], SeungWoo Jung[1], Anne A. Wooldridge[1], Sue H. Duran[1], William Ravis[2]
[1] Department of Clinical Sciences, Auburn University, AL, USA
[2] Department of Drug Discovery and Development, Auburn University, AL, USA

**Introduction**. Torsemide is a loop diuretic agent more potent than furosemide. Its oral administration has demonstrated high bioavailability across species. The aim of the study was to determine the pharmacokinetic profiles of oral torsemide in the horse.

**Methods**. Torsemide was administered intragastrically at a single dose of 6 mg/kg to six healthy adult mares. Blood samples were collected at predetermined time points over a period of 48 hours. Plasma torsemide concentrations were measured by the use of high performance liquid chromatography, and serum biochemical profiles were examined. Student's *t*-test with Bonferroni correction was used to identify differences in pharmacokinetic and biochemical parameters between baseline (pre) and 24 hour post drug administration (post). Statistical significance was set at $p < 0.05$.

**Results**. Pharmacokinetic analysis revealed peak concentration ($C_{max}$) of $11.05 \pm 5.26$ µg/mL, time of maximum concentration ($T_{max}$) of $2.83 \pm 1.33$ h, area under the curve (AUC) of $92.2 \pm 48.9$ µg*h/mL, and an elimination half-life ($T_{1/2}$) of $9.11 \pm 1.5$ h. Significantly increased concentrations of creatinine (pre: median of 1.65 mg/dL and range of 1.5-1.8 mg/dL, post: median of 2 mg/dL and range of 1.9-2.3 mg/dL; $p = 0.02$) and bicarbonate (pre: median of 22.8 mmol/L and range of 17.6-25.3 mmol/L, post: median of 30.7 mmol/L and range of 27.6-34 mmol/L; $p = 0.0007$) were noted. Chloride concentrations were markedly decreased (pre: median of 99 mmol/L and range of 96-103 mmol/L, post: median of 87.5 mmol/L and range of 85-88 mmol/L; $p = 0.0007$).

**Conclusions**. Oral torsemide successfully reached therapeutic concentrations in blood, and resulted in mild pre-renal azotemia and electrolyte changes. These results suggest that oral torsemide may be employed as a clinically useful diuretic agent for management of congestive heart failure and fluid retention in the horse.

# Use of Accelerometers to Evaluate Behavior Changes in Cattle Administered Continuous, Low-dose Endotoxin

Jenna E. Bayne[1], Thomas Passler[1], Brad J. White[3], Miles E. Theurer[3], Edzard van Santen[4], and Paul H. Walz[2]

[1] Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2] Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3] Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, KS
[4] Department of Agronomy, University of Florida, FL

**Introduction:** Administration of lipopolysaccharide (LPS) elicits an acute phase response (APR) and sickness behaviors in cattle. In this study, continuous, low-dose administration of endotoxin was achieved using subcutaneously placed osmotic mini-pumps (OMP). Technologies that monitor cattle behavior and activity offer remote, real-time collection of objective data with the capacity to detect changes indicative of disease. The objectives of this study were to evaluate sickness behavior in cattle using accelerometers during continuous, low-dose endotoxin administration, in conjunction with traditional markers of inflammation.

**Methods:** Twenty mixed-breed beef calves were maintained as a single group on a biosecure pasture for the 28-day study. Calves were randomly assigned to principal (LPS; n = 10) and control (CON; n = 10) groups. Calves were fitted with an accelerometer and pedometer to continuously monitor activity. On day 0, all calves were implanted with OMPs (ALZET 2ML1 Osmotic Pump; Durect Corp., Cupertino, CA). LPS calves were administered endotoxin (*E. coli* O55:B5, Sigma-Aldrich, St. Louis, MO) at a dose of 30 µg/kg/24h for 7 days. CON calves received an equivalent volume of physiological saline alone. OMPs were removed on day 7. On days -7, 0, 7, 14, and 21, physical examination, clinicopathologic evaluations, and data retrieval were performed. Clinical illness scores (CIS) were assigned daily.

**Results**: All calves were clinically normal (CIS 1) during the 7 days prior to OMP placement. A CIS of 2 was observed in one LPS calf on day 1, with all calves having CIS of 1 thereafter for the remainder of the study. OMP were well tolerated by all calves. Differences between LPS and CON calves were found for physical examination, clinicopathologic, and behavioral indices parameters. Changes in behavioral indices were observed in both LPS and CON calves following OMP placement and subsequent removal, compared to respective baselines. Comparing changes from baseline behavior between groups, LPS calves spent more time lying and less time standing on several days post-OMP removal (p <0.05), and increased walking on day 15 compared to CON.

**Conclusions:** Use of accelerometers demonstrated the capacity to detect subtle behavioral changes in cattle undergoing a continuous, low-dose endotoxin infusion in a subclinical disease model. Use of OMP for prolonged, low-dose LPS administration is unique to this study. The OMP devices were well tolerated by cattle.

## Ultrasound Elastography as a Measure of Whole-Body Therapy for GM2 Gangliosidoses

Elise Diffie[1,2], Robert Cole[3], Heather L. Gray-Edwards[1], Miguel Sena-Esteves[4] and Douglas R. Martin[1,2]

[1]Department of Anatomy, Physiology, and Pharmacology; [2]Scott-Ritchey Research Center, [3]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL [4]Horae Gene Therapy Center, University of Massachusetts, Worcester, MA

### Introduction
Sandhoff disease (SD) and Tay-Sachs disease (TSD) are two clinically indistinguishable neurologic syndromes of GM2 gangliosidoses. They are lysosomal storage disorders that, due to a deficiency in the heterodimeric enzyme Hexosaminidsase (Hex), cause fatal neurologic disease in children. There is no efficacious therapy for these diseases, but preclinical adeno-associated viral (AAV) gene therapy experiments in mouse and feline models of SD have shown promise. Ultrasound elastography is a relatively new diagnostic imaging technique that maps the elastic properties of soft tissue, or tissue stiffness, using propagation of shear waves (SW) induced by acoustic radiation force impulse (ARFI). The aim of this study is to determine the effectiveness of this imaging modality for peripheral organ evaluation as a measure of whole-body therapy in cats with SD.

### Methods
Cohorts of cats were selected as follows: 6 normal adult cats (age 9 months-3 years), 6 normal kittens (age 2-4 months), and 2 SD untreated kittens (age 3.5-4 months). Cats were either sedated or placed under general anesthesia for elastography. Blood work (complete blood count and serum chemistry) was performed at the time of elastography; normal cats were excluded from the study if liver enzyme values or total white blood cell counts were increased above reference range. Three regions of interest were selected in each target organ (liver, spleen, kidney, pancreas, skeletal muscle). Within the selected regions, 6 measurements of elasticity (kPa) and shear-wave speed (m/s) were obtained. For each cat, all measurements from regions of interest in each target organ were averaged.

### Results
There was a significant difference (p<0.001) between the liver values obtained from normal adults and each kitten group. A significant difference (p<0.05) was noted between liver values of normal kittens and the SD kittens. For the spleen, a significant difference (p<0.05) was observed between normal adults and each kitten group. There was a significant difference (p<0.05) between the skeletal muscle values obtained from normal adults and normal kittens. Additionally, a significant difference was noted (p<0.05) between the normal adult group and 2 adult cats that were excluded from the study due to increased liver enzymes on serum chemistry blood work.

### Conclusions
Based on these results, ARFI elastography can potentially be used to monitor the effectiveness of whole-body gene therapy for GM2 gangliosidoses in the liver. Further development will explore the utility of this approach for other organs.

### Acknowledgments

# Intravenous delivery of AAV gene therapy in GM1 gangliosidosis

Amanda L Gross[1,2], Heather Gray-Edwards[1], Miguel Sena-Esteves[3,] Douglas R Martin[1,2]

[1] Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA

[2] Department of Anatomy, Physiology, & Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA

[3] Department of Neurology and Gene Therapy Center, University of Massachusetts Medical School, Worcester, Massachusetts, USA

**Introduction**. GM1 gangliosidosis is a hereditary lysosomal storage disease caused by a deficiency of lysosomal β-galactosidase (β-gal). The most common form of GM1 gangliosidosis affects children, is fatal by 4 years of age, and is characterized by rapidly progressing and fatal neurological disease. Outside of palliative and supportive care, there is no effective treatment for GM1. AAV, or adeno-associated viral therapy has proven effective in a well-characterized feline model of GM1 gangliosidosis, demonstrating a greater than 10-fold increase in lifespan after injection to the thalami and deep cerebellar nuclei. However, this injection route is invasive, so intravenous delivery was studied to circumvent the surgical risk while potentially increasing cortical and systemic biodistribution.

**Methods**. AAV9 was delivered at a total dose of 1.5e13 vector genomes/kg body weight at approximately 1 month of age. The six animals included in the study were divided into two cohorts: 1) a long term group, which was followed to humane endpoint, and 2) a short-term cohort, with samples collected 16-week post treatment. Animals were assessed using a clinical rating score to determine disease progression. After the designated time point, biodistribution of β-gal and vector were assessed, using a synthetic enzyme substrate and qPCR, respectively. Biomarkers of disease progression were studied in the CSF, and brain metabolites were analyzed using magnetic resonance spectroscopy (MRS).

**Results**. The long term group (n=2) had an average 5.3-fold increase in life expectancy, with both animals showing limited neurological signs. The short term cohort (n=4) also showed amelioration of clinical symptoms to 16 weeks post-treatment. In both cohorts, there was an increase in the distribution and activity of β-gal, reaching normal levels in some areas of the CNS and peripheral tissues. Also, secondary biomarkers of lysosomal function were improved, and vector was detected throughout the CNS and periphery. Analysis of CSF biomarkers and brain metabolites showed a normalization in comparison to untreated animals.

**Conclusions.** Restoration of β-gal to levels just 0.1 fold normal has shown therapeutic effect, and with IV gene therapy there is an even greater restoration of β-gal activity. Additionally, the biomarkers and metabolites indicate amelioration of cell damage, gliosis, and demyelination, as well as other functions. Taken together, this data strongly supports the use of IV injection of AAV gene therapy as a safe and effective treatment for GM1 gangliosidosis.

# Characterizing the Effects of Heat Stress on Olfactory Response Kinetics

Samantha Hagerty, Melissa Singletary, Ludmila Globa, Oleg Pustovyy, Edward Morrison, Iryna Sorokulova, Vitaly Vodyanoy
Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

**Introduction.** Temperature is one of the most challenging stressors affecting human and animal health. Extreme heat stress has been shown to damage the gastrointestinal mucosa protecting the internal environment of the body from harmful bacteria and endotoxins. Dysfunction of this protective barrier increases intestinal permeability and diffusion of toxic bacterial components from the gut lumen into the blood. Given the extensive interactions between the GI tract and CNS function, there is a likely link between intestinal function and olfactory sensory neurons. Olfaction may be one of the many physiological functions affected by heat stress, and potentially prevented. Our goal is to investigate whether exposure to heat stress affects olfactory responses to odorant stimulation. In this ongoing work, we examine the effects of both environmental and metabolic heat stress on the kinetic properties of rat olfactory sensory neurons *in vitro* using electroolfactogram (EOG) recordings from isolated olfactory epithelium (OE).

**Methods.** There have been three models of heat stress used in these experiments, including environmental and metabolic forms of stress. In one method, animals were exposed to a temperature of 45°C in a climatic chamber for 25 minutes, and olfctory function was assessed after 4 hours. In another method, animals did not undergo whole-body exposure to elevated temperatures, but the extracted OE was placed in a controlled heat chamber specifically designed for attachment to the EOG, in which the temperature of the OE itself was manipulated during assessment. The final method involved rats placed on an exercise treadmill for 25 minutes, and olfactory function was assessed after 4 hours. All controls remained at room temperature (25°C) and did not undergo heat stress exposure. After euthanization, OE was surgically removed for EOG recording and trunk blood was collected for biochemical analyses. EOG were evoked by an odorant mixture of ethyl butyrate, eugenol, and (+/−) carvone. Pulses were 0.20s in duration at 20s aintervals, with one series of 10 pulses representing a single EOG recording. The biochemical properties of the animal blood related to heat stress were characterized by measuring cytokines, LPS, and high resolution light microscopy of erythrocytes.

**Results.** Exposure of rats to high temperatures resulted in a significant increase of body temperature. The mean body temperatures before and immediately after heat stress were $36.7\pm0.07$ °C and $40.3\pm0.17$ °C, respectively. OE within the temperature-controlled EOG chamber were exposed to increments ranging from 17-33 °C. At this time, calculations for the kinetic properties of electrical responses to odorant have only been carried out for rats undergoing whole-body exposure. Kinetics were significantly changed in animals subjected to heat stress. The mean half-rise ($t_r$) and half-decay times ($t_d$) of control OE were $328\pm11$ and $660\pm20$ ms, respectively. The rise and decay of EOG peak amplitude after heat stress were much faster. The $t_r$ and $t_d$ reduced by 175%, and 72%, respectively. Heat stress did not result in a change of IL-1β, IL-6, TNF-α, or INF-γ cytokines level. However, a significant increase of the IL-10 level was found in rats exposed to heat. The level of lipopolysaccharides (LPS) significantly increased in serum of heat-stressed animals compared to control animals. The concentration of free vesicles in animal blood increased after exposure to heat stress from $(1.4\pm0.2)\times10^6$ to $(3.8\pm0.3)\times10^6$ vesicles $\mu l^{-1}$, indicating a faster conversion of erythrocytes into echinocytes

**Conclusions.** The kinetic effects demonstrated in this work are consistent with that of the cultured mouse olfactory neurons in similar work. Additionally, detrimental effects of elevated temperatures on olfaction have been shown in other animals. The change in kinetic properties of olfactory receptors resulting from elevated temperature is consistent with those in other neurons. Cytokines were found to cause the neurodegeneration of olfactory bulb. We conclude that heat stress causes a significant modulation of olfactory responses that must be further investigated to understand the molecular changes occurring.

# Pharmacokinetics of Approved and Compounded Extended Release Levetiracetam After Single Oral Dose Administration in Cats

Erik R. Johnson[1], Amanda R. Taylor[1], Dawn M. Boothe[2], Heather L. Gray-Edwards[3], and Doug R. Martin[2,3]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

[2]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL

[3]Scott-Ritchey Research Center, Auburn University, Auburn, AL

**Introduction.** The half-life of levetiracetam (LEV) is short in cats (approximately 3 hours), prompting the search for an extended release (ER) LEV formulation that might allow for longer dosing intervals. The purpose of this study was to describe and compare the pharmacokinetics of LEV in both the plasma and CSF in cats after a single dose of approved ER LEV and compounded ER LEV.

**Methods.** Using a non-randomized cross-over design, nine clinically healthy cats received a single dose of 500 mg of approved ER LEV PO. Thirteen blood and ten CSF sampling periods were then collected over 24 hours for pharmacokinetic analysis. After a one week washout period, a single oral dose of 500 mg compounded ER LEV was administered in the same nine cats and the same blood and CSF samples were collected.

**Results.** Although significant differences were noted between plasma $C_{max}$ and $t_{max}$, both approved and compounded ER LEV maintained concentrations above the human therapeutic concentration for a 12-hour time period. All other available pharmacokinetic values were not shown to be statistically different between the two groups. Pooled CSF LEV concentrations mirror plasma LEV concentrations when plotted on a scatter plot.

**Conclusions.** This study demonstrates that both approved and compounded ER LEV maintains human therapeutic LEV concentrations in healthy cats 12 hours after oral administration, allowing for twice a day dosing. This study also demonstrates that plasma LEV monitoring can be used as an accurate representation of CSF LEV concentrations in cats.

# The Implications of Drugs Utilized for Assisted Breeding in Managed Rhinoceros

Jack J. Kottwitz[1] DVM, Monica Stoops[2] PhD, Roy Harmon[1] BS, and Dawn M. Boothe[1] DVM, PhD, DACVIM, DACVCP

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**Introduction**. Assisted breeding of captive rhinoceros is developing into a significant conservation endeavor for preservation of these threatened and critically endangered species. Previous studies from this lab have determined that the drugs utilized for sedation and anesthesia for assisted breeding have negative effects on sperm motility *in vitro*. Opioid receptors have been detected on the head, neck, and tail of sperm cells of humans, horses, and boar. The mu, kappa, and delta opioid receptors modulate sperm motility, however, interspecies variation exists and the exact function of each receptor is still being determined. Butorphanol stopped sperm motility in human and domestic animals in *in vitro* studies, while other opioids, such as fentanyl, had partial inhibitory activity. The prevalence of the use of opioid drugs in rhinoceros anesthesia has justified further investigation into these drugs in those species. There is little data in the veterinary literature about distribution of parenterally administered drugs into semen. It is hypothesized in this study that drugs administered intravenously or intramuscularly as part of the anesthesia protocols utilized for semen collection are detectable at levels in the seminal plasma that may alter sperm motility.

**Methods**. Stored seminal plasma fractions from Southern White, (*Ceratotherium simum simum)* Black (*Diceros bicornis*) and Greater One-horned (*Rhinoceros unicornis*) receiving etorphine alone or etorphine and butorphanol as part of the sedation/anesthesia protocol for semen collection were initially screened for etorphine utilizing a Siemens Dimension Clinical Chemistry System Flex Reagent Cartridge. These samples and additional paired serum and seminal plasma fractions from Southern White, (*Ceratotherium simum simum)* and Greater One-horned (*Rhinoceros unicornis*) were screened utilizing a Neogen Butorphanol screening assay.

**Results**. While the Flex Reagent Cartridge appeared to have detectable results, it was not possible to quantify etorphine concentrations. The Neogen Butorphanol assay showed detectable and quantifiable amounts of butorphanol in serum (0.01-0.08 µg/ml) and seminal plasma (range: 0.001-0.1 µg/ml) in the subjects that received butorphanol intravenously or intramuscularly. The Greater One-horned rhinos that received IV butorphanol also had decreased sperm motility (75% progressive motility) compared to the one that did not (90% progressive motility).

**Conclusions**. While these results represent pilot data, butorphanol is detectable in rhino seminal plasma at levels equal or greater than serum levels. Analysis of additional samples is necessary to establish if there is a dose dependent relationship and the degree of concentration of butorphanol in seminal plasma. These detectable drug levels suggest that there is a possibility of a negative effect on sperm motility when butorphanol is utilized as part of the anesthesia protocol. Further study is needed to determine the extent of these effects.

# Experimental Infection of Pregnant Pigs with Bovine Viral Diarrhea Virus (BVDV) 1b

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**Introduction**. Bovine viral diarrhea virus (BVDV) lacks strict host-specificity and can infect many ungulate species including cattle, sheep, goats, camelids, and swine. BVDV infections in naïve pregnant hosts can be deleterious and cause severe reproductive losses. Limited information exists regarding changes introduced in the BVDV genome during infection of heterologous hosts. Using a BVDV-1b isolate previously demonstrated to cause persistent infection in cattle, sheep, goats, and white-tailed deer, this study investigated the outcome of serial infections in BVDV-naïve pregnant gilts.

**Methods**. Six pregnant gilts between 27 and 40 days of gestation were utilized. The first gilt was inoculated with $1\times10^6$ $CCID_{50}$ of the BVDV-1b isolate AU526. The 2nd, 3rd, 4th, 5th, and 6th gilts were inoculated with 1mL of BVDV-positive serum from the preceding gilt. Blood samples were collected for virus isolation and RT-PCR (days 0, 5, 7 after inoculation) and virus neutralization (day 0 and every 28 days until farrowing).

**Results**. BVDV infection was confirmed in all gilts by virus isolation, RT-PCR, and seroconversion. Initially, clinical signs were mild (lethargy and inappetence) and pregnancies proceeded to full term. BVDV infection resulted in fetal mummification, stillbirths, and birth of persistently infected piglets. BVDV was successfully isolated from serum samples obtained the gilts on day 5 or 7 after inoculation and from tissue samples obtained from PI piglets. The aforementioned viruses were submitted for whole genome sequencing to evaluate the changes introduced in the viral genome during serial infections in pregnant swine.

**Conclusions**. Serial infection of pregnant swine with the BVDV-1b isolate AU526 resulted in severe reproductive losses and birth of persistently infected piglets as previously described in cattle, sheep, goats, and white-tailed deer. Genomic BVDV changes introduced during serial infections of pregnant swine are under investigation.

## Short-Term Pioglitazone in Equids Increases High Molecular Weight Adiponectin Concentrations and Decreases Insulin Response to Oral Sugar

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**Introduction:**

Decreased adiponectin concentrations are linked with the laminitis prone phenotype in equids with insulin dysregulation and equine metabolic syndrome (EMS). The high molecular weight (HMW) form of adiponectin is closely linked with insulin sensitivity, and thiazolidinedione drugs increase total and HMW adiponectin concentrations and improve insulin sensitivity in humans. The thiazolidinedione drug pioglitazone is orally absorbed in the horse and is safe and affordable for long-term management. The hypothesis was that pioglitazone would increase insulin sensitivity and HMW adiponectin concentrations in horses and ponies.

**Methods:**

Two cohorts of equids, 7 healthy horses and 8 ponies, were treated with pioglitazone (2 mg/kg PO q24 h) for 28 days. Serum HMW adiponectin concentrations were measured by ELISA at 0, 14, and 28 days after treatment. Oral sugar tests (OST) were performed at days 0 and 28. Differences between baseline and study time points were analyzed with a two-way repeated measures ANOVA with Sidak's multiple comparisons test. Cohorts compared were the ponies, horses, and the insulin dysregulated (ID) equids (defined as horses or ponies having insulin concentrations > 65 μU/ml during the OST at ≥ 60 minutes).

**Results:**

The drug was well-tolerated. Insulin concentrations were significantly lower after pioglitazone at the 90 and 120 minute time points of the OST in ponies [$P=0.0035$] and ID equids [$P=0.0028$], but not the horses [$P=0.4970$]. HMW adiponectin was significantly higher after treatment in horses [$P<0.01$, baseline 2.5 ± 1.0 μg/ml; endpoint 4.9 ± 2.9 μg/ml], ponies [$P<0.05$, baseline 1.6 ± 2.6 μg/ml; endpoint 3.3 ± 4.4 μg/ml], and ID equids [$P=0.0272$, baseline 1.0 ± 8.0 μg/ml; endpoint 2.3 ± 1.8 μg/ml].

**Conclusions:**

The lower insulin concentrations during the OST and increased HMW adiponectin concentrations in response to treatment indicate positive effects of pioglitazone for treatment of metabolic derangements in equids with EMS.

# Image Analysis of Sub-Gross Stains for a Feline Neurodegenerative Disease

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**Introduction**. GM2 gangliosidosis is an autosomal recessive storage disease that results in the deficiency of β-hexosaminidase (Hex) enzyme and subsequent accumulation of GM2 ganglioside in lysosomes. The feline model most closely represents the infantile form of the human disease, which typically causes the death of affected children between 3-5 years of age. To treat affected cats in preclinical studies, feline Hex was delivered via intracranial injections of adeno-associated viral (AAV) vector into the thalamus and deep cerebellar nuclei. Tissues from the central nervous systems (CNS) of treated cats are routinely stained with Naphthol (for Hex activity), and PAS (Periodic acid-Schiff, for ganglioside storage) to determine the impact of treatment in specific CNS regions. However, due to the monochromatic nature of these stains, quantitative and qualitative image analysis is indicated to maximize the understanding of gene therapy treatment.

**Methods**. Naphthol and PAS-stained slides of CNS tissues from normal, untreated, and treated cats were scanned with no magnification on a standard flatbed scanner, and the resultant images were imported into ImageJ software.
Quantitative analysis: Each pixel underwent two categorization processes based on separate intensity thresholds: first to sort into a "stained" or "unstained" category, second to sort into a "tissue" or "background" category. The percentage of "tissue" pixels that were also "stained" was then calculated for each tissue section.
Qualitative analysis: The brightness and contrast of each image was auto-enhanced by ImageJ (with an internal standard), then a thermal Lookup Table was applied to create a "heat map".

**Results**. Quantitative analysis: Untreated GM2 cats had a significantly lower percentage of Naphthol-stained pixels than normal and treated cats in most CNS sections analyzed. The comparison of the percentage of stained pixels between treated and normal cats was variable throughout the CNS, but sections from treated cats had widespread distribution of enzyme activity. Regression analysis was used to validate this method by comparing results to specific activity of Hex previously determined by quantitative assays with a synthetic substrate.
Qualitative analysis: Heat map images were generated for all CNS sections of representative treated cats.

**Conclusions**. Image analysis of sub-gross stains is a valid and useful method for describing the pathology of GM2 gangliosidosis and determining the success of efforts to treat it. Heat maps enhance qualitative perception of stained tissue by emphasizing subtle differences in stain intensity.

**Will anesthesia of the median nerve ameliorate lameness caused by pain in the cubital (elbow) joint?**

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**Introduction**.
Lameness examinations are performed to determine the site of pain causing lameness in horses. Nerve blocking typically begins distally in the limb and advances proximally until lameness is ameliorated. The median nerve is often anesthetized along with the ulnar nerve to desensitize the limb distal to the cubital joint. Local anesthetic has been shown to migrate both distally and proximally within the neurovascular bundle after perineural injection. Because the median nerve is blocked slightly distal to the cubital joint, some clinicians believe that proximal migration of the local anesthetic may desensitize this joint thus complicating interpretation of this nerve block. We hypothesized that a median nerve block will desensitize the cubital joint and ameliorate a lameness caused by pain in this joint. The objective of this study was to determine if a median nerve block can desensitize the cubital joint.

**Methods**.
Six horses were evaluated for lameness using body mounted inertial sensors (Lameness Locator) that detect asymmetry of gait by detecting abnormal head movement. Thermographic images of both front legs were obtained. After horses were sedated, a cubital joint was injected with 100ng of interleukin IL-1β. Accuracy of needle placement was verified by aspiration of synovial fluid. Horses were periodically monitored until they displayed Grade 3 out of 5 lameness (AAEP lameness scale). A post interleukin administration lameness score was determined using the Lameness Locator, and thermographic images of both front limbs were again obtained. The median nerve of the lame leg was anesthetized using 10ml of 2% mepivicaine hydrochloride. The efficacy of this nerve block was assessed by pinching the skin using hemostatic forceps over the dermatome associated with this block (the medial aspect of the pastern and fetlock), and comparing this response to the response elicited by similar stimulation of skin on the contralateral limb. Efficacy of the block was also evaluated by assessing thermographic images for an increase in skin temperature of both the desensitized and untreated limb at 20, 40 and 60 minute intervals following the median nerve block. At these times, all horses were trotted for at least 30 strides in a straight line and gait was evaluated with the Lameness Locator. Statistical analysis of the data was performed using statistical software package JMP 11.0.0 (SAS Institute, Cary, NC). Normality was assessed by visual appraisal of frequency distributions and the Shapiro-Wilk test. A full-factorial repeated measures ANOVA was used to compare treatment effect across time.

**Results**.
Administration of IL-1β resulted in moderate transient lameness in all horses. The median nerve block was confirmed to be successful in all horses at 20 minutes post block by loss of skin sensation and increased temperature on thermographic images over the expected dermatome. Administration of the median nerve block did not result in statistically significant improvement of lameness as quantified by the mean head max vector sum. (p=0.3234)

**Conclusions**.
A volume of 10ml of local anesthetic was successful in desensitizing the median nerve in all horses. Desensitization of the median nerve did not ameliorate lameness originating from the cubital joint. This result has relevant clinical application as it suggests when performing a lameness examination, it is unlikely that blocking the median nerve with a low volume of local anesthetic will ameliorate a lameness originating from the cubital joint.

# Ante-mortem and Post-mortem diagnosis of Ovarian Follicular Dysplasia in Florida Beef Herds through Utilization of Ultrasonography

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**Introduction:** Studies commissioned by the Florida Cattleman's Association in 2007 and 2016 found ovarian follicular dysplasia (OFD) as a primary cause of infertility in Florida beef cows. Ovarian Follicular Dysplasia is a slowly progressive bilateral abnormal growth and/or development of ovarian follicles eventually transforming into Sertoli-form Granulosa Cell Tumor. Later stages of OFD, grades III and IV can be detected via ultrasound examination of the ovaries when using a 6.2MHz linear probe. The objective of this study was to examine the variation in ante-mortem and post-mortem ultrasound examination utilizing a 6.2MHz ultrasound probe for ante-mortem diagnosis versus that of the 8.5MHz linear probe for post-mortem. Our hypothesis was that the 8.5MHz probe would provide a more defined image allowing for detection of earlier stages of OFD detection.

**Methods:** Twenty-eight cows and heifers with subfertility and two "control" (fertile) females from two Florida beef herds underwent trans-rectal ultrasound of both ovaries. All images where recorded and evaluated for mineralization score and follicular numbers. The 30 animals were followed to slaughter. All ovaries, uteruses, and oviducts were collected post-mortem. Fixed ovaries were measured, sectioned para-sagittal through the hilus, photographed, and arranged in histology cassettes for complete examination (histology) of the cut surface. The ovaries were then graded for presence of OFD (0–IV) and other diseases. Ovarian ultrasound images and gross morphology (histology) of fixed sagittal sections were blindly compared for OFD grades in 25 individual ovaries.

**Results:** Of the ranches sampled, 86% of the sub-fertile cattle were OFD positive. At the first ranch, 10 animals had grade I OFD and at the second ranch, seven were grade I, five were grade II and the two were grade III. There was a 94% agreement between the ultrasound and histological diagnosis of OFD. There was 100% (25/25) agreement of when evaluating OFD diagnosis between the two ultrasound probes. The 8.5MHz had greater resolution, resulting in a higher OFD score 92% (23/25) of the time versus that of the 7MHz ultrasound.

**Conclusions:** Early grades of OFD (I & II) can be diagnosed via ultrasound, utilizing 8.5MHz or greater ultrasound probe. The higher resolution ultrasounds may be able to do a more reliably grade OFD. However, additional studies are necessary. Ovarian ultrasound is a useful tool for on-farm diagnosis of ovarian follicular dysplasia.

## Evaluation of the Cancer-Specific Functionality of Canine Promoters to Expand Precision Medicine Approaches for Canine Tumors

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Cancer gene therapy is an approach to treating cancer that aims to deliver therapeutic genes to kill cancer cells. However, the feasibility of the routine use of cancer gene therapy in clinics has been restricted as the therapeutic gene may generate an off-target effect in normal cells resulting in normal cell toxicity. Thus, it is important to explore mechanisms to achieve targeted expression of therapeutic genes to cancer cells with reduced or no toxicity to normal healthy cells. Several studies have shown that transcriptional targeting can be a successful strategy to promote targeted expression of the therapeutic gene in various cancers such as lung, breast and prostate cancer. This is accomplished by utilizing tumor-specific upregulated promoters to drive the expression of therapeutic genes in a tissue- or tumor-specific manner. Although studies showed high levels of tumor-specific expression of promoters, including human telomerase reverse transcriptase (hTERT), survivin and chemokine receptor 4 (CXCR4) in a variety of human cancers and murine models, none of these promoters have been investigated for their activity in dogs. Our goal is to identify and investigate the activity of promoters in various canine tumors. To accomplish this goal, we measured the expression level of promoters in canine tumor cells/ tissues by employing RT-qPCR for endogenous promoters including CXCR4, TERT, and Survivin. Results showed negligible expression differences between canine normal and hematopoietic and non-hematopoietic tumor cells for the TERT promoter, although this promoter showed increased tumor-specificity in the human and mouse models. However, canine Survivin (cSurvivin) showed markedly higher expression in some canine tumor cells when compared with most normal cells and tissues but showed little expression in canine lymphoma cells/tissues. Canine CXCR4 showed little or no expression for most of the hematopoietic and non-hematopoietic cancer cells and tissue except for a T cell lymphoma cell line and tissues. To further validate these findings, we cloned the sequences of these promoters into GFP reporter plasmids to evaluate exogenous promoter activity in canine tumors using cellular transfection experiments followed by measuring of GFP expression through flow cytometry. Expression was normalized to CMV-GFP expression. Results showed that the percentage of cells expressing the tested promoters was at least double that of normal cells (NCF) and mean fluorescent intensity (MFI) for GFP expression was similar in both cancer cells and normal cells for all of the tested promoters. These findings suggest that, since individual promoter activity varies from cell to cell, there is a need to design individualized gene therapies for each patient by selecting patient-specific promoters to drive the activity of therapeutic gene in a patient specific manner.

# Polyethylene glycol coating changes microbial effects of zinc nanoparticles

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**Introduction**. Polyethylene glycol (PEG) is a coiled polymer with multiple ethylene ether units that are soluble in water. PEGylation is a chemical process involving the passivation of the surface of nanoparticles by PEG which can decreases the tendency of nanoparticles to oxidize, thereby offering protection and stabilization. It can also provide an increased affinity to the target protein provide reduced cytotoxicity of nanoparticles. Zinc nanoparticles have been isolated within protein nucleating centers endogenously present in human and animal blood. A distinct subset of zinc nanoparticles mimicking the endogenous subset ranging in size from 1-2 nm and in a non-oxidized and non-ionic state have been studied in their influence on multiple bacterial taxa growth. These preliminary microbiological studies *in vitro* have demonstrated a differential antimicrobial effect on bacteria of various taxa. Present study evaluates the changes in zinc nanoparticle influence on bacteria *in vitro* after zinc nanoparticle stabilization with PEG.

**Methods**. Zinc metal nanoparticles were produced using an underwater spark-discharge method and the 1.2 nm non-oxidized, non-ionic zinc metal nanoparticles (ZnNPs) were coated with 400 g/mol molecular weight PEG. PEGylated and non-PEGylated ZnNPs were analyzed by transmission electron microscopy (TEM), atomic force microscopy (AFM), and x-ray photoelectron spectroscopy (XPS) to confirm size and oxidation state. In the present study, growth of *Bacillus subtilis* and methicillin resistant *Staphylococcus aureus* (MRSA) strains 1, 2, 5, 13, 26, 34, and 45 were cultured with PEGylated ZnNPs. Relative growth patterns were determined by spectrophotometric analysis at a 24-hour time point.

**Results**. Results included an observed trend that ZnNPs were generally MRSA suppressive, with an approximate 40% maximum reduction in viability ($p < 0.05$), with exception of growth in MRSA-26. PEGylation of the ZnNPs resulted in a similar suppressive trend though not significance for any MRSA strains. Conversely, in observing ZnNP influence on growth for *B. subtilis*, showed an approximate 30% maximum increase in viability, though not significance. However, PEGylation of the ZnNPs resulted in a significant suppressive response on growth as compared to non-PEGylated ZnNPs ($p < 0.05$).

**Conclusions**. These initial results demonstrate a change in antimicrobial effects on *B subtilis* growth between a general non-significant increase in viability with ZnNPs to a significant growth suppression when ZnNPs are PEGylated.  The stabilization of ZnNPs with PEG may increase their toxicity toward beneficial bacterial, such as *B subtilis* and requires consideration in potential PEGylated ZnNP applications. Concurrent and future studies are on the influence of PEG on bacteria *in vitro* and *in vivo* studies to evaluate the effects of ZnNPs and PEGylated ZnNPs on microbiota. Further evaluation is needed to determine the mechanism of action for small non-PEGylated and PEGylated non-oxidized zinc nanoparticle growth effects on multiple bacterial taxa.

**A study evaluating the ability of epinephrine to potentiate and prolong lidocaine's ability to attenuate pain when administered for a palmar digital nerve block**

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**Introduction.** Administration of a highly efficacious local anesthetic agent would help to improve interpretation of the results of diagnostic anesthesia by eliminating poor potency of the local anesthetic agent as a possible cause of incomplete resolution of lameness.
In human medicine, epinephrine added to local anesthetic solution prolongs the analgesic effect of the local anesthetic by counteracting the vasodilatory nature of most local anesthetics and intensifies the analgesic effect of a nerve block. We theorized that by mixing epinephrine with lidocaine, the analgesic effect of lidocaine would be intensified as well as prolonged.

**Methods**. Six naturally lame horses that had previously been identified as having foot pain were used for the study. The horses were assigned to three groups in a crossover design study. Group 1 horses were to receive a palmar digital nerve (PDN) block using 2% lidocaine. Group 2 horses were to receive a PDN block using 1% lidocaine, and Group 3 horses were to receive a PDN block using 1% lidocaine to which epinephrine had been added.  For all three groups, gait at a trot was analyzed using a computerized lameness analysis system (Lameness Locator) at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 min after applying a PDN block to the lame limb. The amplitude of the vector sum (VS) obtained from the gait analysis correlates with severity of lameness. Heart rate and skin sensation between heel bulbs were evaluated at each time point.

**Results**. During the first 30 minutes, there was no statistical difference in vector sums (VS) of horses in each of the 3 groups. However, after 30 minutes there were significant differences between horses treated with 1% lidocaine to which epinephrine had been added, and horses blocked using 1% lidocaine (P=0.0004) or 2% lidocaine (P=0.0001). In Group 3, 3 of 6 horses had a 100% improvement of the lameness for the 2 hours' duration of the study. In groups 1 and 2, skin sensation did not correlate with an improvement in gait. In group 3, reaction to skin sensation correlated with improved gait. The amount of pressure that had to be applied to the skin to elicit a reaction during the study for horses blocked with 1% lidocaine spiked with epinephrine were also statistically different from pressure applied to the skin of horses blocked with 2% and 1% lidocaine. No changes in horse's heart rate were observed during the study.

**Conclusions**. Epinephrine in a 1:200,000 concentration with 1% lidocaine provides longer duration of a PDN block in the horse. When a mixture of 1% lidocaine and epinephrine was used, lameness scores were improved for longer periods compared to 1% and 2% lidocaine solutions. Also, the addition of epinephrine to lidocaine for a PDN block increases the likelihood of having a positive correlation between loss of skin sensitivity and an accurate PDN block. Epinephrine combined with lidocaine as a 1:200,000 solution seems to be a safe drug since no cardiovascular or skin reactions were observed during or after the study.

**Notched QRS complexes in dogs with and without structural cardiac disease: 85 cases**

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**Introduction**. Notching of QRS complexes reveals important diagnostic and prognostic information in human cardiology. However, little is known about notched QRS complexes in veterinary medicine. The objectives of this study were to describe the signalment and cardiac disease diagnosis in dogs with notched QRS complexes of normal duration, as well as to describe the specific leads and number of leads with notched QRS complexes on ECG.

**Methods**. A retrospective review of digitally stored ECGs and associated medical records of dogs with a recorded ECG as part of routine clinical evaluation was performed. Medical records were reviewed for signalment and cardiac disease diagnosis in dogs with notched QRS complexes identified.

**Results**. The age at time of ECG recording was 9.15 +/- 3.38 years for the 85 dogs with notched QRS complexes in at least 1 ECG lead. Most dogs (78.8%) had 3 or less ECG leads with notched QRS complexes. Most dogs (69.4%) with notched QRS complexes in at least 1 lead had cardiac disease. The odds ratio of a dog having cardiac disease if more than 1 lead was identified with notched QRS complexes was 3.97. The most common cardiac disease identified was chronic atrioventricular valvular degeneration (CVD), and the majority of these dogs (80%) had 2 or less leads with notched QRS complexes.

**Conclusions**. In conclusion dogs with and without cardiac disease can have notched QRS complexes, and the likelihood of a dog having cardiac disease that has more than 1 ECG lead with notched QRS complexes is significant which should warrant diagnostic evaluation.

# Veterinary Student Platform Presentations

**Evaluation of Arthritic Lesions in Giant Anteater Feet: Pilot Data**

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**Introduction**: The giant anteater (*Myrmecophaga tridactlya*) is the only fully terrestrial member of the order Pilosa. The giant anteater's thoracic limb has evolved specialized adaptations to aid in the excavation of termites and ants. While there are anatomical descriptions of the proximal foreleg, there are no detailed references for the manus. One such adaptation is elongated claws on the digits 1-3. Exostoses of the distal aspect of metacarpal 4 and the associated phalanges with additional bony changes to metacarpal 5 were noted following maceration of a thoracic limb acquired by the Auburn University College of Veterinary Medicine. Subsequent CT scans and radiographs of two additional specimens indicated similar exostoses present in the same region of the manus. These observations have led to the hypothesis that the changes seen in captive giant anteater forelimbs were environmentally related. It is possible that the gait of the giant anteater, walking on the lateral side of the manus, may also predispose to the osteological changes observed.

**Methods**. Facilities accredited by the AZA (Association of Zoos and Aquariums) and museum collections with known giant anteater specimens are the source of specimens in this study. Both wild and captive populations are planned to be sampled to determine if the observed exostoses is a natural phenomenon or somehow related to captive husbandry. Data for this project is being obtained via 4 X-ray views of each foreleg: (1) straight cranio-caudal view, (2) a straight latero-medial view with the toes split as much as possible, (3) and (4) both oblique views or CT analysis of the forelimb. Participating institutions are requested to fill out a questionnaire providing information on the husbandry of living animals or detailed information on the origin of museum specimens.

**Results**. Currently, there are 34 accredited institutions that house Giant Anteaters in North America. To date, 15 AZA facilities are providing radiographs or CT images and 1 museum has provided access to multiple specimens. At this time, 7 individuals with lesions present have been identified. It is important to note that when communicating with these facilities, it has been discovered that radiographs of the forelimbs may not be part of a routine examination. Data collection and analysis is ongoing.

**Conclusions.** It was surprising to note that many institutions housing live giant anteaters do not routinely radiograph the feet. This may indicate that arthritic lesions are present, but not being identified.

## Stability of Compounded Transdermal Meloxicam Gel.

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**Introduction.** Transdermal application of medication via compounded pluronic lecithin organogels (PLO) has been marketed as an effective method of systemic drug delivery despite lack of scientific evidence of quality, efficacy, and safety of these unapproved products. Very few compounded transdermal products have reported therapeutic plasma drug concentrations using this delivery method.

**Methods.** Transdermal meloxicam PLO compounded by the institutional pharmacy at 3mg/mL, 12mg/ mL and 20mg/ mL was tested for accuracy. Three aliquots at each concentration were dissolved in methanol and meloxicam concentration measured via high-pressure liquid chromatography.

**Results.** Preliminary results indicate that there was significant deviation ($p < 0.05$) from the reported concentration at all three concentrations. At 3mg/mL the average dose was 2±0.12mg/mL, at 12mg/mL the average dose was 7.27±0.69mg/mL and at 20mg/mL the average dose was 15.40±4.12mg/mL.

**Conclusions.** The variation in accuracy between the compounded concentrations highlights the challenges in the use a non-approved administration formulation. While novel administration techniques may offer perceived ease-of-administration benefits, the clinician must make every effort to determine whether the described concentration is actually achieved by submitting appropriate plasma or serum to a clinical pharmacology laboratory before and after therapy has begun.

# Chronic Diseases and the Organ Systems Affected in Free-Ranging Captive Lemurs

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**Introduction:** Lemurs are nonhuman primates with over 100 species all of which are endemic to Madagascar. They are the most threatened group of mammals on earth due to habitat loss. Zoos and sanctuaries are preserving lemur populations through captive breeding programs, scientific research, and education programs. The diseases of free-ranging captive lemurs, which are allowed to roam in forested natural habitat enclosures, may be indicative of wild lemur health and the diseases they can obtain in the wild. In captivity some species are long lived and excellent models of aging and human disease. For example, the mouse lemur is the world's smallest primate; however, they can live up to 18 years in captivity, up to six times longer than the similarly sized mouse. They also exhibit human symptoms of aging, such as gray hair, cataracts, and Alzheimer's-like neurodegeneration, making them the only non-human primates to develop such neurodegeneration. The purpose of this study was to provide the first comprehensive description of the chronic diseases of free-ranging captive lemurs and the organ systems most commonly affected; and evaluate the recurring diseases based on age, sex, and species. We hypothesize that the larger species of lemurs will live longer and be less affected by chronic diseases and that older lemurs will have more organ systems affected due to degenerative changes associated with aging.

**Methods:** Species360 is an international organization that maintains a database for captive animals. Members of this organization are allowed to use the Zoological Information Management System, or ZIMS. This system is used for medical record documentation and zoological data collection for *ex situ* conservation efforts, including breeding programs and research. The software ZIMS was used to retrieve medical records for all free-ranging lemurs at the Duke Lemur Center, which is the largest sanctuary for prosimian primates in the world. Retrospective information pertaining to the health of free-ranging captive lemurs was collected from previously published research articles. The chronic diseases in the medical records were documented and compared among individuals and across species. The organ systems affected by the chronic diseases were also documented and compared.

**Results:** Over 10,000 medical records were analyzed for 13 species of free-ranging captive lemurs. Overall, the musculoskeletal, integumentary, and digestive systems were affected most frequently. Approximately 30% had musculoskeletal abnormalities, 29% had diseases of the digestive tract, and 18% had integumentary diseases. The organ system least affected was the lymphatic system, abnormal in only 2% of lemurs. In lemurs that were 20 years of age or older, 74% had 5 or more chronic diseases, 32% developed bilateral renal cysts and 21% obtained bilateral lenticular sclerosis. Only 17% of lemurs less than 20 years of age had 5 or more chronic diseases. When comparing males and females, 30% of both sexes had 5 or more chronic diseases. *Eulemur macaco* and *Eulemur rubriventer* were affected most with 100% of individuals with 5 or more chronic diseases, while *Eulemur rufus* had 60% affected, all of which are smaller species weighing 2 kg on average. *Lemur catta* had 0% and *Propithecus coquereli* had 23% chronically affected, both of which are larger lemur species weighing 4 kg or greater.

**Conclusions:** In this study, we examine the known chronic diseases found in free-ranging captive lemurs. Our results support the hypothesis that older lemurs would be affected more by chronic diseases. Our results also support the hypothesis that larger lemur species would be less affected by chronic diseases compared to the small species of lemurs. Since lemurs are more closely related to humans than mice, lemurs are a great model for human aging and disease research. Evaluating chronic diseases in free-ranging captive lemurs can be indicative of human and nonhuman primate diseases and aging.

# Mapping the Mad Dogs

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**Introduction**. Madagascar, an island off the southeastern coast of Africa, is best known for its immense biodiversity and unique collection of indigenous fauna. A majority of Malagasy wildlife is endangered due to the ever-growing effects of humankind on the ecosystem. Rises in human populations, effects of urbanization and agriculture, as well as the introduction of non-native carnivores such as dogs (*Canis familiaris*) are contributing to environmental decline. In a recent One Health effort to preserve wildlife and promote public health in Malagasy communities, research efforts have begun to address the impact of non-native carnivores like the dog on the ecosystem. As a developing nation, there are limited veterinary resources available. Most dogs go without ever receiving vaccinations, preventatives, or being desexed. As a result, "unhealthy" feral canid populations are spreading. These animals live in close association with humans and roam freely in protected forests coming in to frequent contact with lemurs, small mammals, chameleons, amphibians, and other endemic wildlife. However, little is known about the potential zoonotic implications of introduced carnivores on the health of humans and wildlife. Here, in collaboration with The Mad Dog Initiative (MDI), we investigated the health and medical profiles of both feral and domestic canid populations in Andasibe, Madagascar with specific focus on vector-borne zoonotic pathogens.

**Methods**. Male and female canids were presented for desexing and rabies vaccination. A physical exam was performed to gather basic vitals. As most patients were fear aggressive, sedation with intramuscular Ketamine and Dexdomitor was administered before the exam could take place. Patients were monitored closely and vitals were collected as soon as the patient could be safely handled. Photographs were taken of each individual, surgical procedures performed, rabies vaccines administered, and post-operative injections of Ivermectin and Metacam were given. Each patient also received the following blood work-up: 2mL to 3mL of blood was drawn from the jugular vein or accessory cephalic vein and placed in a 3ml EDTA tube. Heartworm rapid diagnostic tests were performed. Dried blood cards were prepared and stored for future molecular analysis. Blood smears were made, stained with quick-diff, and fixed with Permount. Microscopy was performed on all smears to evaluate cell morphology and to identify the presence of microfilaria as well as intracellular parasites. Fecal and ectoparasite samples were also collected for molecular analysis a part of a long-term collaborative study. All research was conducted under research and export permit #102/16MEEMF/SG/DGF/DCB/SAPT/SCBT and in keeping with IACUC regulations.

**Results**. A total of 38 canids were brought to MDI voluntarily by local villagers. Average body condition score observed was 2 of 5. Common concerns included malnutrition and heavy endoparasite and ectoparasite burdens. The prevalence of ectoparasites observed within the sample population were: fleas 87%, Ticks 5%, Lice 2%. All heartworm tests were negative. Evidence of anemia (anisocytosis and polychromasia) was frequently observed in erythrocytes. The detection of vector-borne zoonotic pathogens is currently being pursued.

**Conclusions**. A considerable amount of research still needs to be conducted to map the zoonotic threats vectored by canids in Andasibe. The observed dog population appears in questionable health. Because canine heartworm and other microfilards have been confirmed in other Malagasy villages, negative heartworm tests in Andasibe may suggest that currently there are not enough hosts for heartworm to thrive. However, the threat of heartworm remains a concern. With the high frequency of fleas, we hypothesize that molecular analysis may demonstrate a prevalence of flea vectored pathogens such as tapeworm (*Dipilydium caninum*), Typhus (*Rickettsia typhi*), *Acanthocheilonema reconditum*, and *Bartonella spp*.

# A Ketogenic Diet Induces Changes in Gene Expression of Hepatic Drug-Metabolizing Enzymes and Drug-Efflux Pumps in Rats

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**Introduction:** Variation in the expression of hepatic drug-metabolizing enzymes and drug-efflux pumps can alter therapeutic response to a variety of clinical drugs and lead to adverse drug interactions. It is known that diets, such as a western diet, induce changes in the levels of hepatic drug-metabolizing enzymes and drug-efflux pumps, leading to potential food-drug interactions. However, it is unknown whether a ketogenic diet induces such changes in the hepatic drug metabolism. Given the use of ketogenic diets for health benefits, such as weight loss, it is important to determine whether ketogenic diets have the potential to induce undesired food-drug interactions. Here, we studied whether a ketogenic diet affects gene expression of hepatic drug-metabolizing enzymes and drug-efflux pumps in rats. Additionally, we determined the effect of an exercise, alone or in combination with a ketogenic diet, on the hepatic drug-metabolizing enzymes and drug-efflux pumps.

**Methods:** Male Sprague-Dawley rats (~10 wk & ~300 g) were fed for 6-wk with isocaloric amounts of a ketogenic diet (KD) [20% protein, 10% carbohydrate, 70% fat], a western diet (WD) [15% protein, 43% carbohydrate, 42.0% fat] or a control standard chow diet (SC) [24.0% protein, 58.0% carbohydrate, 18% fat]. Each dietary group consisted of a wheel-running exercise (EX) and sedentary (SED) subgroups. qRT-PCR experiments were performed on the rat livers to determine gene expression of major drug-metabolizing enzymes (CYP3A1, CYP2B1 & UGT1A1) and drug-efflux pumps (MDR1 & MRP2). We also studied the expression of key xenobiotic receptors (PXR, CAR & AHR), which regulate the gene expression of the major drug-metabolizing enzymes & drug-efflux pumps.

**Results:** Feeding with the KD resulted in an increased gene expression of CYP3A1 and CYP2B1 in the EX rats, UGT1A1 in the SED rats, and MRP2 in the EX and SED rats. The exercise decreased the expression of CYP3A1 and CYP2B1 in the rats fed SC, while increasing the expression of CYP3A1 and MDR1 in the KD fed rats, and MRP2 in the SC and KD fed rats. Likewise, the KD and EX induced changes in the expression of PXR and AHR. Together, these preliminary results suggest that KD and EX alone or in combination induce changes in the expression of the hepatic drug-metabolizing enzymes/drug-efflux pumps/xenobiotic receptors.

**Conclusions:** The preliminary results are consistent with the conclusion that the KD/EX induce changes in gene expression of the major drug-metabolizing enzymes and drug-efflux pumps. These results caution the use of KD/EX in conjunction with medications metabolized/transported via CYP3A1, CYP2B1, UGT1A1, MDR1 or MRP2. Future studies will determine the effect of KD/EX on protein expression and function of the hepatic drug-metabolizing enzymes and drug-efflux pumps.

# Localization and Quantification of Cannabinoid Receptors in Canine Tissue

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**Introduction**. The endocannabinoid system (ECS) is comprised of endogenous signaling molecules known as endocannabinoids, and the G-protein-coupled receptors to which they bind. In recent decades, research has found relationships between the ECS and memory, nociception, inflammation, appetite, metabolism, and more.

In the interest of providing a framework for the eventual development of safe and effective cannabinoid therapies in veterinary species, this paper sought to characterize the two predominant cannabinoid receptors—CB1R and CB2R—by quantification and localization. While many findings from previous studies were confirmed, such as the high concentration of CB1R in gray matter and the high concentration of CB2R in blood and lymph nodes, the study also found unexpectedly high quantities of CB2R in both the male and female gonads. Additionally, unexpectedly low levels of CB2R expression were found in the lung and the liver compared to human and mouse models.

**Methods**. Tissue samples were acquired from living adult dogs that presented to the AU-SATH Surgery Department for procedures related to the tissues obtained. Following collection, the tissue samples were placed into either RNA*later*® or formalin. RNA*later*® samples were stored until processing. Formalin samples were submitted to histopathology for processing. In all, 35 tissue samples were collected in RNA*later*®, and 12 were collected in formalin.

RNA was extracted from tissues stored in RNA*later*®, converted to cDNA, and quantified using quantitative PCR (qPCR). The data were reported as ratios of *CB1R* or *CB2R* gene expression to the constitutively-expressed housekeeping gene *B2M*.

Immunohistochemistry was run using previously-described antibodies, and selective staining was verified by Western blot.

**Results**. Previously undescribed findings immunohistochemistry were limited; CB2 receptors stained most darkly in the endothelial cell membranes of most tissues, with less-significant staining scattered throughout the parenchyma, localized microscopically to the cell membranes.

Quantification showed high expression of *CB1R* gene in the blood, brain, testicles, ovary and uterus, but low expression in kidney, lung, liver and lymph node. Expression values for the *CB2R* gene have been somewhat limited but show high expression in blood and lymph nodes.

**Conclusions**. These results invite future investigation into the reproductive applications of cannabinoids, as well as possible upregulation following exogenous exposure to explain the relative low expression of *CB1R* in liver and lung tissues. Quantification of specific regions of the canine brain may also prove useful in the development of pharmaceutical cannabinoids.

**Therapeutic benefit of a turmeric extract in a nutrionally and oxidatively induced rat model of non-alcoholic steatohepatitis.**

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**Introduction:** We sought to determine the prophylactic and therapeutic effects of a turmeric extract on liver pathology and serum biomarkers in a rat model of non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH).

**Methods:** For Aim I, twenty-four female Wistar rats were randomly assigned to an 8-wk Western Diet (8WD) or 8-wk WD with 0.2% turmeric extract (8WDT) to evaluate the prophylactic efficacy of a turmeric extract in the development of NAFLD/NASH.  For Aim II, twenty-four female Wistar rats were randomly assigned to 12-wk WD (12WD) or 8-wk WD followed by 4-wk WDT (8WD4T) to evaluate the therapeutic efficacy of the same turmeric extract. 15% fructose drinking water was provided and rats received intraperitoneal $CCl_4$ injections at weeks 1, 3, 5, and 7. The turmeric extract (BCM-95, DolCas Biotech, Landing, NJ USA) consisted of 86% curcuminoids and 7-9% essential oils. Rats were euthanized at the end of weeks 8 or 12 and characteristics, humoral and hepatic biomarkers and hepatic pathology assessed.

**Results:** Relative to Aim I, lobular inflammation scores were reduced in the 8WDT group compared the 8WD group (-49.6%, $P=0.008$), but hepatic steatosis, hepatocyte ballooning, NAFLD activity (NAS) and fibrosis scores were not different ($P>0.05$). Serum panels revealed lower aspartate transaminase (AST; -25.4%, $P=0.035$) in the 8WDT group, though alkaline phosphatase (ALP) values were not different ($P>0.05$). Finally, serum chemokine/cytokine assessment revealed higher IL-2 (+80.0%, $P=0.020$) and IL-13 (+83.2%, $P=0.038$) concentrations in 8WDT rats.  Relative to Aim II, hepatic steatosis (-24.1%, $P=0.050$), lobular inflammation (-38.9%, $P=0.003$) and the NAS (-25.8%, $P=0.004$) were reduced in 8WD4T rats compared to 12WD rats. However, hepatocyte ballooning and hepatic fibrosis scores were not different between groups ($P>0.05$). Serum blood panels indicated lower ALP (-26.9%, $P=0.002$) and AST (-29.8%, $P=0.005$) values in 8WD12T rats. Moreover, in 8WD12T rats serum fractilkine, IL-13, 1L-17A and IL-2 chemokines/cytokines were higher (+80.6-191.7%; $P<0.05$) while RANTES was lower (-22.1%, $P=0.016$).

**Conclusion:** Collectively, lobular inflammation scores and IL-13 cytokine concentrations support an anti-inflammatory effect of turmeric in this rat model of NAFLD/NASH. Moreover, reduced AST levels in both turmeric extract supplemented groups suggests a positive effect on liver health. This is further supported by reduced ALP and hepatic steatosis and NAS scores in the 8WD4T rats. Interestingly, pathology and liver function tests were not improved to the same degree in the preventative aim of the study compared to the treatment aim.  We posit that the $CCl_4$, due to oxidative potency, may have 'masked' potential benefits in the 8WDT rats. Further studies should characterize the prophylactic value of turmeric extract in a more physiologically relevant model of NAFLD/NASH (e.g. metabolically induced)

# Utilizing serum antibody levels in post vaccinated piglets to evaluate maternal antibody interference and vaccination compliance

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**Introduction.** Understanding maternally derived antibody (MDA) levels in peri-weaned pigs is critical in determining appropriate vaccination timing within the herd. This information is valuable, as MDA show to be a better indicator than age for vaccination interference. Measuring MDA levels is especially important in herds that utilize pre-farrowing vaccination in sows for determining when levels are low enough not to interfere. The study objective was to evaluate serological responses to a porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae* (MHP) combination vaccine in weaned pigs following two, one mL injections at three and six weeks of age (WOA). In addition, this study analyzed if MDA levels at the time of the first vaccination influenced post-vaccination antibody levels. The overall purpose was to determine if post-vaccination serology results are useful in determining if pigs received both vaccinations.

**Methods.** Comparison of maternally derived antibodies from eight sow farms determined site inclusion. Four sites administered a pre-breeding vaccination (PBV) while four had no pre-breeding vaccination (N-PBV) protocol. Based on MDA, one sow farm from each category were selected for the study. Thirty litters from each chosen sow farm were selected and categorized by parity (P) into one of three groups of 10:P1, P2-3, and P4+. Blood sample collection and vaccination was completed one day before weaning and prior to when the farm staff routinely vaccinate litters. Within each litter, one of the biggest pigs was selected for blood sample collection and tagging, followed by administration of one mL of vaccine to the full litter. After weaning, the pigs from each sow farm were placed at two separate nurseries. At three weeks of placement in the nursery (six WOA), a second one mL vaccination was given. Three weeks following the second vaccination (nine WOA), a blood sample was collected for comparison and analysis.

**Results.** The initial sow farms selected based on their MDA levels showed the following antibody titer results (>= 1280 cut-off for significant levels): 3 of 30 (geo-mean 100.8) and 28 of 30 (geo-mean 3880.2) for the N-PBV and PBV groups, respectively. S/P ratios for MHP were assessed and yielded 13 of 30 samples positive (0.428 avg.) for the N-PBV group and 24 of 30 samples positive for the PBV group (0.918 avg.). Following both vaccination events, serology yielded the following results at nine WOA for PCV2 (>= 1280 cut-off for showing response to vaccine): N-PBV 19 of 21 responded, 2 did not; PBV 7 of 30 responded, 7 did not, 16 were unknown. Unknown was categorized for being above the threshold for response, yet the same or higher than titers at weaning, which could be persisting MDAs. For MHP results on serology at nine WOA, S/P ratios showing response (>0.400) were as follows: N-PBV group 18 of 21 responded; PBV group 25 of 30 responded.

**Conclusions.** These results indicated MDA interference for the PCV2 vaccination while MHP vaccination was able to induce an antibody response despite high levels of MDAs at weaning. This is significant when considering timing and monitoring of both PCV2 and MHP vaccination. Also, these results indicate that post vaccination monitoring is better accomplished through measuring antibodies, as it is difficult to monitor post vaccination antibodies for PCV2 when pigs have high levels of MDA at the time of vaccination.

# Faculty Platform Presentations

**High resolution computed tomography evaluation of BV, AV, and BA ratios in T. cati infected cats**

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### Introduction
*Toxocara cati* infection results in lung pathology which is evident on pulmonary computed tomography (CT). Bronchial to vertebral artery (BV) and pulmonary artery to vertebral body (AV) ratios more accurately detect disease of the bronchi and pulmonary arteries than the previously described bronchial to pulmonary artery (BA) ratio. The use of BV and AV ratios to characterize *T. cati* infection in cats with and without preventative treatment was evaluated, and the performance of the BV, AV, and BA ratios in *T. cati* diseased lung was compared.

### Methods
Archived CT images from 4 groups of cats from baseline (D0) and day 64 post-infection (D64) were reviewed, and BV, AV, and BA ratios were calculated. Groups (n=6 per group) included: prepubertal, untreated, infected (UI); adult, untreated, infected (AUI); prepubertal, treated, infected (IT); prepubertal, uninfected, untreated control (UU). Treated cats were pre-treated with topical moxidectin and imidacloprid monthly, initiated two months prior to infection. Ratios and % change from baseline were compared for each lung lobe using a Kruskal-Wallis ANOVA on ranks with pairwise multiple comparison analysis by Dunn's method.

### Results
BV ratios were significantly different between the AUI and UU groups in the right cranial (P=0.003), left cranial (cranial) (P=0.002), right middle (P=0.002), and left cranial (caudal) (P=<0.001) lobes and between the IT and UU groups in the right middle (P=0.002) and left cranial (caudal lobes) (P=<0.001). AV ratios were significantly different between the AUI and UU groups and between the IU and UU groups in the right middle (P=0.001) and left cranial (caudal) (P=0.003) lobes. Significant differences in BA ratios were only noted between the AUI and UU groups in the right (P=0.017) and left cranial (cranial) (P=0.006) lobes. Percent change from baseline was significantly different for BV ratios between AUI and UU groups in the right cranial (P=0.002), left cranial (cranial) (P=0.008), and left cranial (caudal) (P=0.009) lobes and between IT and UU in the left cranial (caudal) (P=0.009) lobe. Percent change from baseline of AV ratios was significantly different when comparing all infected groups (UI, AUI, IT) and the UU group in the right middle lobe (P=0.002) and between the AUI & UU groups and UI & UU groups in the left cranial (caudal) (P=0.003) lobe. No differences were noted in BV, AV or BA ratios between infected groups in any lobes. Abnormal ratios were more frequently noted in the pulmonary arteries. Changes in BV and AV ratios were more frequent in the right middle and left cranial (caudal) lobes.

### Conclusions
Bronchial and pulmonary artery changes are seen in cats infected with *T. cati*; however, pulmonary artery changes are more pronounced. BV and AV ratios suggested a heavier distribution in the right middle and left cranial (caudal) lobes. Pretreatment with moxidectin and imidacloprid does not prevent bronchial and pulmonary artery changes in cats infected with T. cati per os.

**Enhancing Veterinary Student Learning with Mulitmedia 3D Models: a Pilot Study**

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**Introduction**. Veterinary student education is currently limited by the lack of available technology to enhance spatial understanding of anatomy and disease processes in patients. The aims of this study were to determine whether 3D modeling in different media formats (video and still images) resulted in improved student ability to answer questions regarding anatomy and neurological disease.

**Methods**. Twenty questions (multiple choice and true false) were provided with either a 2D image, 3D image, or 3D video to view prior to answering the question. Image manipulation software (Osirix$^{®}$) was utilized to create the 3D images and videos. Students (n=8) took the 20 question quiz over 25 minutes. For each question, a percentage of correct answers variable (Percent Correct) was calculated. Statistical analysis was performed with a Shapiro-Wilk test and identified a normal distribution for Percent Correct, and mean +/- SD was reported.

**Results**. The Percent Correct for questions which utilized 2D images was 60.1% +/- 36.1%. The Percent Correct for questions which utilized 3D still images was 65.7% +/- 16.3%. The Percent Correct for questions which utilized 3D video images was 59.5% +/- 16.7%. An ANOVA revealed that there was no difference in Percent Correct between these groups (p=0.918).

**Conclusions**. Additional evaluation using more students is warranted to determine whether 3D models aid in student learning.

# Faculty Poster Presentations

**Comparison of Serum microRNA Between Dogs with and without Canine Mammary Carcinoma by Deep Sequencing and Comparison to Histopathologic Characteristics**

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**Introduction:** Canine mammary carcinoma prognosis depends on factors such as histologic subtype, grade, and presence of lymphatic invasion, which require invasive biopsies. Serum microRNA (miRNA/miRs) have generated interest as minimally invasive cancer biomarkers. Objective: To compare the serum microRNA profile between dogs with and without mammary carcinoma, and correlate expression of specific miRs to histologic subtypes and grades.

**Methods:** RNA was extracted from serum and submitted for miRNA deep-sequencing (Illumina HiSeq2500). Tumor biopsy samples were blindly assessed for histologic subtype, grade, and presence of lymphatic invasion. Bioinformatic and univariate statistical analyses comparing miRNA between groups were performed.

**Results:** 10 healthy females (5 spayed, 5 intact) and 10 female dogs with mammary carcinoma were included. Mammary carcinoma samples varied by histologic subtype and grade (n=4 Grade I; n=3 Grade II; n=3 Grade III). 6/10 had lymphatic invasion. 452 unique miRNA were identified in the serum deep-sequencing dataset. Comparing the neoplastic group to controls, 65 serum miRs were significantly upregulated >1.5-fold, while 12 miRs were significantly down-regulated >1.5-fold. A signature of upregulated miRNA predicted to regulate key hormone and tumor suppressor pathways (previously identified in canine mammary tumor cell culture exosomes) was identified, including miR-18a, miR-19b, miR-29b/c, miR-34c, miR-181c, miR-215 and miR-345. Of these, serum miR-18a was significantly higher in dogs with lymphatic invasion (p=0.0141) and Grade III tumors (p=0.0255).

**Conclusion:** Results suggest serum microRNA are differentially expressed in dogs with mammary carcinoma. Expression of serum miR-18a was significantly higher in dogs with tumor lymphatic invasion and Grade III mammary carcinomas

# Mineral analysis of Florida beef cattle diagnosed with ovarian follicular dysplasia

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**Introduction:** Ovarian Follicular Dysplasia (OFD) is a slowly progressive bilateral abnormal growth and/or development of ovarian follicles eventually transforming into Sertoli-form Granulosa Cell Tumor. Cattle with OFD are often infertile leading to culling from the herd. The leading cause of infertility in Florida beef cows is OFD. Definitive diagnosis of OFD is made post-mortem. The purpose of this study was to further describe OFD through mineral analysis in order to lay the groundwork for ante-mortem identification of OFD. An ante-mortem diagnostic test would dramatically improve reproductive efficiency by early detection and culling of OFD affected cows before investing in these infertile animals. Mineral analysis may also provide evidence of inciting factors surrounding the development of OFD

**Methods:** Twenty-eight cows and heifers with subfertility and two "control" females from two Florida beef herds were sampled. A total of 30 Florida beef cows and heifers were followed to slaughter. Ovary, uterus, oviduct and liver were collected post-mortem and graded for presence of OFD(0–IV) and other diseases. The liver samples were sent to Kentucky State Diagnostic Laboratory for micro- and macro-mineral analysis.

**Results:** Histological analysis of the ovarian tissues from these cattle indicated that OFD was diagnosed in 10/15 (66.7%) heifers and 14/15 (93%) cows in the first and second ranches, respectively. At the first ranch 10 animals had grade I OFD and at the second ranch seven were grade I, five were grade II and the two were grade III. Additionally, sodium, arsenic, cobalt, cadmium and barium were all found to be positively associated with increasing severity of OFD. Grade III OFD cattle had significantly higher concentrations of sodium, arsenic, cadmium, and barium in relation to non-OFD cattle, and grade I OFD cattle. Cobalt, cadmium and barium levels were significantly increased in livers of grade II OFD cattle as well. However, copper and lead levels in the liver were negatively associated with the severity of the OFD. The grade II and III OFD cattle had significantly lower quantities of copper and lead when compared to that of the non-OFD cattle. There were no significant differences in any of the remaining mineral analysis.

**Conclusions:** Of the ranches sampled, 86% of the sub-fertile cattle were OFD positive. Levels of sodium, cadmium, copper, and barium have potential for diagnostic value in identifying cows affected with OFD. There is a correlation between specific liver mineral concentrations in OFD cattle, but it is unknown if this is a cause or an effect of OFD.

# Graduate Student Poster Presentations

**Pharmacokinetics of fenbendazole in canine CSF and plasma: A pilot study**

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**Introduction**. Benzimidazoles are among the drugs demonstrated to have *in vitro* efficacy against gliomas due inhibition of tubulin polymerization and disruption of microtubule formation. Studies have demonstrated fenbendazole has *in vitro* anti-tubulin effects in canine glioblastoma cells at a mean $IC_{50}$ (MIC) of 150 ng/ml. However, before fenbendazole is used to treat gliomas, a dose necessary to achieve detectable concentrations in CSF needs to be determined for dogs. The specific aims of our proposed study were to describe the time course of fenbendazole in canine plasma and CSF after oral administration, and to determine an oral dose of fenbendazole necessary to achieve potentially effective concentrations (150 ng/ml).

**Methods**. High performance liquid chromatography was used to detect fenbendzole in canine plasma and CSF.

**Results**. Fenbendazole was administered to two dogs at 50mg/kg, but was below the limit of detection in both plasma and CSF. Subsequently two dogs were administered fenbendazole at 100mg/kg and 200mg/kg (respectively). Although fenbendazole concentrations appear to be dose dependent in plasma (compared to 50 mg/kg), CSF concentrations were comparatively low and inconsistent.

**Conclusions**. The standard dose of 50mg/kg is insufficient to achieve detectable concentrations in canine CSF and plasma. Although higher doses of 100mg/kg or 200mg/kg yield detectable CSF concentrations, their relevance to efficacy for treatment of gliomas is unknown.

**Synopsis of Broiler and Broiler Breeder Diseases Diagnosed at Alabama State Diagnostic Facilities, 2016-2017**

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**Introduction:**  In April 2016 to March 2017, a total of 480 poultry cases were submitted to the Alabama Veterinary Diagnostic Labs (AVDL) from 6 commercial broiler companies in Alabama. An overview of the major diseases that were diagnosed from these commercial broiler (290) and broiler breeder (190) cases have been presented here.

**Methods:** All cases were necropsied according to the standard operating procedures developed at AVDL. In every case, a complete necropsy was performed, visible gross findings were identified, and samples were collected for further laboratory analysis. Collected tissues, blood, and swab samples were analyzed through bacterial culture, ELISA, PCR, cell culture, and histopathology.

**Results:** One-day to 9-weeks old broilers were submitted with a history of high flock mortality. However, 1-day broiler chicks were also submitted (30 submissions) for routine microbial and histological analysis. The lesions and/or pathogens identified in these 1-day-old chicks were - dermatitis (18 cases*), Aspergillus fumigatus* (21), yolk sacculitis (1), omphalitis (1), nephropathy (1), *E. coli* (1), *Salmonella* Enteritidis group D (1), and *Enterococcus fecalis* (3). Important diseases or lesions diagnosed in broiler necropsies were colisepticemia (49), necrotic enteritis and coccidiosis (17), bursal lesions (37), infectious bronchitis (41), infectious laryngotracheitis (75), salmonellosis (2), reoviral arthritis (20), and infectious coryza (4). Major diseases diagnosed from broiler breeders were yok peritonitis (32 cases), bacterial synovitis (20), Histomoniasis (14), Coccidiosis (13), reoviral tenosynovitis (11), fowl cholera (6), yolk pneumonia (6), infectious coryza (2), Pox (2), and mycotoxicosis (1).

**Conclusions:** The distribution of disease does not reflect the actual incidence and is influenced by submission bias of owners. Dermatitis in 1-day old chicks was observed only microscopically and possibly developed from vaccine reaction. Although *A. fumigatus* often cultured from the lungs of 1-day old chicks, gross and microscopic lesions were rare. Mixed infections were identified in many cases especially in broiler breeders. Mortality was associated with secondary bacterial infection influenced by immune suppression in broilers and reproductive status in broiler breeders.

**Evaluating Cepalexin MIC to Cephalotin in *Escherichia coli***

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**Introduction.** *Escherichia coli* is a very common veterinary pathogen. Cephalexin is among the drugs used to treat *E. coli.* However, another first generation cephalosporine, cephalothin is used as the standard when determining Minimum Inhibitory Concentration (MIC) of first generation cephalosporins, including cephalexin. No study has demonstrated the accuracy of cephalothin when predicting *E. coli* susceptibilitly to cephalexin. For cephalothin the breakpoints are $\leq$8 ug/ml is Susceptible where 16 ug/ml is Intermediate. The Resistant breakpoint is $\geq$32 ug/ml. The purpose of this study was to determine the cephalexin MIC of *Escherichia coli* isolates and compare those to the MIC of cephalothin.

**Methods.** *Escherichia coli* strains were obtained from the Clinical Pharmacology Laboratory at the Veterinary Teaching Hospital at Auburn University. Hundreds of *Escherichia coli* strains were collected by the Clinical Pharmacology Laboratory and frozen at -80ºC, for this study 100 strains were randomly selected for the MICs to be determined. Protocols established by the Clinical Laboratory Standards Institute were followed. Previously frozen strains were grown out using TA, Tetrazolium and Arabinose, these strains were TB broth and compared to a 0.5 McFarland Standard to test turbidity. Using a micropiptter, 100 ul of normal TB broth was placed into a 96-well plate and then 100 ul of the *Escherichia coli* were also placed into the wells. Then varying amounts of the three drugs, cephalothin, cephalexin and cefazolin, were placed into the wells and incubated at 37ºC for 18 hours. The following day the wells were read to determine the MIC of the *Escherichia coli* strains of the different drugs.

**Results.** The MICs of the *Escherichia coli* strains were found to be comparable between the two drugs, cephalothin and cephalexin. The MIC50, or the MIC where fifty percent of the isolates are susceptible, of the one hundred strains that were tested was found to be 8ug/ml and the MIC90 was 16ug/ml.

**Conclusions.** Cephalexin was found to have comparable MICs to cephalothin in regards to this study. Due to this, it would be determined that cephalothin was a good indicator for cephalexin per CLSI guidelines.

**High Performance Liquid Chromatography (HPLC) method development for Fenbendazole in canine plasma and Cerebrospinal fluid (CSF).**

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**Introduction**. Fenbendazole is among the benzimadazole parasiticides that have been demonstrated *in vitro* and in rodent models to have anticancer properties. Among the potential targets are gliomas. This study describes the development of a specific reverse phase high performance liquid chromatographic (HPLC) with UV detection method for the identification and quantification of Fenbendazole in canine plasma and cerebrospinal fluid (CSF).

**Methods**. Based on previous studies about the Fenbendazole (FBZ), and considering the chemical structure, physical properties, sample type (canine plasma and CSF), sample size, level of accuracy and precision expected and the method analysis High Performance Liquid Chromatography (HPLC-UV). We set up 3 different methods to develop the optimal chromatographic conditions for identification and quantification of Fenbendazole, 3 different chromatographic columns, 3 buffers (potassium phosphate ammonium acetate and ammonium formate), pH, flow, detection (254 nm, 295 nm), then we worked with the sample preparation procedure to clean the sample (remove the matrix) and extract the Fenbendazole to be analyzed using precipitation, and drying under nitrogen to concentrate the sample. With the optimal conditions found up to date the next step will be the method validation to get a robust quantification in terms of accuracy and precision.

**Results**. The optimal chromatographic conditions found for Fenbendazole in plasma and CSF were: separation was done with a Spherisorb C6, 5 um, 250 x 4.6 mm column, using 45:55 (v/v) ammonium formate, pH 3 : methanol mobile phase, flow rate of 1.0 mL/min, detection at 295 nm. For the sample extraction acetonitrile was used for plasma and CSF canine protein precipitation to remove the matrix, follow by drying under a current of nitrogen to concentrate the sample. The sample was reconstituted with mobile phase, and finally a 100 μL injection volume for plasma samples and 150 μL for CSF samples was injected into the HPLC respectively.

**Conclusions**. For this specific reverse phase high performance liquid chromatographic (HPLC) with UV detection method, the lower limit of detection (LOD) for canine plasma and canine CSF samples were 10 ng/mL and 5 ng/mL respectively. The lower limit of quantification (LOQ) for canine plasma and canine CSF samples were 15 ng/mL and 10 ng/mL respectively.

The main objective for now was to develop the HPLC method, finding the optimal conditions that will allow us to validate the method.

# Prebiotic Prevents Disruption of Gut Barrier Integrity

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**Introduction**. Stressors, such as temperature, can affect an organism's health. Exposure to high temperatures can lead to certain pathological conditions as heat stress can alter the morphology of the gut and, as a result, causes the loss of the gut barrier integrity. The gut integrity controls the translocation of the luminal antigens (bacteria and endotoxins) into the circulation. Increased translocation leads to systemic inflammation, which could result in tissue injury, multi-organ dysfunction, and death by causing systemic stimulation of inflammatory responses. The increased permeability could be a result of a change in expression of tight junction proteins, which are responsible for keeping the integrity of intestinal epithelial cells. There is strong evidence, which suggests that stress compromises the gut microbiota and, as a result, decreases the immune capacity of the organism. Special diets and probiotics, among others, are different approaches to counter the adverse effects of heat stress. However, there is no data regarding the efficacy of prebiotics in the mitigation of stress-induced complications. The main aim of this study was to assess the efficacy of a yeast (*Saccharomyces cerevisiae*) fermentate (EH), having prebiotic activity, in the prevention of heat stress-related complications.

**Methods**. Male Sprague–Dawley rats were used to evaluate the protective effects of the prebiotic compound EH during heat stress. Animals were treated by oral gavage with EH or PBS once daily for 14 days. On the 15th day, half of the rats of each group were exposed to heat stress conditions ($45^\circ$ C, relative humidity 55% for 25 min), while the other half of the rats remained at room temperature. Histological changes in the small intestine (villi height, total mucosal thickness, number of goblet and Paneth cells), serum lipopolysaccharide levels (LPS), and tight junction protein expression were analyzed and compared between groups.

**Results**. The results indicate that there was a significant decrease in the expression of tight junction proteins in the animals, which received PBS before exposure to heat stress. Expression of tight junction proteins in heat-stressed rats administered with PBS showed significant reduction in expression in comparison with rats that were pretreated with EH. There was also a significant reduction of the small intestinal villi height and total mucosal thickness in the heat stressed rats pre-treated with PBS. Goblet and the Paneth cell counts were also significantly decreased in this group of rats. LPS levels significantly increased in the rats that exhibited a reduction in the tight junction protein expression. Treatment of EH before heat stress conditions prevented the damaging effect of heat stress on intestinal morphology.

**Conclusions**. Administration of EH before heat stress conditions was effective in preventing heat stress-related complications, such as changes in the expression of tight junction proteins, morphological changes, and increases in serum LPS levels.

# Antimicrobial activity of rifampin in *Staphylococcus intermedius* group in the dog

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**Introduction.** Methicillin-resistant staphylococcal pyoderma in dogs, particularly *Staphylococcus pseudintermedius* is increasing in prevalence (Eckholm et al. 2013, Bryan et al. 2012, van Duijkeren et al. 2011). Emerging resistance has led to increased use of high-tier antibiotics, such as rifampin, in veterinary medicine. Although rifampin exhibits bactericidal activity against *Staphylococcus* sp. in humans this has not been studied in dogs. This study's hypothesis and objectives are to determine if rifampin exhibits bactericidal versus bacteriostatic activity against canine *Staphylococcus intermedius* Group (SIG) organisms, and to determine if it is a concentration versus time-dependent antibiotic for this organism.

**Methods.** Two methicillin-susceptible SIG isolates with a minimum inhibitory concentration (MIC) of 0.003 µg/ml and 0.008 µg/ml and two methicillin-resistant SIG isolates with a MIC of 0.003 µg/ml and 0.012 µg/ml were used for the time-kill studies. Liquid agar (Mueller Hinton Broth) was supplemented with rifampin at 0, 0.5, 1, 2, 4, 8, 16, and 32 times the MIC of the respective isolate. Each solution was vortexed gently for five seconds and incubated at 30°C. Collection time points occurred at 0, 2, 4, 10, 16, and 24 hours. The number of viable colony forming units (CFU) in each sample was determined using the BacTiter-Glo™ Microbial Cell Viability Luciferase for each time point.

**Results.** Rifampin activity against all four SIG isolates was consistent with time-dependent response because the magnitude of decrease in CFU was unchanged regardless of antimicrobial concentration. All four SIG isolates exhibited bactericidal activity because there was greater than three logarithmic$_{10}$ reductions in CFU/ml at 24 hours when compared to the starting inoculum.

**Conclusions.** This study describes the activity of rifampin against SIG in dogs as a time-dependent and bactericidal antibiotic.

# Canine mammary tumor susceptibility: studying purebred pedigrees and whole genomes for inherited risk factors

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**Introduction**. Canines and humans are genetic homologs, and due to limited intra-breed heterogeneity from canine breeding practices, canine samples provide a beneficial tool in identifying genetic markers for diseases and serve as an excellent genetic model for human diseases. Few genetic research studies have focused on identifying the inherited risk factors for canine mammary tumor (CMT), and as such, there is great potential to discover overlap between known and yet-to-be-discovered human and canine breast cancer susceptibility genes. With ~70% of human hereditary breast cancer cases currently genetically unsolved, CMT susceptibility could aid in new discoveries applicable to both species. This study attempts to identify genetic overlaps between hereditary breast cancer cases and hereditary CMT cases.

**Methods**. DNA samples from 85 CMT affected canines (3 males and 82 females) were extracted from blood/buccal swabs. This cohort includes 32 different canine breeds representing six of the seven American Kennel Club (AKC) recognized groups. The most represented breeds are Golden Retrievers (n=20), Siberian Huskies (n=8), and Standard Schnauzers (n=7). Pedigree analyses were carried out to build breed-specific pedigrees and identify common ancestors. Upon pedigree analysis, 14 samples were chosen for whole genome sequencing (WGS) from four different pedigrees, including Golden Retriever (n=5), Siberian Husky (n=3), Standard Schnauzer (n=3) and Dalmatian (n=3). After WGS, orthologs of human breast cancer susceptibility genes were initially investigated to detect potential risk variants in our CMT pedigrees.

**Results**. Pedigree analyses identified twelve different canine pedigrees corresponding to twelve different breeds and representing 72% of the CMT cohort.
The most impressive pedigrees comprise 20/20 Golden Retrievers, 7/8 Siberian Huskies, 7/7 Standard Schnauzers, 5/5 Welsh Springer Spaniels, 4/4 Newfoundlands, 4/4 Golden Setters, 3/3 Dalmatians, and 3/3 Doberman Pinschers. WGS analysis of orthologs of human breast cancer susceptibility genes has identified a number of coding variants, some of which are predicted to be pathogenic. The pathogenic variants appear to only segregate in certain branches of the pedigrees.

**Conclusions**. The CMT pedigrees composed through pedigree analysis highlight the characteristics of hereditary disease and exhibit promise to identify breed or kennel specific mutations. Our preliminary WGS results suggest CMT risk variants have been identified in orthologs of human breast cancer susceptibility genes. Currently, we are validating these variants through PCR and Sanger sequencing and carrying out variant segregation analysis using all CMT samples from the affected pedigree. This study can serve to prove the viability of using purebred canines with CMT as a model for hereditary breast cancer and improve studies with breast cancer research.

# Phage-GnRH constructs for population control of feral animals: evaluation in cats

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**Introduction.** The overpopulation of cats is a problem in the United States and a growing concern worldwide. Feral cats are a public health concern due to their ability to act as a reservoir for a number of zoonotic infectious diseases. They are also a problem because they prey on wildlife species, such as endangered or threatened birds, mammals, and amphibians. The focus of our research is development of anti-fertility vaccines composed of whole phage particles carrying peptides with contraceptive properties for use in feral animals. The vaccines are designed to trigger antibody production against gonadotropin releasing hormone (GnRH). The antibodies inactivate GnRH, causing reduced release of gonadotropic hormones and gonadal atrophy. Phage-GnRH constructs with potential contraceptive properties were generated via selection from a phage display library. When tested in mice, these constructs invoked the production of antibodies against GnRH and suppressed serum testosterone. The goal of this study is to evaluate the potential of these vaccines in cats.

**Methods.** Five sexually mature male cats were characterized as to their reproductive parameters and then immunized with a phage-GnRH vaccine. Anti-GnRH antibodies and testosterone in serum, testicular volume by ultrasound, and quality and quantity of sperm were evaluated monthly during a 7-month period following immunization.

**Results.** All cats developed anti-GnRH antibodies of varying levels following immunization. Serum testosterone was suppressed in four cats at three time points post-immunization. The total testicular volume decreased in four cats by a range of 22-42%. All five cats produced sperm at month seven with a 14-38% decrease in normal sperm cells, indicating potential gonadal atrophy.

**Conclusions.** This study demonstrated the potential of phage-GnRH vaccines for immunocontraception of cats. This study is ongoing.

**In Vitro Evaluation of the Mechanical and Physical Properties of the Forwarder Knot exposed to Fluid Media Using Large Gauge Suture.**

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**Introduction**. Suture material, pattern, and knot configuration are important factors affecting strength of ventral midline celiotomy closure in horses. The knot is the weakest part of the suture-celiotomy construct, with 71-100% of constructs failing adjacent to the knot in an ex vivo study. Standard closure is performed with a simple continuous pattern using a surgeons' knot at both the start and end of the suture line. Self-locking knots have been shown to have higher knot holding capacity (KHC), higher relative knot security (RKS) and lower knot weight and volume compared to surgeons' knots. When used to close a continuous suture line, a forwarder knot is used to start and an Aberdeen knot is used to end. The Aberdeen knot has been tested under dry and wet (media) conditions in vitro, however the forwarder knot has only been tested under dry conditions. The objective of this study was to evaluate the effect of dry and wet conditions on the KHC, RKS, knot weight and knot volume of the forwarder knot compared to the surgeon's knot. We hypothesized that wet forwarder knots would have a higher KHC and RKS and lower weight and volume than wet surgeons' knots. In addition, we hypothesized that wet forwarder knots would have significantly higher weight and volume than dry forwarder knots.

**Methods**. Forwarder knots were tested linearly on a universal testing machine. Strands of 3 USP polyglactin 910 suture material were soaked in media for 15 minutes prior to testing, control suture remained dry. Forwarder knots were completed with 2, 3 and 4 throws with a total of 10 knots/test group. KHC was calculated in Newton's (N) and RKS was determined as a percentage of KHC. An additional 5 knots from each group were used to assess knot weight and volume. The KHC, RKS, weight and volume of these knots were compared to data previously collected at our laboratory for a surgeon's knots. Normality of data was determined by an Anderson-Darling test. Parametric testing was performed for normally distributed data using separate analyses of variance, followed by Tukey's post hoc analysis for those comparisons with significant differences, to evaluate the mean difference in KHC and RKS between number of throws, media exposure, and type of knot. Significance was set at $P<.05$

**Results**. The wet forwarder knot had significantly higher KHC and RKS and significantly lower weight and volume compared to the wet surgeons' knot ($P=<0.0001$). There were no significant differences in KHC, RKS, weight and volume between wet and dry forwarder knots (RKS and KHC $P=0.09$, weight $P=0.94$, volume $P=1.00$). The strongest knot was the wet forwarder knot completed with 2 throws (RKS 94.14%).

**Conclusions**. Exposure to media did not significantly alter the mechanical and physical properties of the forwarder knot. Forwarder knots had a high RKS (94.14%) compared to the surgeon's knot (59.6%). Based on our results the forwarder knot is a superior knot to start a continuous suture line compared to a surgeon's knot using 3 USP polyglactin 910 suture. Further in vivo testing is required prior to clinical use in ventral midline celiotomy closure.

# Hypothalamic paraventricular nucleus AngII-mediated microglial activation through AT1r-TLR4 crosstalk in neurogenic hypertension

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**Introduction**. Neurogenic hypertension is characterized by pathologic sympathetic outflow from cardioregulatory centers of the brain. Within these centers, such as the hypothalamic paraventricular nucleus (PVN), angiotensin II (AngII) disrupts physiologic humoral responses and mediates inflammation. Studies consistently show an association among dysregulation of AngII and enhanced production of pro-inflammatory cytokines within the PVN, supporting the role of AngII as a modulator of sympathoexcitation. To date, the exact mechanism by which AngII signals cellular and/or molecular targets within cardioregulatory nuclei is undetermined. It has recently been demonstrated that toll-like receptor 4 (TLR4), expressed by microglial cells, interacts with AngII type-I (AT1r) receptors to mediate microglial activation in an AngII-dependent manner within the PVN of normotensive rats (PMID26637556). It is therefore our goal to elucidate whether similar AngII-dependent TLR4 signaling of microglial cells within the PVN contributes to the maintenance of neurogenic hypertension.

**Methods**. Spontaneously hypertensive rats (SHR-Los) were treated by oral gavage with Losartan (AT1r antagonist; 20mg/kg/day for 4 weeks) or vehicle (SHR). Age-matched Wistar Kyoto (WKY) rats were used as control. Tail-cuff blood pressure measurements were taken once a week over the treatment period. Real time PCR was performed TLR4 gene expression in isolated PVN microglia. Immunohistochemistry using anti-TLR4, anti-IBA1, and anti-VP, followed by density analysis using ImageJ was conducted for protein expression quantification. Data is presented as mean$\pm$SD at a significance level of $\alpha = 0.05$.

**Results**. After 4 weeks, SHR mean arterial pressure (MAP) was significantly greater than that of WKY (146.9$\pm$3.19 vs 100$\pm$3.08mmHg). Losartan treatment significantly reduced MAP compared to SHR (112$\pm$3.19 mmHg). Real time PCR analysis showed TLR4 mRNA expression in isolated PVN microglial cells to be 2.56-times greater in SHR, as compared to WKY. Immunohistochemistry quantification in PVN slices showed TLR4 protein density to be increased in SHR compared to control WKY (1.81$\pm$0.02 vs 1.31$\pm$0.11 AU) as well as increased density of IBA1 protein (23.05$\pm$3.03 vs 13.68$\pm$0.6 AU). Co-localization of TLR4 and IBA1 was also significantly higher in SHR compared to WKY (66$\pm$0.9 vs 1.32$\pm$0.2 AU). SHR-losartan protein density was reduced as compared to SHR (TLR4 density: 1.30$\pm$0.05; IBA1 density: 14.9$\pm$0.92 AU).

**Conclusions**. Blocking AT1r ameliorated the elevated TLR4 expression seen in hypertensive SHR, as well as the extent of microglial activation (indicated by IBA1 density), to levels close to those of normotensive controls. These findings support the role of AT1r in mediating AngII-dependent microglial activation via TLR4 in hypertension. Moreover, this data suggests that the sympathoexcitation characteristic of neurogenic hypertension may result, at least in part, from low-grade chronic inflammation due to AT1r-TLR4 crosstalk facilitating microglial activation by AngII.

**Successful restoration of pelvic limb perfusion following manual balloon thrombolysis of distal aortic thrombosis in a dog.**

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**Introduction.** Distal aortic thrombosis (AT) is a serious condition in dogs, and if not fatal, often causes a high rate of morbidity. Distal AT can lead to significantly decreased or complete absence of perfusion to distal abdominal organ systems, such as the renal system and pelvic neuromuscular system. Medical therapy consists of antiplatelet and anticoagulant drugs, but even with medical therapy, it often takes days to weeks for clinical improvement. The risk of permanent damage and even death increase with the duration of compromised perfusion. Because of the common sequelae of AT, prolonged hospitalization with supportive care is common, which can become a financial burden for owners. For these reasons, it is imperative to treat these patients at the time of diagnosis with an attempt to restore perfusion as soon as possible. Along with medical therapy, surgical options are commonly used in humans, including invasive techniques (surgical thrombectomy) and interventional techniques (mechanical thrombectomy, balloon thrombectomy). Interventional techniques are less invasive and allow immediate disruption and/or thrombectomy, with concurrent medical therapy used to lyse the remaining thrombi and/or prevent recurrence. This case report describes manual balloon thrombolysis of a distal aortic thrombus in a dog.

**Methods.** A 1.5-year-old intact female Pomeranian presented for acute pain in the left pelvic limb. Femoral pulses were absent bilaterally (this was expected in the right pelvic limb as the right femoral artery was ligated during patent ductus arteriosus occlusion one month prior). Abdominal ultrasound confirmed a thrombus at the aortic trifurcation. General anesthesia was used to gain access to the right carotid artery via a 4 cm superficial skin incision. A 5 Fr angiographic catheter was advanced to the distal aorta under fluoroscopic guidance. Non-selective aortic angiography was performed, revealing complete obstruction of arterial blood flow at the level of the aortic trifurcation. The catheter was advanced through the thrombus and into the left common iliac artery. Angiography was performed, which revealed appropriate perfusion of the left pelvic limb distal to the thrombus. The catheter was replaced with a 0.035″ guidewire and a 4 mm x 2.5 cm x 150 cm angioplasty balloon catheter was advanced over the guidewire and inflated twice at the level of the thrombus. Subsequent angiography at the level of the previous thrombus revealed complete blood flow to the left common iliac artery, consistent with restored left pelvic limb perfusion. A small filling defect (10% of the arterial lumen) consistent with thrombus adherence to the vascular wall, remained at the level of the aortic trifurcation. Tissue plasminogen activator (t-PA; 0.1 mg/kg) was administered locally at the level of the thrombus via the angiographic catheter. All catheters and guidewires were removed and the incision was closed in routine fashion.

**Results.** Prior to balloon thrombolysis, hyperlactatemia was present (14.1 mmol/L left pelvic limb, 10.4 mmol/L left thoracic limb; N < 1.5) with a discrepancy in glucose concentrations between the left thoracic and left pelvic limbs (386 mg/dL and 194 mg/dL, respectively; N 76-116)). Immediate post-operative bloodwork revealed hyperphosphatemia (10.0 mg/dL; N 2.6-7.9) and hyperkalemia (7.1 mmol/L; N 3.6-4.9). Electrolytes were rechecked four hours post-operatively, revealing normal potassium and lactate concentrations (4.26 mmol/L and 1.50 mmol/L, respectively). The left femoral pulse was normal and synchronous with the heart beat immediately post-operatively. No immediate complications with the procedure or t-PA administration were present.

**Conclusions.** This case report highlights successful manual balloon thrombolysis of AT in a dog. Perfusion parameters (palpable femoral pulse, electrolyte concentrations) returned to normal within four hours after the procedure. These almost immediate results are unexpected with medical therapy alone, showing that balloon thrombolysis could be a beneficial procedure in the future for dogs with AT. Additional cases are necessary to determine long-term outcome of these patients.

# Central and peripheral mechanisms of antihypertensive effects of probiotic kefir in spontaneously hypertensive rats

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**Introduction**. Hypertension is one of the main causes of cardiovascular diseases, which accounts for one in every three deaths in the United States. Evidence supports a role for gut microbiota in regulation of blood pressure. In this context, gut microbiota dysbiosis has been associated to hypertension. Long-term use of probiotics have been shown to decrease blood pressure, improve baroreflex, and ameliorate endothelial dysfunction in Spontaneously Hypertensive Rats (SHR). However, the mechanisms are not well understood. Here, we evaluated the effects of the probiotic kefir on gut anatomy and composition, and within the paraventricular hypothalamic nucleus (PVN) of SHR rats.

**Methods**. Wistar Kyoto (WKY) and SHR male rats were divided into three groups. WKY treated with milk (WKY), SHR treated with milk (SHR), and SHR-kefir treated with milk fermented by the grains of Kefir (SHR-Kefir; oral gavage, daily, 9 weeks). We evaluated blood pressure by tail cuff method weekly. After treatment, we collected feces for gut microbiota analysis and a portion of the small intestine was used for histological analysis (goblet and Paneth cells). Lipopolysaccharide (LPS) content in serum was analyzed via a chromogenic quantitation kit. Protein expression for tyrosine hydroxylase (TH), which is a precursor of cathecolamine, was evaluated via immunofluorescence within the PVN.

**Results**. After 9 weeks of treatment, SHR-kefir presented a lower mean blood pressure (152.3 ± 2.2 mmHg) compared to SHR (173.1 ± 1.8 mmHg, P<0.05 n=6). SHR basal gut microbiota composition differ from that observed for WKY, suggesting dysbiosis. Treatment with kefir restored the normal pattern profile of *Lactobacillus, Staphylococcus, Brucella and Bifidobacterium spp*. Total number of goblet cells were increased in SHR (257.8 ± 4.6) compared to WKY (222.9 ± 4.0). Conversely, the number of Paneth cells/crypt was decreased in SHR (1.59 ± 0.12) compared to WKY (2.99 ± 0.3). LPS was found to be increased in SHR (0.66 ± 0.03) compared to WKY (0.48 ± 0.06 EU/mL), suggesting increased intestinal permeability during hypertension. Treatment with kefir normalized both cell intestinal profiles (SHR-Kefir goblet: 232.4 ± 3.8; Paneth: 2.23 ± 0.15, respectively) and LPS serum content (SHR-Kefir: 0.53 ± 0.01). Within the PVN, chronic treatment of Kefir normalized the observed overexpression of TH in SHR rats (WKY: 10.1 ± 0.7; SHR: 12.69 ± 0.39 and SHR-Kefir: 8.79 ± 0.68, arbitrary units), suggesting decreased production of cathecolamines, which is associated with diminished sympathetic activity.

**Conclusions**. The data suggests that the partial antihypertensive effects of probiotics in SHR rats involves restoration of the gut microbiota composition, improvement in intestinal structure, and diminished cathecolaminergic signaling within the hypothalamus. Altogether, our data suggests that probiotics benefits mechanisms involves *gut* microbiota–*brain* axis communication during hypertension.

**Functions of the DRY motif and intracellular loop 2 of human melanocortin-4 receptor**

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**Introduction:**

The melanocortin-4 receptor (MC4R) is a G protein-coupled receptor (GPCR) primarily expressed in the hypothalamus. MC4R is a critical molecule in regulating energy homeostasis as well as several other physiological functions. Activation of MC4R activates Gs, which subsequently increases adenylyl cyclase activity to produce cyclic AMP (cAMP). Activation of MC4R has also been known to induce extracellular signal-regulated kinases (ERK) 1/2 phosphorylation. DRYxxI motif and intracellular loop (ICL) 2 have been shown to be important for receptor functions in several GPCRs. However, systematic study on DRYxxI motif and ICL2 of MC4R is still lacking.

**Methods:**

MC4R mutants were generated by QuikChange site-directed mutagenesis kit. HEK293T cells were transiently transfected with WT or mutant MC4Rs. Receptor cell surface expression was measured by flow cytometry. Ligand binding assays were performed on intact cells using $^{125}$I-NDP-MSH with or without different concentrations of unlabeled NDP-MSH. cAMP levels were measured by radioimmunoassay. The levels of ERK1/2 phosphorylation were measured by western blotting.

**Results:**

1. One mutant (T150A) had impaired cell surface expression. Four mutants (A154G, Q156A, Y157A, and M161A) had increased cell surface expression.
2. Eight mutants were defective in ligand binding, with either increased $IC_{50}$ (T150A) or decreased $B_{max}$ (D146A, Y148A, Y153A, Q156A, Y157A, M161A, and T162A).
3. Six mutants were defective in NDP-MSH-stimulated Gs-cAMP production, with either increased $EC_{50}$s (D146A, T150A, I151A, L155A, Q156A, and Y157A) or reduced $R_{max}$ (T150A). Six mutants (D146A, Y148A, F149A, F152A, Y153A, and H158A) were identified as constitutively active mutants.
4. All mutants had significantly increased NDP-MSH-stimulated pERK1/2 levles except for five mutants (D146A, Y148A, Y153A, Y157A, and M161A). Two mutants (I151A and Y153A) had significantly increased basal activities.

**Conclusions:**

1. One residue (T150) was important for receptor synthesis or trafficking to cell membrane.
2. Eight residues (D146, Y148, T150, Y153, Q156, Y157, M161, and T162) were critical for ligand binding.
3. Six residues (D146, T150, I151, L155, Q156, and Y157) were important for NDP-MSH-stimulated Gs-cAMP pathway. Six resides (D146, Y148, F149, F152, Y153, and H158) were involved in constraining receptor in inactive state.
4. Five residues (D146, Y148, Y153, Y157, and M161A) played significant roles in NDP-MSH-stimulated ERK1/2 pathway.
5. Some mutants were biased to Gs-cAMP (D146A, Y148A, Y153A, Y157A, and M161A) or ERK1/2 (T150A) pathway.

## Increased Population Homogeneity in ArkDPI Vaccine Precludes Emergence of Subpopulations after Challenge

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**Introduction.**
In previous work we have confirmed that adaptation of an embryo-attenuated infectious bronchitis virus (IBV) Arkansas (Ark) Delmarva Poultry Industry (DPI)-derived vaccine to chicken embryo kidney (CEKp7) cells shifted the virus population towards homogeneity in spike (S) and non-structural protein (NSP) genes. The typical Ark vaccine subpopulations emerging in chickens vaccinated with commercial Ark vaccines were not detected in chickens vaccinated with CEKp7, indicating that kidney-cell adaptation presented a selective bottleneck narrow enough to drastically increase the stability of this vaccine virus population. In a protection trial the second embryo back-passage of CEKp7 (CEKp7e2) was compared with protection conferred by an attenuated commercial ArkDPI-derived vaccine different from which the CEK-adapted virus originated after challenge with a virulent IBV ARK strain. Another trial consisted in vaccinating chickens with the CEK-adapted vaccine or a commercial vaccine and assessing differences in antibody production and cell populations of the immune response.

**Methods.**
The methods used were qRT-PCR, PCR, Sanger sequencing, ELISA, Avidity tests and Flow Cytometry.

**Results.**
In CEKp7e2 vaccinated chickens viral subpopulations different from the challenge virus were detected after challenge in a marginal number (7-8%) of chickens. In contrast, IBV S1 sequences that differed from the predominant population in the challenge virus were detected after challenge in a large number (77%) of chickens vaccinated with the commercial Ark attenuated vaccine. There were no apparent differences between the vaccines in terms of avidity or T cell populations. The B cells populations were increased significantly in the commercial vaccine compared to the CEKp7e2.

**Conclusions**. Increased homogeneity of an ArkDPI-derived vaccine after CEK adaptation reduces the emergence of Ark-like viruses after challenge. Evaluation of B and T cell responses, as well as IgG avidity for IBV, did not provide a clear explanation for this phenomenon.

# Veterinary Student Poster Presentations

## Toll-like Receptor 4 Signaling in Equine Mesenchymal Stem Cells

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**Introduction.** The use of mesenchymal stem cells (MSCs) in equine veterinary medicine has historically been for repair of musculoskeletal injuries, through regulation of the immune system. In order to be immunomodulatory, MSCs have to be exposed to an inflammatory environment. This environment however, can also cause the MSCs to be immunogenic. Primed MSCs can influence both the innate and adaptive immune systems. One of the important mechanisms that MSCs can be primed to influence the innate immune system is through toll like receptor (TLRs) signaling. TLR 4 is a very important TLR in horses due to release of its ligand, Lipopolysaccharide (LPS) and the production of an inflammatory cytokine storm that can result in marked patient morbidity. The objective of this study was to evaluate how TLR4 signaling affects immunomodulation of equine bone marrow derived mesenchymal stem cells (BMSCs).

**Methods.** Bone marrow was harvested via aseptic sternebral aspiration from two healthy adult horses. BMSCs were isolated via plate adherency and cultured in DMEM 4.5 g/L glucose and sodium pyruvate without L-Glutamine supplemented with 10% fetal bovine serum, 0.05% L-Glutamine, and 1% penicillin/streptomycin at 37°C and 5% $CO_2$. BMSCs were characterized via tri-lineage differentiation and immunophenotyped via flow cytometry. BMSCs were plated 20,000 cells/well in 12 well plate then treated with 10ng/mL, 50ng/mL, and 100ng/mL LPS. Total RNA was extracted from BMSCs using the EZNA total RNA kit, cDNA was synthesized with iScript cDNA synthesis kit. Primers and probes were designed with commercial software. PerfeCTa SYBR Green Kits were used to measure relative expression. mRNA expression was normalized to two internal mRNAs (18s and GAPDH). Relative expression was calculated by the comparative threshold cycle (ΔΔ method), and the fold change relative to controls were calculated. Expression of genes (TLR4, MHCI, MHCII, IL-6, IL-8, IL-10, CXCR4, COX2, TGFβ, and Jagged 1) was reported.

**Results.** At 24 hours with a treatment of 10ng/mL of LPS, IL-6 showed an increase in expression by 496.08%. At the same time point and treatment, Cox-2 expression increased by 33.03%. Both of these genes also showed an increase at 48 hours with 50ng/mL of LPS.

**Conclusions.** IL-6 and Cox-2 are two major immunomodulatory mediators in the horse. The increase in their expression is an interesting find that indicates priming of equine BMSCs through TLR4 may stimulate BMSC immunomodulation. There were a few limitations to this preliminary study. This included an inconsistent housekeeping gene through different treatments, primers that did not anneal to the specific cDNA sequence, and too low of a cell seeding density. In future research, we plan to test different housekeeping genes under the same conditions to see if we can find a more stable reference gene, increase the seeding density to 100,000 cells/well, and redesigning primers.

# B-lymphocyte inflammatory response to PEG-fibrinogen microsphere encapsulated endothelial colony forming cells (ECFCs) in equine distal limb wounds

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**Introduction:** Endothelial colony forming cells (ECFCs) are endothelial progenitor cells that are believed to have therapeutic effect in the wound healing process through migration to the injured area, promotion of neovascularization, and vessel repair. We hypothesized that the engraftment, survivability and efficacy of the ECFCs could be improved by combining them with a biomaterial. Further, we hypothesized that ECFCs encapsulated in a Poly(ethylene) glycol (PEG)-fibrinogen (PF) hydrogel microsphere (MS) would not induce a significant inflammatory response equine distal limb wounds.

**Methods:** Two full thickness wounds with an area of 6.25 $cm^2$ were made over the dorsal metatarsus and metacarpus in 3 adult horses and baseline skin samples collected. Four treatments were randomly assigned to the eight wounds: ECFCs alone (2 wounds), ECFCs encapsulated in PF-MS (2 wounds), PF-MS alone (2 wounds), and serum (2 wounds). One wound from each treatment was biopsied at the leading edge weekly for 4 weeks. The remaining 4 wounds were biopsied at the center and leading edge only at week 4. Formalin fixed paraffin embedded biopsy sections were stained for the B-lymphocyte paired box protein (PAX)-5 and digitally quantified (Visiopharm). Tissues quantified were both Inner Wound and Wound Periphery areas from week one through week four biopsies.

**Results:** First, by a two way ANOVA analysis, no statistical difference was found in the amount of Pax5 identified among baseline tissues between different horses and limbs (p=0.821). Second, there was a statistically significant difference in Pax5 stain density among the weekly Inner Wound biopsies, with the greatest amount of Pax5 being identified in the week four tissues (p=0.0041). Finally, by comparing the individual treatments in Wound Periphery, it was found that the greatest amount of Pax5 stain was in the EPC/MS group (p=0.0392).

**Conclusion:** The amount of Pax5 stain, thus the amount of B cells, was increased in the EPC/MS treatment. The significance of this type of inflammatory reaction is still under investigation.

**Medical Students' Attitudes Regarding the Impact of a Therapy Dog Program on Stress Reduction**

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**Introduction.** Animal-assisted therapy (AAT) in the form of therapy dogs have become extremely common in both the medical and educational settings. Evidence supports AAT as a beneficial therapeutic alternative for individuals suffering from physical disabilities and psychological disorders. Particularly, in the medical school setting, the use of therapy dogs has been markedly increasing on campuses due to an existing serious, and documented, problem of anxiety, depression, and suicide among medical students. However, few studies have been conducted to evaluate the impact of alternative therapeutic interventions on the anxiety level of medical students. No research has examined the impact and effectiveness of a therapy dog program on the anxiety level of medical students.

**Methods.** A six-question pre-survey was administered to first and second year students at the Via College of Osteopathic Medicine (VCOM). The survey analyzed self-reported, student anxiety levels during the year and, specifically, before and after an exam. Following completion of this survey, a certified therapy dog was introduced in the morning before each exam. After 10 weeks, a post-survey was administered to students to analyze the impact of the therapy dog on anxiety levels before the exam and the continuation of the program overall.

**Results.** One hundred eighty-three students responded to the pre-survey and 150 students responded to the post-survey. The pre- and post-survey demonstrated that a large majority of students (pre: 79%; post: 74%) rated their anxiety level prior to an exam as a 5 or higher. However, after interacting with the therapy dog prior to an exam, the post-survey demonstrated that only 35% of students rated their anxiety level as a 5 or higher. Additionally, 93% of students that interacted with the therapy dog reported that the dog had an impact on their level of anxiety prior to the exam and improved their learning and testing environment. Overall, 96% of students recommended the implementation of a permanent therapy dog program for medical school campuses.

**Conclusions.** This study is the first to assess the impact of a therapy dog on anxiety levels of medical students prior to an exam. The students were near unanimous regarding a positive impact on their level of anxiety and continuation of a permanent therapy dog program not only at their medical school but every campus.

**In vitro effects of pitavastatin in combination with benzimidazole anthelmintics on canine glioblastoma cell lines**

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Gliomas are the most common primary central nervous system tumor in the human adult and are the second most common intracranial neoplasm in dogs, with an incidence of 32%. Gliomas arise from normal glial progenitor cells (astrocytes, oligodendrocytes, and ependymal cells). Glioblastoma is the most invasive and malignant subtype with a grade IV classified by the World Health Organization. The median survival time is 14.6 months in humans, and dogs have been reported to have similar survival times. The use of canine patients with naturally occurring glial tumors as an intermediate animal model of the human disease is still in its infancy, and there is currently no published work examining the use of statins augmented with chemotherapies in this model. Treatment for intracranial tumors is limited due to the inability of most drugs to cross the blood-brain barrier. The benzimidazole drug mebendazole (MBZ) has this ability due to its lipophilic nature. Previous work in our lab has shown that the canine GB cell lines J3T, G06A, and SDT-3G treated with MBZ have a half-maximal inhibitory concentration (IC50) value well within the range of achievable biologic concentration of the drugs. Cholesterol is crucial in the development and maintenance of the central nervous system in both normal and cancerous tissue. Statins are well-known 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that reduce production of cholesterol by reducing production of the precursor mevalonic acid. Suppressing HMG-CoA reductase allows for decreased levels of mevalonate and downstream lipid intermediates.

The purpose of this study was to evaluate the cytotoxic effects of treatment on cell growth and viability using the MTT assay to establish the IC50 of the pitavastatin and MBZ treatments on canine GB cells and to evaluate the effects of these drug via immunocytochemistry.

We were able to determine IC50 values for pitavastatin for the J3T and SDT-3G cell lines using the MTT assay. We were also able to calculate statistical significance of MBZ plus pitavastatin treated cells. Lastly, we were able to perform immunocytochemistry of all three cell lines treated with MBZ, pitavastatin, and pitavastatin plus MBZ. Our results were significant, and we were able to kill the cell lines using the drugs at therapeutic concentrations. However, the next step would be to do the same experiment in a different cell culture environment such as hypoxia.

**Evaluating Therapeutic Potential of Carnitinoid Compounds in Models of Mitochondrial Dysfunction**

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**Introduction**. Mutations in mitochondrial DNA (mtDNA) can impair oxidative phosphorylation and are especially detrimental to cells with high energy demands. Mitochondrial disease in human patients progressively affects a subset of tissues with high energy demands (CNS, heart and muscle) once the mtDNA mutation load exceeds a threshold of dysfunction. In prior experiments, small molecule carnitinoid "PMX" compounds were shown to protect against neurodegeneration of dopaminergic neurons in the substantia nigra *pars compacta* in rotenone-treated Lewis rats. Additionally, PMX compound co-treatment improved neuromotor and cognitive abilities in a rotenone-treated mouse model.

**Methods**. We sought to create an *in vitro* model that will allow us to assess mitophagy in PMX-treated neuronal cells by examining the cellular localization of two key proteins involved in mitophagy, parkin and PINK1. We also tested the feasibility of a biomarker assay that would detect cell-free mtDNA in the plasma of rotenone-treated mice as a useful assay to provide quantitative supportive data of PMX therapeutic activity.

**Results**. Preliminary data is inconclusive and research is ongoing.

**Conclusions**. PMX compounds are meant to be actively taken up by the mitochondria to target reactive oxygen species and mediate recycling of defective mitochondria, which should be reflected in the rotenone-treated mouse model and the localization of key proteins in mitophagy in neuronal cells.

# Identification of a MircoRNA Expression Profile as a Biomarker for Cancer

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**Introduction**. MicroRNAs (miRNAs) are small noncoding RNA gene products that modulate gene expression at the post-transcriptional level by negatively regulating the translational efficiency of their target mRNAs. Alterations in the expression of miRNAs has been linked to the pathogenesis of many types of cancer. Mechanisms for this altered expression vary by cancer type; possible mechanisms include deletions, amplifications, epigenetic silencing, and dysregulation of transcription factors. MicroRNAs may act as either oncogenes or as tumor suppressors, thereby effecting tumorigenesis. Malignant cells are dependent upon the dysregulated expression of microRNA genes, indicating miRNA expression profiles could be a useful diagnostic tool for specific cancers. Several previous reports explored the use of blood tests to isolate specific miRNAs that have up-regulated or down-regulated expression in specific cancers as a diagnostic tool. This experiment involved a retrospective analysis of previously published miRNA biomarkers for cancer, along with the identification of new biomarkers, in order to identify a single miRNA or a small group of miRNAs that would show dysregulated expression on a blood test and could be used as a general identifier for cancer.

**Methods**. Total exosome isolation was performed on cell lines representative of several cancer types. These exosomes were sequenced and a full miRNA profile with expression rates for each exosome was compiled. The dysregulated miRNAs from each exosome were identified. A retrospective analysis was performed on previously published miRNAs that are dysregulated in specific cancers. Both sets of data were compared to identify the miRNAs that are most likely to be dysregulated in the most types of cancer. Separate whole blood samples were then collected from a normal dog and from a dog known to have cancer. Both samples were allowed to clot and the serum was used for total exosome isolation. PCR was then used to identify presence or absence of miRNAs that were anticipated to be dysregulated.

**Results**. miRNA 16 is a regulator of gene expression and should be present on all normal exosomes. All of the samples displayed expression of miRNA 16, which indicates the successful isolation of exosomes. A number of additional miRNAs were upregulated in many types of cancer and a few were downregulated. These may prove to be reliable indicators of cancer. Further profiling of the dysregulated miRNAs is ongoing.

**Conclusions**. The expression of miRNA 16 in the isolated exosomes indicates our protocol is successful. More blood samples from patients with various types of cancer need to be collected and exosome extracted. PCRs for the predicted dysregulated miRNAs will then be performed on the samples to determine if those miRNAs are consistently dysregulated. If one miRNA, or a group of miRNAs, can be determined to be consistently dysregulated when a malignancy is present, then those miRNAs could be used as a general marker for cancer. In the future a simple blood test could be taken and assayed for the cancer biomarkers in routine physical exams. If the test shows dysregulation of the biomarker miRNAs, then a diagnosis for cancer could be made and further diagnostics would be warranted.

**In Vitro Measurements of Friction Used to Observe the Drying Properties of Intact Equine Articular Cartilage**

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**Introduction**. Articular cartilage (AC) is one of the main components of a synovial joint that allows both static and dynamic movement to occur in a low friction environment. Specifically, equine AC of the carpal joint undergoes a cyclical loading motion, predisposing said joint to various osteopathologies such as osteoarthritis and degenerative joint disease [5]. The goal of this study was to collect and analyze data using a previously developed reciprocating friction protocol on the carpus in hopes to gain a better understanding of natural joint lubrication, cartilage drying times, and the development of pathology in diseased joints. Testing determined the coefficient of friction (COF) of a migrating contact area – the second carpal bone – which was brought into contact with the fixated articular cartilage of the distal medial radius. India Ink staining and compound light microscopy were used to examine the post-testing surface morphology in order to observe any changes to the microenvironment of the samples [1]. It was hypothesized that at a force of 10N over a test duration of 5 hours, there will be an increase in the coefficient of friction. Moreover, as a result of the reciprocating friction testing and increase in coefficient of friction over time, there will be a disruption in the surface architecture of the horizontal test track. Significant increases found over time and the better understanding of drying on the microenvironment of cartilage can both be used to further model studies on joint lubrication and friction that will help clinicians move toward new advances in veterinary orthopedics with future research.

**Methods**. AC samples were collected from four male castrated horses that were euthanized for purposes outside of the study. No horses had a previous history of lameness or degenerative joint disease localized to the carpal joint. Samples were further dissected to remove excessive muscle and connective tissue and AC from the distal medial radial trochlea and second carpal bone were isolated. Cartilage was then inspected grossly for any surface disruptions that would affect testing. Each set of viable test materials were refrigerated in appropriately sized containers and submerged in phosphate buffered saline (PBS) [6,7,9]. Tribological properties were measured using a Bruker UMT-3 mechanical test instrument. Samples of the distal medial radius cartilage served as a stationary flat surface, and the second carpal bone samples served as the vertical loading assembly. The convex surface of the second carpal bone was used to apply the normal force of 10N at a sliding velocity of 1mm/s over a horizontal sliding distance of 2.5mm on the distal medial radius. Each test was conducted for (18000s) 5 hours [9]. All samples were allowed to dehydrate over the testing period. Post-testing, India Ink was chosen to dye cartilage samples. Once stained, samples were then placed into a glass petri dish and viewed using a stereoscope and ring light [1,2].

**Results**. Testing showed variability in the starting and ending COF. However, the majority of the samples did increase over the five hour period. Specifically, there increase changes ranging anywhere from 10 to 400%. The average time of samples to breach a threshold 10% greater than the starting COF was approximately 1000 seconds. Five samples breached a threshold of a coefficient of friction of 0.05, signifying the switch from fluid pressurization lubrication to boundary lubrication. This threshold occurred at approximately 11000 seconds. Survey and magnified images of a carpal sample in India Ink showed visible compression of the normal architecture of the cartilage surface that was exposed to the reciprocating friction testing.

**Conclusions**. Testing proved the first hypothesis true that there was indeed an increase in the coefficient of friction over time. It was also noted that the articulating surfaces of the testing samples maintained a level of wettability and lubrication thus motivating future research to further look into cartilage lubrication research. Secondly, post-testing surface morphology showed a clear wear tract visible on the cartilage surface that showed compression of the natural "peaks and valleys" architecture. Finally, despite the varying initial COF values, all samples breached a threshold COF that was 10% higher than their starting values. Due to the variability in sample age, discipline, hydration status and individual surface topography, research testing protocol needs to be refined.

**Long-term co-culture of equine synovial membrane and articular cartilage explants as an *in vitro* model of osteoarthritis.**

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**Introduction.**
Osteoarthritis (OA) is the leading cause of musculoskeletal pain worldwide and is the most common cause of reduced or lost performance in horses Current treatment for OA are designed to modify the symptoms of disease through reduction of inflammation via corticosteroids or viscosupplementation. However, newer therapies are becoming available that target OA through disease modification by limiting disease progression. Historically, in vitro experiments evaluating OA treatments have evaluated the effect of treatments on one type of articular tissue or cultured cell. However, cross-talk between tissues such as the synovium, articular cartilage, and subchondral bone is integral in the progression of OA. Therefore, the objective of this study is to establish an in vitro co-culture model of OA that will allow cross talk between synovial membrane and articular cartilage to more accurately evaluate the anabolic effects of OA treatments.

**Materials and Methods.**
Tissue Culture: Articular cartilage and synovial membrane were aseptically harvested from the stifle joints of fresh equine cadavers. Synovial membrane and articular cartilage were aseptically harvested and 4 mm explants were obtained from tissues using 4 mm diameter biopsy punch Synovial membrane alone, articular cartilage alone and synovial membrane co-cultured with articular cartilage using 0.4mm transwell inserts cultured in either Dulbecco's modified culture medium containing 5% fetal bovine serum and 1% penicillin/streptomycin or serum free media with 1% penicillin/streptomycin. Tissues were cultured at 37°C, 5% $CO_2$ and maintained for 3, 7 and 14 days. Media will be harvested and snap frozen on days 3, 7, and 14 for assessment of prostaglandin E2 (PGE2) and Hyaluronan (HA). Cellular viability will be assessed at days 0, 3, 7, and 14 days using LIVE/DEAD viability/cytotoxicity kits and images were obtained via confocal microscopy and counted with Image J software. Synoviocyte and chrondrocyte apoptosis will be determined using the TUNEL method. Histopathology will be performed by a blinded board-certified pathologist and scored based on OARSI scoring system.
RT-PCR: For gene expression analysis of IL-1b and TNF-alpha, tissues were homogenized and RNA extracted using RNeasy Fibrous Tissue Mini kit (Qiagen). Total RNA was extracted using qScript cDNA synthesis kits and SYBR green kits used to measure inflammatory and catabolic gene expression with RT-PCR.

**Results.**
Preliminary results have shown viable synovial membrane and articular cartilage following 3 days of culture in complete medium. Preliminary gene expression analysis of all tissues has been performed to ensure proper primer design. Further results are currently pending.

**Identification of a single base deletion in the glycoprotein IIb gene causing Glanzmann Thrombasthenia in a Golden Retriever**

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**Introduction.** Glanzmann thrombasthenia (GT) is an intrinsic platelet disorder that results from a quantitative or qualitative defect in the fibrinogen receptor on platelet membranes. The fibrinogen receptor is made of two subunits encoded by separate genes; glycoprotein IIb (GPIIb) and glycoprotein IIIa (GPIIIa), that combine to form the GPIIb-IIIa complex. GT can result if is a mutation in either of these two genes. Patients presenting with GT typically have platelet-type bleeding with normal platelet counts, coagulation times, and von Willebrand factor (vWF) antigen levels. Because of the lack of fibrinogen receptor expression on platelet surfaces, patients' platelets are unable to aggregate and perform clot retraction. The purpose of this study was to perform mutation analysis of the coding regions of the GPIIb and GPIIIa genes in a young Golden Retriever with GT. Previous studies in dogs and horses have identified causative mutations in the GPIIb gene, whereas in people causative mutations in both genes have been documented. GT in veterinary medicine has been documented in Otterhounds, Great Pyrenees, two related mixed breed dogs and several horse breeds. This study involved a 2-month-old, intact, female Golden Retriever puppy that presented to her animal hospital for petechial and ecchymotic hemorrhages and prolonged oral bleeding.

**Methods.** Initial diagnostics including a CBC, coagulation panel, vWF antigen levels were performed, along with flow cytometry on patient platelets using antibodies to GPIIIa. Genomic DNA of the patient was isolated from whole blood and subjected to PCR to amplify the coding regions of both the IIb and IIIa genes. Specific coding areas of interest in the IIb gene was compared from the patient to both parents, three siblings, and two of the dam's sisters. Some regions were also compared to five control Golden Retrievers that had no bleeding disorders.

**Results.** CBC, coagulation testing, vWF antigen and platelet counts where all within normal limits. Flow cytometry showed significantly reduced CD61 (GPIIIa) expression on platelet surfaces. A single base pair deletion was discovered in exon 19 GPIIb gene in the affected dog. This mutation results in a premature stop codon encoded 24 bases downstream. Both parents were heterozygous for this mutation.

**Conclusion.** This is the first clinical description of GT and associated mutation in the gene encoding GPIIb in Golden Retrievers. Together with previously published characterizations of GT in 3 other breeds of dog, our findings suggest that GT should be included in the differential for platelet-type bleeding in any dog breed. As in human populations, GT is likely to be mutationally heterogeneous in dogs.

## Mission Thyroidectomy

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**Introduction**. Limited access to basic surgical care is endemic in developing countries.[1] Research has demonstrated that as much as 40% of the public health burden in underdeveloped countries is surgical in nature[1]. American surgical teams have responded to this need in great numbers for many years. For example, Operation Giving Back (OGB), the volunteerism initiative of the American College of Surgeons (ACS), serves as a comprehensive resource center to find information on surgical volunteer opportunities and is affiliated with 29 surgical humanitarianism groups that conduct surgical mission trips throughout the year.[2]

Goiter, an abnormal enlargement of the thyroid gland due to iodine insufficiency, is a relatively common diagnosis in patients residing in countries served by mission surgical teams and mission hospitals[4]. Unfamiliarity with local resources could result in unexpected complications, including hypothyroidism, hypocalcemia, and wound problems. The scarcity of reports of complications related to humanitarian thyroidectomy suggests underreporting. We believe there may be a lack of professional guidance for the performance of a thyroidectomy in an austere humanitarian environment. Research has shown that surgical checklists decrease rates of morbidity and mortality from surgeries,[16,17] and we believe that a Mission Thyroidectomy Patient Safety Checklist would be valuable tool for a humanitarian surgical team.

**Methods**. We created a checklist of thirty supplies and experiences commonly considered necessary for the safe performance of a thyroidectomy for goiter. We then surveyed 28 surgeons with thyroid surgery experience in US hospitals and austere environments. For each item, the surgeons could choose: essential, not-essential but lack of item could impact patient outcome, or not necessary.

**Results**. Of thirty items on the Mission Thyroidectomy Patient Safety Checklist, there were fourteen items that the majority of surgeons felt were essential for the safe performance of thyroidectomy in a humanitarian or austere environment and another four items that the surgeons felt were not absolutely essential but lack of could impact the patient's outcome.

**Conclusions**. Humanitarian surgical teams working in an austere environment will likely encounter patients in need of thyroidectomy for endemic goiter.[5] Performing a thyroidectomy in a setting of limited resources or unknown resources may be a new and challenging experience for some humanitarian surgical teams. Checklists in medicine and surgery has been shown to markedly improve patient safety. We believe that our Mission Thyroidectomy Patient Safety Checklist can serve as a valuable guide for the humanitarian surgical teams operating in an austere environment by providing a comprehensive list of items that are critical to the safe performance of thyroidectomy for goiter. We also believe that our checklist will assist humanitarian team medical directors in choosing the appropriate patients for this challenging surgical procedure.

# Effect of Heartworm Disease and Heartworm Associated Respiratory Disease (HARD) on the Right Ventricular Papillary Muscle of Cats

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**Introduction**. Dogs and cats worldwide can be infected with *Dirofilaria immitis*, a filarial nematode. Severe and potentially fatal clinical signs have been documented in dogs, but increasing awareness of feline heartworm disease has warranted evaluation of this disease in this species. Cats infected with heartworms exhibit different clinical signs than dogs, which is in part caused by the immune response of the cat at two important times. The first is the arrival of the heartworms in the pulmonary arteries approximately 70 – 90 days post infection. Clinical signs associated with this time point are known as Heartworm Associated Respiratory Disease (HARD). The other time point at which cats exhibit severe clinical signs is following death of adult heartworms, which can cause thromboembolism and fatal lung injury. The aim of this study was to assess myocardial changes in the right ventricular papillary muscle of cats affected with HARD and with adult heartworm disease.

**Methods**. Cardiac and pulmonary tissue were obtained from six groups of cats eight or eighteen months following infection with the infective larval stage (L3) of *D immitis*. Two groups were untreated and allowed to develop adult heartworms. Two groups were treated with selamectin one month post infection, preventing development of L5 or adult heartworms. Two groups were treated with ivermectin three months post infection, allowing time for immature adult heartworms to develop and induce HARD, but preventing development of mature adults. A seventh group of uninfected cats was included as a negative control. Lung pathology was objectively assessed in each cat and a ratio of right ventricular (RV) to left ventricular (LV) weight was obtained for each cat. Samples of RV papillary muscle were obtained and assessed for collagen content.

**Results**. Uninfected cats in the negative control group were found to have the greatest amount of collagen in the RV papillary muscle compared to all other groups. Infected, untreated cats at eighteen months post infection had significantly decreased (p = 0.04) RV papillary muscle collagen content in comparison with uninfected cats. There was no significant effect of severity of pulmonary parenchymal and arterial pathology on RV papillary muscle collagen content (p = 0.81 and p = 0.35, respectively). RV papillary muscle collagen content was not affected by increase in RV/LV ratio (p = 0.16).

**Conclusions**. The data suggest that cats infected with heartworms of any stage experienced decreased collagen content of the right ventricular papillary muscle, however those affected with adult heartworms had the greatest loss of collagen. This decrease in collagen content was not influenced by severity of pulmonary pathology or changes in RV size.

**Tracking PEG-Fibrinogen microsphere encapsulated endothelial colony forming cells after injection into equine distal limb wounds**

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**Introduction.** Endothelial colony forming cells (ECFCs) are a subdivision of endothelial progenitor cells (EPCs) that aid in angiogenesis. Therefore, these cells are thought to be important for repair of ischemic wounds such as equine distal limb wounds that are predisposed to form excessive granulation tissue. When injected into an equine distal limb wound, the ECFCs were hypothesized to localize and incorporate into blood vessels with enhanced localization and retention when cells were protected by encapsulation in polyethylene glycol-fibrinogen (PEG-fibrinogen) microspheres.

**Methods.** Two full thickness dermal wounds were created on the distal limbs of three horses, each wound measuring 6.25 $cm^2$ (eight wounds per horse). Wounds then received one of four randomized treatments by subcutaneous injection: serum, PEG-fibrinogen microspheres (MS), naked ECFCs (EPC), or ECFCs encapsulated in PEG-fibrinogen microspheres (EPC/MS). Wounds were biopsied at baseline and weekly for four weeks. Immunofluorescent staining for von Willebrand Factor (vWF) and quantum nanodot (Qtracker 655) labelled ECFCs was performed on biopsies of EPC and EPC/MS treated tissue. These tissues were then analyzed for fluorescent signal using confocal microscopy.

**Results.** Labeled cells were found in 2/3 EPC treated wounds and 3/3 EPC/MS treated wounds at week 1, 1/3 EPC and 1/3 EPC/MS treated wounds at week 2, and 1/3 EPC and 1/3 EPC/MS treated wounds at week 3. No labeled cells have been found at week 4. Week 3 and week 4 analysis is still ongoing. These visible signals indicate the ECMCs remained viable after the injection process. Labelled ECFCs were found in clusters in earlier weeks. Later in the study they were found near the newly formed capillaries, but none had been incorporated into the vessel walls.

**Conclusion.** These preliminary data show ECFCs remain viable through the injection process, and they remain present in the tissue during angiogenesis for up to 21 days.

**Effect of Acidosis and Storage Time on Platelet Aggregation in Horses**

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**Introduction —** The Multiplate platelet function analyzer provides a method to measure the extent of platelet aggregation in whole blood in response to an agonist (primary hemostasis). In human medicine, there is an established 30 minute–2 hour post-blood draw time window within which accurate results can be obtained with this instrument. One goal of this study was to evaluate the effect of blood storage time on results obtained for horses. Additionally, we used the Multiplate to study the effects of acidosis on primary hemostasis by adjusting the pH of blood from healthy horses through the addition of lactic acid.

**Methods —** A prospective study was performed using blood from 39 clinically healthy horses (29 mares, 10 geldings; aged 9–28 years). Twenty-five mL of blood was collected from each horse and distributed into collection tubes as follows: 1 EDTA, 3 lithium heparin, and 5 sodium citrate. A CBC, chemistry profile, and coagulation profile were performed for each horse to determine if any abnormalities were present. The agonist used to initiate platelet aggregation in our study was adenosine diphosphate (ADP). The time window component of the study used blood stored in lithium heparin and sodium citrate for Multiplate analysis at both 30 minutes and 2 hours post-blood draw. To evaluate the effect of acidosis on platelet aggregation, Multiplate analysis was additionally performed after adding 7 or 12 µL of 1M lactic acid to 1 mL heparinized blood to assess the effects of lactic acidosis. The pH was obtained for each blood sample before and after addition of lactic acid (NOVA biomedical pHOx Ultra). Descriptive statistics were generated and preliminary data comparisons were performed using a paired T-test.

**Results —** There was a significant difference ($p < 0.05$) in platelet function between equine blood stored in sodium citrate for 30 minutes and 2 hours but not for blood stored in lithium heparin. There was a significant difference in platelet function when 12µL of lactic acid was added to heparinized blood (mean pH 7.12) but not when 7µL lactic acid was added (mean pH 7.28).

**Conclusions —** Platelet aggregation decreases as storage time increases when equine blood is stored in sodium citrate. There is no apparent effect on platelet aggregation when equine blood is stored in lithium heparin for up to 2 hours. There is a measurable decrease in platelet aggregation at very low blood pH (mean pH 7.12), suggesting a hypocoagulable state at this level of acidemia.

## Anesthesia enhances subthreshold critical slowing-down in a stochastic Hodgkin-Huxley neuron model

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Critical slowing-down (CSD) emerges spontaneously in subthreshold voltage tracings from stochastic neuron models on approach to spiking threshold and is predicted to occur in populations of neurons influenced by general anesthetics. Numerical simulation was used to investigate the subthreshold behavior of a type-I integrator Hodgkin-Huxley model endowed with stochastic descriptions of ion channel kinetics and subject to the influence of phasic $GABA_A$ inhibition. The model was driven by multiple Poisson distributed trains of inhibitory GABA impulses and investigated for various model parameterizations as a function of proximity to spiking threshold via application of a constant excitatory input current. Reduction of the $GABA_A$ conductance decay rate was used to model the influence of propofol anesthesia. Transmembrane potential tracings were numerically simulated and power spectral densities, autocorrelation functions, and membrane potential histograms of the zero-mean fluctuations were computed to characterize subthreshold behavior. For a given reduction in $GABA_A$ conductance decay rate, nonlinear growth in amplitude simultaneous with decay in frequency and increasing temporal persistence of transmembrane voltage fluctuations were observed as distance to spiking threshold was reduced. The magnitude of these statistical signatures of CSD increased in conjunction with increased anesthetic effect. In a Hodgkin-Huxley model equipped with stochastic ion channel kinetics and phasic $GABA_A$ inhibition, anesthesia enhances the magnitude of critical slowing-down on approach to spiking threshold.

# Concentration of rifampin in the sebum and buffy coat of dogs

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**Introduction**. Bacterial skin infections in the dog and cat are most commonly caused by the commensal organism *Staphylococcus pseudintermedius* of the *Staphylococcus intermedius* group (SIG). Antibiotic exposure can induce SIG organisms to develop methicillin resistance. Rifampin (RFP) is an antibiotic that may be used to treat methicillin-resistant SIG infections; however, very limited pharmacokinetic and pharmacodynamic information exists for its use in dogs. Antimicrobial therapeutic success is facilitated by achieving effective drug concentrations at the site of the infection. The objective of this study was to measure the concentration of RFP in the sebum and buffy coat of dogs after multiple oral dosing.

**Methods**. Twelve healthy client-owned dogs weighing more than 15kg and ranging in ages from 1-7 years were enrolled in the study: seven males and five females. Dogs received 5-10mg/kg/day of RFP by mouth for 13 consecutive days. On day 14, all dogs received an equivalent single intravenous dose. Sebutapes™ were used to measure RFP concentration in the sebum. Sebum was collected at 0 hours (before RFP given), and at days 1, 7, and 14 at peak plasma concentration (Cmax). Sebutapes™ were applied to the skin for two hours prior to each sampling time. Sebutapes™ were removed at each sampling time and stored in red-top tubes at 20°C until analyzed.

In addition to sebum samples, whole blood was collected into heparin tubes on day 14 at times 0hr, 0.5hr, 4hr, 8hr, 12hr, 36hr, and 48hr. Samples were centrifuged at 2500 rpms for 15 minutes to separate the white blood cell (WBC)-containing buffy coat, which was harvested and stored in 0.2mL PCR tubes at 20°C for later HPLC analysis.

RFP was quantitated in all samples using high performance liquid chromatography (HPLC).

**Results.** Preliminary results from 2/12 dogs revealed that RFP concentrations (µg/ml) in sebum on days 1, 7, and 14 were $24 \pm 11.7$, $105 \pm 96$ and $16 \pm 2.3$ resulting in a sebum to plasma ratio of 1.1, 3.3, and 0.6, respectively. Sebum RFP concentrations were more than 3000-fold higher than the reported minimum inhibitory concentration ($MIC_{90}$) of RFP for SIG organisms regardless of methicillin resistance. Buffy coat samples are undergoing analysis.

**Conclusions**. These preliminary results suggest that RFP administered at 5-10 mg/kg/day orally for 13 days in dogs achieves concentrations in sebum that well exceeds the $MIC_{90}$ of RFP for SIG. This suggests that RFP should be a reasonable, if not preferred, antibiotic choice for treatment of methicillin-resistant SIG skin infections in dogs.

**Sheep Natural History**: **A Study of Tay-Sachs Disease in Jacob Sheep**

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**Introduction.** The purpose of the study was to observe the natural disease progression of sheep with Tay-Sachs disease over time and correlate clinical signs with histopathologic changes. To do this we determined how different cell populations (neurons, oligodendrocytes, astrocytes, and microglial cells) respond to Tay-Sachs disease over time in various areas of the brain. The ultimate goal is to understand how Tay-Sachs disease progresses to help guide delivery routes and optimal times for treatment of novel therapeutics.

**Methods.** A cohort of 16 sheep were used for the study. The CNS sections evaluated were the thalamus, hippocampus, parietal cortex, cerebellar hemispheres, cervical and lumbar intumescences. The stains utilized were H&E, GFAP (for astrocytes), OLIG2 (for oligodendrocytes), IBA1 (for microglial cells), MAP2 (for neurons), and Luxol Fast Blue (for myelin). Image analysis was conducted using Visiopharm 6.9.2.3050.

**Results.** The 16 sheep cohort consisted of 8 normal and 8 Tay-Sachs sheep. They were necropsied and evaluated at 3 months (n=3 per group), 6 months (n=3 per group), and 9 months (n=2 per group) of age. Clinical signs in sheep consist of knuckling (earliest detectable sign), gait disturbances, limb paresis, limb contracture, wide-based stance, vision deficits, proximal limb paresis, and tremors. Neurological examinations showed postural reaction deficits starting as early as 3 months, decreased flexor withdrawal starting at 5 months, and decreased menace response starting at 6 months of age. The clinical rating scale, which is composed of gait, posture, and ocular motor abnormalities shows deviation from normal starting at 3 months of age increasing progressively to the 9 month time point. OLIG2 staining showed significant decreases in oligodendrocyte density in the thalamus and cerebellum for all time points, and in the hippocampus at 3 and 6 months. IBA1 showed significant increases in microglial density in the parietal cortex and cerebellar hemispheres at 9 months. GFAP results show significant increase in astrocytic density in the thalamus at the 6 month time point. MAP2 results showed significant decrease in neuronal density at 6 months in the hippocampus and cerebellar hemispheres.

**Conclusions.** A global decrease in oligodendrocyte density was observed by 3 months of age. In the thalamus and parietal cortex, there were increases in astrocytic & microglial density with decreases in myelin and MAP2 densities by 6 months of age. The cerebellar hemispheres showed increased astrocytic and microglial densities with a decreased MAP2 density. Overall, this data suggests a reduction in neuronal staining, myelination, astrocytosis, and microgliosis in the Tay-Sachs sheep, which is worse in the thalamus, but present to a lesser degree in the cerebellum.

# EGFR and AR regulated genes: profiles in novel African American and Caucasian prostate cancer cell lines.

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**Introduction**. Prostate cancer is one of the most common cancers seen in men. Race is one of the risk factors for this cancer; African American men have a higher risk of prostate cancer than Caucasian men do. In 2010, researchers at Tuskegee University developed a new African American prostate cancer cell line called RC-77T/E. The purpose of this research was to characterize this cell line and compare it to Caucasian cell lines, including LNCaP, PC-3, and DU-145. The expression of EGFR and AR regulated genes were examined, as well as the effects of EGF and androgen on these cell lines.

**Methods**. Several laboratory techniques, including qPCR, RT-PCR and gel electrophoresis, western blotting, immunocytochemistry (ICC), and MTT assay, were used to compare the African American RC-77T/E prostate cancer cell line to the Caucasian LNCaP, PC-3, and DU-145 cell lines. For PCR, mRNA was extracted and expression of the following genes was examined: AR, ARA70, NKX3, PSA, EGFR, and Kaiso. For ICC, anti-Kaiso and anti-AR secondary antibodies were used. For western blotting, anti β-actin, anti-AR, and anti-EGFR secondary antibodies were used. For the MTT assays, cells were either untreated, treated with R1881, or treated with EGF.

**Results**. AR was expressed in LNCaP and RC-77T/E, but not in PC-3 or DU-145. EGFR and Kaiso were expressed in all four cell lines. Treatment with both R1881 and EGF increased the expression of EGFR and AR regulated genes (ARA70, NKX3, and PSA) in the PC-3 cell line. Treatment with R1881 and EGF increased LNCaP proliferation.

**Conclusions**. AR and EGFR are critical in prostate cancer progression and are likely to regulate each other. Like androgen dependent LNCaP, RC-77T/E expresses biologically active AR and EGFR as well as downstream regulated genes, PSA and Kaiso. The RC-77T/E cancer cells provide novel tools to study the molecular and genetic mechanisms of prostate carcinogenesis, especially for high rick African Americans.

# Undergraduate Student Poster Presentation

**Expression of the INK4AB/ARF tumor suppressor transcription factor MSK1 in canine breast cancer: Quantification through QrtPCR and correlation with established phenotypes.**

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**Introduction**. Canine and human mammary cancers have many similarities, allowing canine samples to be used as effective models of human disease. As cancer is a heterogeneous disease, the ability to determine and later predict the precise mechanisms promoting neoplasia would allow the advancement of therapeutic targets/strategies to combat cancer directly. Mitogen- and stress-activated kinase 1 (MSK1) is a gene investigated for its downstream regulation of crucial tumor-suppressor proteins p15 and p16, and is therefore upregulated during oncogenic stress resulting in a failure to suppress cancer cell proliferation. Due to its regulation of a pro-survival pathway, MSK1 is of great interest as a target for cancer vaccine therapy.

**Methods**.
Cell culture: Six established canine mammary tumor cell lines (CMT 9, 12, 27, 28, 47, 119) and one primary canine mammary epithelial cell culture (CMEC) were grown in Alpha-MEM supplemented with 10% FBS, 100 µg/ml penicillin, and 100 µg/ml streptomycin in a 5% $CO_2$ 100% humidity atmosphere at 37°C.

RNA extraction: Total cellular RNA was extracted from the cell lines using the High Pure RNA isolation kit according to the manufacturer's protocol.

Qrt-PCR: RNA product was amplified by QrtPCR and analyzed using a quantitative reverse transcriptase PCR assay with a SYBR Green fluorescent marker. Component input was standardized at 1 ug for cDNA and 0.5 uL (uM?) of forward and reverse primers for MSK1 per well.

**Results**. In previous study of these CMT and CMEC cell lines, the MSK1 PCR DNA product was detected on ethidium bromide-stained agarose gels revealing the predicted 450 bp amplicon. The specificity of the amplified product was confirmed in all CMT and CMEC cell lines by DNA sequencing. The amounts of amplicon recovered, using this semi-quantitative assay, suggested enhancement of expression in neoplastic cells. To confirm this data, QrtPCR was performed and the results supported the previous data demonstrating increased expression of MSK1 in CMT cell lines compared to normal CMEC cells.

**Conclusions**. Enhanced expression of MSK1 in neoplastic cells confirmed the attempts by these cells to suppress proliferation but then fail in this effort due to defects in the p15/p16 encoding INK4AB/ARF locus. To our knowledge, there are no studies that have analyzed the expression of MSK1 in canine mammary gland cancers or to access its value as a therapeutic target in canine breast cancer patients. Further exploration of the p16, MSK1 pathway will focus on downstream targets of MSK1, including the CDK1 and CDK2 cell cycle integration complex proteins, utilizing western blotting, flow cytometry, and fluorescent microscopy.