# PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 6, 2019

# 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 6, 2019

**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 6, 2019 – VETERINARY EDUCATION CENTER

## 8:00: Opening Statement

Dr. Frank F. Bartol,
Alumni Professor and Associate Dean for Research & Graduate Studies,
Auburn University College of Veterinary Medicine

## 8:15-11:00 MORNING Presentations - Overton Auditorium
## Moderator (Morning Sessions): Dr. Tom Jukier

### Undergraduate Student

| | | |
|---|---|---|
| 8:15 | Courtney Garrett | Cardiovascular pathology in feline GM2 gangliosidosies before and after AAV gene therapy |

### Veterinary Students

| | | |
|---|---|---|
| 8:30 | R. Jordan Farrell | A Retrospective Analysis on Determinants of Litter Size |
| 8:45 | Hannah Himmelmann | The Influence of Probe Radius of Curvature on the Articular Cartilage Friction |
| 9:00 | Ali Perregrino | Serologic evidence of select vector-borne pathogens in unowned dogs |

### Graduate Students

| | | |
|---|---|---|
| 9:15 | Ferrin Antony | IFN-λ regulates early events in HSV I-induced corneal immunopathology |
| 9:30 | Lauren Charnock | Ocular blunt force trauma: A retrospective observation of 44 equine cases |
| 9:45 | Anne Maguire | A Meta-Analysis of Myelin Pathology in Neurodegenerative Lysosomal Storage Diseases |
| 10:00 | Amanda Gross | Intravenous delivery of AAV gene therapy in GM1 gangliosidosis |
| 10:15 | Jenny Howard | Documenting Secondary Bacterial Infection In Canine Aspiration Pneumonia |
| 10:30 | Bamidele Jeminiwa | Endocrine disrupting effects of dietary soy isoflavones in pubertal male rat |
| 10:45 | Shari Kennedy | Evaluation of Bovine Viral Diarrhea Virus Genetic Changes in Congenitally Infected Piglets |

***The Presentation of the Zoetis Research Award will precede the 11:00 AM Lecture**

**11:00: Presentation of 2019 Zoetis Research Award Auburn Faculty Award**

## 11:05: Joy Goodwin Lecture-101 VEC
**Dr. Edward Breitschwerdt**
**"Pathobiome of Blood and Complex Disease Expression"**

## 12:00–12:45 POSTER Presentations-VEC Lobby

## 12:45-3:45 AFTERNOON Presentations - Overton Auditorium
**Moderator (Afternoon Sessions): Dr. Kathy Gerken**

### Graduate Students (continued)

| | | |
|---|---|---|
| 12:45 | Jiwoong Her | Relationship between admission variables in dogs with brain herniation: A retrospective study in 54 dogs |
| 1:00 | Steven Kitchens | Movement and Persistence of *Salmonella* in a Veterinary Teaching Hospital |
| 1:15 | Maxime Derré | Retro-Oesophageal Scoping Approach (ROSA): Description of an Original Nasopharyngeal Endoscopic Technique and Comparison to Conventional Retroflexed Endoscopic Approach |
| 1:30 | Gisela Martinez-Romero | Mammaglobin-A immunohistochemistry and protein expression in canine mammary tumors |
| 1:45 | J. Fletcher North | Susceptibility of Swine to Human Influenza A Viruses and the Emergence of Zoonotic Viruses |
| 2:00 | Claudia Reyner | Effect of Tumor Necrosis Factor Alpha and Interleukin-1 Beta on Equine Endothelial Colony Forming Cell Function in Culture |
| 2:15 | Jenna Stockler | Evaluation of health and performance of dairy calves supplemented with colostrum replacer during the pre-weaning period |
| 2:30 | Ana Velloso Alvarez | Effects of autologous protein solution, autologous conditioned serum, and triamcinolone on inflammatory and catabolic gene expression in equine cartilage and synovial explants treated with IL-1β in co-culture |
| 2:45 | Kathleen Weatherall | Comparison of Tensile Strength and Early Wound Healing of Self-Locking and Surgeon's Knots for Closure of Ventral Midline Celiotomy in Horses |

## Faculty

| | | |
|---|---|---|
| 3:00 | Erik Hofmeister | Non-participant student observation of faculty classroom teaching |
| 3:15 | Allisandra Rha | Ratiometric pH probe for the identification of acidic compartments along the endo-lysosomal network |
| 3:30 | Melissa Singletary | Incorporation of Virtual Reality to Enhance the Teaching of Small Animal Gross Anatomy in the Veterinary Curriculum |
| 3:45 | Bruce F. Smith | Gene expression profiles in individual canine patient osteosarcomas |

## 4:00 KEYNOTE LECTURE-Overton Auditorium

## 5:00 INDUCTION AND AWARDS ANNOUNCEMENT

**INDUCTION of new Phi Zeta Members**

**Research Awards Presentations**

## 5:30 RECEPTION - VEC Lobby

# PHI ZETA KEYNOTE SPEAKER AND JOY GOODWIN LECTURER

## "Bartonellosis: A One Health Approach to an Emerging Infectious Disease"

**Edward Breitschwerdt, DVM, DACVIM**
The Melanie S. Steele Distinguished Professorship in Medicine
Professor, Internal Medicine
College of Veterinary Medicine
North Carolina State University

Dr. Edward B. Breitschwerdt is a professor of medicine and infectious diseases at North Carolina State University College of Veterinary Medicine. He is also an adjunct professor of medicine at Duke University Medical Center, and a Diplomate, American College of Veterinary Internal Medicine (ACVIM). Dr. Breitschwerdt directs the Intracellular Pathogens Research Laboratory in the Comparative Medicine Institute at North Carolina State University. He also co-directs the Vector Borne Diseases Diagnostic Laboratory and is the director of the NCSU-CVM Biosafety Level 3 Laboratory.

A graduate of the University of Georgia, Breitschwerdt completed an internship and residency in Internal Medicine at the University of Missouri between 1974 and 1977. He has served as president of the Specialty of Internal Medicine and as chairperson of the ACVIM Board of Regents. He is a former associate editor for the *Journal of Veterinary Internal Medicine* and was a founding member of the ACVIM Foundation.

Breitschwerdt's clinical interests include infectious diseases, immunology, and nephrology. For over 30 years, his research has emphasized vector-transmitted, intracellular pathogens. Most recently, his research group has contributed to cutting-edge research in the areas of animal and human bartonellosis. In addition to authoring numerous book chapters and proceedings, Dr. Breitschwerdt's research group has published more than 350 manuscripts in peer-reviewed scientific journals. In 2012, he received the North Carolina State University Alumni Association Outstanding Research Award and in 2013, he received the Holladay Medal, the highest award bestowed on a faculty member at North Carolina State University. In 2017, Dr. Breitschwerdt received the American Association of Veterinary Medical Colleges Outstanding Research Award and the American Canine Health Foundation Excellence in Research Award. In 2018, he was named the Melanie S. Steele Distinguished Professor of Medicine at North Carolina State College of Veterinary Medicine.

# Posters

## Undergraduate Students

| | |
|---|---|
| John Abrams | Comparison of Lysosomal Enzyme Activity in Cat Tissue with or Without Saline Perfusion |
| Greer Cauthen | Expression of Xrra1 gene in mouse adrenal gland |
| William Kretzschmar | CRISPR/Cas9 Mediated Genetic Modification of Adenovirus Type II |

## Veterinary Students

| | |
|---|---|
| Seth Bowden | Altered astrocytic Nrf2 protein regulation in spontaneously hypertensive rats |
| Lucia Cresci | Identification of disease diagnostic and prognostic biomarkers in the equine gastrointestinal microbiome |
| Chloe Haynes | Engineering a bispecific molecule to simultaneously inhibit and co-stimulate immune checkpoints for combination immunotherapy of canine cancer |
| Natalie Heape | Regulation of immune surface markers by CDK4/6 inhibitors in gammaherpesvirus-infected tumor cells |
| Marisa Hernandez | Elective cesarean sections: effects of perioperative variables on Neonatal Survivability |
| Micaela Ludwig | Relative Quantification of Cannabinoid Receptor mRNA in Equine Tissue |
| Kara Maneval | Equine origin vascular endothelial growth factor effects on equine endothelial colony forming cells |
| Ana Portales | Modeling Developmental Trichloroethylene Toxicity in the Zebrafish |
| Laura Raines | Maternal serology as a prenatal test for detecting Bovine viral diarrhea virus persistent infection |
| Ronald Sams | Development of Bispecific T Cell Engagers (BiTEs) Against Rhabdomyosarcoma Targeting FGFR4 |
| Joshua Trumble | Alfaxalone Cross-Reactivity affecting Progesterone Concentrations in Cats |

## Graduate Students

| | |
|---|---|
| Sophie Boorman | Presentation, Treatment and Outcome of Aural Hematoma in Horses: 7 cases (2008-2019) |
| Sophie Boorman | Clinical Osteochondritis Dissecans in Standardbred Yearlings: Lesion Specific Impact on Racing Performance and Longevity |
| Samantha Bradley | Investigation of endocrine disrupting properties of per- and polyfluoroalkyl substances in male rats |
| Wenqi Cao | Maturation of gut microbiota and dramatic shift of microbial composition during canine puppy development |
| Jeff Daniel | Spatiotemporal expression profile of embryonic and adult Ankyrin and EF-hand containing protein 1-encoding genes ankef1a and ankef1b in zebrafish |
| Taylor Flaat | Hunting mast cell progenitors in normal canine blood |
| Jiwoong Her | Relationship between admission variables in dogs with brain herniation: A retrospective study in 54 dogs |
| Yoshimi Iwaki | Canine myxosarcomas, a retrospective analysis of 32 dogs (2003-2018) |
| Xiaolei Ma | Whole-genome shotgun metagenomic analysis of chicken gut microbiome under healthy conditions and coccidia infection |
| Saba Omer | Anti-Proliferative Effects of Cannabinoids in Canine Lymphoma |
| Damien Ruiz | Development of an E3 Modified Canine Oncolytic Adenovirus |
| Julia Salamat | Gefitinib, at Its Clinically Relevant Concentrations, Inhibits Rifampicin-Induced CYP3A4 Gene Expression in Human Hepatocytes |
| Haolong Wang | Evaluating the efficacy of marker-assisted selection (MAS) using disease resistance and growth traits QTLs in catfish |
| Xiao Xiong | Characterizing the genetic and genomic architecture of eight laboratory opossum strains using ddRAD-seq technology |
| Ramon Zegpi | Protection Conferred by IBV S-ectodomain Expressed from Recombinant NDV LaSota |
| Huifei Zheng | Overexpression of Foxl2 leads to ovarian-like adrenal glands |
| Yihang Zhou | Single-cell transcriptome analysis of lung cancer brain metastatic cells in the cerebrospinal fluid |

## Faculty/Staff

| | |
|---|---|
| Dawn Boothe | The Disposition of Cannabidiol in Dogs after Single Dose Oral Administration |
| Dawn Boothe | Therapeutic Drug Monitoring of Cannabadiol (CBD) and THC Concentrations in Dogs Receiving "Hemp Oil" Products |
| Crisanta Cruz-Espindola | High Performance Liquid Chromatography (HPLC) method development and validation for Mebendazole in canine plasma and Cerebrospinal fluid (CSF) |
| James Gillespie | Phage-peptide constructs for stimulation of anti-cancer immune responses against CD47 |
| James Gillespie | Evolution of landscape phage library in a mouse model of breast cancer |
| Qiongxia Liu | The sexually dimorphic response of the mouse adrenal inner cortex to thyroid hormone treatment |
| Jonathan Marable | Nanobody-based anti-CTLA4 immune checkpoint blockade therapy for canine cancer |
| Rachel Moon | Effect of Visual Arts Training on Veterinary Student Radiographic Observation, Description and Interpretation Skills |
| Mariano Mora Pereira | Sorting equine endothelial colony forming cells based on low-density lipoprotein uptake |
| Rebecca Riggs | Characterization and Differentiation of *Salmonella enterica* Typhimurium, *Escherichia coli* and *Listeria monocytogenes* using Hyperspectral Imaging |
| Vicky van Santen | Intestinal tropism of an infectious bronchitis virus isolate is not explained by spike protein binding specificity |
| Jinbin Wang | Rapid detection of P-35S and T-nos DNA elements in genetically modified organisms (GMO) by recombinase polymerase amplification combined with a lateral flow strip |

# Undergraduate Student Platform Presentations

### Cardiovascular pathology in feline GM2 gangliosidosies before and after AAV gene therapy

Courtney Garrett[1], Amanda Gross[1,2], Douglas Martin[1,2], Raymond Wang[3]

[1]Scott-Ritchey Research Center, [2]Department of Anatomy, Physiology, & Pharmacology, College of Veterinary Medicine, Auburn University, AL
[3]Division of Metabolic Disorders, Children's Hospital of Orange County Children's Specialists, Orange, CA

**Introduction.** GM2 gangliosidosies collectively refers to three genetic disorders that each lead to beta-hexosaminidase deficiency within lysosomes. Beta-hexosaminidase breaks down a glycolipid called GM2 ganglioside and without this enzyme, GM2 ganglioside accumulates in lysosomes and causes pathology throughout the body. GM2 patients usually begin to show symptoms of neurological disease during infancy and most do not survive past 5 years of age. Currently, palliative care is the only option for GM2 gangliosidosis, but intravenous adeno-associated viral (AAV) gene therapy has proven effective in mouse and feline models. The cardiovascular effects of GM2 were compared in phenotypically normal cats, untreated GM2 cats, and GM2 cats that were treated with intravenous AAV therapy. More specifically, elastin content and intima-media thickness (IMT) were quantitated in the ascending aorta, descending aorta, and carotid artery using Visiopharm and Aperio ScanScope software. Cats with GM2 gangliosidosies are known to have reduced elastin content and thicker intima-media and AAV therapy was expected to ameliorate these cardiovascular issues.

**Methods.** Tissue slides of the ascending aorta, descending aorta, and carotid artery were made from normal cats, untreated GM2 cats, and AAV-treated GM2 cats. Normal cats were an average age of 5.3±3.4 months, untreated GM2 cats were 1.9±1.9 months, and the AAV-treated GM2 were 6.6±1.6 months. These slides were then stained with haematoxylin-eosin, and elastin content was quantitated using Visiopharm software. Intima-media thickness was measured on the same scans with Aperio ScanScope by taking the average of six random measurements across the arterial tissue. Statistical analyses were performed to determine if elastin content and intima-media thickness were significantly different between the three populations.

**Results.** Arterial elastin content was lowest in untreated GM2 cats, and highest in normal cats, with intermediate levels in treated GM2 cats. Mean intima-media thickness was reduced in aorta tissue when comparing both normal (p-value=0.3295) and AAV-treated GM2 cats to untreated GM2 cats (p-value=0.2316). No statistical differences across cohorts were in IMT of the other vessels analyzed.

**Conclusions.** This data suggests that intravenous AAV treatment of GM2 cats prevents the loss of elastin content in cardiovascular tissues. AAV treatment appears to reduce intima-media thickness in the aorta, but not in the other vessels analyzed. Future studies could determine if ganglioside accumulation in cardiovascular tissue is diminished by intravenous AAV therapy.

**Acknowledgments.** This research was funded by the PSF Research Investigator Award to R.W. and private donations to the research program of D.R.M.

# Veterinary Student Platform Presentations

### A Retrospective Analysis on Determinants of Litter Size

R. Jordan Farrell[1], Robyn Wilborn[1], Pamela Haney[2] and Jamie M. Douglas[1]

[1]Department of Clinical Science, College of Veterinary Medicine, Auburn University, AL
[2]Canine Performance Sciences, College of Veterinary Medicine, Auburn University, AL

**Introduction.** The mission of Canine Performance Sciences (CPS) Breeding Program is to improve canine technologies through intentional breeding of superior quality detection dogs. Detection dogs enhance national security and are the most effective tool for the detection of emerging threats. Canids are a monoestrous species, meaning their physiology mandates a six- to eight-month wait between breeding attempts. Therefore, in purpose bred dogs, maximizing the number of successful breedings and litter size is important. Expenses associated with missed breeding attempts include the cost of food, housing, and health care, in addition to the cost of breeding and the loss of productivity, which led to the question, "What are the key factors affecting pregnancy and litter size in the CPS breeding colony?" The purpose of this research study was to standardize the breeding process to achieve consistent litter sizes in purpose bred detection dogs.

**Methods.** A retrospective study of the CPS breeding population examining 55 breeding cycles across 26 dams was conducted. Within each breeding cycle, multiple factors were examined. A multiple regression was run to analyze the age of the dam at the time of breeding, semen type used for insemination, insemination method, and total number of inseminations per litter.

**Results.** The average litter size of a 30-month old dam when using fresh semen, artificial insemination, and no induction drugs was 5.45 puppies per litter. A multiple regression model examining the above factors was statistically significant, $F_{(7, 47)} = 9.08$, $p < 0.001$, $R^2 = 0.5116$. This regression model explained 57% of the variance in litter size. Based on the analyses, the addition of one transcervical insemination (TCI) during the peak fertile window significantly increased litter size ($p = 0.005$). Similarly, the addition of one live cover mating during the peak fertile window increased litter size ($p = 0.07$). Litter size was impacted by the type of semen utilized, the use of frozen semen significantly decreased the litter size ($p_{frozensemen} < 0.001$). There was no statistically significant effect of age of dam or the use of estrus management drugs on litter size or conception rate ($p_{ageofdam} = 0.44$, $p_{estrusdrugs} = 0.90$).

**Conclusions.** In the CPS breeding colony, litter size was greatest when fresh semen was used at breeding. Frozen semen used for breeding produced the least successful litter size. Fresh and cool-shipped semen inseminations averaged litters between 6 and 8 puppies. Scrutiny of type of insemination revealed that the addition of one live cover mating increased litter size by 2 puppies, while the addition of one TCI mating increased litter size by 3 puppies. In summary, data from this study supports the incorporation of one live coverage and TCI utilizing fresh semen to increase average litter size in the CPS breeding program.

**Acknowledgments.** Boehringer Ingelheim Summer Scholars Program, Auburn University Theriogenology Services, Auburn Canine Performance Sciences, Auburn College of Veterinary Medicine Office of Research.

## The Influence of Probe Radius of Curvature on the Articular Cartilage Friction

Hannah K. Himmelmann, Reid Hanson[1], Robert L. Jackson[2], Kyle D. Schulze[3]

[1]Department of Equine Sports Medicine and Surgery
[2]Department of Mechanical Engineering
[3]Department of Mechanical Engineering

**Introduction.** Joints are well known for their ability to withstand high loads with minimum damage. Articular cartilage is an essential element providing lubrication in joints. Multiple lubrication mechanisms are observed within joints. It is important to understand how joint friction mechanisms work for disease purposes. Measuring the coefficient of friction (COF) of a sample of cartilage under specific conditions can help explain friction mechanisms in a joint. Determining which pressure regime a joint resides in at a different pressure is also important in understanding how joints work. The Hertz pressure regime is what is expected. The Hertz regime considers laterally coupled elasticity, while the Winkler regime assumes that the vertical stiffness is laterally isolated. If pressure continues to increase, the pressure regime will change from Hertz to Winkler's pressure regime. Last year, we found that under a constant force and varying pressures, a smaller radial contact area will produce a larger COF. We also found that under varying forces and pressures, a smaller radial contact area will still produce a larger COF. The hypothesis for this study was when applying a constant pressure and varying forces to the articular cartilage, the smaller radial contact area will still have a higher coefficient of friction.

**Methods.** First, articular cartilage samples were collected from horses euthanized for reasons unrelated to this study. Articular cartilage was collected from the left and right distal, medial aspect of the articular surface of the radius. This side was cut into roughly a 4 $cm^2$ sample which was used for testing. Tests were run on the samples within 24 hours of cutting. The sample was mounted and a phosphate buffer saline solution filled the bowl until the articular cartilage sample was slightly submerged.
Five tests, each five minutes in length, were run on each articular cartilage sample. Each test involved a different sized steel ball. Steel balls sized 1/16 in., 1/8 in., ¼, in., ½, in., and 1 in. were used. The study used a Bruker Universal Mechanical Test (UMT) machine. The reciprocating friction data was processed so that an average coefficient friction for each test was found.

| Diameter of Steel Ball (in) | Force (N) |
|---|---|
| 1/16 | 0.314 |
| 1/8 | 1.25 |
| ¼ | 5 |
| ½ | 19.9 |
| 1 | 78.5 |

**Results.** The 0.314 N (1/16 inch) measurement and the 1.25 N (1/8 inch) measurement are in the Hertz pressure regime and have high COF. The 5 N (1/4 inch) measurement is transitioning between Hertz and Winklers pressure regime and has the lowest COF. The 19.9 N (1/2 inch) and 78.5 N (1 inch) measurements are in Winklers pressure regime and the COF is high.

**Conclusion.** The transition from the Hertz to the Winkler pressure regime seems to correlate with the increase in COF due to an indirect increase in force. The Hertz pressure regime correlates with a low COF and minimal cartilage wear. However, more tests need to be conducted to fully understand the results observed.

**Acknowledgments.** Thank you to Cole Baker and everyone in necropsy and the Boehringer Ingelheim Veterinary scholarship program for funding.

## Serologic evidence of select vector-borne pathogens in unowned dogs

Ali N. Perregrino[1], Lindsay Starkey[2], and Sarah Zohdy[2,3]

[1]Class of 2021, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3]School of Forestry and Wildlife Sciences, Auburn University, AL

**Introduction.** In the United States, and especially the southeast, vector-borne pathogens (VBP) associated with ectoparasitism are of concern for domestic animal health. Currently there are many gaps in the surveillance, reporting, and subsequent epidemiological data regarding VBP in companion animals, especially free-roaming or unowned animals. The purpose of this study was to test for the presence of four vector-borne pathogens in a subset of free-roaming/unowned dogs in Alabama and Georgia.

**Methods.** A pre-existing program at Auburn University's College of Veterinary Medicine temporarily admits dogs from county humane societies in Alabama and Georgia for health exams and castration surgeries. Anticoagulated blood samples are drawn by clinicians for standard pre-surgical diagnostics; any samples with remaining volume were available for opportunistic retrieval and analysis for this study. The 4Dx SNAP Plus (IDEXX Laboratories, Inc.), which is an antigen test for *Dirofilaria immitis* and an antibody test for *Borrelia burgdorferi, Ehrlichia canis, E. ewingii, Anaplasma phagocytophilum,* and *A. platys,* was utilized for serologic testing. Blood smears were prepared and visually analyzed; any residual whole blood was preserved for future DNA extraction and PCR. Ectoparasites visualized during physical exam were noted, and if possible, removed and preserved in ethanol for species identification and analysis.

**Results.** From May to October, 2019, 114 canine patient samples were available for opportunistic retrieval. Out of 114 samples, 35.1% (40/114) were seropositive for one or more VBP: *Dirofilaria immitis* (20.2%; 23/114) and *Ehrlichia* spp. (20.2%; 23/114) with six of the VBD-positive dogs (15%) testing positive for both *D. immitis* and *Ehrlichia* spp. No dogs tested seropositive for either *Borrelia burgdorferi* or *Anaplasma* spp. (0%; 0/114). Microfilariae (MFF) of *D. immitis* were present in blood smears of 7/23 dogs; no MFF were seen in any dog testing negative for *D. immitis* antigen. Morulae of *Ehrlichia* or *Anaplasma* spp. were not identified in any blood smear. Out of the 114 patients, 19.3% (22/114) were documented as infested with fleas and 9.6% (11/114) infested with ticks. Out of the 11 patients where ticks were detected, 13 adult and 13 nymphal ticks were collected from 8 dogs and identified as adult and nymphal *Amblyomma americanum* (92.3%; 24/26), adult *Amblyomma maculatum* (3.8%; 1/26), and adult *Dermacentor variabilis* (3.8%; 1/26).

**Conclusions.** The four VBP examined in this study pose health risks for not only unowned and free-roaming animals, but also pets and humans. Over 35% of the dogs evaluated in this study tested seropositive for one or more VBP which is higher than the canine results for *D. immitis* and *Ehrlichia* spp. currently reported for 2019 provided through the Companion Animal Parasite Council website (Alabama = 3.58–4.06%; Georgia = 2.2–3.57%). These data indicate that VBP risk in Alabama and Georgia may be higher than reported by the current VBP data, and that the reservoir potential of domestic animals, especially free-roaming animals, warrants further investigation.

**Acknowledgments.** Boehringer Ingelheim Summer Scholars Program. Hoerlein spay and neuter program.

# Graduate Student Platform Presentations

### IFN-λ regulates early events in HSV I-induced corneal immunopathology

Ferrin Antony and Amol Suryawanshi

Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

**Introduction.** Herpes simplex virus-1 (HSV) infection of the eye causes chronic inflammatory lesion in the cornea called herpetic stromal keratitis (HSK). HSK is highly painful condition and a leading cause of infectious blindness worldwide. The lesion is considered to be immuno-pathologically orchestrated by neutrophils and IFN-γ producing $CD4^+$ T (Th1) cells. Currently, HSK is mainly controlled by combination of therapies that include topical/oral antiviral, long-term corticosteroids and corneal transplantation. Unfortunately, these therapies are moderately effective and can cause severe side effects such as aggravated corneal ulcer, ocular hypertension, thinning and perforation of the cornea. Thus, there exists an unmet need to develop novel therapies against HSK. Accordingly, the selective induction of strong anti-viral response with minimal activation of immuno-pathological responses embodies a powerful treatment approach for HSK patients. Recent studies have identified IFN-λ as the predominant anti-viral cytokine and first line of defense at epithelial surfaces during several acute and chronic viral infections. Interestingly, IFN-λ lacks the pro-inflammatory side effect of IFN-α/β and plays a non-redundant role in host protection during viral infections. Since IFN-λ effectively control viral replication with minimal activation of inflammatory innate and adaptive immune responses, we propose that activating IFN-λ response during corneal HSV infection will be an ideal therapeutic strategy to treat HSK patients.

**Methods.** The mice strain C57BL/6J was used in our study and experiments were conducted under specific-pathogen-free conditions. Mice were anesthetized and infected with the virus strain HSV-1/RE (5000 pfu/eye) by scratching the cornea. For the treatment group, we administered rIFN-λ topically and used sterile water as the vehicle control. The progression of infection was clinically graded from 1 to 5. +1, mild corneal haze; +2, moderate corneal opacity or scarring; +3, severe corneal opacity, but iris visible; +4, opaque cornea, iris not visible; +5, necrotizing stromal keratitis. Mice were euthanized for collection of cornea, spleen and periocular draining lymph nodes which was used for immune cell analysis by flow cytometry and real time PCR.

**Results.** In this study using mouse model of HSK, we characterized the differential induction of type I and type III IFN responses and evaluated the therapeutic potential of recombinant IFN-λ (rIFN-λ) during ongoing corneal HSV infection. There was decrease in viral titer upon early rIFN-λ administration. The severity of lesions were reduced in the treatment group which was evident by the microscopic examination of the infected eye and H&E staining of the cornea. Further, a decrease in HSK immunopathology was observed in rIFN-λ treated group through reduced infiltration of Th1, neutrophil and macrophages into the cornea and secondary lymphoid organs. Additionally, we observed a decreased expression of ISG-15 and CXCL-1 (neutrophil chemokine) upon rIFN-λ treatment indicating reduced neutrophil activation.

**Conclusions.** From the results obtained, we conclude that corneal HSV-I infection induces both type I and type III IFN responses. Further, IFN-λ treatment during corneal HSV infection suppresses the HSK pathology through regulation of innate and adaptive immune responses. The early rIFN-λ treatment also suppresses the viral replication and corneal infiltration of pro-inflammatory innate immune cells (neutrophils and macrophages collectively).Topical rIFN-λ administration represent a promising therapeutic approach to treat HSK, the most common cause of infectious blindness in the Western world.

**Acknowledgments.** This research work was funded by the Department of Pathobiology, CVM, Auburn University Start-up Fund and the Presidential Graduate Research Fellowship.

## Ocular blunt force trauma: A retrospective observation of 44 equine cases

Lauren Charnock[1], Richard J. McMullen Jr.[1,2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Equine Clinic Munich-Riem, Munich, Germany

**Introduction.** Blunt ocular trauma is frequently suspected in horses and may be associated with a wide variety of clinical signs. These signs include corneal ulceration, corneal edema, hyphema, uveitis, cataract formation, lens (sub-)luxation, and visual changes, but are commonly present with other ocular diseases, making it a challenge to diagnose blunt ocular trauma. The objectives of this study were to identify patterns of clinical signs associated with blunt ocular trauma, and to report other clinical findings that are suspected to be pathognomonic for equine blunt ocular trauma.

**Methods.** Medical records of horses presenting for ophthalmic examination to the equine ophthalmology services of the Equine Clinic Munich-Riem, Munich, Germany and Auburn University 2013 and 2018 were reviewed. The following data was recorded: age, sex, breed, laterality, clinical findings on ophthalmic examination, and whether an observed traumatic incident was observed (confirmed) or unobserved (suspected).

**Results.** Blunt ocular trauma was confirmed in 12/44 or suspected in 32/44 of the horses. Frequent ocular findings included cataract formation (29/44), one or more components of uveitis (22/44) [including hyphema (10/22)], corneal edema (20/44), peri-papillary depigmentation (15/44), lens subluxation/luxation/loss (14/44), avulsed corpora nigra (10/44), iridodialysis (9/44) and secondary glaucoma (9/44).

**Conclusions.** Cases of equine blunt ocular trauma often present with a distinct collection of clinical signs, allowing for differentiation from other ocular diseases. The footprint of ocular signs described in this manuscript may serve as a useful source when assessing chronic ocular lesions during pre-purchase examinations, and to differentiate blunt ocular trauma from equine recurrent uveitis.

## Retro-Oesophageal Scoping Approach (ROSA): Description of an Original Nasopharyngeal Endoscopic Technique and Comparison to Conventional Retroflexed Endoscopic Approach

Maxime Derré[1], Elisabeth Snead[2], Adrien Aertsens[3], Hugues Beaufrere[4]

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**Background.** In cats, posterior rhinoscopy is limited by the size of the pharynx making biopsy difficult and sometimes samples non-representative.

**Objective.** To describe a new minimally invasive endoscopic technique for performing posterior rhinoscopy using an esophageal approach, described as Retro-Oesophageal Scoping Approach (ROSA), and to compare this technique to the traditional Retroflexed Endoscopic Approach (REA).

**Design.** Prospective randomized controlled crossover evaluation study.

**Animals.** Thirty-six feline cadavers used by 36 participants in a veterinary teaching hospital, between March 2019 and May 2019.

**Procedures.** An esophagostomy was performed to introduce a bronchoscope and perform a full exploration of the nasopharynx. The order of the ROSA and REA procedures was randomized for each participant. Two landmarks (choanae and edge of the soft palate) had to be identified and then biopsied. The time to complete each procedure was recorded for each participant. They were asked to rate the perceived ease of use of both techniques, as well as the quality of images of the two landmarks collected during the study. Clinical application on a client-owned feline patient and management of naso-pharyngeal stenosis on a cadaver using the ROSA, are reported as well.

**Results.** Fifteen participants (15/36) were unable to visualize the edge of the soft palate and/or the choanae using the REA whereas 35 participants were able to visualize and biopsy the edge of the soft palate and the choanae using the ROSA. The quality of the images of the soft palate using the ROSA was estimated higher ($p<0.001$), with a median score and interquartile range of 7.5/10 (1.25), compared to the REA at 4.5/10 (2.1). The ROSA was quicker and seemed easier as compared to the REA ($p<0.001$).

**Conclusions.** This cadaveric study describes an alternative endoscopic technique (ROSA) that is quick and easy to perform, and provides a superior assessment of the feline nasopharynx compared to the more traditional REA.

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## Intravenous delivery of AAV gene therapy in GM1 gangliosidosis

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**Introduction.** GM1 gangliosidosis is a fatal neurodegenerative disease caused by a deficiency of lysosomal β-galactosidase (β-gal). In its most severe form, GM1 causes death by 4 years of age, and no effective treatments exist. Previous work has shown that injections of brain parenchyma with adeno-associated viral (AAV) therapy provides pronounced therapeutic benefit in a feline GM1 model. To develop a less invasive treatment for the brain and increase systemic biodistribution, intravenous injection of AAV9 was evaluated.

**Methods.** AAV9 expressing feline β-gal was intravenously administered at 1.5e13 vector genomes/kg body weight at approximately one month of age to six GM1 cats. The animals were divided into two cohorts: 1) a long-term group, which was followed to humane endpoint, and 2) a short-term group, with samples collected 16-weeks post treatment. Clinical assessments included neurological exams, cerebrospinal fluid (CSF) and urine biomarkers, and 7T magnetic resonance imaging (MRI) and spectroscopy (MRS). Postmortem analysis included β-gal and virus distribution, histological analysis, and ganglioside content.

**Results.** Untreated GM1 animals survived 8.0±0.6 months while IV treatment increased survival to approximately 3.5 years. Neurological abnormalities, which in untreated GM1 animals progress to the inability to stand and debilitating neurological disease by 8 months of age, were mild in treated animals. CSF biomarkers such as aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were normalized, indicating decreased central nervous system (CNS) cell damage in the treated animals. Urinary glycosaminoglycans, known to be elevated in GM1 patients, decreased to normal levels in the long-term cohort. MRI revealed the preservation of brain architecture in AAV-treated cats, and MRS revealed correction of glycerophosphocholine and phosphocholine, a marker of demyelination. β-gal activity was increased throughout the CNS, reaching normal levels in the CSF, cerebellum and spinal cord (cervical, thoracic and lumbar regions). Peripheral tissues such as heart, skeletal muscle and sciatic nerve also had normal β-gal activity in treated GM1 cats. GM1 histopathology in the CNS was largely corrected with treatment, with little to no evidence of storage lesions. There was no evidence of tumorigenesis or toxicity.

**Conclusions.** Restoration of β-gal activity in the CNS and peripheral organs by IV gene therapy led to profound increases in life span and quality of life in GM1 cats. This data supports the promise of IV gene therapy as a safe, effective treatment for GM1 gangliosidosis and has enabled a recently initiated clinical trial at the NIH.

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**Relationship between admission variables in dogs with brain herniation: A retrospective study in 54 dogs**

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**Introduction.** Brain herniation is one of the most frequent life-threatening neurological emergencies. This study evaluated the clinical utility of admission systolic blood pressure (SBP), heart rate (HR), and Modified Glasgow Coma Scale (MGCS) score to help distinguish dogs with brain herniation compared to dogs without brain herniation with neurological signs.

**Methods.** Medical records from a veterinary teaching hospital were compiled to identify all dogs presenting primarily for neurological signs that underwent a brain MRI between January 2010 and May 2019. For entry into the study group, each dog had evidence of brain herniation on MRI. Based on MRI findings, dogs were divided into two groups: herniated and not herniated. The two groups were compared for HR/SBP/MGCS score/SBP-HR on admission, age, and weight.

**Results.** A total of 54 dogs with brain herniation fulfilled the inclusion criteria. The control population consisted of 40 dogs without brain herniation. Dogs with brain herniation had significantly higher SBP ($P = 0.0078$), greater SBP-HR difference ($P = 0.0006$), and lower MGCS score ($P < 0.0001$) compared to control dogs. A cut-off value of SBP > 178 mmHg, SBP-HR > 60, and MGCS score < 14 provide a specificity of 90 to 98 percent to identify dogs with brain herniation.

**Conclusions.** High SBP, a greater difference between SBP and HR, and low MGCS score were highly predictive of brain herniation in dogs presenting with neurological signs upon admission. Early recognition of these abnormalities may help veterinarians to suspect brain herniation and determine timely treatment.

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## Documenting Secondary Bacterial Infection In Canine Aspiration Pneumonia

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**Introduction.** Aspiration pneumonia (AP) is an inflammatory pulmonary disorder in dogs resulting from inhalation of gastric contents or other substances. Despite the unknown prevalence of secondary bacterial infection, AP is routinely treated with antibiotics potentially contributing to antimicrobial resistance. Our study aims to identify the prevalence of bacterial infection in dogs with AP using bronchoalveolar lavage fluid (BALF) cytology and culture, and to evaluate agreement between these two modalities in detecting infection. Refuting widespread antimicrobial use, we hypothesized <50% of dogs with AP would have cytological evidence of bacterial infection and be culture positive.

**Methods.** Cases (n=21) were recruited retrospectively from the University of Missouri CVM & Auburn University CVM between 01/01/09-10/01/19. Inclusion criteria were as follows; a radiographic diagnosis of AP and ≥ 1 clinical risk factor, a CBC, thoracic radiographs and BALF cytology and culture collected within 48 hours of presentation. Dogs receiving >1 dose of antibiotics 7 days prior to BALF collection were excluded.

**Results.** Alveolar and interstitial patterns were identified in dependent lung lobes in 16 and 5 dogs, respectively. Eight dogs (38%) had concordant results of cytology and culture supporting secondary infection; 2 dogs (9%) had negative results for both. Most dogs had either positive cytology with negative culture (8 dogs; 38%) or negative cytology with positive culture (3 dogs; 14%) making it unclear if infection was present. Agreement between BALF cytology and culture was 47%.

**Conclusions.** Results highlight the diagnostic challenge of confirming secondary bacterial infection in AP. A multimodal approach may be required.

**Endocrine disrupting effects of dietary soy isoflavones in pubertal male rat**

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**Introduction.** Increased consumption of soy-based diets in the population is associated with putative health beneficial effects. Soybeans are a good source of protein (32-42%) containing most essential amino acids and low amounts of saturated fats. However, soybeans contain 0.1- 5 mg of isoflavones per gram. The predominant isoflavone in soybeans are genistein (50-55%) and daidzein (40-45%). Isoflavones (also known as phytoestrogens) are thought to either mimic and / or antagonize endogenous hormones. Endocrine disrupting compounds are substances present in the environment with capacity to interfere with the endocrine axis. Previous work from our lab and others have focused largely on the effects of isoflavones occurring in the immediate perinatal period. However, the increased use of soy-based diets in school lunch programs and USDA promotion of the nutritional benefits of soy has renewed interests on effects of soy diets in young individuals. Also, it is not clear that effects of soy-based diets on the male reproductive axis are due to the singular effects of genistein, daidzein or to both compounds acting together. Therefore, we investigated the effects of soy-based diets at concentration that are environmentally relevant on male reproductive development. We also determined whether effects of isoflavones are due to genistein, daidzein or both isoflavones.

**Methods.** Pubertal male rats at 35 days of age were maintained on control diet (Con), whole soybean diet (SBM) or the control diet supplemented with genistein and daidzein (G+D) for 14 days. In a separate experiment, male rats at 35 days of age were maintained on control diet (Con), or the control diet supplemented with daidzein (DAI) or genistein (GEN) or both genistein and daidzein (G+D) for 14 days. At sacrifice in both cases, serum testosterone (T) concentrations and testicular and Leydig cell T secretion were assayed by RIA. In addition, pituitary and gonadal tissues were processed for western blot analysis of proteins mediating isoflavone effects in the pituitary-gonadal axis.

**Results.** Results showed that in pubertal male rats, feeding of the SBM and G+D diets decreased serum T concentrations (Con: 6.1±0.5, SBM: 4.7±0.3, G+D: 4.7±0.4 ηg/mL; $p< 0.05$ ), inhibited testicular T (Con: 6.1±0.5, SBM: 4.7±0.3, G+D: 4.7±0.4 ηg/g; $p< 0.05$), and Leydig cell T production (Con: 13.6±0.7, SBM: 7.4±0.4, G+D: 7.5±0.4 ηg/ $10^6$ cells/ 3 h; $p< 0.05$) compared to the control diet. Furthermore, expression of Müllerian Inhibiting Substance (MIS) and inhibin Bβ protein were decreased in testes of rats maintained on the SBM and G+D diets compared to control ($P<0.05$). In contrast, pituitary FSHβ and LHβ subunit proteins were greater in rats from the SBM and G+D diet groups than in control animals ($P<0.05$). In the second experiment, daidzein and genistein acting singly or together inhibited Leydig cell T secretion (Con: 10.7±0.6, DAI: 8.7±0.6 GEN: 7.3±0.4, G+D: 6.6±0.4 ηg/ $10^6$ cells/ 3 h; $p< 0.05$). In addition, the DAI and GEN diets, alone or together, increased pituitary FSHβ and LHβ subunit protein compared to the control group ($P<0.05$).

**Conclusions.** Together, the data suggest that isoflavones present in soybeans can impact testicular androgen secretion and affect the gonadal-pituitary axis in pubertal rats. Thus, immature stage of development in the rat is sensitive to the endocrine disrupting effect of dietary isoflavones.

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## Evaluation of Bovine Viral Diarrhea Virus Genetic Changes in Congenitally Infected Piglets

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**Introduction.** Bovine Viral Diarrhea Virus (BVDV) is a disease that results in disease in not only cattle, but wild and domestic animals such as sheep, goats, deer, alpacas, and pigs. As BVDV does not possess strict host specificity, heterologous, or non-cattle, infections provide generation of viral diversity. It has been proposed by multiple researchers that BVDV infections in heterologous hosts such as in pigs may pose a significant reservoir of infection for cattle. The long-term goal of our research is to further the understanding of BVDV infections in heterologous species. We have previously demonstrated that heterologous infections result in greater genetic variability than infections in cattle. Based on this previous work, we hypothesize that change in the BVDV allows some congenitally infected piglets to clear BVDV, a remarkable phenomenon, not known to occur in persistently infected ruminants.

**Methods.** 4 pregnant BVDV naïve gilts were purchased from Auburn University Swine Research Facility at approximately 23 days of gestation. On day 26 or 27 days of gestation, gilts were inoculated with the non-cytopathic isolate of BVDV-1b (AU526) that had been previously pass through pregnant pigs. Inoculation was performed using approximately 5 x 10^6 median cell culture infective dose (CCIDv50) intravenously. Infection was verified by performing Virus Isolation (VI) and RT-qPCR on serum and buffy coat on day 7 post-infection. Virus neutralization (VN) to monitor seroconversion and transabdominal ultrasound pregnancy evaluations were performed every 28 days until farrowing. Piglets were evaluated for viremia via ear notch Antigen Capture ELISA (ACE) and qPCR at birth, then are monitored weekly for viremia or seroconversion via VI and VN on buffy coat and serum, respectively, until approximately 6 months of age. Whole genome sequencing will be performed on virus obtained from persistently infected and the time point prior to seroconversion in chronically infected piglets, initial inoculum, and from gilts prior to seroconversion.

**Results.** Gilts were confirmed to be naïve to BVDV via negative VI and VN for BVDV-1b at -21 and 0 days of pre-inoculation. Successful inoculation and seroconversion was demonstrated by a zero serum neutralizing antibody titer on day 7 and rising titer at day 28 post-infection. Antigen Capture ELISA demonstrated that 7/28 were positive (viremic) at birth. Multiple congenital abnormalities including stillborn, mummification, polydactyl, arthrogryposis, and small and weak piglets were observed. Pending results of VI and whole genome sequencing.

**Conclusions.** Successful inoculation with BVDV-1b (AU526) and seroconversion of BVDV naïve gilts during early gestation can result in a congenital infection that demonstrated similar syndromes to congenital infections in cattle. Contrary to what is observed in cattle, it appears that piglets born viremic, such as chronic or persistently infected, can also possess congenital abnormalities.

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## Movement and Persistence of *Salmonella* in a Veterinary Teaching Hospital

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**Introduction.** *Salmonella* is the most commonly associated agent responsible for nosocomial outbreaks in veterinary teaching hospitals. Previous studies conducted in our lab at the Auburn University College of Veterinary Medicine (AUCVM) found *Salmonella* contamination in the environment. The current study was conducted to determine if dairy calves on the AUCVM campus were shedding *Salmonella*, and if so, to define the frequency and duration of fecal *Salmonella* shedding. Because these dairy calves are located adjacent to the AUCVM Equine Reproduction Center (ERC), we performed a parallel study focused on sampling the ERC environment, hypothesizing that we would find the same *Salmonella* strains in the ERC that were isolated from the calves.

**Methods.** Samples were processed for *Salmonella* culture and isolation as described in the USDA FSIS *Microbiology Laboratory Guidebook*. *Salmonella* serogrouping was performed onsite and *Salmonella* isolates were submitted to the National Veterinary Services Laboratories (NVSL), Ames, IA, or Biovet, Inc., for serotyping. Pulse field gel electrophoresis was performed at the NVSL. Data was analyzed with Statistical Analysis System (SAS).

**Results.** Calves sampled from 2017 through 2018 had intermittent and sporadic shedding of *Salmonella*, primarily of serotypes Muenster and Cerro (serogroups E and K, respectively). In the summer of 2018, environmental samples from the ERC contained serotypes Muenster and Cerro, along with serotype Muenchen, (*Salmonella* serogroup $C_2$). Before closure of the ERC in September, 2018, due to environmental *Salmonella* contamination, calves at the AUCVM started shedding *Salmonella* serotype Muenchen which was indistinguishable from the strain isolated from the ERC. Environmental cultures of samples from the ERC in the spring of 2019 remained positive for *Salmonella* Muenster.

**Conclusions.** The isolation of the identical *Salmonella* Muenchen strain from the dairy calves and the ERC environment shows movement of these strains between adjacent AUCVM units. These findings support the need for implementation of intervention strategies that increase biosecurity at the AUCVM. And the persistence of *Salmonella* serotype Muenster in an environment over two seasons after animals were removed suggests this serotype is very resilient in the environment or the source of contamination is an undiscovered source.

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## A Meta-Analysis of Myelin Pathology in Neurodegenerative Lysosomal Storage Diseases

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**Introduction.** Though recent advances in gene therapy of neurodegenerative lysosomal storage diseases (LSDs) have dramatically improved central nervous system (CNS) function, some clinical signs persist. Myelin, a lipid-rich substance that enhances action potential transmission down axons, is often deficient in neurodegenerative LSDs, a secondary pathology that has proven especially refractory to treatment. This meta-analysis examined two major causes of myelin deficits: dysmyelinogenesis (pathology in oligodendrocytes, the glial cells responsible for CNS myelin production) and primary demyelination (direct destruction of myelin after formation). We compared developmental markers of oligodendrocytes and myelin quantified by 4 primary studies across 3 neurodegenerative LSDs (Niemann-Pick type A, Niemann-Pick type C, Sandhoff Disease).

**Methods.** After an initial systematic title and abstract screening, we examined the full text of 34 articles and selected 4 papers to include in the analysis. We extracted means and variances from bar graphs comparing the mRNA, protein, or immunohistochemistry staining of myelin and oligodendrocyte markers between affected animals and their age-matched normal controls. We also recorded the following modifiers for each data point: disease, species, age, substance measured, CNS region, and specific marker. We performed the meta-analysis using a random effects model with the natural log response ratio as the effect size.

**Results.** *Developmental markers:* We extracted a total of 105 independent observations and found a response ratio of 0.58, meaning that affected animals had, on average, 58% normal levels. The effect was still apparent with only the myelin (n=72) or oligodendrocyte (n=33) markers (54% and 68%, respectively).
*Modifier analysis (meta-regression)*: After standardizing the age at each observation to the humane endpoint of its corresponding disease, the mixed effects model found that very little of the observed disease effect was due to age ($R^2$ = 0.0076).
*Modifier analysis (subgroups)*: Overall, we found that very little heterogeneity was explained by any one modifier. We did note that the response ratio in white matter tracts (0.35) was half that of the cerebrum (0.70), which correlates to the anatomical localization of myelin to white matter.

**Conclusions.** The dramatic decrease in developmental oligodendrocyte and myelin markers in mice affected with neurodegenerative LSDs confirms myelin pathology observed in previous studies. Since this decrease was also significant in the independent myelin and oligodendrocyte marker analyses, we conclude that both dysmyelinogenesis and primary demyelination play a role in the observed myelin pathology. One unexpected finding, the lack of correlation between age and response ratio, implies that myelin deficits are already prevalent almost immediately post-partum. Future studies should distinguish gray matter from white matter.

**Acknowledgments.** Dr. Alan Wilson provided invaluable guidance for the analysis.

**Mammaglobin-A immunohistochemistry and protein expression in canine mammary tumors**

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**Introduction.** Mammaglobin-A (Mam-A) is highly expressed in breast cancer and due to its tissue specificity it is considered a target for cancer immunotherapy. In our laboratory we found that the expression of Mam-A is expressed in five established canine mammary tumor (CMT) cell lines and phenotypically normal canine mammary epithelial cells (CMEC). The aim of this study was to analyze the expression of Mam-A by western blot on CMT cells lines and by immunohistochemistry on paraffin-embedded canine mammary tumor tissues.

**Methods.** Twenty formalin-fixed, paraffin-embedded tissues from canine mammary gland carcinoma cases were evaluated histologically and with immunohistochemistry (IHC) for Mam-A. Primary polyclonal antibody was used at 1:250 dilution. To detect Mam-A protein, cell lysates from five established CMT cell lines and CMEC were analyzed by Western blot using Mam-A polyclonal antibody at a concentration of 2 ug/ml. Signals were quantified and normalized to the GAPDH protein level.

**Results.** We studied Mam-A expression in 20 cases of mammary gland carcinomas of various histological grades and types. Positive immunolabeling for Mam-A was seen in 50% of mammary gland carcinoma cases. The staining pattern was multifocal to diffuse and cytoplasmic. The anti-Mam-A antibody detected a band with an apparent molecular weight of approximately 45-kDa by Western blot in four CMT cell lines. The Mam-A expression was higher in the CMT cell lines when compared to CMEC. Unpublished results from our laboratory indicate that CMT cell lines have increased expression of Mam-A by rt-PCR.

**Conclusions.** Mam-A protein is overexpressed in CMT cell lines. Immunostaining for Mam-A expression in 50% of the mammary gland carcinomas was similar in all grades and subtypes.

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**Susceptibility of Swine to Human Influenza A Viruses and the Emergence of Zoonotic Viruses**

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**Introduction.** Swine are natural hosts of Influenza A viruses (IAVs). Clinical disease and immune responses during infection closely resemble human infection, making swine a good model for IAV research. Human IAVs (huIAV) occasionally spillover into swine, allowing opportunities for the viruses to reassort with swine IAVs (swIAV), resulting in antigenically novel viruses with zoonotic potential. However, wholly human IAVs are rarely detected in pigs, bringing into question the spillover rate of huIAVs into swine, as well as their overall fitness and transmission, as well the rate of reassortment.

**Methods.** In this study we compared the replication kinetics and immune responses of swine and human IAVs in pigs. Groups of 8-week-old influenza seronegative piglets were infected intranasally with $10^{7.0}$ $TCID_{50}$ of either A/sw/NC/KH1552516/2016 (a swine H3N2) or A/TX/50/2012 (a seasonal human H3N2). Nasal swabs were collected daily from challenge to day 14 post infection (PI). Virus titers were determined by virus isolation in MDCK cells and M-gene qPCR. On days -2, 2 and 4 PI bronchoalveolar lavage (BAL) fluids were collected for virus titration, cytology and multiplexing for porcine cytokines and chemokines.

**Results.** Pigs infected with swIAV exhibited standard clinical symptoms such as increased respiratory rate, fever, and anorexia, while those infected with huIAV exhibited no clinical signs for the duration of the study. swIAVs replicated at much higher titers in the nasal swabs and BAL fluids compared to the huIAV, and were detectable up to 10 days PI, while huIAVs were only shed for up to 6 days. Cytological staining of the BAL fluid revealed a high rate of neutrophil infiltration into the interstitial space of the lungs during swIAV infection, which was notably absent in samples collected from huIAV-infected piglets.

**Conclusions.** Infection with swIAV resulted in greater respiratory disease, virus replication, and shedding in comparison to huIAV. The lower rate of replication, as well as the lessened duration of illness and subclinical nature of the infection, may explain why human viruses are so rarely isolated during surveillance. The significantly shorter duration of infection may also play a role in determining the rate of reassortment, as both viruses must coinfect the same animal for reassortment events to occur. More research is needed to determine the rate of reassortment events and how these emeging viruses may develop zoonotic attributes.

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**Effect of Tumor Necrosis Factor Alpha and Interleukin-1 Beta on Equine Endothelial Colony Forming Cell Function in Culture**

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**Introduction.** Endothelial colony forming cells (ECFCs) facilitate vasculogenesis in adult life. The ability of ECFCs to undergo vasculogenesis makes them attractive for treatment of ischemic diseases in horses such as laminitis, intestinal disease, and chronic wounds. During inflammation, pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β) likely influence function of circulating ECFCs. An improved understanding of the effect of inflammation on ECFC function could be helpful in the development of targeted ECFC based therapies in horses.

**Methods.** ECFCs were isolated from the peripheral blood of three different healthy adult horses. Function testing was performed through adhesion, wound healing, tubule formation, and uptake of 1, 1'dioctacdecyl 3,3,3', 3'-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein (DiI-Ac-LDL uptake) assays. IL-1β (0, 0.1,1, and 10 ng/mL) and TNF-α (0, 0.5, 5, and 50 ng/mL) were assessed at three different exposure times (6, 24 and 48 hours). Cell proliferation was determined by 2, 3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide assay.

**Results.** Stimulation of ECFCs with IL-1β for 48 hours in the tubule formation assay resulted in a decreased percent change from control in number of segments, branches, mesh formation, junctions, tube length, and mesh area compared to 6 hour exposure times. Stimulation with IL-1β for 48 hours in the wound healing assay resulted in a decreased percent change in wound area from control compared to 6 hour exposure times. Higher concentrations of IL-1β (10 ng/mL) at 48 hour exposure time resulted in decreased change in wound area at 6 hours compared to lower concentrations of IL-1β. IL-1β did not significantly influence ECFC adhesion, DiI-Ac-LDL uptake, or proliferation of ECFCs. TNF-α did not significantly influence adhesion, wound healing, vascular tube formation, DiI-Ac-LDL uptake, or proliferation of ECFCs.

**Conclusions.** These results suggest an important role for IL-1β in downregulating ECFC function in vitro. Functional changes in ECFCs following stimulation with IL-1β do not appear to be due to changes in proliferative capacity.

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## Evaluation of health and performance of dairy calves supplemented with colostrum replacer during the pre-weaning period

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**Introduction.** Bovine colostrum has been successfully used as an adjunct therapy with antimicrobials in cases of infectious diseases of the gastrointestinal tract in people. Results of previous studies indicate that continuous administration of colostrum after gut-closure (>24h of age) could benefit overall dairy calf health during the pre-weaning period and reduces the need for antibiotic therapy. The objective of the proposed study was to evaluate the effects of colostrum supplementation during the pre-weaning period on health, antibiotic therapy, and performance of dairy heifers from birth to weaning.

**Methods.** Thirty Holstein-cross dairy heifers were separated from their dams immediately after birth and received 3 doses (300 grams) of a commercial colostrum replacer within 6 hours of birth. After colostrum feeding calves were randomly assigned to 1 of 2 treatment groups and moved to individual calf hutches for the initial study period of 75 days. Calves in the MR group (n=15) received 454 g of a commercial milk replacer twice daily until weaning (day 75). Calves in the CS group (n=15) received 150 g of dried bovine colostrum powder containing 32 g of IgG added to 304 g of milk replacer powder twice daily until weaning. Health events, antibiotic therapy, and individual body weights were recorded daily by personnel blinded to treatment allocation from birth to weaning in each calf and compared between the groups.

**Results.** All calves had more than adequate transfer of passive immunity (>20g IgG/L). The number of diarrhea events and the number of calves treated with antibiotics before weaning was greater in the MR group compared with the CR group. Mortality events were not observed in MR or CR groups during this study.

**Conclusions.** Supplementation of the milk replacer ration with a colostrum replacer powder for the first 75 days of life could reduce dairy calf morbidity associated with diarrhea and antibiotic therapy before weaning.

**Acknowledgments.** Saskatoon Colostrum Company

**Effects of autologous protein solution, autologous conditioned serum, and triamcinolone on inflammatory and catabolic gene expression in equine cartilage and synovial explants treated with IL-1β in co-culture**

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**Introduction.** Autologous protein solution (APS) and autologous conditioned serum (ACS) are newer therapeutic options for osteoarthritis (OA). Few studies have directly compared the effects of APS and ACS from the same individual on reduction of inflammatory mediators nor have these studies directly compared these products to the standard treatment, corticosteroids. Co-culture of interleukin-1β stimulated cartilage and synovium demonstrates a similar response to naturally occurring OA. The study objective was to investigate the effects of APS, ACS, and triamcinolone (TA) on inflammatory and catabolic gene expression in a co-culture model. We hypothesized that TA would reduce $PGE_2$ concentrations, but ACS and APS would protect matrix gene expression.

**Methods.** Blood was collected and processed for APS and ACS from six horses prior to euthanasia, and then articular cartilage (AC) and synovium membrane (SM) explants were harvested from the stifle. Explants were placed in duplicate co-culture and treated as: (1) unstimulated control, (2) stimulated control, (3) ACS at 25% v/v (4) ACS at 50% v/v, (5) APS at 25% v/v, (6) APS at 50% v/v, (7) TA ($10^{-6}$ M). Groups 2-7 were stimulated with interleukin-1β (10 ng/ml). Cultures were maintained at 37°C with 5% $CO_2$ for 96 hours. Media concentrations of $PGE_2$ were measured by ELISA. qPCR analysis was used to evaluate tissue inflammatory cytokines (IL-1 β, TNF-α, IL-6 and IL-8), matrix degrading enzyme (MMP-3), anti-inflammatory cytokine (IL-10), and extracellular matrix proteins (type II collagen and aggrecan) gene expression.

**Results.** IL-1β stimulation produced a significant increased gene expression of IL-1β ($p=0.029$), IL-8 ($p=0.011$) and MMP-3 ($p=0.043$) in SM; and increased gene expression of IL-1β ($p=0.003$) and TNF-α ($p=0.001$) in AC. TA significantly downregulated the expression of IL-β in SM and AC ($p=0.009$ and $p= 0.002$, respectively). APS and ACS at 50% showed a chondroprotective effect downregulating more efficiently IL-1β expression than TA in AC ($p= 0.001$ and $p=0.0004$). APS and ACS showed a trend to upregulate IL-10 in SM, and type II collagen and aggrecan in AC. All treatments reduced $PGE_2$ concentrations compared to stimulated control ($P=0.0009$); APS was most effective (13-fold decrease) and TA was the least effective (2.3-fold decrease). This study was limited by the ex vivo model and inherent variation of biological products and tissues between horses.

**Conclusions.** TA downregulated IL-1 β in SM and AC. However, ACS and APS reduced more efficiently the expression of this gene in AC, and decreased $PGE_2$ concentration in media compared to TA. This anti-inflammatory effect associated with a trend to upregulate IL-10 and extracellular matrix proteins have important implications for treatment of naturally occurring OA in horses.

**Acknowledgments.** Project funding provided by the Birmingham Racing Committee and the Auburn University Department of Clinical Sciences. We would like to thank Qiao Zhong for technical assistance for completion of the project.

**Comparison of Tensile Strength and Early Wound Healing of Self-Locking and Surgeon's Knots for Closure of Ventral Midline Celiotomy in Horses**

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**Introduction.** Incisional complications following ventral midline celiotomy (VMC) are a common cause of increased morbidity/mortality. Characteristics of suture/body wall closure are associated with complication risk and the surgical knot shown to be the weakest point of the suture line. Self-locking knots, such as the forwarder and Aberdeen knot, have been investigated in vitro and ex vivo, supporting their superior knot strength, smaller knot volume, and higher bursting pressures when compared to the surgeon's knot. We hypothesized that VMC closure using forwarder and Aberdeen (F-A) self-locking knot combination would have greater tensile strength and improved wound healing when compared to the surgeon-surgeon's (S-S) knot combination in vivo.

**Methods.** Fourteen horses underwent VMC, closed with either F-A or S-S knot combination. Incisions were subjectively graded by blinded evaluators for dehiscence, edema and drainage. Biomechanical testing was performed on three randomized abdominal segments and histopathology on one randomized abdominal segment from each subject following humane euthanasia ten days post VMC. Seven control horses, with no celiotomy, were also euthanized and biomechanical testing performed on abdominal segments in the same manner.

**Results.** Statistical analysis showed no difference between groups for any variable evaluated, including tensile strength ($p=0.98$), location of failure ($p=0.24$), and healing grade ($p=0.60$). However, the F-A group failed more frequently (15/21, 71%) along the rectus sheath versus S-S (6/21, 26%), consistent with the control (28/28, 100%) linea alba.

**Conclusions.** Similar tensile strength and wound healing shows either knot combination provides a secure closure in horses for the early post-operative period following VMC. While this study failed to find statistical difference between the tensile strength and healing scores of these knot combinations, the F-A failed more commonly in a similar manner to normal tissue, along the rectus sheath. Additionally, there was no evidence of impaired healing surrounding the self-locking knots with similar incisional complications seen across both treatment groups. Our study model consisted of physiologic conditions of recovery from general anesthesia and a ten-day post-operative period to mimic stresses and strains applied to these closures unlike the previous in vitro and ex vivo work. Study limitations include the small sample size, use of healthy subjects, and the short study period. Results support a randomized prospective clinical trial to evaluate performance of the F-A self-locking knot combination in clinical cases with long-term follow-up to further support clinical use.

**Acknowledgments.** Funding obtained through the Birmingham Racing Commission and the Department of Clinical Sciences of Auburn University.

# Faculty/Postdoctoral Fellow Platform Presentations

**Non-participant student observation of faculty classroom teaching**

Hofmeister EH

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**Introduction.** Student evaluations are commonly used to evaluate teaching effectiveness. Non-participant observation uses individuals who are not a part of the learning process, but are observers who can create observations about the teaching encounter, possibly with less bias than student evaluators. The purpose of this study was to analyze reports by inexperienced non-participant observers of faculty classroom teaching episodes. The hypothesis was that veterinary faculty have common characteristics in their classroom teaching which are observable by non-participant observers and that these are similar to characteristics observed historically by student evaluators.

**Methods.** This study was a qualitative document analysis of written observations made by senior veterinary students attending pre-clinical classroom lectures by a faculty member. Each written report was analyzed using thematic concept analysis, and the researchers met multiple times throughout the process to discuss the analysis and develop conclusions about themes that were encountered consistently among observations.

**Results.** Common themes that emerged included information formats, PowerPoints, timing, organization, student engagement, and delivery.

**Conclusions.** Non-participant observers may contribute valuable data which may enhance faculty development in pedagogy. Observations may serve to augment data from student evaluations, self-reflection, and peer assessment.

**Ratiometric pH probe for the identification of acidic compartments along the endo-lysosomal network**

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**Introduction.** Compartments along the endo-lysosomal network (ELN) are acidic with pH values strictly regulated by V-type proton ATPases. Although numerous methods exist for the detection of lysosomal pH, detection of variations in pH of different ELN compartments in a single assay is not possible. To design a probe capable of monitoring the pH of multiple compartments simultaneously, we modified an existing probe used to detect autophagic flux. The mRFP-GFP-LC3B probe has been used to develop an unbiased analysis of the autophagy pathway through the identification of autophagosomes and the maturation of autolysosomes. Here, we replaced the LC3B moiety with lysosomal associated membrane glycoprotein 1 (LAMP1), effectively localizing the probe to compartments of the ELN and complementing the previous probe by providing a complete picture of cellular catabolism.

**Methods.** Total RNA was isolated from human normal dermal fibroblasts and reverse transcribed to cDNA. LAMP1 cDNA was then amplified using gene-specific primers and cloned to a plasmid containing the mRFP-GFP fusion by restriction digest. LAMP1 cloning was confirmed by Sanger sequencing. Normal human dermal fibroblasts and HEK293T cells were transfected with the plasmid containing the mRFP-GFP-LAMP1 fusion and visualized by laser scanning confocal microscopy.

**Results.** LAMP1 cloning to the plasmid containing the mRFP-GFP fusion was confirmed by Sanger sequencing. Transfection of mRFP-GFP-LAMP1 to human normal dermal fibroblasts and HEK293T cells was monitored by laser scanning confocal microscopy with proper trafficking and localization confirmed by anti-Lysosomal associated membrane glycoprotein 2 (LAMP2) staining.

**Conclusions.** Through use of the fusion protein mRFP-GFP-LAMP1, we are now able to ratiometrically measure the pH of acidic compartments along the ELN. Identification and quantification of puncta possessing the same pH will be critical to studies examining perturbations to the ELN in diseases affecting cellular catabolism.

**Acknowledgments.** This work was supported by private donations to the research program of D.R.M.

**Incorporation of Virtual Reality to Enhance the Teaching of Small Animal Gross Anatomy in the Veterinary Curriculum**

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**Introduction.** Anatomy is a challenging subject with the quantity of information covered, the establishment of a new vocabulary, and the appreciation of spatial relationship with a goal for the student to attach a functional understanding to the subject. This volume of terminology and topography makes it difficult for students to move beyond memorization-based learning. However, due to its foundational nature in the veterinary curriculum it is key to establish methods to connect memorization-level knowledge holistically to the functional comprehension to enhance the students understanding and application. The use of Virtual Reality as a supplemental educational platform in gross animal anatomy could be such a method that allows bridging in student learning.

**Methods.** We have established a pilot Virtual Reality canine osteology laboratory for the first year veterinary students at Auburn University. Using a software platform developed at Virginia-Maryland College of Veterinary Medicine and the HTC Vive Virtual Reality equipment we established first year veterinary student virtual reality laboratory. A research survey was developed to examine the software for qualitative use and student experience, approved under IRB protocol, #19-303 Singletary.

**Results.** The study survey results rate the student experience as excellent and the feedback is encouraging to support expanding this platform in function and capacity as a student educational resource. One student remarked, "… I believe this equipment could potentially revolutionize the way first year veterinary students excel and learn anatomy that will benefit every single patient they see in their future careers."

**Conclusions.** This project applies new innovative Virtual Reality software and technology to enhance the student learning experience. The study is ongoing with preliminary results encouraging for the use of this platform in expanding its access and in advancing beyond the osteological context and expanding into other systems such as musculature, vasculature, organology, and nervous system structures.

**Acknowledgments.** This project is supported by the Daniel F. Breeden Endowed Grant, Biggio Center, Auburn University.

**Gene expression profiles in individual canine patient osteosarcomas**

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The study of gene expression in tumors has great promise for elucidating both mechanisms of tumorigenesis and identification of therapeutic targets. As technology has improved, the focus of such studies has shifted from identifying similarities in groups of tumors to teasing out unique details of individual masses. This novel approach requires model systems in which both the tumor and the therapeutic response can be assessed. Canine osteosarcoma represents both a significant health problem in larger breed dogs and a highly correlative model of osteosarcoma in humans. Using this model, gene expression profiles were completed for eight canine osteosarcomas using corresponding patient normal bone RNA for comparison. RNA was extracted from tumor and normal, marrow-depleted, phalanges from amputated limbs of patient dogs and sequenced. Sequence data was examined in the context of similarities between the patients' tumors and normal bone cellular constituents, both as a group and as individuals. Approximately 3,000 genes were found to be differentially expressed in tumors as compared to the matched normal tissue. These genes were expressed in both unique and overlapping patterns when comparing individuals. When expression of cellular receptors was examined specifically, the four most commonly upregulated receptors across all tumors were CXCR4, AVPR1, ADGRB1, and TNFRSF4. Expression of these genes was increased in most, but not all tumors. When the most overexpressed receptor in each dog was determined, the pattern was less cohesive, with significant inter-individual variation. Ultimately, these data emphasize both the commonalities and differences among different tumors of the same histological type. The latter supports development of therapeutic approaches based on individual tumor gene expression patterns as potentially the best approach to confronting the variability of tumor gene expression.

**Acknowledgments.** This work was supported by funding from the Scott Shockley Foundation and Auburn University Research Initiative in Cancer (AURIC).

# Undergraduate Student Poster Presentations

### Comparison of Lysosomal Enzyme Activity in Cat Tissue with or Without Saline Perfusion

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**Introduction.** Perfusion, which is the practice of passing saline through the vasculature to remove the majority of blood, is common practice during necropsies. Our laboratory, which studies feline models of two different lysosomal storage diseases, has developed disease-specific procedures for necropsy of animals with each disease, with the primary difference being perfusion. In an effort to minimize animal use and evaluate impact on downstream analyses, lysosomal enzyme activity in perfused and non-perfused normal animals was directly compared.

**Methods.** Banked samples of frozen tissue from perfused and non-perfused normal cats were compared for enzyme activity throughout the central nervous system (CNS). Enzyme activity is determined using homogenates from specific tissues. Homogenates are then incubated with a synthetic fluorogenic substrates specific for each of four lysosomal enzymes: Beta-Galactosidase (β-gal), Mannosidase, Hexosaminidase A, and total Hexosaminidase will be evaluated with their corresponding substrates.

**Results.** Minimal differences in enzyme activity between the perfused and non-perfused samples were seen when testing for Mannosidase, Hexosaminidase A, and total Hexosaminidase. However, β-gal activity decreased in the activity for the non-perfused animals. On average, non-perfused CNS samples have an average β-gal specific activity of $8.79 \pm 3.25$, while the perfused CNS samples have an average β-gal specific activity of $18.89 \pm 8.01$.

**Conclusions.** Perfusion seems to have little impact on the activity of most lysosomal enzymes, but in some instances, there may be a substantial increase in the activity of perfused samples. For example, β-gal activity was more than doubled, on average, in the perfused animals. This difference is important considering that β-gal is the deficient enzyme in GM1 gangliosidosis. We hypothesize that this difference is due β-gal instability. Cold saline is used during perfusion, which rapidly lowers the body temperature and perhaps stabilizes β-gal. Also, constituents of peripheral blood may destabilize β-gal. Additional research is planned to evaluate further differences between perfused and non-perfused samples.

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**Expression of Xrra1 gene in mouse adrenal gland**

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**Introduction.** A remarkable gender difference can be seen in adrenal physiology with females having a predisposition to disease. The molecular reason of this sexual bias is not known at this time. Mouse studies have shown that the adrenal gland is sexually dimorphic at the histological level as well as the gene expression level. Current knowledge of sexual dimorphism in the mouse adrenal gland mainly focuses on the adrenal X-zone, a transitory cellular layer in the inner most adrenal cortex. To further understand the sexual differences of the adrenal gland at the transcriptome level, RNA sequencing was used to obtain the expression of every gene in the adrenal gland across key developmental stages.

**Methods.** RNA sequence data from mice at different developmental stages were compared to observe what genes might be responsible for the sexual dimorphic development of the adrenal gland. Male and female mice of multiple developmental stages were used to identify gender-specific genes as well as age-related genes. To study these novel candidate genes further, double immunofluorescence was used to obtain the cellular expression patterns of the genes across the developmental states. In short, paraffin sections were dewaxed and rehydrated, and antigen retrieval was performed. The slides were then placed in blocking solution and were stained with two primary antibodies at the same time, followed by the incubation with proper secondary antibodies.

**Results.** *Xrra1* was one of the gender and age specific genes with a unique expression pattern at the mRNA level. The RNA sequence results showed an increase in *Xrra1* expression in females over time and a relatively constant *Xrra1* expression in males. The double immunofluorescent results further showed that *Xrra1* is expressed primarily in the zona glomerulosa (zG) and in the medulla. This expression pattern is similar to beta catenin. Beta-catenin is a key gene that controls adrenal gland development.

**Conclusion.** The preliminary data not only separated genes that expressed sexual dimorphism in mouse adrenal, but also identified that *Xrra1* as a key candidate gene that may have an unique function in the adrenal gland. Research suggests *Xrra1* has a role in the response of the X-radiation-mediated DNA damage and may be correlated with cancer development. The colocalization of *Xrra1* and beta-catenin in the adrenal glands, points to further research opportunities to study the interaction of the two genes and mechanism behind the prevalence of adrenal diseases in females.

## CRISPR/Cas9 Mediated Genetic Modification of Adenovirus Type II

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**Introduction**. Current methods of cancer treatment lack the ability to discriminate between healthy and cancerous tissues resulting in collateral damage that can be detrimental to the well being of the patient. Oncolytic viruses, viruses that have been modified to replicate specifically in cancer, are a new method that may offer a significantly more targeted treatment. In adenoviruses, this targeted replication can be achieved by modifying the promoter of the E1A region, which works to begin cascade for gene expression and packaging. ICOCAV15 a modified canine adenovirus type 2 (CAV2) is one such oncolytic virus. In order to track this virus in experimental infections, we sought to modify the virus to express the fluorescent protein dsRed. Previously, adenovirus modification required the identification of appropriate restriction enzyme sites, severely restricting the possible sites to modify or insert genes. We utilized CRISPR/Cas9 to precisely and efficiently cut ICOCAV 15 without impacting the function of the virus. Two different versions of dsRed were used; one with the constitutive CMV promoter and one with a splice acceptor site (IIIA) driven by the viral major late promoter. The CMV version allows dsRed to be transcribed whether or not functional virus particles are made. The IIIA version, is only transcribed if virus particles are replicating properly, due to the requirement of major late promoter activation. This construct is crucial as it allows us to see that our virus is properly replicating and packaging.

**Methods**. A guide RNA was designed for CRISPR/Cas9 digestion between the Fiber and E4 region of the ICOCAV15 genome. The dsRed inserts were prepared by overhang PCR. Forward and reverse primers were designed with overhangs that were complimentary to viral sequence flanking the cut location on ICOCAV15 to allow homologous recombination. The dsRed inserts were inserted into CRISPR/Cas9 digested viral genomes by homologous recombination. Recombinant viral genomes were transformed into competent NEB 5-alpha E. coli on carbenicillin agar plates allowing for selection of colonies that have taken up plasmid. Colony PCR was used to identify colonies with the desired insert. The selected colonies were then expanded in culture and then plasmid purified using a midi prep kit. The plasmid backbone was removed with Not1 enzyme digestion. Purified viral genomes were transfected into DKcre cells using Lipofectamine. Cells were observed for seven days for red fluorescence and pictures were taken to document fluorescence and cytopathic effect. On the 7th day, the DKcre cells were lysed, and the lysate used to infect subsequent round of DKcre cells.

**Results and Consclusions**. Based on our observations, CRISPR/Cas9 technology can be used for the precise genome modification of CAV2. Our reporter constructs CMVdsred and IIIAdsRed have shown fluorescence after transfection, proving the gene was inserted, and CMVdsred has been shown to produce properly packaged infectious adenoviruses. IIIAdsred is currently in the process of testing infectivity.

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# Veterinary Student Poster Presentations

### Altered astrocytic Nrf2 protein regulation in spontaneously hypertensive rats

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Neurogenic hypertension is associated with oxidative stress, chronic low-grade inflammation, and elevated sympathetic tone. Angiotensin II (AngII) is a primary factor driving these alterations, yet the exact mechanisms are not fully understood. One potential is that chronic AngII activation of glial cells, which induces an inflammatory response, also dysregulates Nrf2, the master regulator of antioxidant machinery. We hypothesized that AngII reduces Nrf2 signaling within cardioregulatory nuclei (i.e., the hypothalamic paraventricular nucleus [PVN] and rostral ventrolateral medulla [RVLM]), heightening oxidative stress and neuroinflammation. 12-13 week-old male spontaneously hypertensive rats (SHR) were age-matched with Wistar Kyoto (WKY) controls. Using real-time PCR, no differences were observed in *Nrf2* gene expression between SHRs and WKYs within the PVN or RVLM. Immunofluorescence analyses revealed regional- and cell type-specific alterations in Nrf2 protein expression and localization in SHRs. In both the PVN and RVLM, Nrf2 protein density was decreased in SHR astrocytes compared to WKY (PVN: 2.6±0.3 vs 5.4±0.5%area; RVLM: 2.4±0.2 vs 4.0±0.3%area). In PVN astrocytes of SHRs, both nuclear and non-nuclear Nrf2 protein density was diminished relative to WKY (4.1±0.6 vs 11.9±1.8 and 2.2±0.3 vs 4.6±0.4%area, respectively), while RVLM astrocytes showed reductions in nuclear Nrf2 alone (4.4±0.6 vs 7.0±0.9%area). Our data suggest impaired Nrf2 signaling within the PVN and RVLM during hypertension. Given that nuclear translocation of Nrf2 is necessary for an effective cellular antioxidant response, a deficient Nrf2 response would further oxidative stress, perpetuating neuroinflammation and, ultimately, sympathoexcitation.

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**Identification of disease diagnostic and prognostic biomarkers in the equine gastrointestinal microbiome**

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**Introduction.** Horses have unique gastrointestinal (GI) tracts that rely on hindgut fermentation by an extensive microbiome. The equine GI microbiome is suspected to influence disease etiology. Specifically, idiopathic GI diseases, such as colic, are a leading health problem of horses and yet there is little known about the relationship between the microbiome and the etiology of colic. The objectives of this study are to determine if an association between the equine gut microbiome (GM) and primary organ system affected (e.g., respiratory, GI, orthopedic disease, etc.) exists, and whether there is a difference in the GM between decompensated colics that end in euthanasia or colics that resolve and are discharged. We hypothesize that the equine GM will form distinct clusters based on general disease etiology or affected organ system, reflecting a relationship between disease and the microbiome population. We also hypothesize that decompensated colics ending in euthanasia will possess microbiomes with lower richness relative to colics that resolve. Fecal samples were collected from hospitalized equine patients (and healthy controls) at presentation to one of four participating Veterinary Teaching Hospitals, and every 1 to 2 days thereafter.

**Methods.** Samples underwent DNA extraction, 16s rRNA PCR amplification and sequencing, and statistical analysis to compare richness, diversity, and composition between groups.

**Results.** Kruskal-Wallis one-way ANOVA on ranks, with Dunn's post hoc, using 'healthy' as control showed a significant difference in α-diversity between healthy equine GMs and GMs of horses presenting for gastrointestinal disease. Bray-Curtis PERMANOVA also showed significant differences in β-diversity between healthy GMs, GI GMs, and non-GI GMs. Other than GI disease, no significant association between disease group/affected organ system and GM diversity or richness was found. Contrary to our hypothesis, no difference in richness in the GM between decompensated colics that end in euthanasia or colics that resolve and are discharged was found.

**Conclusions.** We expected to find an association between disease etiology and composition of the GM, at presentation, and a difference in GM richness between positive and negative outcomes in cases of acute colic. However, we only found a significant correlation between GM richness and diversity for cases of gastrointestinal disease. Many GI etiologies are linked to GM disturbances. Findings, as the correlation above, will allow us to identify prognostic indicators or possible therapeutic interventions to better treat GI disease in the future.

**Acknowledgements.** We wish to acknowledge IDEXX-BioAnalytics and Boehringer Ingelheim Summer Scholars Program for providing stipend support for Lucia Cresci. Research support provided by the MU Metagenomics Center. We also wish to thank Giedre Turner and Rebecca Dorfmeyer for laboratory support. We also wish to thank the participants of the Equine Gut Group for their support and participation in the program.

**Engineering a bispecific molecule to simultaneously inhibit and co-stimulate immune checkpoints for combination immunotherapy of canine cancer**

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**Introduction.** Cancer immunotherapy using monoclonal antibodies (mAbs) as inhibitors or co-stimulators of immune checkpoints have shown remarkable success in several human malignancies. Blocking mAbs for inhibitory immune checkpoints such as PD-1 and CTLA-4 have been approved by the FDA for treatment of several human cancers. However, the efficacy of monotherapy PD-1/PD-L1 blockade seldom exceeds 40% in most cancers due to the high rate of primary or acquired resistance. This failure of checkpoint immunotherapies is mainly attributed to the immunosuppressive tumor microenvironment (TME) that inhibits optimum migration and activation of anti-tumor $CD8^+$ T cells. To overcome this, alternative approaches are currently being explored to combine the blocking antibodies with agonistic antibodies for co-stimulatory molecules such as OX40, a member of the TNF receptor superfamily (TNFRSF). Agonistic antibodies against OX40 overcome dendritic cells (DCs)-mediated immunosuppression by direct activation of tumor-specific $CD8^+$ T cell responses in TME. *The primary objective of this study is to engineer a nanobody-based bispecific molecule that will block and inhibit the canine CTLA4/B7 pathway while simultaneously activating the OX40/OX40L pathway.* We believe a combination therapy of CTLA4 inhibition and OX40 co-stimulation will synergistically initiate the development, proliferation and maintenance of anti-tumor T cell responses.

**Methods.** We have previously identified an anti-CTLA4 nanobody from our yeast nanobody library. This nanobody (CTLA4.Nb6) binds to canine CTLA4 on the surface of transiently transfected HEK-293 T cells and canine peripheral blood mononuclear cells (PBMCs). We have also successfully conjugated this nanobody (CTLA4.Nb6) to the hinge and Fc region of the canine IgG. For this study, we engineered a bispecific molecule (CTLA4.Nb6-cFc-OX40$L_{ECD}$) by linking our previously identified anti-canine CTLA4 nanobody (CTLA4.Nb6) through the Fc region of canine IgG to the extracellular domain (ECD) of OX40 ligand. The open reading frame encoding the CTLA4.Nb6.cFc.OX40$L_{ECD}$ sequence was synthesized from the Gene Universal and cloned into the mammalian expression vector pcDNA3.1/$Hygro^+$ containing N-terminal Strep II Tag. The recombinant CTLA4.Nb6.cFc.OX40$L_{ECD}$ protein was expressed and purified from the ExpiCHO-S cells using the StrepTrap HP column by AKTA explorer. Two additional molecules, CTLA4.Nb6.Fc and other Fc.OX40L were also constructed.

**Results.** The open reading frame encoding the CTLA4.Nb6.cFc.OX40$L_{ECD}$ sequence was successfully synthesized from the Gene Universal and cloned into the mammalian expression vector pcDNA3.1/$Hygro^+$. An N-terminal mouse IgG kappa signal sequence was cloned to direct secretion of the bispecific molecule. Two monospecific molecules, CTLA4.Nb6.Fc and Fc.OX40L were also amplified and cloned in the pcDNA3.1/$Hygro^+$ containing N-terminal Strep II Tag. Recombinant plasmids were transiently transfected in ExpiCHO cells and bispecific molecule and CTLA4.Nb6.Fc were successfully purified from supernatant using AKTA explorer. The reduced form of the bispecific molecule migrated at the predicted molecular weight of ~65kDa. These blots also revealed that CTLA4.Nb6.cFc.OX40$L_{ECD}$ form dimers under non-reducing condition. The presence of canine Fc domain was confirmed by binding of the bispecific molecule to protein A/G column. The purified CTLA4.Nb6.cFc.OX40$L_{ECD}$ successfully bind to the canine CTLA4 expressed on HEK-293T cells.

**Conclusion.** We have successfully engineered, expressed and purified a bispecific molecule, CTLA4.Nb6.cFc.OX40$L_{ECD}$ to simultaneously inhibit and activate CTLA4/B7 and OX40/OX40L pathways, respectively. This molecule retains the ability to fold properly, and has the functional ability to bind to canine CTLA4. Currently, we are investigating the binding of the CTLA4.Nb6.cFc.OX40$L_{ECD}$ to canine OX40. We will also evaluate the functional activity of the CTLA4.Nb6.cFc.OX40$L_{ECD}$ and compare it to monospecific molecules.

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**Regulation of immune surface markers by CDK4/6 inhibitors in gammaherpesvirus-infected tumor cells**

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In addition to direct anti-tumor effects, cyclin dependent kinase 4 and 6 (CDK4/6) inhibitors have been shown to upregulate expression of surface immunologic proteins such as major histocompatibility complex class I (MHC-I) in cancer cells, as well as enhance activation of T cells. Viruses have evolved mechanisms downregulate surface immune proteins, such as MHC-I, ICAM-I, and B7-2, to evade T cell and natural killer (NK) immunity. We explored the ability of three clinically approved CDK4/6 inhibitors, abemaciclib, palbociclib, and ribociclib, to upregulate expression of PD-L1, ICAM-1, B7-2, and MHC-I on the surface of tumor cell lines infected by chronic herpesviruses, including Kaposi sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV). We investigated three primary effusion lymphoma (PEL) cell lines (BCBL-1 and BC-3: KSHV+; JSC-1: KSHV+, EBV+) and two Burkitt lymphoma lines (Akata and Raji: EBV+). At clinically relevant doses, CDK4/6 inhibitors increased surface expression of B7-2 (1.45 to 16.2 fold) and ICAM-1 (1.48 to 4.34 fold increase, except in BC-3). Enhancement of ICAM-1 and B7-2 can potentially enhance NK killing of the tumors. Expression of PD-L1 was substantially increased (1.66 to 460 fold) and may blunt T cell control; however, this suggests that these drugs might best be used in combination with anti-PD-1 or anti-PD-L1 antibodies. Planned next steps include quantifying the effects of CDK4/6 inhibitors on MHC-I expression, exploring effects on KSHV and EBV activation, and examining effects on NK and T cell function. These results support the idea that CDK4/6 inhibitors warrant further investigation in the clinical treatment of virally induced malignancies.
Research Grant: Intramural Research Program of the NIH, National Cancer Institute
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**Elective cesarean sections: effects of perioperative variables on neonatal Survivability**

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**Introduction.** Elective Cesarean sections (c-section) are often used in breeds prone to dystocia. In 2014, the Canine Performance Sciences detection dog program at Auburn University replaced the natural whelping process with elective c-sections to shorten the duration of the delivery, decrease stress on mother and pups, and improve neonatal survivability. Effects of elective c-section on the survivability of the litters had not yet been studied. This study aimed to discover the difference in duration of delivery and puppy survivability between c-sections and natural whelping, while determining whether certain perioperative variables affected the survivability of the pups.

**Methods.** Records on 51 litters from 27 dams were analyzed over a period of 6 years; 23 deliveries were natural whelpings and 28 were elective c-sections.

**Results.** Results revealed that c-section delivery was significantly quicker (47 ± 11 minutes, n=17) when compared to natural whelping (237 ± 66 minutes, n=12). Puppy survivability did not significantly differ between the two methods, $p<0.14$. Perioperative variables significantly influenced puppy survivability, $F_{(7,15)}=8.509$, $p<0.001$. The use of propofol (vs. alfaxalone) for induction decreased the neonatal survivability rate by 79%, $p<0.001$. Also, a post-operative epidural increased neonatal survivability rate by 58%, $p<0.001$.

**Conclusions.** In summary, either elective c-section or natural whelping can be used with good results; however, if elective c-section is chosen, based on the data analysis, the ideal combination of drugs used for an elective c-section in this population would consist of hydromorphone, alfaxalone, sevoflurane and a post-operative epidural.

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**Relative Quantification of Cannabinoid Receptor mRNA in Equine Tissue**

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**Introduction.** The discovery of the endocannabinoid system (ECS), comprising the G-protein coupled cannabinoid 1 (CB1R) and 2 receptors (CB2R), and their endogenous and exogenous ligands has been found in physiological and pathological functions such as seizures, nociception, inflammation, cell growth, appetite, metabolism, memory, and more. Use of cannabidiol (CBD), an exogenous cannabinoid, has increasing popularity in human medicine for the treatment of a variety of chronic clinical conditions such as epilepsy, pain, cancer, and anxiety. Moreover, CBD also interacts with TPRV-1 receptors which are known to mediate pain sensation and inflammation. Several research studies in clinics show positive effects of CBD including anti-epileptic, antinociceptive, and anti-inflammatory effects. A few studies evaluating the safety and efficacy of CBD in dogs with osteoarthritis showed positive pain-modulating effects. While additional CBD studies using laboratory animals have been published, no equine studies have been reported. This paper sought to characterize the two predominant cannabinoid receptors—CB1R and CB2R— and also TPRV1 mRNA by relative quantification.

**Methods.** Eight client-owned horses were euthanized for reasons including poor quality of life, intractable pain, or chronic progressive debilitating disease with a poor prognosis at the JT Vaughan Large Animal Teaching Hospital. Immediately after euthanasia, tissues including brain (frontal cortex and pituitary gland), abdominal adipose, mesenteric lymph node, liver, lung, kidney (cortex and medulla), spleen, synovium, and large intestine were collected from these horses. The tissue samples were placed in an RNA stabilizing agent and stored until processing. RNA was extracted from the tissues, converted to cDNA, and quantified using quantitative PCR (qPCR). The data was reported as a cycle threshold (CT) ratio of *CB1R*, *CB2R, or TPRV1* mRNA expression to the constitutively-expressed housekeeping gene *B2M*.

**Results.** Relative quantification of CB1 and *TPRV1* mRNA was normalized with the CT value from the frontal cortex of brain. The CT ratio of *TPRV1* mRNA expression showed high expressions in most collected tissues except for pituitary gland, liver, and kidney. For *CB1R* mRNA, the CT ratio is presented with the highest expression in the frontal cortex of brain and followed by adipose tissue, mesenteric lymph node, spleen, and synovium, from high to low ($P<0.05$). Expression values for the *CB2R* mRNA have been somewhat limited but show some levels of expression in spleen, synovium, and frontal cortex of brain ($P<0.05$).

**Conclusions.** These results invite future investigation into the pain management applications of cannabinoids, as well as possible upregulation following exogenous exposure to explain the relatively low expression of *CB1R and TPRV1* in the pituitary gland, liver, and kidney. While many findings from previous studies were confirmed, such as the high expression of *CB1R* in brain and CB2R in lymph tissue, the study also found unexpectedly high *TPRV1* expression in most tissue and low quantities of CB2R expression were found in the lung and the liver compared to human and mouse models. Quantification of specific regions of the equine brain or spinal cord related to the pain pathway may also prove useful in the development of better understanding and leading to pharmaceutical cannabinoids.

**Acknowledgments.** The staff at JT Vaughan Large Animal Teaching Hospital were assisting in collecting the tissues.

**Equine origin vascular endothelial growth factor effects on equine endothelial colony forming cells**

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**Introduction.** Endothelial colony forming cells (ECFCs) play a central role in both vasculogenesis and angiogenesis. As such, ECFCs have potential therapeutic value in ischemic diseases. To optimize use of ECFCs, further characterization of their function within microenvironments is warranted. The objective of this study was to evaluate the effect of equine recombinant vascular endothelial growth factor alpha (EqVEGFα) on equine ECFC function in vitro. We hypothesized that EqVEGFα would increase tubule formation and cell migration with no cytotoxic effects.

**Methods.** Equine ECFCs from 2 healthy adult horses were grown in culture for use in wound healing and in vitro tubule formation assays. Cells were exposed to 1 of 5 types of endothelial growth media: serum-free (SF) and VEGFα-free, standard (STD, 10% equine serum) with 2ng/mL human VEGFα (hVEGFα), STD with 2ng/mL EqVEGFα, SF with 2ng/mL hVEGFα, or SF media with 2ng/mL EqVEGFα. Cytotoxicity in response to increasing concentrations of EqVEGFα (0-200ng/mL) was evaluated with tetrazolium dye (XTT) assays. For the wound healing assay, percent gap closure was measured at 3 and 6 hours after wound formation. Images of in vitro tubule formation taken at 5, 18, and 24 hours were scored (1-4) by 2 observers and the results averaged. Data were analyzed using ANOVA ($P<0.05$).

**Results.** No significant differences between treatments were observed in the wound healing or XTT assays. A significant decrease was detected in tubule formation between cells exposed to STD versus SF media at 5 and 18 hours, but the presence or source of VEGFα did not have an impact.

**Conclusions.** EqVEGFα does not have cytotoxic effects on equine ECFCs up to 200 ng/mL, nor does EqVEGFα influence ECFC migration in wound healing assays. The presence and source of VEGFα does not have an impact on in vitro tubule formation; however, the presence of serum significantly decreased tubule formation at 5 and 18 hours. This data merits the need for further description of equine ECFCs and their response to various sources of VEGFα and other factors present in equine serum.

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**Modeling Developmental Trichloroethylene Toxicity in the Zebrafish.**

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**Introduction.** Trichloroethylene (TCE), historically used as an industrial solvent and degreaser, is a known environmental toxicant that contaminates over half of Superfund sites and is a human carcinogen. Zebrafish (*Danio rerio*) are used in toxicology screening assays, primarily due to the 80 percent similarity of protein-coding genes related to disease to humans, making the information collected from zebrafish translational to humans. The purpose of this study is to investigate the toxicodynamics of embryonic TCE exposure in the zebrafish biomedical model. We hypothesize that embryonic exposure to environmentally relevant levels of TCE will alter the normal growth and development of zebrafish

**Methods.** Zebrafish embryos were collected from an adult breeding colony and exposed in groups with TCE concentrations of 0 parts per billion (ppb; control), 5 ppb (maximum contaminant level for drinking water), 50 ppb, and 500 ppb. Embryonic survival and hatching were monitored every 24 hours until 120 hours post fertilization (hpf). Embryonic photomotor response was evaluated in 24 hpf embryos using an Olympus SZX10 stereomicroscope and DanioScope software. Embryo were recorded over thirty second periods and monitored for photomotor response to one second intervals of spotlight exposure. A larval Visual Motor Response Test evaluated locomotion and behavior in 120 hpf larvae using a Noldus DanioVision Observation Chamber and EthoVision software. Larval movement was tracked and recorded over a 50 minute period using the white light routine, which consisted of alternating ten minute intervals of dark and light. Survival, hatching, and behavioral data was analyzed with Minitab18 software and an ANOVA with Fisher's Least Significant Difference post hoc test when $p<0.05$ determined differences between groups.

**Results.** Although there was no significant difference in survival, all TCE exposures had a significant delay in hatching at 48 hpf. In the 24 hpf Photomotor Response Test, all TCE exposures had significantly increased inactivity compared to controls, and the 500 ppb exposure had a significant decrease in burst count. In the 120 hpf Visual Motor Response Test, the 500 ppb had a significantly altered turn angle and angular velocity compared to controls, which was predominantly attributed to altered activity in the third dark period.

**Conclusions.** The alterations in percent hatch, 24 hpf behavior, and 120 hpf behavior suggest developmental TCE toxicity is still a concern at regulatory concentrations and that TCE should remain a priority environmental toxicant.

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**Maternal serology as a prenatal test for detecting Bovine viral diarrhea virus persistent infection.**

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**Introduction.** *Bovine viral diarrhea virus* (BVDV) is recognized as one of the most important infectious viral diseases of cattle. This infection of the developing fetus and the subsequent generation of PI calves delivers the most significant reservoir of BVDV. The purpose of this study was to evaluate prenatal testing methods for BVDV persistent infection. We hypothesize that pregnant cows carrying PI fetuses have continual elevations in BVDV-specific antibodies as the gestation progresses.

**Methods.** We obtained archived serum samples from cows known to have given birth to PI calves (principal) and cows that gave birth to calves that were not persistently infected (control). Antibody titers were measured at three time points on each cow. Sera were tested for neutralizing antibodies against the viral strains that caused the fetal persistent infection in the dams (homologous strain), as well as cytopathic reference strains (BVDV 1a strain NADL and BVDV 2 strain 125c). For analysis, the reciprocal of serum antibody titers was log2-transformed, which were then back-transformed as geometric means for presentation of results. If differences were detected over time between principal and control cows, the geometric means of the reciprocal of the antibody titers were compared at each time point using repeated measures ANOVA.

**Results.** Statistically significant results were obtained when comparing the back-transformed geometric means of the homologous strain and 125c between the two groups ($p<0.001$, $p<0.001$). No statistical significance was seen with NADL.

**Conclusions.** BVDV-specific neutralizing antibody titers are elevated in dams carrying PI fetuses. Those antibody titers continue to rise as the gestation progresses to term. Antibody titers against current circulating strains of BVDV within a herd are a promising potential prenatal detection method for BVDV persistent infection.

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**Development of Bispecific T Cell Engagers (BiTEs) Against Rhabdomyosarcoma Targeting FGFR4**

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**Introduction.** Rhabdomyosarcoma (RMS) is a rare pediatric cancer that originates from skeletal muscle. Despite multimodal therapy including surgery, radiation and aggressive chemotherapies, relapsed refractory or metastatic RMS remains a lethal disease with no significant improvement in outcome over several decades of clinical trials and therefore novel therapies are needed. FGFR4 is a cell surface receptor tyrosine kinase that is highly expressed in all types of RMS but is minimally expressed to absent in normal humans tissues. FGFR4 is highly expressed in RMS and in myoblasts. Recently we and others have shown that FGFR4 is a direct target and strongly induced by the fusion gene driver PAX3-FOXO1 found in alveolar RMS. We therefore hypothesized that FGFR4 will provide a rational target for antibody-based immunotherapy in RMS with high FGFR4 expression.

**Methods.** Using a yeast display library, we identified 10 human binders with FGFR4 specificity. m412 was one of the binders that recognized FGFR4 with a binding affinity at nanomolar range. This binder was further developed into Bispecific T Cell Engagers (BiTE) in which scFvFc of m412 and an anti-C3 antibody (UTCH1) were subcloned into "Knob" and "Hole" expression vectors respectively and successfully made *in vitro* by the ExpiCHO expression system. Protein A affinity chromatography was used to purify m412 BiTE to 93.3% as measured by bioanalyzer.

**Results.** FACS analysis showed m412 BiTe recognized FGFR4 and CD3. We found that FGFR4 expressing cell lines including RH30 showed enhanced specific cytotoxicity when incubated with T cells in the presence of m412 BiTE. Further validation of the m412 BiTE is being performed with additional RMS cell line *in vitro*. Confirmatory *in vivo* experiments are planned using RMS patient derived xenografts.

**Conclusions.** We have thus developed a FGFR4 BiTE that may provide effective immunotherapy for RMS as well as other cancers exhibiting an overexpression of FGFR4.

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## Alfaxalone Cross-Reactivity affecting Progesterone Concentrations in Cats

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**Introduction.** Alfaxalone is a popular sedative and anesthetic in small animals because of its ease of use. In cats, alfaxalone can be used as an intramuscular or intravenous agent to achieve clinically useful sedation or anesthesia. On clinical observation, intact female cats given alfaxalone that were not recently in estrus serum progesterone ($P_4$) serum concentrations measured using the Immulite 1000 suggestive of luteal activity. This led to the question: is alfaxalone cross-reacting with the progesterone assay.

**Methods.** Eight neutered male, DSH cats were administered 3mg/kg of alfaxalone IM. Blood samples were collected at set time points, and serum concentrations of $P_4$ were determined using an automated immunoassay system (Immulite 1000).

**Results.** Statistical analysis was performed by Repeated Measures ANOVA with statistical significance was set at $p=0.05$. Serum $P_4$ was significantly elevated at 30 minutes, 1 hour, and 3 hours ($p<0.05$) when compared to baseline $P_4$. Progesterone had returned to baseline at 6 hours.

**Conclusions.** This study demonstrates a confounding factor when using alfaxalone as a sedative. Use of this sedative may be associated false elevation in blood concentrations of $P_4$ in cats.

# Graduate Student Poster Presentations

### Presentation, Treatment and Outcome of Aural Hematoma in Horses: 7 cases (2008-2019)

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**Introduction.** Aural hematoma is a commonly diagnosed condition in small animal practice and is defined as hemorrhage occurring between the skin and cartilage of the concave pinna. The exact etiology is unclear, but theories include trauma, hypersensitivity diseases, ectoparasitism, increased capillary fragility (e.g. hyperadrenocorticism), and autoimmune diseases. There is often a concurrent diagnosis of otitis externa. This condition can be usually combatted by addressing the underlying primary cause in combination with needle drainage, with or without corticosteroid administration. Surgical treatments, such as incision and placement of compressive sutures, are usually reserved for cases that reoccur. Conversely, aural haematomas are rare in horses, though theoretically the same principals of treatment could be applied.
The aim of this case series was to report the presentation, diagnosis, treatment and outcomes of aural hematomas in horses.

**Methods.** Medical records were reviewed for all horses with a diagnosis of aural hematoma at the JT Vaughan Large Animal Teaching Hospital at Auburn University, College of Veterinary Medicine between 2008 and 2019. Data retrieved included signalment, pertinent historical information, presenting clinical signs, diagnostic procedures including dermatologic assessment, and treatments. Case outcome was determined via documentation of subsequent visits in the medical record or via telephone communication with owners or referring veterinarians (rDVM).

**Results.** Three of the horses presented after reoccurrence of the aural hematoma following treatment by the rDVM. Four horses had a previous history of allergic skin disease prior to aural hematoma development. The majority (6/7) of patients were unilaterally affected. Diagnostic modalities employed included otoscopic evaluation (3/7), ultrasound (3/7), cytological evaluation (3/7) and histopathology (1/7). Eight pinna were treated in total: two by nonsurgical needle drainage (one with concurrent corticosteroid injection), and the remaining six by surgical incision and placement of compressive sutures. Surgical incision techniques included a stab incision at the most dependent portion of the pinna (3), two parallel linea incisions (2), several incisions made with a $CO_2$ laser (1) and an 'S' shape incision (1). All surgical cases utilized compressive sutures with non-absorbable suture and sterilized buttons as stents. Follow-up was available for six horses and all affected pinnae were lichenified with four horses having permanent drooping of the pinna. Two horses reportedly had recurrence of the aural hematoma, one horse developed a hematoma in the opposite pinna one year after hospital discharge.

**Conclusions.** Equine aural hematoma is a rare condition. The main principle of treatment is drainage, treatments commonly utilized in small animal practice can be successfully applied to horses. Owners should be aware that a change in the cosmetic appearance of the pinna is likely.

**Acknowledgments**. The authors thank Kassie Kearns and Patricia Recklet.

## Clinical Osteochondritis Dissecans in Standardbred Yearlings: Lesion Specific Impact on Racing Performance and Longevity

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**Introduction.** Osteochondrosis is a developmental orthopaedic disorder defined as a disturbance of endochondral ossification. In the horse the location of osteochondrosis lesions are anatomically predictable: the distal intermediate ridge of the tibia, the lateral trochlear ridge of the stifle etc. There is some indication in the literature that both the anatomical site of the lesion and the gait of the horse have an impact on performance and longevity, but these studies have been underpowered. The aim of this study was to evaluate the short and long-term racing performance (including longevity) of a large population of Standardbred racehorses. The hypothesis was that horses with osteochondrosis would have decreased racing success compared to horses without and that different lesion locations would differ in impact on performance.

**Methods.** Retrospective cohort study. Every horse born on one Standardbred breeding farm between 2009 and 2017 which survived to racing age was included. Horses was classed as clinical osteochondrosis positive if lesions were confirmed arthroscopically. Race records were obtained from the United States Trotters Association. Multiple linear regression models were utilized to test the hypotheses.

**Results.** There were 2711 horses that were included in the study. 78.5% (2128/2711) had at least one qualifying racing start. 14.1% (382/2711) were treated for clinical osteochondrosis; there were 829 total osteochondral lesions. Osteochondrosis affected horses had 4.04 less starts than horses that were not affected by osteochondrosis (95% CI = 1.22-6.86, P = 0.0051). They had 0.44 less wins (95% CI = 0.00-0.88, P = 0.0491), 0.59 less places (95% CI = 0.15-1.02, P = 0.0082), 0.74 less shows (95% CI = 0.32-1.16, P = 0.0006) and 0.62 less total number of 1st – 3rd finishes (95% CI = 0.55-2.99, P = 0.0045). Osteochondrosis non-affected horses had a longer career than osteochondrosis affected horses, racing 0.15 years longer (95% CI = 0.04-0.25, P = 0.0049). Horses with a lesion on the distal intermediate ridge of the tibia (DIRT) or on the lateral trochlear ridge of the talus (LTR) had decreased racing performance compared to horses without these lesions.

**Conclusions.** Horses with clinical osteochondrosis performed worse over their career than horses without clinical osteochondrosis in a large population of horses over an 8 year period. Horses were only radiographed if they displayed clinical signs (joint effusion, lameness etc.) so it is unknown if the healthy controls had clinically silent osteochondrosis lesions. Osteochondrosis-affected horses had less starts and shorter careers, this could be due to osteochondrosis having long-term implications for joint health in racing Standardbreds.

**Acknowledgments.** Race records were provided by the United States Trotters Association. This work was funded by a Raymond Firestone Trust Research Grant.

**Investigation of endocrine disrupting properties of per- and polyfluoroalkyl substances in male rats**

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**Introduction.** Per- and polyfluoroalkyl substances (PFASs) are a group of chemicals that are used in the manufacture of fire extinguishers and fire-safety foams, photo imaging, and in non-stick dishware. Unregulated disposal of PFASs into landfills has caused contamination of groundwater sources for drinking and agriculture. These activities result in contamination of produce and chemical exposure of the population. Toxicity concerns have resulted in the enactment of new regulations prohibiting the use of long chain PFASs. In this regard, there are suggestions that short chain PFASs, such as perfluorobutanoic acid (PFBA) and perfluorobutane sulfonic acid (PFBS), may be safer alternatives to long chain perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) because they are less toxic and are more readily degraded in the environment. However, PFBS and PFBA, with highly degradation-resistant carbon-fluorine bonds, are thought to accumulate in body tissues. Of interest, there is little to no information on hormonal activity and/or endocrine-disrupting properties of short-chain PFASs. Therefore, we performed experiments to compare the effects of short-chain PFASs to their long-chain counterparts in the male gonad.

**Methods.** First, male Long-Evans rats were administered test chemicals (PFOA, PFOS, PFBA, and PFBS) in drinking water at 1µg/L from 21 to 35 days of age. In a follow-up experiment, male Long-Evans rats were administered PFOA and PFBA in drinking water at graded doses (1, 10, 100, and 1000 ng/L) for 14 days from postnatal days 21 to 35. At the end of both experiments, serum was separated from blood collected at sacrifice. Also, testicular tissue was incubated in DMEM/F-12 culture medium without (basal) and with 100 ng/mL ovine LH (LH-stimulated) for 3 h. Androgen secretion was assessed by RIA measurement of serum, basal and LH-stimulated testicular T concentrations.

**Results.** Results of the first experiment showed that exposure of male rats to PFOA and PFBA, but not PFOS and PFBS, decreased ($p<0.05$) serum T concentrations compared to control animals (Control, 6.66; PFOA, 1.62; PFOS, 7.45; PFBA, 3.96; PFBS, 5.70 ng/mL). In the second experiment, we observed that male rats exposed to 10 ng/L PFBA in drinking water exhibited increased serum T concentrations compared to control (control, 13.19; PFBA, 22.82 ng/mL, $p<0.05$), but this effect was not seen in any other treated group ($p>0.05$). Although basal testicular T concentrations showed a slight dose-dependent decrease after treatment with varying concentrations of both PFOA and PFBA, these changes were not significant ($p>0.05$). LH-stimulated T concentrations were similar in control and all treated groups ($p>0.05$).

**Conclusions.** These data demonstrated that alkyl-containing PFOA and PFBA may exert dose-dependent effects on serum testicular testosterone secretion. However, more exposure paradigms warrant to be tested in order to fully identify differences between long- and short-chain PFASs.

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**Maturation of gut microbiota and dramatic shift of microbial composition during canine puppy development**

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**Introduction.** The gut microbiota is the collection of commensal microorganisms including bacteria, archaea, eukaryotes and viruses. It affects every aspect of animal physiology. After birth, the microbial diversity increases in human infant gut and converges toward an adult-type microbiota by the end of 3 years of age. Major life events, such as weaning, introduction of formula or solid food, antibiotics usage and vaccination also impact the developing infant gut microbiome. Canine microbiome resembles human more than other examined species in taxonomic composition and functional capacity. The colonization and maturation of the gut microbiome have not been investigated in dogs.

**Methods.** In this study, we collected fecal samples in two male and two female puppies in the Auburn University Canine Performance Sciences program, at 16 developmental time points during the first year after birth. Fecal samples were homogenized using Qiagen PowerLyzer24 instrument and microbial DNA samples were extracted using Qiagen Allprep PowerFecal DNA/RNA kit. 22 metagenomic sequencing libraries were made with NEBNext Ultra II DNA Library Prep kit and sequenced on an Illumina NovaSeq6000 sequencer. Approximately 200 million 150 base pair (bp) reads were generated for each sample. A metagenomic analysis pipeline was developed for the data analysis.

**Results.** *De novo* metagenomic assembly discovered a total of 881,618 microbial contigs (total length 1.15 billion base pairs). 1,740,555 bacterial genes were predicted in the gut microbiome. Relative abundance in the canine microbiota was determined at phylum and genus level at each developmental time point. We discovered that from birth to first 3 weeks, *Proteobacteria* are dominant in the puppy gut microbiota and the whole gut microbiota exhibits low diversity. There is a rapid turnover and *Firmicutes* become the most abundant phylum at 3 to 4 weeks of age. Another dramatic shift in microbial composition occurs at 4 to 6 weeks, with more than 60% *Bacteroidetes* and an increase microbial diversity. From 6 to 12 weeks, transition to the adult-types gut microbiota happens gradually and later data points are needed to determine to time of complete maturation of canine gut microbiota.

**Conclusions.** In conclusion, using high coverage whole-genome shotgun metagenomic sequencing, we characterized the canine gut microbiome, investigated the composition of core gut microbes, their relative abundance and the microbial gene catalog at multiple developmental stages after birth. We significantly advanced the knowledge in canine microbiome developmental dynamics and will inform strategies to improve canine health and detection dog training success.

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**Spatiotemporal expression profile of embryonic and adult Ankyrin and EF-hand containing protein 1-encoding genes ankef1a and ankef1b in zebrafish**

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**Introduction.** Recent human next-generation sequencing (NGS) studies indicate a correlation of ANKEF1 (ankyrin repeat and EF-hand domain containing protein 1) expression and cilia formation or function. Additionally, a single study conducted in the African clawed frog (Xenopus laevis) has indicated ankef1 is down-regulated after pharmacological fibroblast growth factor (FGF) inhibition and plays a role in protocadherin-mediated cell protrusion and adhesion. That study also revealed a critical role for ankef1 in the embryonic development of the frog, with morphants exhibiting phenotypes including spina bifida and a shortened body axis. Interestingly, while little is known about ANKEF1 function in other vertebrate systems, recent proteomic data has shown ANKEF1 is enriched in ciliated cells. Likewise, publicly available EST profile databases indicate ANKEF1 expression in multiple human tissues, including high levels in the testes. Together, these previous studies suggest an important role for ANKEF1 in ciliated tissues and during embryonic development. Here, we have cloned zebrafish (Danio rerio) *ankef1a*, as well as its paralog, *ankef1b*, and conducted expression analyses by whole-mount in situ hybridization (WISH) and quantitative polymerase chain reaction (qPCR) during embryonic development and in adult tissues.

**Methods.** Whole-mount in situ hybridization. Cryosections. Quantitative RT-PCR.

**Results.** WISH revealed that both forms are ubiquitously expressed early in development, with more discrete expression of both transcripts in embryonic tissues known to precede or possess motile cilia, including dorsal forerunner cells (DFC) and the otic vesicles, respectively. Additionally, both transcripts are enriched in the developing pharynx and swim bladder. Our qPCR results revealed enhanced expression in the testes, along with increased expression in brain.

**Conclusions.** Certainly, our experiments in the zebrafish model system with *ankef1a* and *ankef1b* provide a solid foundation for future studies to uncover the molecular pathways through which Ankef1 acts in both healthy and disease states.

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## Hunting mast cell progenitors in normal canine blood

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**Introduction.** Mast cells are widely considered to be a multi-functional master cell, with involvement in histamine based allergic reactions, wound healing, tissue remodeling and innate immunity. They are strongly associated with the tumor micro environment as well as pathologies such as anaphylaxis, allergic rhinitis and infantile asthma as well as a myriad of other pathologies. Mast cells are found in nearly every tissue of the body except the central nervous system and the retina of the eye, but originate in the bone marrow as hematopoietic stem cells. After release from the bone marrow, they circulate in the blood as uncommitted mast cell progenitors (MCp) which are $CD117^+$, $CD34^+$, $Fc\varepsilon RI^+$ and $CD90^-$ before being recruited to the peripheral tissues where they mature. Malignant transformation of mast cells results in mast cell tumor, which is a common tumor of dogs. Canine mast cell tumors are most commonly found in the skin, but may also occur in the liver, intestines, spleen and elsewhere. Normal mast cells from canine mast cell tumor patients are needed to compare transcriptomes to patient mast cell tumors using deep sequencing/single cell sequencing and from this data, therapeutic targets may be selected. The current project seeks to identify and obtain appropriate normal mast cell precursors from patient animals with minimal additional morbidity. Circulating MCp's represent a population of approximately 0.1-0.5% of a cell types throughout the blood, making them a difficult target to isolate.

**Methods.** Herein we describe two methods for isolating a $CD117^+$ cell population from whole blood. The first is a method of magnetic activated cell sorting (MACS) to deplete $CD90^+$ cells and enrich for $CD117^+$ cells followed by flow cytometric analysis. The second uses MACS to deplete $CD90^+$ cells first followed by fluorescence activated cell sorting (FACS) to isolate $CD117^+$ cells for downstream use in single cell sequencing.

**Results.** For the first method we start with roughly 3x10^6 cells and finish with roughly 5x10^4 cells. Cytospin has shown this to be a very heterogeneous population consisting of very few granulated cells. Flow cytometric analysis has also shown this to be a very heterogeneous population with only 1-2% $CD117^+$ cells. Method two using FACS yields a much smaller cell population of about 8,000 cells.

**Conclusions.** Method 1 yields a very impure population that cannot be used for further downstream analysis via single cell sequencing. Method 2 yields a very low cell number but may be of higher purity so single cell sequencing may still be an option. We are currently troubleshooting both methods to increase purity enough for downstream usage in single cell sequencing.

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## Canine myxosarcomas, a retrospective analysis of 32 dogs (2003-2018)

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**Introduction.** Myxosarcomas are known to be classified as soft tissue sarcomas. However, there is limited clinical characterization pertaining specifically to canine cutaneous myxosarcomas in the literature. The objective of this study is to evaluate the local recurrence rate, metastatic rate and prognosis of canine myxosarcoma

**Methods.** The medical records of dogs diagnosed with histologically confirmed myxosarcoma at five institutions between June 2003 and January 2018 were retrospectively reviewed.

**Results.** A total of 32 dogs diagnosed with myxosarcoma via histopathology were included in this retrospective study. All dogs had surgical resection. No adjunct treatments were performed in 9 dogs, while 22 dogs also received either radiation therapy or chemotherapy, or a combination of both. One dog received only NSAID after surgery. Overall median survival time (MST) was 730 days (range 20-2345 days). The MST of dogs with a tumor mitotic count < 10/10 HPF was 1393 days (range 20-2345 days). The dogs with a tumor mitotic count of 10 or greater/10 HPF had a MST of 433 days (range 169-831 days). There was no significant difference of MST among different treatment modalities. Local recurrence was noted in 13 cases (40.6%) and the median time to recurrence was 115.5 days (range 50-1610 days). The median time to local recurrence in dogs with mitotic count of < 10/10 HPF was 339 days (range 68-1610 days) and in dogs with mitotic count of 10 or greater/10 HPF was 119 days (range 50-378). Metastasis to local lymph node or lung was noted in 8 cases (25%) with median time to metastasis of 158.5 days (range 0-643 days).

**Conclusions.** Based on the results of this retrospective study, myxosarcoma may have a higher local recurrence rate and risk of metastasis to the local lymph nodes compared to other soft tissue sarcomas.

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**Whole-genome shotgun metagenomic analysis of chicken gut microbiome under healthy conditions and coccidia infection**

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**Introduction.** Chicken is the largest component of meat and poultry industry in the United States. *Eimeria spp.* are among the most important pathogens of chickens, causing coccidiosis in animals, which is commonly referred to as coccidia. The annual economic damage is estimated to be 4.54% of the gross revenue from sales of live broilers, which equals 1.4 billion dollars. Currently, *Eimeria* infection in broiler production was controlled by anticoccidial feed additives. Recently, antibiotic-free and organic production became the mainstream. New approaches to coccidiosis management are in urgent need, such as the use of prebiotics and probiotics.

**Methods.** To characterize the influence of *Eimeria* infection on the chicken gut microbiota, we performed whole-genome shotgun (WGS) metagenomic sequencing in fecal samples collected 6-day and 10-day post infection. Specifically, fecal DNA was extracted from jejunal content using the Qiagen PowerFecal DNA/RNA kit. We fragmented the DNA to 300-500 bp insert size on Covaris M220 ultrasonicator. NEB Microbiome enrichment kit was exploited to enrich the microbial DNA and minimize the chicken DNA contamination, before we constructed the WGS metagenomic libraries using NEBnext Ultra II library preparation kit. The final libraries were QCed and sequenced on an Illumina HiSeqX machine. After quality control, the paired-end reads were joined by PEAR program, sequencing adapters were removed using trimmomatic. Filtered reads were mapped to the chicken genome and NCBI viral database to remove host and virus contamination. Unmapped reads were retained for subsequent analysis. MEGAHIT was used for metagenome assembly and Kaiju for gene annotation and taxonomy classification.

**Results and Conclusions.** Before microbial DNA enrichment, about 84~88% of reads were from the chicken genome. After enrichment, the chicken reads have been reduced significantly, with 50-75% microbial reads, suggesting excellent efficacy of prokaryotic DNA enrichment. The viral reads and *Eimeria* reads from 16 metagenomes were smaller than 1%. We assembled a total 1,684,584,503 reads (approximately 503 Gb in length) and got 1,172,817 bacteria contigs, with a total contig length of 656,918,471 bp. Gene annotation pipeline predicted 891,941 prokaryotic genes. The three most abundant orders in the chicken gut microbiota are *Lactobacillales*, *Clostridiales* and *Bacillales*. We identified significant overrepresentation of *Clostridiales* in infected samples 10-day post infection, which is consistent with the 16S rRNA gene sequencing results. In addition, we observed variable amount of Chlamydiae sequencing in the gut microbiome, which is not expected. There Chlamydiae reads may originate from the host intestine cells and we are analyzing the data to determine the taxonomy classification at species and strain level.

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## Anti-Proliferative Effects of Cannabinoids in Canine Lymphoma

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**Introduction.** Non-Hodgkin lymphoma (NHL) is the fifth leading cause of human cancer death and is the second fastest growing cancer with regard to mortality in people. Likewise, lymphoma is one of the most common neoplasms encountered in dogs. It accounts for about 20% of all canine cancers and about 85% of blood cancers, with an annual incidence up to 134 per 100,000 dogs. Canine and human lymphoma are generally characterized by a high rate of initial remission following conventional CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) based therapies; however, 95% of dogs and 30% of humans will succumb to drug-resistant relapse. To date, lymphoma is still a serious condition for which there are unmet medical needs both in humans and dogs. For this reason, it is essential to develop novel strategies to improve the outcome of patients suffering from aggressive or therapy-resistant lymphoma. Cannabinoids have been used in human patients with cancer for their palliative effects (e.g. inhibition of chemotherapy- induced nausea, vomiting, apatite stimulation and pain) since the early 1970s. However, in addition to palliative interventions, cannabinoids have demonstrated anticancer effects on various xenograft animal models of cancer, Cannabinoids appear to decrease tumor progression by at least two mechanisms: potentiating apoptotic death of tumor cells and inhibition of tumor angiogenesis, thus decreasing tumor progression, cell migration and spreading. Cannabinoid receptors and anti-cancer effects of cannabinoids have never been studied in canine and the canine may be an extremely useful model for the study of lymphoma in humans, owing to striking similarities in histology, biology and gene expression. Objective of our study was to evaluate cannabinoid receptors (CB1 and CB2) expression and anti-proliferative effect of cannabinoid in canine lymphoma.

**Methods.** We used two canine B cell lymphoma cell lines (17-71 and CLBL1) and one canine T cell lymphoma cell line CL1 to characterize the expression of cannabinoid receptors using real time PCR to confirm the expression we ran the OCR gel. To find the anti-proliferative effect of cannabinoid we used endocannabinoids (2AG and AE), phytocannabinoids (CBD and THC) and synthetic cannabinoid agonists and antagonists. Cells were incubated for 24 and 48 hours with the drugs in five different concentrations, (100nM, 5000nM, 1μM, 25μM and 50 μM). We used untreated cells in media as a control and looked at the anti-proliferative effect of cannabinoids using MTT assay.

**Results.** We found positive expression of cannabinoid receptor CB1 and CB2 in all lymphoma cell lines. With cell viability assay we found significant dose dependent anti-proliferative effect with AEA, CBD, WIN55-212-22 and synthetic agonist HU-210. Our results suggest that cannabinoids could be developed as novel therapeutic agents for the treatment of canine and human lymphoma.

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**Development of an E3 Modified Canine Oncolytic Adenovirus**

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As we advance our understanding in immunology and cancer biology, it is inevitable that increasing numbers of therapeutic genes or targets will emerge for cancer-targeted therapies. One of the most salient features of many tumors is their ability to subvert host T-cell immune response by recruiting various immune checkpoints. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is an important immune checkpoint molecule upregulated on the cell surface of naïve T cells during their initial priming in the secondary lymphoid organs (e.g. lymph nodes). Engagement of CTLA4 with B7-1/B7-2 inhibits the TCR downstream phosphorylation cascade, attenuates T cell activation and induces T cell tolerance by decreasing T-cell proliferation, secretion of interleukin-2 (IL-2) and trans-endocytosis and degradation of the B7 ligands on antigen-presenting cells. Cancer cells often exploit CTLA4/B7 pathways to avoid the antitumor immune response. Our lab has previously developed an anti-CTLA-4 canine heavy chain-only antibody (CTLA4.Nb6.cHcAb) that specifically binds to canine CTLA-4 receptor. We hypothesize that CTLA4.Nb6.cHcAb will block the CTLA-4/B7 pathway and result in generation of an antitumor T-cell response by blocking T-cell inhibitory signals. Unlike many studies, which involve systemic delivery of monoclonal antibodies as the therapeutic modality, we have developed an E3 modified canine oncolytic adenovirus for direct gene transfer of CTLA4.Nb6.cHcAb. *Thus, the primary objective of this study was to develop a canine conditionally replicating oncolytic adenovirus (cCRAd) to for intratumoral or systemic delivery of CTLA4.Nb6.cHcAb*. As an added benefit, oncolytic adenovirus will cause direct tumor oncolysis, which will prime an immune response against tumors due to the production of cytokines and release of tumor antigens. Because canine adenovirus is not as well characterized as human adenovirus, we first determined if an E3 deleted CAV2 (cCRAdΔE3) would be viable in cell culture. To construct the E3 deleted CAV2, a PCR amplified green fluorescent (GFP) expression cassette was produced and cloned into the CAV2 via homologous recombination in yeast. The recombinant adenoviral plasmid from yeast was isolated and amplified in E. coli. The cCRAdΔE3.GFP plasmid was electroporated into Dkcre cells and cCRAdΔE3.GFP virus was isolated via three freeze thaw cycles after 85-90% of the Dkcre cells began fluorescing. The crude viral lysate collected was used to infect Dkcre cells, and after two rounds of amplification, an adenoviral cytopathic effect (CPE) was observed. The cCRAdΔE3.GFP virus was amplified and purified using iodixanol gradient using standard techniques. Like ICOCAV15, cCRAdΔE3.GFP virus was able to infect and kill osteosaroma cancer cell lines (D17 and CF11). Furthermore, cCRAdΔE3.GFP was also successful at infecting and killing primary osteosarcoma cells, COC3 and COC4. In this study, we have successfully demonstrated that a replication-competent oncolytic adenovirus can be produced after deleting and replacing E3 region of canine adenovirus serotype 2. Additionally, we have shown that our modified virus retained the oncolytic activity against canine osteosarcoma cell-lines. We have also replaced the open reading frame of GFP with CTLA4.Nb6.cHcAb in cCRAdΔE3.GFP plasmid. In future studies, we will purify this virus and evaluate its efficacy in *in-vitro* and *in-vivo* studies.

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**Gefitinib, at Its Clinically Relevant Concentrations, Inhibits Rifampicin-Induced CYP3A4 Gene Expression in Human Hepatocytes**

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**Introduction.** Pregnane xenobiotic receptor (PXR) has been shown to induce chemoresistance during multidrug chemotherapy via upregulation of PXR target genes such as cytochrome P450 3A4 (CYP3A4). CYP3A4 contributes to the metabolism of over 50% of clinically active drugs. Therefore, during multidrug chemotherapy, drug induction of hPXRmediated CYP3A4, can affect the therapeutic response of coadministered drugs, leading to chemoresistance. It is possible to overcome a PXR agonist–induced chemoresistance with a PXR antagonist. Gefitinib (GEF), a tyrosine kinase inhibitor approved for the treatment of advanced non-small cell lung cancer, and used in combinational chemotherapy treatment, is a promising candidate due to its PXR ligand-like features. We therefore sought to determine whether GEF would behave as a PXR antagonist in human primary hepatocytes human hepatocells, and HepG2 human hepatocellular carcinoma cells.

**Methods.** Rifampicin, an agonist of PXR, was used to activate PXR-mediated CYP3A expression. Reporter gene assays were conducted to determine PXR transactivation of CYP3A4
promoter activity. Cell viability assays were performed to assess cytotoxicity. Quantitative RT-PCR assays were conducted to study endogenous CYP3A4 gene expression. PXR molecular docking studies were carried to predict binding affinity of GEF to PXR. Finally, competitive ligand binding and Steroid receptor coactivator-1 (SRC-1) coactivator recruitment assays were performed to examine GEF ability to bind to PXR and alter SRC-1 recruitment to PXR.

**Results.** GEF, at its therapeutic concentrations, repressed rifampicin-induced endogenous CYP3A4 gene expression in human primary hepatocytes and human hepatocells. Additionally, GEF inhibited rifampicin induction of PXR-mediated CYP3A4 promoter activity. These findings indicate that GEF could inhibit an agonist-activated PXR-mediated CYP3A4 gene expression. Molecular docking studies predicted that GEF can bind to multiple sites on PXR including the ligand binding pocket. Indeed, GEF bound to the PXR and attenuated the PXR agonist-induced SRC-1 interaction, suggesting that GEF directly interacts with multiple sites on the PXR.

**Conclusions.** The results suggest that GEF, at its clinically relevant therapeutic concentration, can antagonize PXR agonists-induced CYP3A4 gene expression. Thus, GEF could be a potential candidate for use in combinational chemotherapies to combat PXRmediated chemoresistance. Future studies are required to examine GEF selectivity towards PXR, and to determine GEF suppression of PXR agonists-induced chemoresistance.

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## Evaluating the efficacy of marker-assisted selection (MAS) using disease resistance and growth traits QTLs in catfish

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**Introduction.** Disease resistance is one of the most important traits for aquaculture industry. Enteric septicemia of catfish (ESC, *Edwarsiella ictaluri*), columnaris (*Flavobacterium Columnare*) and motile *Aeromonas* septicemia (MAS)are three major diseases in catfish, causing huge economic damage to the catfish industry. Channel catfish is the primary cultured species and has greater resistance to columnaris and aeromonas diseases compared to a lesser used species, blue catfish. However, blue catfish has almost total resistance to ESC in the culture environment. The hybrid between female channel catfish and male blue catfish exhibits heterosis with superior phenotypes compared the two parental species, including faster growth, enhanced disease resistance and greater fillet yield. Therefore, combining the genomes of blue catfish and channel catfish could increase the overall disease resistance and obtain better growth trait of US grown catfish.

**Methods.** We propose to test the effectiveness of marker-assisted selection (MAS) for improving the resistance to bacterial pathogens in comparison to the traditional mass selection approaches. In the phenotype-based selection, response to selection was variable and the genetic enhancement is relatively slow. Based on the genetic underpinnings in disease resistance, marker genotyping assays will be designed to assist parent selection in the channel-blue synthetic breed of catfish. The resistance of the progeny will be systematically evaluated to measure effectiveness in genetic gain and improvement of the breeds. Allele-specific pyrosequencing assays will be designed for rapid, robust and accurate genotyping for marker-assisted selection.

**Results and conclusions.** A total of 89 SNPs were selected from previously identified QTLs (quantitative trait loci) identified in Genome-wide Association studies (GWAS) in catfish by Dr. Dunham's team. These candidate QTLs include 37 SNPs for ESC resistance, 10 SNPs for columnaris resistance, 26 SNPs for Aeromonas resistance and 16 SNPs for superior growth phenotype. Many of these loci are involved in immune functions in catfish. Therefore, our study has the potential not only to improve the current breeding schemes, but also to increase our understanding of disease resistance mechanisms and fish immunogenetics. The expected outcome is the development of a new synthetic breed of catfish with superior disease resistance to currently grown channel catfish and channel catfish female X blue catfish male hybrids. Additionally, the synthetic breed will be crossed to blue catfish males, which will result in a new hybrid with both enhanced bacterial disease resistance as well as heterosis for other economically important traits.

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**Characterizing the genetic and genomic architecture of eight laboratory opossum strains using ddRAD-seq technology**

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**Introduction.** The gray, short-tailed opossum, *Monodelphis domestica* (also known as the 'laboratory opossum') is firmly established as the predominant laboratory-bred research marsupial species. Compared to other marsupial models, *M. domestica* is much smaller (60-150g), easier to maintain, and able to breed year-round and reach sexual maturity relatively rapidly. It serves as a key species for comparative genomic research, providing the pivotal phylogenetic outgroup for studies of derived vs. ancestral states of genomic/epigenomic characteristics for all eutherian mammal lineages. Therefore, it is widely used as a comparative "alternative mammal" in investigations that span many topics relevant to animal development, physiology, and disease susceptibility. 20 genetic stocks have been developed after imported from Brazil and Bolivia in 1978 and the *M. domestica* genome was sequenced in 2007. Charactering the genetic architecture is critical to understand the evolutionary relationship of these strains.

**Methods.** Genomic DNA samples were extracted using 78 individuals from eight laboratory opossum strains (AH11L, ATHHN, ATHL, LSD, FD2M1, FD2M4, LL1 and FD8X) derived from animals collected in three different geographic locations. Double digest restriction-site associated DNA sequencing (ddRAD-seq) protocol was used to characterize the genetic and genomic architecture in these genetic strains. Illumina sequencing reads were aligned to the *Monodelphis* reference genome version 5.0. *De novo* SNP calling was performed using BAM files generated from genome alignments. SNP density, average heterozygosity and other population genetics parameters were computed using the polymorphism data.

**Results and Conclusions.** A total of 66,640 high quality SNPs from eight autosomes and the X-chromosome were identified with the minimum sequencing depth of 6X. Based on the analysis, we found that the inbred strains had a deficit of heterozygotes SNP positions, which is consistent with their breeding history. Almost no heterozygous SNP loci were identified on the X chromosome in male individuals, indicating low frequency of false positives. Principal component analysis (PCA) and clustering analysis revealed that the population genetic structure is in accordance with the geographic locations of the founder animals of the eight strains. The overall genetic diversity of these strains reflects breeding history. In conclusion, our ddRAD-seq data is sufficient to identify the genetic structure and population differentiation in the eight opossum strains.

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**Protection Conferred by IBV S-ectodomain Expressed from Recombinant NDV LaSota**

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**Introduction.** We previously demonstrated protection against IBV infection in chickens following subcutaneous vaccination with recombinant soluble trimeric recombinant spike-ectodomain protein. We now demonstrate proof-of-principle that vaccination with recombinant NDV LaSota expressing Ark-type IBV S-ectodomain protein confers protection against IBV infection. Vaccinated chickens challenged with virulent Ark-type IBV exhibited lower incidence of respiratory signs and reduced tracheal damage compared to unvaccinated controls.

**Methods.** To study protection against challenge of recombinant NDV LaSota expressing Ark-type IBV S-ectodomain protein (rLS/IBV.Se), two trials were developed.
Trial 1. SPF chickens were vaccinated with rLS/IBV.Se ($10^5$ $EID_{50}$ /bird) at one day of age (DOA), boosted with the same vaccine at 14 DOA, and subsequently challenged 14 days post-vaccination (DPV) with an Ark-type virulent strain.
Trial 2. Because vaccination at 1 DOA may result in suboptimal immune responses, in trial 2 chickens were vaccinated at 1 or 10 DOA with rLS/IBV.Se ($10^7$ $EID_{50}$ /bird) and challenged 20 DPV with virulent Ark. In both trials, chickens were challenged with $10^4$ $EID_{50}$ /bird of Ark-type virulent strain AL/4614/98 (GenBank accession DQ458217). Protection was determined five days post-challenge by respiratory signs, viral load in tears (IBV RNA by qRT-PCR) and tracheal histopathology and histomorphometry.

**Results.** Protection measured 5 DPC following rLS/IBV.Se priming at 1 DOA and boosting at 14 DOA compared to unvaccinated controls reduced incidence of respiratory rales, mucosal thickness and lymphocytic infiltration, and marginally reduced viral loads.
Protection measured 5 DPC following rLS/IBV.Se vaccination at 1 or 10 DOA showed respiratory signs significantly reduced when vaccinated at 10 DOA compared to unvaccinated controls. No clear effect of vaccination on viral load in tears was observed.

**Conclusions.** Prime and boost with rLS/IBV.Se vaccine reduces IBV clinical signs and lesions in the trachea following IBV challenge. Postponing vaccination with rLS/IBV.Se beyond day 1 of age confers improved protection. Vaccination with rLS/IBV.Se did not reduce viral loads in tears following IBV challenge.

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**Overexpression of Foxl2 leads to ovarian-like adrenal glands**

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**Introduction.** Adrenal gland, testis and ovary share a common primordium (adrenal gonadal primordium). Adrenocortical cells, Leydig cells, Sertoli cells, granulosa cells, and theca cells are not only coming from the same origin but also express *Sf1*, which controls the formation of these three organs. However, the molecular regulations driving the differentiation of *Sf1*-positive cells remain unclear. Here we found that the suppression of *Foxl2*, the most conserved regulator of ovarian granulosa cell identity, in *Sf1*-positive cells is required for the development of a normal adrenal gland.

**Methods.** The *ROSA-Foxl2* mice were crossed with *Sf1-Cre* mice, resulting in ectopic induction of *Foxl2* in *Sf1-Cre*-expressing cells. Adrenals were collected and analyzed at several developmental stages from both genders.

**Results.** Overexpression of *Foxl2* in *Sf1-Cre* expressing cells affects adrenal gland development as early as at postnatal day 0 (P0). Mutant adrenals were small with an under-developed adrenal cortex. Most mutant mice die after birth. Adrenals in surviving mice failed to develop a proper zonation of the cortex and the medulla at all developmental stages we have analyzed. Adrenals in 7-month old mutant mice showed clustered cell structures similar to ovarian follicle and corpus luteum. These ovarian-like adrenals did not contain germ cells nor express other ovarian markers such as AMH.

**Conclusions.** Our finding indicates that the suppression of *Foxl2* is required for the development of a normal adrenal gland. Ectopic expression of *Foxl2* in adrenocortical cells leads to ovarian-like adrenal glands in adult mice.

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## Single-cell transcriptome analysis of lung cancer brain metastatic cells in the cerebrospinal fluid

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**Introduction.** Non-small cell lung cancer (NSCLC) is the main type of lung cancer, accounting for 85% of lung malignancies, and its 5-year survival rate is less than 15%. Brain is the most common metastatic site of NSCLC and the incidence of brain metastases, including leptomeningeal metastasis (LM) is as high as 22%-54%, which can occur in different stages of tumorigenesis, especially in advanced patients. Although the diagnosis and prognosis of NSCLC-LM patients have improved in recent years, the overall treatment outcomes are still not ideal. In NSCLC-LM patients, a small number of circulating tumor cells (CTCs) are present in the cerebrospinal fluid (CSF), whose cell composition is primarily lymphocytes, therefore the standard bulk RNA-seq method failed to detect these metastatic CTCs.

**Methods.** To establish the cell type profile in normal CSF, we investigated diagnostic test samples from patients with other brain diseases that do not affect the CSF cell composition, and performed single-cell RNA-seq (scRNA-seq) on 344 cells in 3 patients in these nearly normal CSF samples. In the CSF samples of five enrolled NSCLC-LM patients, we flow sorted 1,776 CD45(-) cells and performed scRNA-seq in individual CTCs following the SMART-seq2 protocol. The scRNA-seq libraries were sequenced on an Illumina HiSeqX machine to achieve on average 300,000 reads per cell. After quality control and filtering, high quality transcriptome data from 1,152 cells were retained for the subsequent analyses.

**Results.** We found that approximately 20% of the normal CSF cells are monocytes and the rest 80% belong to T cells, which is consistent with what is previously reported in the literature. Clustering analysis in NSCLC-LM patient samples revealed a number of lung adenocarcinoma markers, including SFTPA, SFTPB, SFTPC, NAPSA and KRT family members. These genes are highly expressed these CD45(-) cells, which is in sharp contrast to the CSF lymphocytes. These results indicate that these cells are indeed lung cancer metastatic cells in CSF samples (CSF-CTCs). Substantial among patient heterogeneity and intratumor heterogeneity have been discovered in the single-cell transcriptome profiles.

**Conclusions.** This research is the first systematic and comprehensive characterization of normal CSF cell profile as well as lung cancer brain metastatic CTCs in CSF samples at single-cell transcriptome level. We discovered that the intratumor heterogeneity varies at patient level. This study will provide essential knowledge and inform diagnosis and treatment of lung cancer brain metastasis.

**Acknowledgments.** Y.Z. is supported by the Auburn University Presidential Graduate Research Fellowship and College of Veterinary Medicine Dean's Fellowship. We thank Auburn University Research Initiative in Cancer (AURIC) for funding. For all sample materials used in this research, prior patients' consents and approval were obtained from the Ethics Committee of Huashan Hospital, Fudan University School of Medicine.

# Faculty/Visiting Scholar/Staff Poster Presentations

### The Disposition of Cannabidiol in Dogs after Single Dose Oral Administration

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**Introduction.** Cannabidiol (CBD) is one of two major cannabinoids found in marijuana. Because CBD is void of the psychotropic effects typical of THC, it increasingly is used for the same indications for medical marijuana, such as drug refractory epilepsy, pain and anxiety. These indications are relevant to companion animals, including dogs. Paramount to the well-designed clinical trial demonstrating efficacy in dogs is administration of an appropriate dose. In humans, the oral disposition of CBD is complicated by first pass metabolism and increased absorption with food. The purpose of this study was do determine the oral disposition of CBD in dogs when administered orally with or without food or as a soft chew.

**Methods.** Normal, apparently healthy beagle dogs (n=8) were studied three times, each time after a single dose of 2 mg/kg dose of CBD orally, using a non-randomized triple cross-over design with an 7 day washout period between trials. The first trial was CBD administered as an oil extracted from industrial hemp; the second trial was the same but dosing was immediately followed with feeding, and the third trial was administration as a soft chew formulation. Prior to dosing, an indwelling catheter was placed in the jugular vein for blood collection. Blood was collected prior to, and at 0.5, 1, 2, 4, 6, 8, 12 and 24 hrs. Plasma was harvested from each sample and frozen at -20$^{o}$C until analyzed. Plasma CBD and THC were quantitated using LC-MS/MS using ESI positive mode. Samples were analyzed using MRM. The assay was validated in canine plasma; the LOQ was 2 ng/ml for both THC and CBD. Precision and accuracy were both within 15%. Data was subjected to noncompartmental analysis (Phoenix WinNonLin®) using the log-linear trapezoidal rule.

**Results.** Key parameters include (fasting followed by fed and soft chew): maximum plasma concentration ($C_{max}$; ng/ml) 109$\pm$ 60 vs 290 $\pm$ 126 vs 272$\pm$ 130 at $T_{max}$ (time to maximum concentration [hr]) of 3.5 $=$ 1.4 vs 2.2 $\pm$ 0.7 vs 3.75$\pm$ 0.7; area under the curve (AUC; ng/ml/hr) 1672$\pm$ 2543 vs 1292 $\pm$ 592 vs 1100 $\pm$ 379; and elimination half-life (hr) of 6.5$\pm$ 3.3 vs 6.2$\pm$ 1.7 vs 5.0 $\pm$ 2.The relative bioavailability (F, or %) of CBD in fed vs fasted animals was 1.78 $\pm$ 0.89, indicating feeding enhances oral absorption in dogs. Relative bioavailability of oil with food compared to soft chews was similar (1 $\pm$ 0.3). THC was detectable in all preparations but Cmax was less than 30 mcg/ml.

**Conclusions.** Although feeding did increase bioavailability in this study, the increase is not as dramatic as in humans for which a 3 to 5 fold increase in AUC may occur. A therapeutic range for CBD has not yet been established, although in humans, concentrations higher than 100 ng/ml commonly are reported and in drug resistant epileptic children, a starting dose of 5 mg/kg divided q 12 hrs Epidolex® (an FDA approved CBD product) results in a $C_{max}$ of 241 ng/ml. As such, an oral dose of 2 mg/kg may be a reasonable starting dose in dogs, but higher doses may be necessary for clinical trials.

**Acknowledgments.** Nutramax Laboratories® provide both financial support for this study as well as data collection.

**Therapeutic Drug Monitoring of Cannabadiol (CBD) and THC Concentrations in Dogs Receiving "Hemp Oil" Products**

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**Introduction.** Cannabidiol (CBD) is one of two major cannabinoids found in marijuana. Because CBD is void of the psychotropic effects typical of THC, it increasingly is used for the same indications for medical marijuana, such as drug refractory epilepsy, pain and anxiety. These indications are relevant to companion animals, including dogs. Although CBD derived from marijuana remains a schedule I product according to the Drug Enforcement Agency, CBD derived industrial hemp has been legalized in the USA and most states. Not surprisingly, many companies are producing supplements labeled to contain CBD. These can be purchased through a variety of sources by lay persons. Companion pet owners are administering products labeled to contain CBD to their pets, often without veterinary supervision. Because supplements undergo no premarket regulatory consideration, the content of these products is often not stated on the label. An important question that should be answered when assessing their clinical efficacy is how much CBD is reaching the target tissues. The purpose of this study was to measure the plasma concentrations of CBD and THC in dogs receiving a CBD (hemp-oil) product.

**Methods.** Samples were solicited by the Therapeutic Drug Monitoring Laboratory(TDML) of the Auburn College of Veterinary Medicine's Clinical Pharmacology Laboratory through the TDML, the Veterinary Information Network and the American College of Veterinary Internal Medicine's list serve. Inclusion criteria included any dog receiving any hemp-oil based product for any indication. Serum CBD and THC were quantitated using LC-MS/MS using ESI positive mode. Samples were analyzed using MRM. The assay was validated in canine plasma; the LOQ was 2 ng/ml for both THC and CBD. Precision and accuracy were both within 15%. Plasma CBD was measured at no cost to the client. Data was reported out descriptively (median and range).

**Results.** 180 samples were analyzed from different dogs between January of 2016 and June of 2019. Patients were receiving products from at least 63 different manufacturers. The most common formulation was oil (90%) followed by capsule, biscuit or chew. The most common indications was epilepsy (78%) followed by pain (13%). The reported dose was not always provided, in part because the amount of CBD on the product labels was not given, but most patients were receiving 1 to 2 mg/kg. The median CBD concentration was 21.75 ng/ml, but the range was 0.5 to 929 ng/ml. For THC, the median was 1.18 but the range was 0.34 to 87 ng/ml.

**Conclusions.** This study demonstrates a marked variability in the plasma concentrations of CBD in patients receiving hemp oil products. It is not clear if the variability demonstrated here reflects inappropriate labeling of the product, differences in dose, differences in oral bioavailability or other factors such as drug interactions. The cause for this variability needs to be identified. One of the difficulties in implementing a controlled clinical trial with a hemp-oil CBD based product is demonstrated here: marked variability in cannabinoid concentrations (CBD or THC) may mitigate ability to detect significant differences in patient response. Among the approaches to minimize this problem in patients is routine monitoring of CBD.

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

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**Introduction**. Mebendazole is an anthelmintic drug used to treat parasitic worms both in humans and in animals. It functions by blocking microtubules formation and nutrient uptake in helminthes. Recently it has been demonstrated to have antineoplastic effects for selected cancers including glioblastomas. This study describes the development and validation of a specific reverse phase high performance liquid chromatographic (HPLC) with UV detection method for the identification and quantification of Mebendazole in canine plasma and cerebrospinal fluid (CSF).

**Methods**. Previously reported methods about the Mebendazole (MBZ), the chemical structure, physical properties, sample type (canine plasma and CSF), sample size, level of accuracy and precision expected were considered to develop the High Performance Liquid Chromatography (HPLC) with UV detection. Three different methods to develop the optimal chromatographic conditions for identification and quantification of Mebendazole were tested, different chromatographic columns (Spherisorb C6, C18, Gemini C6-Phenyl) and mobile phases (50 mM ammonium formate-methanol and 20 mM ammonium formate-acetonitrile) were tested. The pH, flow, and detection (295 nm, 314 nm) also were tested. For sample preparation, an extraction method with methanol, acetonitrile and ethyl acetate (remove the matrix) was tested, then the supernatant was dried under nitrogen to concentrate the sample. With the optimal conditions established the next step was to validate the method to get a robust quantification in terms of accuracy and precision.

**Results**. Results. Mebendazole plasma and CSF samples were analyzed by HPLC using a reverse-phase Gemini C6-Phenyl, 5 um, 150 x 3.0 mm column at 40 °C. The mobile phase was 20 mM Ammonium formate buffer pH 3 and acetonitrile, with a flow rate of 1.5 mL/min and UV detection at 314 nm. Protein precipitation procedure with acetonitrile and centrifugation was used to extract the mebendazole and to remove the matrix, following by dry under a current of nitrogen, and finally, the residue was reconstituted with the mobile phase. The injection volume was 100 µL. The linear coefficient was 0.999. The lower limit of detection (LOD) for canine plasma and canine CSF samples was 5 ng/mL. The lower limit of quantification (LOQ) for canine plasma and canine CSF samples was 10 ng/mL. The Precision (CV %) for Mebendazole at 14, 26, 60, 120 and 800 ng/mL was 1.95%, 0.40%, 8.45%, and 2.17% respectively. The Accuracy (% Recovery) for Mebendazole at 25, 60, 125 and 350 ng/mL was 104.34%, 99.40%, 99.38%, 100.28 and 101.64% respectively.

**Conclusions**. Mebendazole an analytical method by reverse-phase High-Performance Liquid Chromatography and UV detection in canine plasma and CSF was developed and validated. This assay will be used to quantify the Mebendazole in a pharmacokinetic study in dog plasma and CSF.

**Acknowledgments**. Department of Anatomy, Physiology and Pharmacology.

**Phage-peptide constructs for stimulation of anti-cancer immune responses against CD47**

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**Introduction.** CD47 is a cell surface protein that belongs to the immunoglobulin superfamily and demonstrates high homology in the exposed extracellular-domain between species. It binds several proteins including signal-regulatory protein-alpha (SIRP$\alpha$) expressed on phagocytes. Binding of CD47 to SIRP$\alpha$ leads to inhibition of phagocytosis. In this respect, CD47 plays a role of a "don't eat me" signal for phagocytic cells, making cells expressing CD47 resistant to phagocytosis. Many types of cancers escape clearance by the immune system by up-regulation of CD47 expression. One strategy to disarm the "don't eat me" signal on cancer cells is to block CD47 with neutralizing antibodies, preventing the CD47-SIRP$\alpha$ interaction. The present study is designed to generate and characterize phage-CD47 constructs to stimulate production of blocking antibodies against CD47 epitopes using phage display.

**Methods.** Phage-CD47 constructs were obtained via selection from phage display libraries for binding to CD47 antibodies as selection targets. Two types of CD47 antibodies (B6H12 and BRIC126) and three phage display libraries (Ph.D.-C7C, Ph.D.-7, and Ph.D.-12) were used for enrichment of CD47-mimicing peptide sequences. After the final round of selection, ~80 clones were randomly isolated for Sanger sequencing and the unamplified portion of each fraction amplified by qPCR with P3-specific primers for analysis of the population by next generation sequencing (NGS). Sequences obtained from Sanger sequencing or NGS were analyzed for their ability to map to the surface of a CD47 crystal structures obtained from the protein database (PDB) using the PepSurf epitope mapping algorithm.

**Results.** After 3-4 rounds of selection with each heptapeptide library, we observed a successive increase in yield from the linear library suggesting population enrichment of phages from the linear library. Candidate peptides were identified based on their high number of occurrence from the sequenced clones or were found at the proposed interaction site between CD47 and SIRP$\alpha$. In total, 12 peptides were identified and synthesized as peptides. Peptides were adsorbed to an ELISA plate and tested for their performance to bind either CD47 antibody in an indirect ELISA assay.

**Conclusions.** We discovered 3 peptides with relatively high binding activity to both CD47 antibodies tested suggesting the antibodies may share a common structural epitope. The outcomes of the proposed study should provide a platform for continued characterization of phage-CD47 constructs for anti-cancer efficacy in various models of human cancers and in dogs with cancers such as spontaneous lymphomas. This research may lead to the development of novel, active anti-cancer immunotherapies applicable to both human and veterinary patients.

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## Evolution of landscape phage library in a mouse model of breast cancer

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**Introduction.** Peptide phage displayed libraries are billion-clone collections of diverse chimeric bacteriophage particles, decorated by genetically fused peptides built from a random composition of natural amino acids. Studying the evolution of phage displayed libraries in mammalian model systems, using *in vivo* phage display techniques, can provide invaluable knowledge about the physiology of the vasculature system, allows recognition of organ- and tissue-specific networks of protein-protein interactions, and provides ligands for targeted diagnostics and therapeutics. Recently, we discovered that landscape phage libraries, a specific type of multivalent mosaic peptide phage display libraries, expose on their surface comprehensive collections of elementary binding units (EBUs), which can form Short Linear Motifs (SLiMs) that interact with functional protein domains of physiologically important proteins. Because of their unique structural and functional features, mosaic landscape phages can use an alternative mechanism of directed molecular evolution – combinatorial avidity selection. These discoveries fueled our interest in revisiting the *in vivo* evolution of phage displayed libraries using another format of display – landscape phages.

**Methods.** In this study, we monitored the evolution of the landscape phage library in a mouse model with and without an implanted human breast cancer tumor xenograft. We studied the physiologically tolerated dosage of library required to allow recovery of tissue-selective phage particles while reducing toxicity. Peptide structures distributed into each tissue were determined using p8-targeted amplicon sequencing. Tissue-selective EBUs were determined by counting the frequency of each 3-mer motif recovered from each tissue. Motif selectivity was determined by comparing the frequency of each motif in the tissue to the frequency in the unselected library. Finally, we used network analysis to study interactions of tissue-selective motifs with each other to identify important peptides with the potential for tissue selective migration.

**Results.** As expected, the multivalency and mosaic architecture of landscape phages provided strong selectivity and a huge diversity of tissue penetrating chimeric particles. We identified several types of EBU interactions evolved during the course of tissue distribution including interactions of EBUs with all tissue types, those that interact selectively with specific organs or tissues with shared gene expression profiles or functionalities, or others that interact in a tissue-selective manner.

**Conclusions.** We demonstrated that landscape phage libraries are a rich collection of unique nanobioparticles that can be used to identify functional organ and tissue-binding elements after a single round of *in vivo* phage display. This work presents new opportunities for phage-derived particles to be studied as a protein-based nanocarrier for development of novel nanomaterials/nanomedicines.

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**The sexually dimorphic response of the mouse adrenal inner cortex to thyroid hormone treatment**

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**Introduction.** Evolution has resulted in intense differences between males and females that extend to non-reproductive organs. Mouse studies have shown that the adrenal gland, which makes a variety of hormones that are necessary to maintain the normal function of the body, is also sexually dimorphic at different levels including the transcriptome, histology and cell renewal. Although the gender bias in adrenal diseases has been noticed for a long time, the mechanism behind the high prevalence of adrenal diseases in females is unclear. The purpose of this study is to understand how the adrenal gland responses to the same external cue in a gender-specific manner.

**Methods.** The thyroid hormone (T3) has been known to directly elicits its function on the adrenal inner cortex by changing the cell fate of this population. We used RNA-seq to demonstrate how male and female adrenal glands respond differently to T3 treatment. Also, multiplex immunostaining is used to see how T3 induces the changes in morphology and the expression of adrenal cortical markers at the histological level.

**Results.** By comparison of male and female with T3 or saline treatment, we found that female adrenals are more responsive to T3 treatment at the transcriptome level. We also identified *Cyp2f2* as the most sexually dimorphic T3-responsive gene, which is specifically expressed in the adrenal inner cortex. Also, under T3 treatment, a unique cell population surrounding the adrenal X-zone formed only in females. Moreover, immunostaining results confirmed the top sexually dimorphic T3-responsive genes are mainly expressed in the adrenal inner cortex.

**Conclusions.** Our findings not only identify novel marker genes for adrenal inner cortex but also highlight the significant sexually dimorphic response of thyroid hormone action in the adrenal gland. Our data provide a genome-wide evidence to show the sexually dimorphic response of the adrenal inner cortex to the same external cue.

**Acknowledgments.** This work was supported by NIH R00 HD032636.

## Nanobody-based anti-CTLA4 immune checkpoint blockade therapy for canine cancer

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**Introduction.** In recent years, monoclonal antibody-based immune checkpoint blockade (ICB) therapy has made a paradigm shift in cancer treatment. Checkpoint inhibitors targeting cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed death-1 (PD-1) pathways have yielded unprecedented results in significant percentages of human cancer patients. However, such therapies are currently not available for canine cancer patients due to lack of effective canine-specific monoclonal antibodies (mAbs). Moreover, mAb-based immunotherapeutics are highly expensive. Conversely, nanobodies (representing the variable domain of heavy chain only antibodies (HcAbs)) are small in size and inexpensive to produce in bulk quantities. Nanobody-based immunotherapeutics perform at the same level as their corresponding mAbs. Thus, *the primary goal of this project is to develop a nanobody-based anti-CTLA4 ICB therapy for the treatment of canine cancer patients.* We hypothesize that anti-canine (c) CTLA4 nanobodies will bind and block CTLA4/B7 pathway, thus initiating an antitumor immune response.

**Methods.** We used a yeast nanobody library to identify anti-canine CTLA4 nanobodies. The canine CTLA4 protein labeled with either biotin or Alexa Flour-647 fluorophores was used to enrich canine CTLA4 specific binders through MACS selection. We selected three single yeast clones (CTLA4.Nb2, CTLA4.Nb3 and CTLA4.Nb6) showing excellent binding specificity for in-depth characterization. The nanobody encoding DNA sequences were retrieved from the single yeast clones, cloned in pET22b vector, expressed and purified from BL21 (DE3) *E. coli*. We also conjugated the CTLA4.Nb6 sequence to the hinge and Fc domain of the canine IgG to construct a heavy chain only antibody (CTLA4.Nb6.HcAb). The chimeric heavy chain only antibody was expressed and purified from ExpiCHO-S cells. The HEK-293T cells transiently expressing canine CTLA4 were used to evaluate the binding ability of different anti-CTLA4 nanobodies and CTLA4.Nb6.HcAb. We also evaluated the binding ability of anti-CTLA4 nanobodies against canine peripheral blood mononuclear cells (PBMCs).

**Result.** In this study, we have identified several anti-CTLA4 nanobodies by MACS selection using our yeast nanobody library. The identified single yeast clones specifically bind to the recombinant canine CTLA4 protein. The nanobody sequences encoding CTLA4.Nb2, CTLA4.Nb3 and CTLA4.Nb6 were retrieved, cloned and expressed in *E. coli* (DE3) cells and purified from the periplasmic fraction using HisTrap HP (precharged Ni sepharose) and Strep II Tag columns by affinity chromatgraphy on AKTA explorer to achieve a near 100% purity. We have also cloned, expressed and purified CTLA4.Nb6.HcAb from ExpiCHO-S cells. This anti-CTLA4 heavy chain only antibody form dimers under non-reducing condition. The anti-CTLA4 nanobodies and CTLA4.Nb6.HcAb specifically bound to HEK293T cells expressing canine CTLA4 protein, while no lableing was observed in untransfected HEK293T cells. Similary, anti-CTLA4 nanobodies also specifically bind to canine CTLA4 expressed on activated (with PMA and ionomycin) peripheral blood mononuclear cells.

**Conclusions.** We confirm that a yeast nanobody library can be used to identify nanobodies against canine CTLA4 protein. The identified nanobodies can be produced and purified at high levels in bacteria. Moreover, the purified anti-CTLA4 nanobodies and nanobody-based heavy chain antibodies successfully bind to canine CTLA4 expressed on HEK-293T cells and activated canine peripheral blood mononuclear cells (PBMCs). We presume that the chimeric heavy chain antibody will have the advantageous feature of Nbs, i.e., high solubility and stability, coupled with the effector functions from the Fc domain of canine IgG. We expect purified nanobodies to block cCTLA4/B7 pathways, and thus offer an inexpensive yet effective ICB therapy for canine cancer patients.

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**Effect of Visual Arts Training on Veterinary Student Radiographic Observation, Description and Interpretation Skills.**

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**Introduction.** The basis for successful radiographic interpretation is astute and unbiased observation and descriptive skills. Unfortunately, these basic skills are not often specifically addressed by the veterinary curriculum. The purpose of this study was to evaluate if there is a positive effect of specific observational skills training on veterinary student radiographic interpretation ability and confidence.

**Methods.** A cohort of third year veterinary student volunteers participated in a museum based visual arts training exercise after which both qualitative (survey) and quantitative (clinical exam performance) data were collected.

**Results.** Results demonstrated a positive impact of the visual arts training on student radiographic observational, descriptive and interpretation skills.

**Conclusions.** Targeted observational training has the potential to enhance veterinary student success in radiology and other clinical skills.

**Sorting equine endothelial colony forming cells based on low-density lipoprotein uptake**

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**Introduction.** Endothelial colony forming cells (ECFC) have the ability to migrate to sites of ischemia, promote vasculogenesis, and improve function of blood vessels. Isolation of equine ECFCs is performed using media selection, so there is potential for a mixture of cell types. Equine ECFCs have been characterized based on their ability to uptake acetylated-low density lipoprotein (Ac-LDL) and *in vitro* vascular tubule formation. The purpose of the study was to optimize a cell sorting protocol for equine ECFCs based on LDL uptake. Our hypothesis was that a subpopulation of LDL-positive ECFCs would perform better at *in vitro* tubule formation.

**Methods.** Cryopreserved ECFCs from three different horses confirmed positive for LDL uptake on flow cytometry and *in vitro* tubule formation were expanded in culture with standard endothelial growth media. Cells ($1.5x10^6$) were sorted based on LDL uptake (positive versus negative) using fluorescence activated cell sorting. Two different sorting buffers were evaluated: one with EDTA and DNAse and the other with Accumax®, EDTA and DNAse. The efficiency of cell sorting was recorded, and sorted cell subpopulations were subcultured and further analyzed for presence or absence of *in vitro* tubule formation and percentage of LDL uptake.

**Results.** Sorting equine ECFCs based on uptake of Ac-LDL was possible and yielded cell populations that successfully grew when subcultured. The protocol using Accumax® as part of the buffer resulted in higher sorting efficiency. LDL-positive cells maintained their *in vitro* tubule formation, while tubule formation was absent in LDL-negative ECFCs. Percentage Ac-LDL uptake after sorting was not significantly different between LDL-negative (96.1 ± 4.9%) and LDL-positive (mean 96.8 ± 2.9%) populations ($P$=0.8194).

**Conclusions.** Equine ECFCs can be successfully sorted based on the uptake of Ac-LDL. The LDL-positive population had better function as demonstrated by *in vitro* tubule function. The addition of Accumax® to the sorting buffer reduced cell clumping and improved ECFCs sorting efficiency. An optimized protocol for sorting ECFCs will allow use of this technique with more specific cell surface markers and improve the characterization and function of these cells. The LDL uptake by negative sorted cells could be due to overgrowth of other cell types or LDL-positive ECFCs included in the negative group.

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**Characterization and Differentiation of *Salmonella enterica* Typhimurium, *Escherichia coli* and *Listeria monocytogenes* using Hyperspectral Imaging**

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**Introduction.** Recent advances in microscopy including the Cytoviva darkfield condenser and hyperspectral imaging technology have created new avenues for diagnostic research. Using the Cytoviva hyperspectral imaging system and software, spectral profiles were elicited from 3 different types of pathogenic bacteria, *Escherichia coli* O157:H7, *Salmonella enterica* Typhimurium and *Listeria monocytogenes*. Using these spectral profiles, a non-statistical model was applied to the data to differentiate between the three organisms. Hyperspectral imaging is a new, exciting area for imaging diagnostics that has the potential for rapid pathogen detection.

**Methods.** All organisms were initially cultured onto Tryptic Soy Agar (TSA) and incubated at 37°C for 18 hours. After growth, the organisms were properly stored at 5°C for temporary storage. Liquid cultures are required for observation under the Cytoviva Hyperspectral Microscope System and were prepared by inoculating 2 mL of Brain Heart infusion with a single colony of the organism. Liquid cultures were also incubated at 37 C in a shaker incubator for 18 hours. Standardized cultures were then created in which each culture in 25 mL of broth in a 250 mL Erlenmeyer flask obtained the same, final concentration of an OD600 equal to 0.02. 0.5 mL of each sample were removed at 18, 21 and 24 hours and washed in 1x PBS prior to 0.8μL of sample is transferred to a sterile poly-L-lysine slide for imaging. The spectra of 10 cells for each organism was obtained at 18, 21 and 24 hours of growth and compared. First, the local maximum method was applied which is a non-statistical method used to analyze spectral differences by establishing 4 distinct points that can be further compared: Peak, Full Width-Half Maximum, Center and Centroid. The averaged parameter was analyzed using ANOVA and the Tukey HSD test to determine statistical differences in the parameters when comparing each of the organisms.

**Results.** The 21-hour time point proved to be the most capable of differentiating the spectra of all three organisms by multiple parameters considering statistical differences were found in the area, FWHM, and center. The centroid was successful at differentiating *Salmonella* and *Listeria* and *E. coli* and *Listeria* but no difference was observed when comparing *Salmonella* and *E. coli.* For the 18-hour timepoint, the only parameter that was statistically different for each of the 3 organisms was the FWHM parameter. When comparing *Salmonella* and *E. coli* the area and FWHM parameters proved to be significantly different from one another. When comparing *Salmonella* and *Listeria* the area, FWHM and center parameter all were successful in differentiating the two organisms' spectra while only the FWHM parameter was successful at differentiating *E. coli* and *Listeria*. Similarly, at 24 hours the only parameter that could be used to differentiate the three organisms was the FWHM parameter. The area and FWHM proved to be statistically different for *Salmonella* and *E. coli* while the area, FWHM and center parameter could distinguish between spectra of *Salmonella* and *Listeria*. Only the FWHM parameter could be used to differentiate *E. coli* and *Listeria*.

**Conclusions.** Hyperspectral analysis is useful for characterizing and is capable of differentiating microorganisms based upon the spectral profiles produced. A protocol for imaging live, metabolically active bacteria was created and using the Cytoviva condenser a single cell imaging technique was utilized. This research was successful in differentiating *Salmonella*, *E. coli*, and *Listeria* at 18, 21 and 24 hour time points.

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**Intestinal tropism of an infectious bronchitis virus isolate is not explained by spike protein binding specificity**

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**Introduction.** The avian infectious bronchitis coronavirus (IBV) is an economically important respiratory pathogen of chickens that affects the performance of both meat-type and egg-laying birds. IBV replicates in the epithelium of the upper respiratory tract, the urogenital tract, and kidneys. Depending on the strain, disease outcomes range from mild respiratory symptoms to severe kidney disease. An IBV (CalEnt) with unusual enteric tropism was isolated from a California broiler flock exhibiting runting-stunting syndrome. IBV was detected in small intestine, but not respiratory tract or kidney. During virus isolation in embryos, it did not replicate in chorioallantoic membrane (CAM) but could be recovered from intestines. Its attachment protein, the S1 subunit of the viral spike protein, had 93% amino acid sequence identity to a California variant isolated in 1999 (Cal99). Intestinal lesions were reproduced following oculo-nasal inoculation of specific pathogen free (SPF) chickens, but respiratory signs and lesions were also present. The virus was detected in both respiratory and intestinal tissues. To determine whether the novel tropism of IBV CalEnt was due to an increased ability of its S1 protein to bind to intestinal epithelium, we compared binding of soluble trimeric recombinant S1 and S1-N-terminal domain (NTD) proteins representing CalEnt and Cal99 to chicken tissues. We also used bioinformatic approaches to explore the possibility that the unique tropism of CalEnt might be a result of functions of the spike protein in cell entry steps subsequent to attachment.

**Methods.** Protein histochemistry was used to evaluate binding to formalin-fixed chicken tissues of recombinant soluble, trimeric S1 proteins expressed in HEK-293T cells from codon-optimized constructs. We used the RDP4 program to detect putative recombination events within the CalEnt spike gene. Protease cleavage sites near the putative recognition site for the protease that activates the S protein for viral envelope fusion with host cell membranes during viral entry were predicted using PROSPER and PeptideCutter servers.

**Results.** Contrary to expectations, recombinant CalEnt S1 and S1-NTD proteins did not bind to small intestine, nor to respiratory epithelium or CAM. Recombination analysis suggests that the genomic region encoding CalEnt's S2 subunit of the spike protein was acquired through a recombination event. In addition, CalEnt S2 has a unique amino acid sequence at the putative recognition site for the protease that activates the S protein for fusion. This unique sequence includes predicted cleavage sites for digestive enzymes.

**Conclusions.** Our results do not support better attachment to intestinal epithelial cells as a reason for CalEnt's extended tropism. These results may reflect shortcomings of the assay, including that it does not detect potential contributions of the S1-C-terminal domain to attachment. However, activation of CalEnt S2 activation by tissue-specific proteases might facilitate CalEnt entry into intestinal epithelial cells and compensate for poor binding by its S1 protein.

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## Rapid detection of P-35S and T-nos DNA elements in genetically modified organisms (GMO) by recombinase polymerase amplification combined with a lateral flow strip

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**Introduction.** Recombinase polymerase amplification (RPA) was first reported in 2006 and has recently been shown to improve the detection of genetically modified organisms (GMOs). PCR assays have been used to detect the 35S promoter of Cauliflower Mosaic Virus (P-35S) or the nos terminator of *Agrobacterium tumefaciens* (T-nos).

**Methods.** In this research we developed a more efficient RPA-based and lateral flow test strip (LFD)-based platform for the detection of P-35S and T-nos for GMOs, circumventing the need for expensive instruments and technicians. The RPA forward and reverse primers were labeled with the fluorophore biotin, digoxin, and FITC at the 5'-end and quickly determined whether the sample contains P-35S and T-nos of GMOs using the RPA-LFD method at room temperature. Nine events of GMOs were collected to determine the specificity of the RPA-LFD method, including KMD1, MON531, Rf1, Kefeng6, RRS, Bt11, MON863, Bt176 and Ms1.

**Results.** The results showed that the limit of detection of RPA-LFD was 50 copies and 100 copies, which was consistent with the limit of detection of RPA-AGE. The specificity and stability among the nine events were consistent. Finally, this detection method was validated in nine double-blind samples with 100% accuracy.

**Conclusions.** In conclusion, we developed a more sensitive, specific and stable field screening method for determining the GMO content which is suitable for field applications.

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