

PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter



November 4, 2020

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE



PHI ZETA RESEARCH DAY FORUM

NOVEMBER 4, 2020 – 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

8:05-11:00 MORNING Presentations - Overton Auditorium

Undergraduate Student

8:05 Kathryn Wolfe Systematic Review of Literature Containing Quality of Anesthetic Induction and Recovery Scales used in Dogs to Assess for Validity and Applicability

Veterinary Students

8:17 Virginia Aida Efficacy and immunogenicity of a novel subunit influenza vaccine in pigs against an H1N2 field strain

8:29 Casey Phillips Investigation of Platelet Parameters in Dogs with Hematological Neoplasia

Graduate Students

8:41 Ferrin Antony Interleukin-27 regulates corneal immunopathology and viral clearance during Herpes Simplex Virus-I infection

8:53 Sophie Boorman Comparison of the efficacy of 2% mepivacaine and a solution of 2% lidocaine/epinephrine administered for median and ulnar nerve blocks in horses with naturally occurring forelimb lameness

9:05 Serena Ceriotti Assessment of age-related β_2 -adrenergic receptors signaling activity in the equine respiratory tract: preliminary results

9:17 Ethan Hefner Comparison of the Image Quality From Optical Coherence Tomography of the Equine Cornea and Retina Using Sedation and General Anesthesia Protocols With or Without the Use of Retrobulbar Anesthesia

9:29 Karly Hicks Genomic and *in vitro* pharmacodynamics analysis of rifampicin resistance at clinically relevant concentrations in multi-drug resistant canine *Staphylococcus pseudintermedius* isolates



PROGRAM

- 9:41 Anna Huskey CEACAM gene family mutations associated with increased risk for inherited breast cancer – a comparative genetics approach in dogs and humans
- 9:53 Zubair Khalid Enhanced Protection by Recombinant Newcastle Disease Virus Expressing Infectious Bronchitis Virus Spike Ectodomain and GM-CSF
- 10:05 Shune Kimura Evaluation of Orally and Rectally Administered Misoprostol in a Low-Dose Endotoxin Challenge in Horses
- 10:17 Steven Kitchens Chicken Embryo Lethality Assay for *Salmonella* Newport
- 10:29 Troy LoBue More Evidence for RNASEL:p.Glu265*, A Potential Genetic Modifier of Early-Onset Breast Cancer for Carriers of High-Risk Mutations
- 10:41 Anne Maguire Clinical metrics and whole-slide image analysis demonstrate that intravenous AAV gene therapy slows neurodegeneration in feline Sandhoff Disease
- 10:53 Cierla McGuire Sams Rare and potentially pathogenic variants in the hydroxycarboxylic acid receptor genes identified in breast cancer cases

***The Presentation of the Zoetis Research Award will precede the 11:10 AM Lecture
11:05: Presentation of 2019 Zoetis Research Award Auburn Faculty Award**

**11:10: Joy Goodwin Lecture-Overton Auditorium
Dr. Douglas Martin
"Gene Therapy Trials (and Tribulations) for
Neurologic Disease"**

12:00–1:30 POSTER Presentations- Online Display

**1:30-4:00 AFTERNOON Presentations –
Overton Auditorium**

Graduate Students (continued)

- 1:30 Mariano Mora Pereira Equine arterial ring assay as an ex vivo model for vasculogenesis in horses
- 1:42 Peter Neasham Developing an *in vitro* system to identify mechanisms of Influenza A virus (IAV) species adaptation using human and swine respiratory epithelial cells



PROGRAM

- 1:54 James North Interspecies Transmission of Influenza A Viruses and the Emergence of Pandemic Viruses
- 2:06 Saba Omer Cannabidiol (CBD) induced inhibition of cancer cell viability in canine B and T-Cell Lymphoma
- 2:18 Vasileios Pliasis Influenza neuraminidase virus-like particle vaccine platform as a candidate universal influenza vaccine in the porcine model
- 2:30 Julia Salamat Pregnenolone 16-alpha Carbonitrile, an Agonist of Rodent Pregnane X Receptor, can Impair Testosterone Biosynthesis in Rodent Leydig Cells
- 2:42 Olivia Stephenson The prognostic significance of exon 8 c-kit mutations in canine mast cell tumors
- 2:54 Huifei Zheng Thyroid hormone upregulates Dhcr24 expression in adrenal gland

Faculty/Postdoctoral Fellows/Staff

- 3:06 Julie Gard Schnuelle Treatment of Bovine *Trichostrongylus axei* with an Extended Release Topical Formulation
- 3:18 Ashley Smith Evaluation of zoledronate for the treatment of canine metastatic osteosarcoma
- 3:30 Alissandra Rha Genome editing as a potential therapeutic strategy for GM1 gangliosidosis
- 3:48 Amanda Gross Effect of administration route and AAV serotype for treatment of feline GM1 gangliosidosis

4:00 KEYNOTE LECTURE-Overton Auditorium

5:00 INDUCTION AND AWARDS ANNOUNCEMENT

INDUCTION of new Phi Zeta Members

Research Awards Presentations



**PHI ZETA KEYNOTE SPEAKER AND
JOY GOODWIN LECTURER**

“Gene Therapy and One Health: Are We There Yet?”



**Douglas Martin, PhD
Professor
Department of Anatomy,
Physiology and Pharmacology
Director of the Scott-Ritchey
Research Center**

Douglas R. Martin, PhD, has 25 years’ experience with developing therapies for fatal neurologic disorders such as Tay-Sachs disease, Sandhoff disease and GM1 gangliosidosis. As co-founder of the Tay-Sachs Gene Therapy Consortium, Dr. Martin works closely with a team of scientists, physicians and families dedicated to treating these disorders. He has been inspired by heroic families and patients and by the heroic animals that enable therapy development. Using gene therapy, the survival of affected cats has increased from 8 months (untreated) to an average of 5 years, with some treated animals alive and well at >8 years of age. Such results have supported the initiation of a first-in-human gene therapy clinical trial for GM1 patients at the National Institutes of Health (ClinicalTrials.gov Identifier NCT 03952637) and a similar trial for Tay-Sachs and Sandhoff diseases at the University of Massachusetts Medical School. At the same time, the disease-causing mutations discovered by Dr. Martin and colleagues have been eradicated from cat breeds such as Korat and European Burmese through an international carrier screening program.

Dr. Martin is a native of northeastern Ohio but has lived in Alabama for many years, having completed both his Bachelor of Science (Biology) and PhD (Biomedical Science) degrees at Auburn University. Currently he is a Professor of Anatomy, Physiology & Pharmacology and Director of the Scott-Ritchey Research Center at the Auburn University College of Veterinary Medicine. His research has generated over \$11 million in extramural funding and licensing fees from sources such as the National Institutes of Health, the National Tay-Sachs & Allied Diseases Association and biotechnology companies. Among Dr. Martin’s chief accomplishments is helping to train the next generation of researchers. Through his laboratory and as an instructor in the professional veterinary curriculum, Dr. Martin teaches numerous graduate, veterinary and undergraduate students. Along with many pets, he lives in Opelika, Alabama with his lovely wife, who is a veterinarian and private practice owner. He has two beautiful, adult step-daughters who reside in Phoenix and New York City.



Posters

Undergraduate Students

Madison Hogans	Towards Elucidation of Canine Adenovirus type 2 tropism
Daniel Patton	Production of Anti-PD1 antibody by modified Canine Adenovirus-2

Veterinary Students

Tom Bunch	Awareness and perception of Breed-Specific Legislation in a College of Veterinary Medicine
Grace Duer	Surgical Correction and Prophylactic Surgical Correction of Third Eyelid Gland Prolapse: A Retrospective Study
Sarah Ezell	Characterization of the Cannabinoid Receptor – 1 Gene in Three Beagle Dogs and Six Cats
Chloe Haynes	Engineering a bispecific molecule to simultaneously inhibit and co-stimulate immune checkpoints for combination immunotherapy of canine cancer
Sarah Hoke	Investigation of Classification Accuracy and Clinical Management Trends of Corneal Ulcer Types Between General Practitioners and Ophthalmologists

Graduate Students

Taylor Abernathy	Trichloroethylene (TCE) toxicity in zebrafish (<i>Danio rerio</i>): a multigenerational approach
Sophie Boorman	Standing jejunal enterotomy for resolution of an ileal impaction
Wenqi Cao	Identification of embryonic genomic imprinting pattern using RNA-seq analysis in a marsupial model <i>Monodelphis domestica</i>
Renlei Ji	Topmouth culter (<i>Culter alburnus</i>) melanocortin-3 receptor: regulation of pharmacology by two isoforms of melanocortin receptor accessory protein 2
Ting Liu	Regulation of Melanocortin-5 Receptor Pharmacology by Two Isoforms of MRAP2 in Swamp eel (<i>Monopterus albus</i>)
Xiaolei Ma	Effects of lactocrine insufficiency from birth on the uterine transcriptome and gut microbiome in neonatal pigs on postnatal day 14
Monica Midon	Invasive blood pressure in anesthetized horses: does the artery site matter?



PROGRAM

Monica Midon	Capnography waveform during Anesthetic Index determination in chickens (<i>Gallus gallus domesticus</i>)
Haolong Wang	Elucidating the immunomodulatory mechanism of growth-promoting biodegradable microparticles using RNA-seq in mouse macrophages
Xiao Xiong	Phylogenomic analysis of the intracellular symbiont <i>Wolbachia</i> in arthropods reveals genome evolution and interclade recombination events
Sandra Zetterström	An ex-vivo study of a minimally invasive technique for tenotomy of the tibial insertion of the semitendinosus muscle in horses
Sandra Zetterström	An ex-vivo Study of Double Hemitenotomy for Lengthening of the Equine Deep Digital Flexor Tendon
Yihang Zhou	Whole-genome shotgun metagenomic analysis revealed gut microbiome changes in beef cattle under endophyte-infected tall fescue toxicosis



Undergraduate Student Platform Presentations

Systematic Review of Literature Containing Quality of Anesthetic Induction and Recovery Scales used in Dogs to Assess for Validity and Applicability

Kathryn L. Wolfe¹ and Erik Hofmeister²

¹Department of Animals Sciences, Auburn University, AL

²Department of Clinical Science, College of Veterinary Medicine, Auburn University, AL

Introduction. During research, collecting accurate measurements is important to a dependable study. Assessing a subjective variable is especially hard, as is seen in literature measuring the quality of induction and recovery in dogs. Measurements can be made using a scale or scoring system, however, in order to produce results that are consistent and reliable, a good method of construction and solid background of validity and reliability testing should be performed on the scale. Current studies that include assessment of induction and recovery use multiple types of scoring systems, including visual analog scales (VAS), numerical rating scales (NRS), and simple descriptive scales (SDS). No research has been done to verify the number or level of validity of these scales in circulation. The aim of this systematic review is to perform a comprehensive assessment of all induction and recovery scales, compare them, and determine the need for a new, validated, scoring system.

Methods. The systematic review was conducted through a search on PubMed. Key phrases used were 'induction scoring systems dogs,' 'recovery scoring systems dogs,' 'anesthetic induction score dogs,' and 'anesthetic recovery score dogs.' The search was limited to 1980 to the present, and included all studies describing either an induction or recovery scoring system, or both. Inclusion was not limited by study size, breed of dog, or type of procedure. Each result was reviewed and initially rejected or accepted based on abstract alone. Then each selected study was read in its entirety and included if the criteria were met. The cited works in each included study were reviewed and additional studies were included. The induction and recovery scales, validation methods, date of publication, study design, and sample size were extracted from the included studies. It was also noted if the scale used was adapted from a previous study. The number of each type of scoring system and number of unique scales within those systems was determined. All studies were assessed using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) system.

Results. The search yielded 267 studies, 80 of which were chosen for review based on abstract. 70 articles were included in the study after removal of nonapplicable studies, 18 of which were included after review of the cited works of the initially chosen studies. The publication year of the included studies ranged from 1994-2019, and sample size ranged from five to 149 dogs of various breeds. 45 total induction scales were counted, 16 were unique SDS scales and one was a VAS. 68 total recovery scales were counted, 18 unique SDS scales, four unique VAS scales, two 0-10 NRS, and two compound NRS scales. Only one study mentioned validation, and no techniques were described.

Conclusions. It was determined that a widely accepted scoring system for measuring quality of induction and recovery in dogs does not exist. There a large number of unvalidated scales being used, producing inconsistent and incomparable results. In order to reduce subjectivity of this measurement and provide accurate measurements for this variable, it is recommended that a quality of induction and/or recovery scoring system be constructed and validated for widescale use.



Veterinary Student Platform Presentations

Efficacy and immunogenicity of a novel subunit influenza vaccine in pigs against an H1N2 field strain

Virginia Aida¹, Vasilis Pliastas¹, Amanda L. Skarlupka², Ji-Hang Yin¹, J. Fletcher North¹, Peter J. Neasham¹, Maria C. Naskou¹, Dylan Wilson¹, Sheniqua Glover¹, Ted M. Ross², Constantinos S. Kyriakis^{1,2}

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL

²Center for Vaccines and Immunology, University of Georgia, Athens GA, USA

Introduction. Influenza A viruses (IAVs) is a chief public health threat to both human and animals worldwide. Hemagglutinin (HA) is the immunodominant antigen in current vaccine formulations. Unfortunately, the frequent antigenic drift and shift in the HA of circulating IAV strains often results in antigenic mismatch with the vaccine HA and attenuated efficacy. The current study assessed the Computationally Optimized Broadly Reactive Antigen (COBRA) recombinant HA (rHA) platform as a candidate universal influenza vaccine in the porcine model.

Methods In the vaccination-challenge study, a total of 20 influenza-seronegative piglets (divided into 4 groups) were utilized. Animals were vaccinated twice (4 week interval) with (a) COBRA Swine-1 rHA, (b) COBRA Swine-2 rHA, (c) an equal mixture of COBRA swine-1 and swine-2 rHA and (d) adjuvant only. Six weeks after boost, pigs were intranasally challenged with A/swine/NC/152702/2015, an H1N2 swine IAV field isolate. Serological protection was assessed by hemagglutination inhibition assay. Nasal swabs were collected daily (day 0 to day 5) post-challenge. Bronchoalveolar lavage fluid (BALF) and tissue samples from the entire respiratory tract were harvested at euthanasia. Protection was evaluated utilizing virus titers in nasal swabs, tissue homogenate, BALF cytology, and respiratory histopathology.

Results. In comparison to unvaccinated controls, pigs immunized with COBRA Swine-1 rHA showed reduced viral titers in the trachea, left lung, and right lung; Animals immunized with COBRA Swine-2 showed reduced BALF neutrophilic infiltration and viral titers in the right lung; Animals immunized with combined COBRA Swine-1 and Swine-2 rHA demonstrated reduced BALF neutrophilic infiltration.

Conclusions. Overall our study revealed that COBRA rHA conferred some protection against IAV challenge however, this platform warrants further investigation to fully characterize its potential as a universal vaccine candidate.

Acknowledgements. The authors want to thank the NIH/NIAID Centers of Excellence for Influenza Research and Surveillance and the Alabama Agricultural Experiment Station for funding of this research.

**Investigation of Platelet Parameters in Dogs with Hematological Neoplasia**

Casey Phillips Maria C. Naskou, Elizabeth Spangler

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

Introduction. Thrombocytopenia has been associated with some neoplastic processes, including hematological neoplasia. Advances in optical-based automated hematology analyzers enable us to make a detailed assessment of platelet status, by recording several platelet related indices that could be valuable in making clinical decisions and monitoring critically ill patients. There is no information regarding specific changes in the platelet indices in dogs with hematological neoplasia compared to healthy dogs. The first objective of this study was to establish reference intervals for the platelet indices in healthy dogs and to evaluate whether differences exist regarding sex and age. The second objective was to compare platelet parameters in dogs with hematological neoplasia to those from healthy dogs. The third objective was to compare these parameters in patients with hematologic malignancies, with or without thrombocytopenia.

Methods. This was a retrospective study. Platelet parameters were determined using the Advia 120 Hematology analyzer when a CBC was performed, and included platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT), mean platelet component (MPC), platelet component distribution width (PCDW), mean platelet mass (MPM), platelet mass distribution width (PMDW), and number of large platelets. Reference intervals were determined with data from 129 healthy dogs. Patients with hematological neoplasia (n=50) were identified through retrospective evaluation of medical records from the Auburn University Veterinary Teaching Hospital, and separated into thrombocytopenic (n=30) and non-thrombocytopenic groups (n=20).

Results. Platelet number and plateletcrit were significantly higher for older dogs compared to younger dogs (P=0.0016, P=0.0107 respectively). Statistically significant differences were identified when comparing healthy dogs and those with hematological neoplasia for PDW (P<0.0001), PCDW (P=0.0001), PMDW (P<0.0001) and number of large platelets (P=0.009). When comparing thrombocytopenic and non-thrombocytopenic dogs with hematological neoplasia, all parameters except large platelets were significantly different between the groups.

Conclusions. Differences in platelet parameters based on patient age must be considered when interpreting CBC results. There are statistically significant differences in platelet parameters when comparing healthy dogs and dogs with hematological neoplasia and when comparing thrombocytopenic and non-thrombocytopenic dogs with hematological neoplasia. The results warrant further investigation for the prognostic value of these platelet parameters and their application in a clinical setting.

Acknowledgments. Auburn University College of Veterinary Medicine Summer Research Scholars Program



Graduate Student Platform Presentations

Interleukin-27 regulates corneal immunopathology and viral clearance during Herpes Simplex Virus-I infection

Ferrin Antony¹, Maninder Sandey¹, Anil Kumar Jaiswal¹, Amarjit Mishra¹ and Amol Suryawanshi¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Herpes Simplex Virus type-1 (HSV-1) infection of cornea is the leading cause of infectious blindness in the United States. The recurrent infection causes a chronic inflammatory condition in cornea called herpetic stromal keratitis (SK). The massive influx of immune cells such as neutrophils, macrophages, Th1 and Th17 cells cause permanent damage to the corneal tissue. Currently, SK is mainly controlled by a combination of therapies that include topical/oral antiviral and long-term corticosteroids. Unfortunately, these therapies are moderately effective and can cause severe side effects. Thus, there exists an urgent need to understand the immunoregulatory mechanisms which could be utilized to treat SK. Interleukin (IL) -27 is an immunoregulatory cytokine which is secreted by antigen presenting cells. IL-27 has pleiotropic properties that can enhance or limit immune response by acting on macrophages, dendritic cells, NK cells and T cells. The activation of its receptor (IL-27R α) recruits Jak family kinases which induces the phosphorylation of STAT-1 and STAT-3. Studies have shown that IL-27 can induce an anti-inflammatory response by inhibiting Th1 and Th17 proliferation. However, how IL-27 regulates SK pathology is unknown. Therefore, in this study we examined whether IL-27 could suppress the inflammation in SK pathology.

Methods. For our study, we used the IL-27R- α knockout (IL-27RKO) mice in C57BL/6NJ background. All the experiments were done in accordance to IACUC protocol. Mice were anesthetized and infected with the virus strain HSV-1/RE (10^4 pfu/eye) by scratching the cornea. 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. We observed an increased level of IL-27 expression at Day 2 post HSV-1 infection. After HSV-1 infection, IL-27RKO mice had a higher clinical score at day 11 and day 15 when compared to the wild-type mice. The severity of lesions were higher in the IL-27RKO mice which was evident by the microscopic examination of the infected eye and H&E staining of the cornea. In addition, the viral titers in IL-27RKO mice were higher at day 3 and day 5 post HSV-1 infection in comparison to the wild-type mice. Further analysis of infected cornea by flow cytometry showed a higher influx of neutrophils, macrophages and Th1 cells in IL-27RKO mice at day 2 and day 15 post HSV-1 infection.

Conclusions. The absence of IL-27 signaling increased the severity of SK lesions and viral titers in cornea. Our findings indicate that impaired IL-27 signaling could cause higher inflammatory cell influx into the cornea and increase corneal immunopathology. Thus, IL-27 could play an important role in regulating the inflammation in cornea and help in the viral clearance.

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



Comparison of the efficacy of 2% mepivacaine and a solution of 2% lidocaine/epinephrine administered for median and ulnar nerve blocks in horses with naturally occurring forelimb lameness

Sophie Boorman¹, Fred DeGraves², John Schumacher¹, R. Reid Hanson¹ and Lindsey H. Boone¹

¹Department of Clinical Sciences, Auburn University, AL

²Department of Agriculture and Food Science, Western Kentucky University, KY

Introduction. The ideal local anesthetic for lameness examinations in horses is highly efficacious for resolving pain, has a reliable onset and duration and is non-irritating to the tissue. Of the two drugs most commonly used, 2% mepivacaine hydrochloride is preferred over 2% lidocaine hydrochloride due its superior potency, longer duration and less irritation to soft tissues; however, mepivacaine is not licensed for use in horses or available at all in some countries. Previously, Alvarez et al. reported that the addition of 5 µg /mL epinephrine to a 1% lidocaine solution improved the efficacy and duration of palmar digital nerve blocks in horses compared to lidocaine alone. It is currently unknown if these results are applicable to anesthesia of the larger, more proximal nerves (i.e. high regional nerve blocks). Therefore, the aim of this study was to determine whether a solution of 2% lidocaine solution containing 1:200,000 concentration of epinephrine would be as efficacious as an equal volume of 2% mepivacaine in ameliorating forelimb lameness in horses when administered as median and ulnar nerve blocks. The hypothesis was that lidocaine/epinephrine would have a potency and duration similar to mepivacaine.

Methods. Six horses with naturally-occurring forelimb lameness were evaluated using an inertial gait sensor system. Following baseline lameness exam, median and ulnar nerve blocks were performed with lidocaine/epinephrine (0.5 mg epinephrine added to 50 mL of 2% lidocaine immediately prior to administration) or an equal volume of 2% mepivacaine. After blocking, horses were trotted at 5 minutes and then at 30-minute intervals for 150 minutes. After at least 24 hours, nerve blocks were repeated using the alternate local anesthetic. Data were evaluated using linear models.

Results. Nerve blocks performed using lidocaine/epinephrine or mepivacaine resulted in similar amelioration of lameness in horses ($P = 0.691$). For five horses, mean time to resolution of lameness (vertical head movement <8.5 mm) was 5 and 9.6 minutes for lidocaine/epinephrine and mepivacaine respectively. For one horse, vertical head movement was not reduced to less than 8.5 mm with either treatment; this horse had the highest vertical head movement before treatments were administered. Improvement lasted for 150 minutes in both treatment groups. Median and ulnar nerve blocks performed with lidocaine/epinephrine or with 2% mepivacaine resulted in equivalent symmetry of gait.

Conclusions. Lidocaine/epinephrine administered for median and ulnar nerve blocks resulted in amelioration of lameness for 150 minutes and was equivalent to mepivacaine, therefore we can accept our hypothesis. The mechanism by which epinephrine potentiates a nerve block is incompletely understood, but one possible mechanism is via the stimulation of vascular alpha-adrenergic receptors, resulting in constriction of the neuronal vasculature and decreasing the clearance of the local anesthetic. The main limitation of the study was the small sample size.

Acknowledgments. The authors thank Jessica Brown for her help.



Assessment of age-related β_2 -adrenergic receptors signaling activity in the equine respiratory tract: preliminary results

Serena Ceriotti¹, Ya-Xiong Tao², Anne Wooldridge¹, and Kara Lascola¹

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

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

Introduction. Administration of β_2 -adrenoceptor agonist bronchodilators is critical in management of equine asthma; however diminished response to treatment is reported with ageing. It is unknown whether age-related changes in β_2 -adrenoceptor function occur in horses. The present study assessed β_2 -adrenoceptor signaling activity in different segments of the healthy equine respiratory tract from varied ages of horses. The hypothesis was that β_2 -adrenoceptor signaling activity would decrease with increasing age, and age-associated changes would be most prominent in the trachea.

Methods. Samples of peripheral lung and tracheal and bronchial smooth muscle with the overlying epithelium were collected *post-mortem* from healthy horses of 3 age groups: 1-5 year-old (young, n=6); 6-19 year-old (middle, n=6); ≥ 20 year-old (old, n=6). Tissues were snap frozen, homogenized, centrifuged, and the supernatant stored at -20°C until batch analysis. Samples were analyzed in duplicate with a competitive cAMP radioimmunoassay. The mean \pm standard deviation (SD) cAMP activity (pmol/g) was calculated for each tissue in each age group and compared using a one-way ANOVA. Level of statistical significance was set at $p < 0.05$.

Results. Cyclic AMP concentration (pmol/g) for young, middle, and old horses was, respectively, 333 ± 86 , $492. \pm 132$, and 409 ± 133 in tracheal samples; 206 ± 91 , 148 ± 67 , and 199 ± 101 in bronchial samples; 139 ± 34 , 131 ± 62 , and 138 ± 62 in lung samples. No significant differences were detected among age groups for any tissue. In each age group, cAMP concentration was significantly higher in tracheal samples, compared to bronchial and lung samples ($p = 0.002$ young; $p < 0.0001$ middle; $p = 0.0002$ old).

Conclusions. No age-related differences in β_2 -adrenoceptor signaling activity were detected in any segment of the respiratory tract. Further studies involving larger number of horses and assessing β_2 -adrenoceptor distribution and density are required. In the present study, higher signaling activity of β_2 -adrenoceptors in equine trachea did not appear to be affected by ageing.

Acknowledgments. We would like to acknowledge the Birmingham Racing Commission for funding the study.



Comparison of the Image Quality From Optical Coherence Tomography of the Equine Cornea and Retina Using Sedation and General Anesthesia Protocols With or Without the Use of Retrobulbar Anesthesia

EM Hefner, HC Lin, RC Cole, PA Moore, RJ McMullen Jr

Auburn University College of Veterinary Medicine, Department of Clinical Sciences, Auburn, AL

Introduction. Optical coherence tomography (OCT) is a non-contact imaging technique that provides cross sectional images of the cornea and retina in near microscopic detail (5-10um). This allows for sequential evaluation of lesions without the invasiveness of a tissue biopsy. Gaze fixation is required for OCT image acquisition in human patients, which is not achievable in awake veterinary patients. Regional anesthesia is necessary for a variety of advanced ophthalmic examination techniques and ocular surgeries and helps to immobilize the globe. Horses undergoing anesthesia are at risk for colic, traumatic injuries, as well as post-anesthetic myositis. Using sedation, retrobulbar block (RB), and supportive head restraint allows many ophthalmic procedures to be performed in horses while eliminating the risks associated with anesthesia. The purpose of this study was to determine if RB can improve the ease of corneal and retinal OCT image acquisition and quality in standing horses similar to that of horses under general anesthesia.

Methods. One randomly selected eye from six horses free of ocular disease were evaluated via OCT under three conditions: 1). Standing sedation without RB ; 2). Standing sedation with RB, and 3). General anesthesia with RB. Five (axial, 12, 3, 6, and 9 o'clock) regions of interest (ROI) were evaluated in the cornea and fundus (optic nerve head (ONH)). Three diagnostic scans of predetermined quality were obtained per anatomical region. Image acquisition times and total scans per site were recorded. Corneal and retinal OCT Image quality were graded on a 0-4 scale (0: non-diagnostic to 4: excellent).

Results. Results are listed in the following order: Standing sedation without RB, standing sedation with RB, and GA. Mean total cornea scan attempts: 24, 23, and 17. Mean total retinal scan attempts: 23, 19, and 19. Mean total cornea scan times: 880, 790, and 550 seconds. Mean total retina scan times: 1150, 550, and 780 seconds. Mean cornea grades: 2.0, 2.3, and 2.5. Mean retina grades: 2.7, 2.9, and 2.5.

Conclusions. Neither total scan time nor number of corneal scan attempts differed significantly between sedation groups. The RB facilitated globe akinesia and improved the percentage of "scans in frame" and ROI accuracy for retinal imaging. Retinal OCT grades were significantly lower in the GA group compared with the RB group.

Acknowledgements. Birmingham Racing Commission Research Agreement 12/11/2018 under AU Fund 250441 and Auburn University, Department of Clinical Sciences.



Genomic and *in vitro* pharmacodynamics analysis of rifampicin resistance at clinically relevant concentrations in multi-drug resistant canine *Staphylococcus pseudintermedius* isolates

Karly Hicks¹, Yongjun Tan², Wenqi Cao³, Terri Hathcock³, Dawn Boothe⁴, Robert Kennis¹, Dapeng Zhang², Xu Wang^{3, 4, 5} and Amelia White¹

¹Department of Clinical Sciences, Auburn University, Auburn, AL 36849

²Department of Biology, Saint Louis University, Saint Louis, MO 63103

³Department of Pathobiology, Auburn University, Auburn, AL 36849

⁴Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL 36849

⁵Alabama Agricultural Experiment Station, Auburn University, Auburn, AL 36849

⁶HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806

Introduction. Antimicrobial resistance is a growing concern and complicates treatment of *Staphylococcus pseudintermedius* dermatitis in dogs. Treatment with rifampicin (RFP) is considered only in *S. pseudintermedius* isolates that are both meticillin-resistant (MR) and multidrug-resistant (MDR).

Methods. Three MR-MDR *S. pseudintermedius* canine skin isolates were studied, with the minimum inhibitory concentration (MIC) of 0.004, 0.008 and 0.016 µg/mL in MR-MDR1, MR-MDR2 and MR-MDR3 isolates, respectively. Whole-genome resequencing and bioinformatic analysis identified nine antimicrobial resistance genes in their genomes. To determine an optimal RFP dosing for MR-MDR *S. pseudintermedius* treatment without the induction of RFP resistance, we conducted time-kill assays in a control isolate (*S. aureus* ATCC® 25923™; MIC 0.008 µg/mL) and three MR-MDR *S. pseudintermedius* isolates at six concentrations (32 to 1,024 times the MIC).

Results. RFP activity against all four isolates was consistent with a time-dependent and bacteriostatic response. RFP resistance was observed in six of the 28 time-kill assays, including concentrations 64 in MR-MDR1 isolates at 24 hours, 32 in MR-MDR2 after 48 hours, 32 in MR-MDR3 at 48 hours, and 256 in MR-MDR3 at 24 hours. Whole-genome resequencing in three RFP resistant strains characterized the base-pair resolution mutational profile and discovered the causal mutations in the coding region of *rpoB* gene.

Conclusions. Our results suggest that a minimum of 512xMIC is recommended to prevent the development of antibiotic resistance to RFP. Studies have shown that 6 mg/kg per os will result in canine plasma RFP concentrations of 600-1000 times of *S. pseudintermedius*, which is within the suggested dosing guidelines for canine pyoderma.

Acknowledgments. Funding for this project was provided by an intramural grant by the Auburn University College of Veterinary Medicine Dept of Clinical Sciences. X.W. is supported by an USDA National Institute of Food and Agriculture Hatch project 1018100, National Science Foundation EPSCoR RII Track-4 Research Fellowship (NSF OIA 1928770), an Alabama Agricultural Experiment Station Enabling Grant, as well as a generous laboratory start-up fund from Auburn University College of Veterinary. D.Z. is supported by the Saint Louis University start-up fund. W.C. is supported by Auburn University Presidential Graduate Research Fellowship, College of Veterinary Medicine Dean's Fellowship, and the Alabama Established Program to Stimulate Competitive Research (EPSCoR) Graduate Research Scholars Program (GRSP). We acknowledge the Auburn University Hopper Cluster for support of this work.

**CEACAM gene family mutations associated with increased risk for inherited breast cancer – a comparative genetics approach in dogs and humans**

Anna LW Huskey^{1,2}, Isaac McNeely¹, and Nancy D Merner¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

²Department of Drug Discovery and Development, Harrison School of Pharmacy, Auburn University, AL

Introduction. Considering that canines are genetic homologues to humans, they serve as an excellent model to enhance disease gene discovery efforts in both species. Canine breeding practices result in large and consanguineous pedigrees with reduced heterogeneity, comparable to isolated human populations, except there is even less genetic variation. Therefore, canine mammary tumors (CMTs) represent useful genetic models for human breast cancer.

Methods. Fourteen purebred CMT-affected dogs from four different families were whole genome sequenced. These dogs were investigated for mutations in known high-penetrant breast cancer risk genes, however the mutation did not fully explain the CMT presence in the families. Therefore, we further investigated shared mutations in the five Golden Retrievers sequenced. A deleterious mutation was found to be shared among the five Golden Retrievers, a frameshifting mutation in *CEACAM24*, which truncates the protein at the 82nd amino acid. This gene is part of the *CEACAM* family of genes. Due to the homology of the *CEACAM* genes to each other and the homology of the dog *CEACAM* gene family to the human *CEACAM* gene family, we further investigated this gene family in human breast cancer cases. The Cancer Genome Atlas (TCGA) is an online repository containing information for people who have been diagnosed with cancer. We analyzed sequencing data from blood derived samples from European and African American people with breast cancer. We identified protein-truncating mutations in the *CEACAM* gene family within TCGA breast cancer patients, and carried out both single variant association tests and an aggregated analysis to determine if protein-truncating mutations in the gene family are linked to breast cancer risk.

Results. Overall, protein-truncating mutations in the *CEACAM* gene family were associated with breast cancer risk in both African Americans and European Americans. In African Americans, protein-truncating mutations in *CEACAM7* and *CEACAM20* appear to play the largest role within the gene family in influencing breast cancer risk. However, in European Americans, not only do *CEACAM7* and *CEACAM20* appear to play a larger role, so does *CEACAM21* in influencing inherited breast cancer risk.

Conclusions. This is the first report identifying *CEACAM* gene family mutations as inherited risk factors for breast cancer. However, the *CEACAM* gene family has already been noted to be aberrantly expressed in multiple different types of cancer such as colon, ovarian and skin in humans. For example, decreased expression of *CEACAM1* was tied to ovarian and lung cancer. In other studies, somatic mutations in *CEACAM1* have been linked to colorectal cancer. Furthermore, high expression levels of *CEACAM* genes were tied to colorectal cancer and mutations within *CEACAM5* specifically were linked with colorectal cancer through tumor mutation analysis. The full implication of mutations within the gene family and what all cancers they effect is unknown. From this analysis, the *CEACAM* gene family was linked to inherited breast cancer risk.

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Enhanced Protection by Recombinant Newcastle Disease Virus Expressing Infectious Bronchitis Virus Spike Ectodomain and GM-CSF

Z. Khalid¹, Q. Yu², C. Breedlove¹, K. S. Joiner¹, H. Toro¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

²USDA Southeast Poultry Research Laboratory, Athens, GA

Introduction. Recombinant Newcastle disease virus LaSota (rLS) expressing infectious bronchitis virus (IBV) Arkansas-type (Ark) trimeric spike ectodomain (rLS/Se) provides limited protection against IBV challenge. We developed rLS co-expressing Se and granulocyte-macrophage colony-stimulating factor (GM-CSF) in an attempt to enhance vaccine effectiveness.

Methods. We first compared protection conferred by vaccination with rLS/Se or rLS/Se.GM-CSF in chickens. Birds were challenged with virulent Ark and protection was determined using signs, viral load and tracheal histomorphometry. In the 2nd experiment, we evaluated enhancement of cross-protection of a Mass live vaccine by booster vaccination with rLS/Se.GM-CSF. Chickens were challenged with Ark and protection was evaluated as described above.

Results. Experiment 1 showed that co-expression of Se and GM-CSF from rLS significantly reduced tracheal viral load and lesions compared to Se expression in chickens challenged with Ark. In experiment 2, greatest reduction of viral loads in trachea and tears was observed in chickens primed with rLS/Se.GM-CSF and boosted with Mass. Consistently, antibody levels 14 DPB measured with recombinant Se-coated ELISA plates were significantly higher in chickens primed with rLS/Se.GM-CSF and boosted with Mass. However, antibody avidity was not significantly different between groups.

Conclusions. Co-expression of GM-CSF from rLS/Se enhances protection. A prime and boost strategy using rLS/Se.GM-CSF and live Mass provides enhanced cross-protection. Thus, rLS-GM-CSF tailored to express Se of different IBV types used in combination with live Mass can be used to protect against differing regional strains.

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**Evaluation of Orally and Rectally Administered Misoprostol in a Low-Dose Endotoxin Challenge in Horses**

Shune Kimura¹, Annette M. McCoy², Anne A. Wooldridge¹, Dawn M. Boothe³, Emily C. Graff⁴, Kara M. Lascola¹

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

²Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL

³Department of Anatomy, Physiology and Pharmacology, Auburn University, AL

⁴Department of Pathobiology, Auburn University, AL

Introduction. Endotoxemia, a sequela in many equine diseases, causes systemic inflammatory response syndrome (SIRS), which impacts case prognosis. Misoprostol demonstrates *in vitro* reductions in pro-inflammatory cytokine production when stimulated by endotoxin. *In vivo* evaluation of this activity in horses is unknown. The objectives of this study were to characterize the pharmacokinetics and pharmacodynamics of a single dose of misoprostol and to evaluate its effects on clinical inflammatory parameters in horses exposed to low-dose endotoxin.

Methods. Endotoxin (30 ng/kg IV) was administered to 6 healthy adult horses (All geldings, various breeds, 9-18 years in age, 454-655 kg bodyweight) randomized in a balanced 3 x 3 Latin square design to receive misoprostol (5 µg/kg) orally (PO) or per rectum (PR), and water (CONTROL). Each horse observed a 28-day minimum washout between treatments. Serial complete blood counts and physical examinations were performed. Misoprostol plasma concentration-versus-time-data were subjected to non-compartmental analysis. Fold changes in mRNA expression were determined using the $\Delta\Delta C_t$ method to determine relative gene expression changes.

Results. Misoprostol PO mean \pm SD maximum plasma concentration (C_{max} ; 5,209 \pm 3,488 pg/mL), and area under the concentration-versus-time curve ($AUC_{0\rightarrow\infty}$; 299,971 \pm 219,907 pg*min/mL), were higher than PR values of C_{max} and $AUC_{0\rightarrow\infty}$ (854 \pm 855 pg/mL and 10,749 \pm 9,315 pg*min/mL respectively). Misoprostol PO mean \pm SD time to maximum concentration (t_{max} ; 25 \pm 12 min), and disappearance half-life ($\lambda_{1/2}$; 39 \pm 20 min) were longer than PR values of t_{max} and $\lambda_{1/2}$ (3.3 \pm 0.8 min and 13.0 \pm 6.4 min respectively). Preliminary results show downregulation of TNF α and IL-6 gene expression between PO vs. CONTROL at select time points. Differences in comfort score AUC, absolute leukocyte, neutrophil, and lymphocyte numbers were not identified among treatment groups. Differences in the magnitude of change from baseline values were not identified among treatment groups for comfort score AUC, physical examination parameters and leukocytes (ANOVA $p < 0.05$).

Conclusions. Evaluation of changes in inflammatory cytokine expression will improve our understanding of the anti-inflammatory activity of misoprostol in horses. Mean C_{max} in this study is more than 8-fold higher than C_{max} values previously reported in adult horses administered misoprostol orally suggesting that endotoxin administration may alter absorption of orally administered misoprostol. Future studies will investigate changes between PR and CONTROL gene expressions, and differences in cytokine production across treatments. A multi-dose misoprostol study is warranted to establish appropriate dosing intervals.

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**Chicken Embryo Lethality Assay for *Salmonella* Newport**

Steven R. Kitchens¹, Stuart B. Price¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. The One Health approach to preventing illness, which recognizes that the health of people is connected to the health of animals and the environment, is important for controlling pathogens such as *Salmonella* Newport which is a zoonotic enteropathogen that causes significant disease in humans and livestock animals. One proposed pre-harvest intervention to reduce sources of foodborne pathogen contamination is the use of specific bacteriophages (phages) to selectively reduce or eliminate susceptible bacteria from selected environments or animals. Bacteriophages are chosen which only target the pathogen of interest and the normal gut microflora are not affected, but this specificity may affect the phages ability to be protective in a living model system. It is essential before conducting controlled clinical trials (targeted animals or humans) to determine the efficacy of phage therapy. We hypothesize that an avian embryo model could be used to evaluate phage efficacy against *Salmonella* Newport. Assessing the efficacy of phage therapy in such an animal model is more relevant than *in vitro* studies. This study is designed to evaluate an *in vivo* model for the treatment of a *Salmonella* Newport infection with bacteriophage using embryonated chicken eggs.

Methods. Specific-pathogen free (SPF) eggs were incubated until day 11 of development. SPF eggs were inoculated with 10^2 CFU/egg of *Salmonella* Newport in allantoic fluid. Eggs were inoculated with 100 μ L of buffer containing specific doses of phage 2 hours after bacterial inoculation. Eggs were candled daily to monitor embryo mortality up to day 16 of development. SAS Studio v.3.8 (SAS Institute Inc., Cary, NC, USA), Kaplan–Meier curves and log-rank test were used to analyze data.

Results. A series of experiments was conducted to determine if lethality of chick embryos occurred from inoculation with *Salmonella* Newport, and if an *S.* Newport-targeted bacteriophage could prevent embryo mortality. *Salmonella* Newport (10^2 CFUs) caused 100% mortality in embryos by 48 hours. Lethality in *S.* Newport-challenged eggs administered phage S50 at a multiplicity of infection (MOI) = 10 and 100 was not different from non-treated eggs. Phage S50 administered at an MOI = 1×10^7 , the maximum achievable S50 phage dose, was significant ($p=0.0067$) in the survival of chick embryos challenged with *Salmonella* Newport. Using a five-phage cocktail composed of S11, S40, S41, S44, and S50 at MOI's = 10, 100, 1000 was not significant in the survival of chick embryos challenged with *Salmonella* Newport compared to controls.

Conclusions. *Salmonella* Newport caused lethality in chicken embryos at a low dose. Neither a single phage nor a cocktail of phages was successful in protecting chicken embryos at MOI's = 10, 100, and 1000. However, phage S50 was shown to increase survivability of chicken embryos at a MOI = 1×10^7 . But because some of the cocktail phages could not be amplified to reach a titer high enough to exceed the MOI = 1000, more studies need to be conducted to determine a baseline MOI that is protective on average using phage targeting *Salmonella* Newport.

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More Evidence for RNASEL:p.Glu265*, A Potential Genetic Modifier of Early-Onset Breast Cancer for Carriers of High-Risk Mutations

Troy LoBue¹, Elizabeth Stallworth¹, Elizabeth Kinnucan², Brandon Johnson², Nancy Merner¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL.

²East Alabama Medical Center, Spencer Cancer Center, Opelika, AL.

Introduction. Breast cancer risk for individuals with a mutation in a clinically relevant breast cancer susceptibility gene is significantly greater compared to the lifetime risk of an average woman in the U.S., which is 13%. Carriers of pathogenic mutations in the high risk breast cancer susceptibility genes, *BRCA1* and *BRCA2*, have greater than a 4-fold lifetime risk of developing breast cancer, while carriers of pathogenic mutations in moderate risk genes have a 2-fold lifetime risk of developing breast cancer. Risk can be further elevated in those individuals by inherited mutations in other genes, known as genetic modifiers. Recently, *RNASEL*:p.Glu265*, a variant known for affecting prostate cancer risk, was identified in a number of early-onset breast cancer cases known to have a pathogenic mutation in a clinically relevant breast cancer susceptibility gene, suggesting potential modifying effects.

Methods. Using the Alabama Hereditary Cancer Cohort (AHCC), gene panel screening was conducted to evaluate the presence of mutations in *RNASEL* as well as clinically relevant breast cancer susceptibility genes.

Results. *RNASEL*:p.Glu265* was identified in an individual diagnosed with a grade II ductal carcinoma at the age of 36. This individual also happened to have a pathogenic mutation in the clinically relevant breast cancer susceptibility gene, *CHEK2*.

Conclusions. This report provides further evidence that *RNASEL*:p.Glu265* may be a genetic modifier of risk for early-onset breast cancer for carriers of a clinically relevant breast cancer mutation. *CHEK2* mutations are typically associated with a moderate risk of developing breast cancer; thus, the estimated lifetime risk for pathogenic *CHEK2* mutation carriers ranges from 20-44% dependent upon the individual having first- or second-degree relatives with breast cancer. Since *CHEK2* mutations have been reported to be infrequent in early onset breast cancer cohorts, the presence of *RNASEL*:p.Glu265* may explain why this *CHEK2* mutation carrier was diagnosed at such a young age compared to the median age of diagnosis for American women which is 62 years. Larger genetic and functional studies are required to validate the specific influence of *RNASEL* on early-onset breast cancer risk.

Acknowledgments. We would like to thank all patients and family members that enroll in the AHCC.

**Clinical metrics and whole-slide image analysis demonstrate that intravenous AAV gene therapy slows neurodegeneration in feline Sandhoff Disease**

Anne S Maguire^{1,2}, Elise B Diffie^{1,2}, Miguel Sena-Esteves^{3,4}, and Douglas R Martin^{1,2}

¹Scott-Ritchey Research Center, ²Department of Anatomy, Physiology & Pharmacology, College of Veterinary Medicine, Auburn University, AL

³Department of Neurology, ⁴Horae Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA

Introduction. Sandhoff Disease (SD) is a fatal lysosomal storage disease that causes progressive neurodegeneration and death of severely affected children before 5 years of age. This autosomal recessive disease, a type of GM2 gangliosidosis, results in the dysfunction of the enzyme β -hexosaminidase A (HexA) and the subsequent accumulation of GM2 ganglioside in neuronal lysosomes. HexA is composed of an α and β subunit requiring the *Hexa* and *Hexb* genes, respectively. In a feline model of infantile SD, intracranial administration of monocistronic adeno-associated viral (AAV) vector quadrupled lifespan and increased quality of life. It has recently been proposed that a bicistronic vector containing both *Hexa* and *Hexb* genes would be even more efficient at correcting affected cells after intravenous (IV) administration. This route could also reduce the risk of intracranial surgery while improving vector distribution. Therefore, the purpose of this pre-clinical study was to evaluate the efficacy of a bicistronic AAV vector delivered intravenously to cats with SD.

Methods. We treated 13 SD cats intravenously with a bicistronic AAV9 vector at one month of age. These cats were divided into 4 groups: low dose (5e13/kg) short-term (n=3), low dose long-term (n=4), high dose (2e14/kg) short-term (n=2), and high dose long-term (n=4). Animals in the short-term group were euthanized 16 weeks post-treatment, while cats in the long-term group were allowed to live to a standard humane endpoint. Cats were neurologically evaluated every 2 weeks and assigned a clinical rating score based on gait and tremor analysis. Cerebrospinal fluid (CSF) was collected 16 weeks post treatment and at necropsy. Hex activity was assessed in 16 separate CNS regions using fluorimetric 4MU assays. Luxol Fast Blue (LFB) and 3 immunohistochemical (IHC) stains (anti-Olig2, anti-GFAP, and anti-Iba-1) were performed on the thalamus and parietal cortex. Whole-slide quantification of blue (LFB) or brown (IHC) color was performed across regions of interest using QuPath software.

Results. While untreated SD cats live to 4.3 \pm 0.2 months, cats treated with the low and high doses lived to 8.3 \pm 1.2 and 12.4 \pm 2.7 months, respectively. In-life assessments revealed partial correction of SD pathology, with the most dramatic improvement seen in the reduction of tremors in treated animals. Bimonthly neurological examinations demonstrated slowed neurodegeneration in a dose-dependent manner. CSF levels of AST and LDH were decreased both at 16 weeks post-treatment and at long-term endpoints, indicating a reduction of cell damage within the CNS. Hex activity was partially normalized, with more dramatic improvement in the high dose group and the spinal cord. Whole-slide image analyses of LFB, anti-Olig2, anti-GFAP, and anti-Iba-1 stains was successful, with preliminary results indicating a mixed response to AAV treatment.

Conclusions. These results support the efficacy of IV delivery of AAV, especially at a high dose, for restoring lifespan, quality of life, and enzyme activity in a feline model of SD. The newly optimized whole-slide stain quantification methods will be applied to tissues from more brain regions of all cats included in this study, and will provide valuable insight into dysmyelination and neuroinflammation.

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Rare and potentially pathogenic variants in the hydroxycarboxylic acid receptor genes identified in breast cancer cases

Cierla McGuire Sams¹, Kasey Shepp¹, Jada Pugh¹, Madison R. Bishop¹, and Nancy D. Merner¹

¹Auburn University, College of Veterinary Medicine, Department of Pathobiology, Auburn, Alabama, 36849.

Background. Three paralogous genes clustered together on human chromosome 12, comprise a group of Hydroxycarboxylic Acid Receptors (HCARs), *HCAR1*, *HCAR2*, and *HCAR3* which encode G-protein coupled receptors that play a role in detecting glycolytic metabolites and controlling fatty acid oxidation. Though better known for lipid metabolism regulation in adipocytes, HCARs have recently been functionally associated with breast cancer proliferation/survival; *HCAR2* has been described as a tumor suppressor, and *HCAR1* and *HCAR3* as oncogenes. The aim of this study was to identify genetic variants in *HCAR1*, *HCAR2*, and *HCAR3* that could potentially be associated with breast cancer.

Methods. Due to the extremely high sequence homology between *HCAR1*, *HCAR2*, and *HCAR3*, primers were carefully designed to amplify each gene separately by way of nested PCRs followed by Sanger sequencing. Screening was conducted on forty-six unrelated breast cancer cases for rare, non-synonymous coding variants.

Results. Upon screening, a total of four variants were identified in four separate cases, each diagnosed with estrogen receptor-positive (ER+) breast cancer. These variants were identified exclusively in *HCAR1* and *HCAR3*. In *HCAR1*, two highly conserved and potentially damaging missense variants were identified, c.58C>G;p.Pro20Ala and c.721C>T;p.Leu241Phe. Statistical analyses revealed that c.58C>G;p.Pro20Ala was in significantly more cases than controls. In *HCAR3*, the missense variant, c.560G>A;p.Arg187Gln, was identified and associated with breast cancer risk. Additionally, a frameshift mutation, *HCAR3* c.1117delC;p.Gln373Lysfs*82, was detected that greatly extends the C-terminus and alters the secondary and tertiary protein structure.

Conclusions. Due to the important role of HCARs in breast cancer, it is crucial to better understand how these genetic variants play a role in breast cancer risk and proliferation, as well as their ramifications on treatment strategies. Due to the small sample size in this study, larger additional studies are necessary to validate these findings. Regardless, the identification of these potentially pathogenic variants supports the need to investigate their functional consequences.

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**Equine arterial ring assay as an ex vivo model for vasculogenesis in horses**

Mariano Mora-Pereira¹, Katelyn Kahler¹ and Anne A. Wooldridge¹

¹Department of Clinical Sciences, Auburn University, AL

Introduction. The ex vivo arterial ring assay allows the study of vasculogenesis in a highly reproducible manner. The purpose of the study was to describe the arterial ring assay technique using equine arteries and to observe the neovascularization process. Following standardization of the technique, the effect of different growth factors in vascular network formation was evaluated. Our hypothesis was that equine arterial rings can serve as a model to study angiogenesis in horses, and that equine-specific growth factors contained in the endothelial basal media will enhance vessel growth.

Methods. A branch of the transverse facial artery was dissected from 5 horses following euthanasia. Arteries were cut into 1 mm rings and embedded in basement membrane matrix. Rings from 3 horses were cultured with endothelial basal media (EBM) with human growth factors and 10% horse serum (HS) (standard endothelial growth medium (EGM)). Rings were imaged daily until matrix lysis (ML) and vascular regression (VR) were observed. Time of appearance of the first sprout (FS), VR and ML were compared between horses. Rings from 2 additional horses were exposed to standard EGM, EBM, EBM with human vascular endothelial growth factor (hVEGF), EBM with 10% HS, or standard EGM containing EDTA. Vascular network area (VNA) and maximum network growth (MNG) were compared among groups at day 7. Means were compared by ANOVA or Kruskal Wallis tests with Tukey's or Wilcoxon rank-sum post hoc tests. $P < 0.05$ was considered significant.

Results. All rings exposed to standard EGM produced new blood vessels. Time of FS, VR, and ML were 5.0 ± 0.9 , 10.7 ± 1.3 , and 12.1 ± 1.0 days, respectively. No significant differences were observed between horses for FS ($p=0.18$), VR ($p=0.07$) or ML ($p=0.14$). Rings exposed to EBM and EDTA did not show any vascular growth. VNA in standard EGM was significantly larger than EBM+hVEGF ($p=0.049$), but no different than EBM+HS ($p=0.620$). The MNG was significantly greater for the standard EGM compared to EBM+HS ($p=0.0019$) and EBM+hVEGF ($p=0.0001$). The EBM+HS had a greater effect on MNG when compared to EBM+hVEGF ($p=0.0431$).

Conclusions. Equine arterial rings produce ex vivo neovascularization. Greater VNA and MNG were observed using the standard EGM, which could be attributed to the larger concentration of growth factors. Equine-specific factors contained in HS increased the MNG compared to xenogenic hVEGF, so the source of growth factors is important.

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Developing an *in vitro* system to identify mechanisms of Influenza A virus (IAV) species adaptation using human and swine respiratory epithelial cells

Peter J. Neasham¹, Vasilios Pliasis¹, J. Fletcher North¹, Virginia Aida¹, Sheniqua Glover¹ and Constantinos S. Kyriakis¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Swine have often been described as the 'mixing vessel' intermediate host species for the evolution of novel IAVs with pandemic potential. This is primarily because they are susceptible to IAVs of swine, human and avian origin, which serves to increase the genetic diversity of IAVs circulating in the swine host and to generate novel reassortant strains with pandemic potential. To study the molecular mechanisms that enable IAVs to adapt to a novel host species, particularly at the human and swine interface, we employed the use of primary fully differentiated normal human (NHBE) and swine bronchial epithelial (PBE) cells. The fully differentiated primary cell lines serve as a physiologically relevant cellular model for IAV research, by mimicking the host's upper respiratory tract *in vitro*. Additionally, immortalized human (Calu-3) and swine (immortalized PBE) respiratory epithelial cells were included in the study, to gain an insight into the molecular mechanisms that contribute to IAV adaptation to the swine or human host.

Methods. Primary NHBE and PBE cells were cultured in a 12-well air liquid interface (ALI) system for approximately 2-4 weeks to promote the formation of pseudostratified structure. Immunofluorescence microscopy was used to identify if the NHBE and PBE cells were fully differentiated and ready for use. Human and swine respiratory cells were infected at an MOI of 0.01 with either swine (A/sw/MN/A01125993/2012 H3N2 and A/sw/NC/152702120055 H1N2) or human (A/CA/07/2009 H1N1 and A/TX/50/2012 H3N2) IAVs. Replication curves for the panel of IAVs were generated by measuring the virus titer from the cell supernatant collected at 12, 24, 48 and 72 hpi by TCID₅₀. The panel of IAVs were serially passaged in the human and swine respiratory epithelial cell lines respectively, and the supernatant collected following each consecutive passage was prepared for Next Gen Sequencing.

Results. The replication kinetics for the panel of IAVs used in this study were characterized in the fully differentiated human and swine cells, and immortalized Calu-3 cells. This has enabled us to begin individually serially passaging the IAV in their respective cell lines, to study the interspecies adaptation and evolution of human and swine IAVs overtime.

Conclusions. We have developed a preliminary system to study the evolution and species adaptation of IAVs of human and swine origin *in vitro*. In the future, we aim to use this system to identify molecular markers in the IAVs genome that could serve to assess the zoonotic and pandemic potential of IAVs currently circulating at the human/swine interface and isolated during surveillance.

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Interspecies Transmission of Influenza A Viruses and the Emergence of Pandemic Viruses

J. Fletcher North¹, Peter J. Neasham¹, Vasileios Pliasis¹, Virginia Aida¹, Dylan S. Wilson¹, Paul H. Walz¹, S. Mark Tompkins², and Constantinos S. Kyriakis^{1,2}

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

²Center for Vaccines and Immunology, University of Georgia, Athens, GA

Introduction. Swine serve as natural hosts of Influenza A Viruses (IAVs), and exhibit very similar disease pathology and immune responses to humans. As such, swine make excellent models for studying human IAV infection. Occasionally, human IAVs (huIAVs) spillover into swine, termed reverse zoonosis, allowing opportunities for reassortment and the evolution of antigenically novel viruses with pandemic potential. However, human viruses are rarely isolated during surveillance efforts, despite recent research showing that reverse zoonosis is a much more common occurrence for IAVs than zoonosis. This brings into question the real rate of these events, and the susceptibility of swine to huIAVs. Without knowing these, it is difficult to gauge the potential risk posed by reverse zoonotic events.

Methods. In this study, groups of 6-week-old, IAV seronegative piglets were infected intranasally with one of five different Influenza A viruses (either swine or human origin H3N2, a swine origin H1N2, a human origin H1N1, or the 2009 pandemic H1N1). Clinical scores were taken daily, viral shedding and replication were assessed in nasal swabs (collected daily) and bronchoalveolar lavage fluid (collected preinfection, and on day 4 post infection). Plasma collected prior to infection and on day 4 post infection was collected for serological examination, including a 13-plex porcine cytokine/chemokine panel, and for hemagglutination inhibition assays.

Results. Pigs infected with swIAV exhibited standard clinical symptoms such as increased respiratory rate, fever, and depression, 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. Interestingly, the pdm09H1N1 was intermediate, but replicated prominently in the lower respiratory tract. Preliminary cytokine analysis revealed a stronger innate immune response in those animals infected with a swIAV.

Conclusions. Animals infected with a swIAV or the pdm09H1N1 exhibited typical clinical disease, including labored breathing, depression, and fever, while those infected with a huIAV showed little to no disease. In agreement with this, replication of huIAVs was significantly decreased in both the upper and lower respiratory tracts, with many animals negative by PCR in BALF. The difference in apparent disease is also partially explained by the reduced immune responses seen in huIAV infected animals. In sum, these results help explain the lack of detection during surveillance, and indicate that huIAVs can go undetected for days in swine populations, pointing to the risk of reassortment in swine populations.

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Cannabidiol (CBD) induced inhibition of cancer cell viability in canine B and T-Cell Lymphoma

Saba Omer¹, Dawn Boothe, Mahmoud Mansour, Satyanarayana Pondugula, Muralikrishnan Dhanasekaran_

¹PhD Student, Department of Anatomy, Physiology, and Pharmacology, Biomedical Sciences, Auburn University, AL

Introduction. In the last two decades, Cannabinoids have been studied extensively for its potential use in various fields of human medicine including oncology. To date, canine lymphoma is still a serious condition for which there are unmet medical needs. The purpose of this study was to demonstrate the antitumor effects of cannabinoids in canine B and T cell lymphomas.

Methods. Canine B cell (1771, CLBL1) and T cell (CL1) lymphoma cell lines and lymphocytes from healthy dogs (control) cultured in RPMI. Expression of cannabinoid receptors studied using qPCR. Based on receptor expression cells were treated with receptor agonist cannabidiol (CBD). Cell viability assessed using MTT assay. Data was analyzed using ordinary one way ANOVA on Prism software and dose response relationship analyzed using SAS software.

Results. Both B and T lymphoma cell lines showed positive expression of CB1 and CB2 receptors. Cell viability assay demonstrated a dose-dependent decrease in cell viability with CBD. No significant effect on cell viability found on normal lymphocytes.

Conclusions. Canine B and T cell lymphoma cells express both cannabinoid receptors like human lymphoma and activating cannabinoid receptors with agonist CBD induces cancer cell death in both canine lymphoma cells. Our results suggest that cannabinoids have an anti-proliferative and pro-apoptotic effect on canine lymphoma cells and support the need for further studies providing evidence of efficacy against both human and canine B cell lymphomas. Discuss the results.

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Influenza neuraminidase virus-like particle vaccine platform as a candidate universal influenza vaccine in the porcine model

Vasilis Pliasis¹, Zach Menne², Virginia Aida¹, Ji-Hang Yin¹, Peter J. Neasham¹, J. Fletcher North¹, Maria C. Naskou¹, Sheniqua Glover¹, Ioanna Skountzou², Constantinos S. Kyriakis^{1,3}

¹ Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA

² Department of Microbiology and Immunology, Emory University, Atlanta GA, USA ³ Center for Vaccines and Immunology, University of Georgia, Athens GA, USA

Introduction. Influenza A viruses (IAVs) constitute a major threat to both human and animal health. Developing effective prophylactic vaccine strategies is pivotal in controlling the disease. Neuraminidase (NA), although commonly neglected in influenza vaccine formulations, is an immunostimulatory protein able to elicit heterologous NA-specific protection. The objective of this study was to assess an influenza NA Virus-Like-Particle (VLP) platform as a candidate universal influenza vaccine in the porcine model.

Methods. In our vaccination-challenge study, we used a total of 18 influenza-seronegative piglets. They were divided into 3 groups of 6 animals per group. They were vaccinated twice with a 3-weeks interval with (a) the N2 VLP vaccine, that contained the NA protein from the A/Perth/16/2009 (H3N2) (b) a commercial swine IAV vaccine or (c) adjuvant only. Four weeks after boost, pigs were intranasally challenged with A/swine/NC/152702/2015, an H1N2 swine IAV field isolate. Amino acid homology between the vaccine and challenge NA was 87.6%. Serological protection was assessed by NA ELISA, Neuraminidase Inhibition, Hemagglutination Inhibition and Virus Neutralization assays. Nasal swabs were collected daily (day 0 to day 5 post-challenge) and Bronchoalveolar lavage fluid (BALF) and tissue samples from the entire respiratory tract were harvested at euthanasia (day 5). Protection was evaluated based on virus titers in nasal swabs and tissue homogenate samples, BALF cytology, and respiratory histopathology.

Results. Although neither vaccines conferred sterilizing immunity, pigs immunized with the NA VLP vaccine showed reduced pulmonary virus titers, BALF neutrophilic infiltration and histopathology compared to unvaccinated controls.

Conclusions. Overall, our study revealed that the NA VLP platform performed comparably to the commercial vaccine and conferred substantial protection against heterologous challenge.

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Pregnenolone 16-alpha Carbonitrile, an Agonist of Rodent Pregnane X Receptor, can Impair Testosterone Biosynthesis in Rodent Leydig Cells

Julia M. Salamat, Elizabeth M. Ortiz, Benson T. Akingbemi, Chen-Che J. Huang, Satyanarayana R. Pondugula

Department of Anatomy, Physiology and Pharmacology, Auburn University, AL.

Introduction. Leydig cells (LC) in testes produce the male sex hormone testosterone (T). Several therapeutic drugs and environmental chemicals are known to disrupt T homeostasis. Some of these drugs and chemicals can activate pregnane X receptor (PXR), which is a ligand-dependent orphan nuclear receptor that regulates the expression of the enzymes/proteins involved in the metabolism of endobiotics and xenobiotics. Notably, PXR activation has been shown to disrupt corticosteroid hormone homeostasis. Therefore, it is possible that PXR is expressed in LC and that PXR activation in LC can lead to impaired T homeostasis. In this preliminary study, we sought to determine whether PXR is indeed present in rodent LC and whether pregnenolone 16-alpha carbonitrile (PCN), an agonist of rodent PXR, affects T biosynthesis in the LC.

Methods. Rat primary LC, isolated from 35 days old male Long Evans rats, were treated with DMSO (0.1%) or PCN (10 μ M) for 24 hours. The LC were then either unstimulated (basal) or stimulated with luteinizing hormone (LH; 100ng/ml) for 3 hours before measuring the concentration of secreted T (intracellular and extracellular) using testosterone radioimmunoassay. Western blot analysis was conducted to study protein expression of PXR as well as to examine the effect of PCN on protein expression of the enzymes/proteins involved in T synthesis in rat primary LC. PXR mRNA expression was also studied in rat primary LC. We also studied mRNA and protein expression of the PXR in MA-10 mouse Leydig tumor cells. Finally, RNA-sequencing analysis was performed in MA-10 cells to determine the effect of PCN (10 μ M; 24 hours) on the transcriptome profile of the genes involved in T synthesis.

Results. PXR was found to be expressed at mRNA and protein level in rat primary LC. Treatment of rat primary LC with PCN resulted in decreased T secretion in both basal and LH-stimulated conditions. Additionally, PCN treatment resulted in the downregulation of protein expression of steroidogenic acute regulatory protein (StAR), cytochrome P450 17A1 (CYP17A1), 3 β -hydroxysteroid dehydrogenase (3 β -HSD), and 17 β -HSD in rat primary LC. PCN, however, did not alter the expression of cytochrome P450 19 (CYP19), which converts T to estrogen, suggesting that PCN-induced reduction of T production in rat primary LC may not be due to increased conversion of T to estrogen. PXR was also found to be expressed at mRNA and protein level in mouse MA-10 cells. Consistent with the effect of PCN on protein expression of the enzymes/proteins in rat primary LC, the RNA-sequencing analysis in mouse MA-10 cells showed that PCN treatment leads to downregulation of StAR, CYP17A1, 3 β -HSD, and 17 β -HSD. In mouse MA-10 cells, similar to rat primary LC, PCN treatment did not change the transcript levels of CYP19 expression.

Conclusions. Together, these preliminary results suggest that PCN, an agonist of rodent PXR, can impair T biosynthesis in rodent LC by downregulating the expression of the enzymes/proteins involved in T biosynthesis. Future studies will be directed to demonstrate whether PCN impairs T biosynthesis in a PXR-dependent manner in rodent LC.

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**The prognostic significance of exon 8 c-kit mutations in canine mast cell tumors**

Olivia A. Stephenson¹, Rebecca G. Newman², and Bridget K. Urie²

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

²Pittsburgh Veterinary Specialty & Emergency Center (PVSEC), Oncology, Pittsburgh, PA

Introduction. Mast cell tumors (MCT) are the most common malignant skin tumor in dogs. Despite being so prevalent, there remains substantial variability in the biological behavior of canine cutaneous MCT. One of the strongest prognostic indicators relied on is tumor grade. Additional prognostic factors exist, including breed of dog, tumor location, and mitotic count. More recent advances in molecular testing have identified that up to 40% of canine MCT harbor a mutation in the *c-kit* gene that codes for KIT. KIT is a receptor tyrosine kinase that plays a central role in the survival, proliferation, differentiation, and migration of mast cells and MCT. Previous studies have found that *c-kit* mutations most commonly occur at exon 11 and the presence of an exon 11 mutation is associated with a poor prognosis. To the authors' knowledge, there are no studies investigating the differences in survival times for dogs with an exon 8 positive MCT versus dogs with an exon 11 positive MCT. The primary objective of this study was to compare the outcome of dogs with exon 8 mutations to those with exon 11 mutations. The secondary objective of this study was to evaluate whether treatment with a tyrosine kinase inhibitor (TKI) resulted in prolonged survival times in dogs with *c-kit* mutations.

Methods. The Pittsburgh Veterinary Specialty & Emergency Center (PVSEC) medical record database was searched for client-owned dogs with a confirmed mast cell tumor who had a *c-kit* mutation identified in either exon 8 or exon 11. Data was available for cases seen between June 2002 to June 2020. The following information was recorded for each patient: signalment, date of diagnosis, location and grade of tumor, whether metastatic disease was present or not, treatment, disease progression, and date and cause of death. Data was analyzed using Kaplan-Meier and Cox linear regression analyses.

Results. A total of 130 dogs were identified who met inclusion criteria. 35/130 (26.9%) were exon 8 mutation positive and 95/130 (73.1%) were exon 11 mutation positive. 28/35 (80%) exon 8 dogs underwent surgical removal of their MCT. The most common grade was low grade 2 (64.3%), followed by high grade 2 (10.7%). 5 dogs (17.8%) who were exon 8 positive had subcutaneous MCT and 2 dogs (5.7%) presented with systemic mastocytosis. 77/95 (81%) exon 11 dogs underwent surgical removal of their MCT. The most common grade was high grade 3 (36%), followed by low grade 2 (28.5%) and high grade 2 (26%). Again, 5 dogs (6.5%) had subcutaneous exon 11 MCT surgically removed. No dogs with an exon 11 mutation presented with systemic mastocytosis. The overall median survival time (MST) for exon 8 positive dogs was 1932 days (95% CI, 8-2286 days), whereas the MST for exon 11 positive dogs was 399 days (95% CI, 31-2301 days). Treatment with a TKI did not influence patient survival for exon 8 nor exon 11 patients.

Conclusions. Canine patients with a mutation in exon 11 had higher grade MCT compared to those with a mutation in exon 8. Exon 11 mutations were associated with a shorter MST, as well, although the range of survival times were similar. Regardless of which mutation was present, treatment with a TKI did not confer a statistically significant advantage in overall survival. Additional data analysis is warranted to determine if TKI treatment has an impact on time to disease progression or development of metastatic disease and a larger study, using data from multiple institutions, should be collected to strengthen our findings.

Conclusions. The authors report no conflict of interest and no third-party funding or support was received for this study.

**Thyroid hormone upregulates Dhcr24 expression in adrenal gland**

Huifei Sophia Zheng, Yuan Kang, and Chen-che Jeff Huang

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

Introduction. Dhcr24/Seladin-1, a crucial enzyme in sterol synthesis, has been identified as a gene whose expression is down-regulated in affected brain areas in patients with Alzheimer's disease (AD). Conversely, high levels of DHCR24/seladin-1 exert protective functions which protect cells from apoptosis. We previously reported that thyroid hormone prevents cells in the adrenal gland inner cortex from apoptosis, a tissue remodeling process during normal development. Our preliminary data showed that *Dhcr24* is upregulated in the adrenal gland under thyroid hormone treatment. Studies using cell lines and the reporter assay also showed that DHCR24 can be upregulated by thyroid hormone. Here, we are deciphering the connection between DHCR24 and the thyroid hormone in the adrenal gland, and their possible cell protective effect on the adrenal gland inner cortex.

Methods. The biotinylated thyroid hormone receptor β (TR β) knock-in mouse model was used to identify TR β binding sites at the genomic level. The cellular expression pattern of DHCR24 in the adrenal gland during development was examined by immunostaining and was confirmed using LacZ reporter mice. To understand how thyroid hormone affects DCHR24(+) domain in the adrenal gland, C57BL/6J mice were administered with 1 μ g of T3 in 30 μ l of saline subcutaneously once daily for 10 days. Control mice were injected with 30 μ l of saline. Adrenals were collected and analyzed using RNAseq and immunostaining.

Results. The Chromatin Affinity Purification next generation sequencing (ChAP-seq) results showed that in mouse adrenal glands, the 5' region of *Dhcr24* contains a TR β binding site, suggesting TR β -mediated direct regulation of *Dhcr24* expression. Immunostaining showed that DHCR24 is highly expressed in the adrenal inner cortex with an age- and sex-dependent expression pattern. Moreover, thyroid hormone treatment experiment showed that thyroid hormone expanded the DHCR24(+) domain and prevented its apoptosis-mediated regression during development.

Conclusions. In the present work, we provided evidence showing the direct effect of the thyroid hormone-mediated *Dhcr24* expression effect and how thyroid hormone affects the cell fate of DHCR24(+) cells.

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Faculty Platform Presentations

Treatment of Bovine *Tritrichomonas foetus* with an Extended Release Topical Formulation

Julie G. Schnuelle,¹ and Sue H. Duran¹

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

There is no approved treatment for *Tritrichomonas foetus* (*T. foetus*) in bulls. Hence, it was our purpose to develop an approved treatment of *T. foetus* in bulls. Three confirmed Trichomoniasis positive Charolais bulls were topically treated for Trichomoniasis on June 3, 2019. A patented mixture of Oxfendazole PLO gel was placed topically on their prepuce and penis. Prior to application of the Oxfen-Pluronic Lecithin Organogel (PLO) gel, all bulls were tailed, and cleaned with dawn soap and water four times to remove any biofilm present. After application, the treated area was dried using a hair dryer. Bulls were serially tested for *T. foetus* via collection of preputial smegma via a preputial scraping utilizing primarily the Pizzel Stick (Lane Manufacturing). The preputial scraping was placed in a vial of Modified Diamond's Media and taken to the Bishop-Thompson-Sparks-Alabama State Diagnostic Laboratory for culture and real time quantitative polymerase chain reaction (RTqPCR). The bulls were serially tested at numerous time points following treatment. A positive reaction for the real time PCR assay is detected by the accumulation of a fluorescent signal. The cycle threshold (CT) is the number of cycles required for the fluorescent signal to cross the threshold to exceed the background level. Hence, the CT levels are inversely proportional to the amount of target nucleic acid in the sample. The lower the CT level the greater the amount of target nucleic acid within the sample. Real time qPCRs undergo 40 cycles of amplification. If CT values are less than or equal to 29 they are considered strong positive reactions that indicate abundant target nucleic acid in the sample. If the CT value lies in the range of 30-37 then it is considered to be a positive reaction indicative of moderate amounts of target nucleic acid. If the CT value lies in the range of 38-40 then it is considered a weak reaction indicative of minimal amounts of target nucleic acid, which can represent possible infection or environmental contamination. All cultures were cultured for a period of 5 days. All samples were evaluated via microscopy for live organisms initially and at the end of five days. All bulls had two treatments of the combination of Oxfen-PLO gel. All bulls were initially negative for culture a following treatment. One bull was consistently negative for live organisms on culture for approximately 5 months from July to the end of November 2019. Further combinations of Oxfen-PLO gels need to be studied in clinical trials in order to get a complete kill of *T. foetus*.



Evaluation of zoledronate for the treatment of canine metastatic osteosarcoma

Ashley A. Smith¹, Gregory T. Almond¹, Stephanie Lindley¹, Noelle Bergman¹, and Annette N. Smith²

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

²VCA Carriage Hills Animal Hospital and Pet Resort, Montgomery, AL

Introduction. Osteosarcoma (OSA) is the most common malignant bone tumor in dogs. Greater than 90% of dogs develop pulmonary metastasis despite treatment with the standard of care. Stage III disease is refractory to chemotherapy (0-17.6% response rate) and associated with poor survival times (median 59 days). Zoledronate (ZOL) is an injectable bisphosphonate conventionally used for cancer-associated bone pain. Direct anti-cancer effects of ZOL have been recently discovered *in vitro* and in rodent models. ZOL reduces OSA cell migration, inhibits angiogenesis, augments innate anti-tumor immunity, and induces apoptosis in OSA cell lines. Phase I trials in dogs have been performed and found that ZOL can rarely cause renal injury and osteonecrosis of the jaw. The goal of the present study was to determine the activity of ZOL for the treatment of canine metastatic OSA and describe any side effects encountered with drug administration.

Methods. A phase IIa clinical trial was performed at Auburn University and enrolled client-owned dogs with stage III OSA. Dogs must have a histopathologic diagnosis of appendicular OSA, control of local disease, and the presence of pulmonary metastasis. Dogs were excluded if they had a pathologic fracture, life expectancy less than 1 month, and/or renal azotemia. ZOL was administered at 0.1 mg/kg IV over 15 minutes once monthly until cancer progression was noted. Monitoring consisted of renal values (blood urea nitrogen, creatinine, urine specific gravity) and three-view thoracic radiographs at baseline and every 30 days until disease progression. Clients were expected to report side effects and their pet's quality of life at each appointment. Dogs were removed from the trial at the time of cancer progression or at the owner's request. This study was approved by Auburn University's Institutional Animal Care and Use Committee (IACUC) and Clinical Research Review Committee (CRRC).

Results. Eleven dogs were enrolled. Two experienced disease stabilization per standardized veterinary oncology reporting criteria (VCOG RECIST), and 9 dogs progressed at 30 days. One responder was removed from the clinical trial at the owner's discretion at 30 days. The other dog with stable disease received 4 doses of ZOL and experienced disease control for 93 days. The median survival time after detection of metastasis was 85 days (range, 46 to 237 days). No alterations in renal values were noted over the course of the study. One dog experienced bilateral conjunctivitis that responded to topical corticosteroids. Another dog developed a fever, lethargy, anorexia, tachypnea, and hypocalcemia resulting in euthanasia 4 days after ZOL administration. Post-mortem histopathologic evaluation of the lungs was performed in 6 dogs and confirmed a diagnosis of metastatic OSA in all cases.

Conclusions. Treatment with ZOL demonstrated a biologic response rate of 18.2% (2 of 11 dogs). Survival times do not appear different from previous data for stage III OSA. Renal injury and osteonecrosis were not encountered. However, we observed two toxicities that have not been previously reported in dogs. These toxicities have been described in humans as a result of pro-inflammatory cytokine release by ZOL-stimulated $\gamma\delta$ T cell lymphocytes. Additional studies are warranted to determine alternative doses and schedules of ZOL, further characterize its side effects, and later investigate its efficacy for canine OSA.

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**Genome editing as a potential therapeutic strategy for GM1 gangliosidosis**

Allisandra K. Rha¹, Douglas R. Martin^{1,2}

¹Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

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

Introduction. GM1 gangliosidosis is a rare autosomal recessive disorder affecting one in every 100,000 to 200,000 live births. Progressive neurodegeneration results from mutations in the *GLB1* gene, which codes for the lysosomal enzyme β -galactosidase (β -gal). Of the three types of GM1 (infantile, late infantile/juvenile, and adult-onset), the infantile form is the most common and severe with persistent neurodegeneration leading to death by \sim 4 years of age. Restoration of β -gal function and amelioration of neuropathology will be essential to curing GM1. Though current FDA-approved treatments for GM1 do not exist, promising work is ongoing. Genome editing remains an unexplored mechanism for the treatment of GM1. CRISPR/Cas complexes, which were first defined as a form of adaptive immunity in bacteria and archaea, have been manipulated for precise genome editing in eukaryotes. The fusion of cytidine or adenine (ABE) deaminases to the Cas9 endonuclease represent robust mechanisms for the correction of single nucleotide polymorphisms (SNP), which make up the majority of GM1 pathogenic mutations. Here, we use CRISPR/Cas-ABE to target pathogenic C>T transitions in human GM1 patient fibroblasts to address our hypothesis that genome editing is an effective therapeutic strategy for the restoration of β -gal activity. Translation of these strategies *in vivo* will be used to further assess efficacy and the treatment of neuropathology.

Methods. Single guide RNAs (sgRNAs) were designed complementary to the thymine-containing strand using Benchling and validated manually through analysis of *GLB1* and the position of the target adenine within the protospacer region. Gene Blocks containing the U6 promoter, tracrRNA, and the sgRNAs were synthesized by IDT with 5'-MfeI and 3'-MluI restriction enzyme sites. The gene blocks were cloned to the Cas9-ABE plasmid by restriction digest. xCas9(3.7)-ABE(7.10) was a gift from David Liu (Addgene plasmid # 108382). Human GM1 patient fibroblasts were obtained from the Coriell Institute (GM05652; GM02439) and cultured in DMEM/10%FBS/1%PSA. GM1 fibroblasts were transfected via nucleofection using Mirus Ingenio electroporation solution and the Bio-Rad GenePulser Xcell, and cultured for 96 hours before analysis. Cells were analyzed by X-gal assay at pH 4.2 to specifically examine lysosomal β -gal. Assay of β -gal activity was performed using the fluorogenic substrate 4-MU-gal.

Results. In untreated GM1 infantile (GM05652) and juvenile (GM02439) patient fibroblasts, X-gal staining was undetectable. Assay of β -gal using 4-MU-gal yielded 0.02 and 0.05 fold-normal β -gal activity for untreated infantile and juvenile lines, respectively. In treated cells, X-gal staining was detectable in both GM1 patient lines, with a modest increase in β -gal activity by 4-MU enzyme assay.

Conclusions. CRISPR/Cas-ABE was able to restore β -gal activity in some cells from both human GM1 patient lines. These preliminary results serve as proof-of-concept for the optimization of future *in vitro* work and translation to *in vivo* GM1 models. With the achievement of this critical first step, further development of CRISPR/Cas-ABE editing of *GLB1* will focus on enhancing efficiency, delivery mechanisms and ultimate application to patients.

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Effect of administration route and AAV serotype for treatment of feline GM1 gangliosidosis

Amanda L Gross¹, Heather L Gray-Edwards², Miguel Sena-Esteves^{3,4}, Douglas R Martin^{1,5}

¹Scott-Ritchey Research Center, ⁵Department of Anatomy, Physiology, & Pharmacology, College of Veterinary Medicine, Auburn University, AL

²Department of Radiology, ³Department of Neurology, ⁴Horae Gene Therapy Center, University of Massachusetts Medical School, Worcester, MA

Introduction. GM1 gangliosidosis is a fatal neurodegenerative disease caused by a deficiency of lysosomal β -galactosidase (β gal). GM1 animals are effective models for studying gene therapy since the therapeutic vector can also act as a reporter construct. Cerebrospinal fluid (CSF) administration of adeno-associated viral (AAV) therapy is hypothesized to be an effective method for treating neurodegenerative diseases. In this study, we evaluated two serotypes (AAV9 and AAVrh10) using CSF delivery via the cisterna magna (CM). Additionally, we compared these results to intravenous administration of AAV9.

Methods. All treatment cohorts received 1.5×10^{13} vector genomes/kg body weight at 1.8 ± 0.5 months of age. Clinical assessments included neurological exams, CSF biomarkers, and 7T magnetic resonance imaging (MRI) and spectroscopy (MRS). Postmortem analysis included β gal distribution.

Results. Untreated GM1 animals survived 8.0 ± 0.6 months while treated animals lived significantly longer. GM1+AAV9 CM (N=3) animals survived 13.9 ± 1.8 months, GM1+AAVrh10 CM (N=2) animals survived 11.3 ± 0.5 months, and GM1+AAV9 IV (N=2) animals survived 42.1 ± 1.8 months. Neurological abnormalities, which in untreated GM1 animals lead to an inability to stand by 8 months of age, were delayed but not halted in both CM treated cohorts and all animals became blind as their disease progressed. The IV treated cohort had mild neurological symptoms, similar to those seen early in disease stages, but no further impairments. Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), biomarkers of central nervous system damage when measured in the CSF, were both reduced in all treatment cohorts. MRI revealed delayed progression of neurodegeneration in both CM cohorts and preservation of brain architecture in the GM1+AAV9 IV cohort. Glycerophosphocholine and phosphocholine (GPC+PCh), a MRS biomarker that increases with loss of myelin integrity, showed no correction in the GM1+AAVrh10 cohort, correction only in the cerebellum of the GM1+AAV9 CM cohort, and correction in several brain locations of the IV cohort. In the CM cohorts, β gal activity was restored in the cerebellum and spinal cord but did not penetrate deep brain structures (such as thalamus). The GM1+AAV9 IV cohorts had increased β gal activity throughout the CNS. All cohorts had some degree of β gal restoration in the heart, liver, and skeletal muscle, and the IV cohort also had normalized levels in the sciatic nerve.

Conclusions. Using a similar vector backbone and same total dose, this study demonstrates AAV efficacy in all treatment cohorts and suggests that IV gene therapy is most effective in the feline model.

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Undergraduate Student Poster Presentations

Towards Elucidation of Canine Adenovirus type 2 tropism

Madison D Hogans¹, Will Kretzschmar¹, Payal Agarwal^{1, 2}, Bruce F Smith^{1, 2}

¹ Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

²Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Adenovirus based vectors are very important for oncolytic gene therapy. Human Adenovirus 5 (Ad5) is the most commonly used Ad vector. However, Ad5 based vectors have limited utility because a variety of cells are refractory to Ad5 transduction. In addition, many people have prior exposure to Ad5, leading to pre-existing immunity that eradicates the virus when it enters the human body. Therefore, identification of alternative tropism for Ad5 could provide both a mechanism to target refractory cells and to evade preexisting immunity. Canine adenovirus type 2 is a vector that has similar characteristics to Ad5. Although the genome of CAV2 is well characterized, its cellular tropism is still unclear. While Ad5 attaches to the cell by the cell surface coxsackie and adenovirus receptor (CAR) and internalizes using cell surface integrin ($\alpha\beta 5$, $\alpha\beta 3$) or a major histocompatibility complex class 1 (MHC-I), CAV 2 can infect in the presence or absence of CAR and integrins making it, at least in some cells, CAR and integrin independent. CAV2 does not have the conserved Arg-Gly-Asp (RGD) or Lys -Thr-Lys-Lys (KTKK) motifs that interact with the $\alpha\beta$ integrins. In addition, we have recently demonstrated that CAV2 can infect canine lymphoma cells which are resistant to Ad5 infection, however, the component(s) responsible for the internalization is, as of now, unknown.

Methods. We have evaluated the infection pattern of green fluorescent protein (GFP) variants of CAV2 and Ad5 (CAV2GFP, Ad5GFP) in a variety of canine and human cell lines and determined the correlation of infection with the expression of $\alpha\beta$, $\beta 3$, $\beta 5$, and CAR. All viral infections were performed for 48 hours and GFP expression was analyzed using flow cytometry.

Results. Our research demonstrates that CAV2GFP can successfully infect cells that are not infected by Ad5GFP, such as canine lymphoma cells (1771, CL-1, and OSW). CAV2GFP can also infect human ovarian cells (SKOV3) at a lower multiplicity of infection than Ad5GFP.

Conclusions. The pattern of infection of CAV2GFP differs from that of Ad5GFP. CAV2GFP can infect cells that are poorly or not infected by Ad5GFP. The CAV2GFP infection pattern is also not dependent on the level of expression of CAR or cell surface integrins. Therefore, the mechanism of CAV2 transduction is different than Ad5 and consequently, different cell surface receptors are involved.

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**Production of Anti-PD1 antibody by modified Canine Adenovirus-2**

Daniel Patton¹, Natalie Stahr¹, Payal Agarwal^{1, 2}, and Bruce Smith^{1,2}

¹Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University

²Department of Pathobiology, College of Veterinary Medicine, Auburn University

Introduction. The PD-1/PD-L1 immune checkpoint pathway functions in the body to peripherally regulate autoimmune responses by CD8+ T cells. Tumor cells often overexpress PD-L1 which allows them to evade the immune system resulting in proliferation of cancer cells. Antibodies to PD-1 have been used to prevent the interaction of tumor produced PD-L1 and T-cell PD-1, which allows the immune system to target the tumor cells. Typically, anti-PD-1 antibody has been given intravenously, leading to some anti-tumor responses along with deleterious systemic side effects. Localized administration of anti-PD1 antibody might generate a more effective immune response and eliminate many of the systemic side-effects. In order to provide delivery of anti-PD-1 antibody in canine tumors, a conditionally replicative Canine adenovirus 2 (CAV2) has been modified to produce a camelid single-chain anti-PD-1 antibody in cancer cells. The goal of this approach is to locally overcome this immune checkpoint and initiate an anti-tumor immune response. This approach should also reduce or prevent the systemic side effects seen with the intravenous infusion of anti-PD-1 antibody.

Methods. A selectively replicating oncolytic CAV2, AU-M1, carrying the DS red marker gene, was selected as the backbone for the anti-PD-1 antibody vector. CRISPR-Cas9 gene editing was used to remove the E3 region of the virus through two cut sites. A camelid single chain anti-PD-1 antibody with a CMV promoter and a 6-His tag was inserted into the plasmid through homologous recombination in yeast. The plasmid carrying the viral genome was then transformed into *E. coli* and verified through PCR and sequencing. The linearized viral genome was transfected into a canine packaging cell line, DKcre, to produce viral particles. The transfected cells were lysed at day 3 and cell lysate was used to infect fresh DKcre. The anti-PD-1 antibody produced by cells was purified both from cell lysate and cell media using a nickel-NTA column. Anti-PD1 antibody secretion was confirmed Western gel using antibody to the 6-His tag.

Results. Nucleotide sequencing confirmed successful excising of the E3 region by CRISPR-Cas9 and anti-PD1 antibody sequence insertion by yeast homologous recombination. Cell lysis due to viral replication was observed 3 days after virus transfection. The presence of virus in the transfection and subsequent infections were indicated by red florescence. Western blot analysis confirmed the production and secretion of anti-PD-1 antibody in the infected DKcre cells.

Conclusions. The virus was successfully modified to produce anti-PD-1 antibody using CRISPR-Cas9 gene editing. The modified virus (AU-M3) was able to infect, replicate, and lyse cells and was shown to produce anti-PD-1 antibody

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Veterinary Student Poster Presentations

Awareness and perception of Breed-Specific Legislation in a College of Veterinary Medicine

Tom Bunch¹, and Vinicia C. Biancardi¹

¹Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. The goal of this pilot study was to determine whether students and faculty at Auburn University College of Veterinary Medicine were aware of breed-specific legislation (BSL) and to gather the overall opinion of said legislation with the college. As a secondary goal, the study aimed to determine whether certain perceptions were influenced by dog ownership and specific breed ownership.

Methods. We used a structured interview as a quantitative research method, which included demographic questions (age, race, ethnicity, educational level) and questions about awareness and perception of BSL. The survey was distributed within the CVM community only. Several questions were asked in the survey which pertained to awareness and perception of BSL, as well as whether participants owned dogs and, if yes, the breed of said dogs. The voluntary and anonymous survey was distributed online within the CVM community via email. The survey was hosted by Qualtrics, an online survey hosting service. The data collected were aggregated for comparison and correlations.

Results. There were 279 total responses to the survey. 81% of respondents identified as female and 19% identified as male. 8.24% of respondents identified as Hispanic, Latino, or of Spanish origin, and 91.76% of respondents identified as not of that origin. 91.64% of respondents identified as White, 2.79% as Black or African-American, 2.09% as American Indian or Alaska Native, 0.70% as Asian, 0.35% as Native Hawaiian or Pacific Islander, and 2.44% as other. 70.25% of respondents identified as 18-30 years old, 10.39% as 31-40 years old, and 17.2% as 41 years or older. 68.95% of respondents were familiar with BSL and 31.05% were not familiar. 73.45% of all respondents indicated that they were strongly opposed to BSL, 13.45% slightly opposed, 6.55% neither opposed or in favor, 4.73% slightly support, and 1.82% strongly support BSL.

Conclusions. Originally the project set out to determine correlation of breed ownership by race or ethnicity, but a much different result was found than what was intended. This population is obviously not indicative of the general public because over 90% of respondents identified as White and 81% of respondents identified as female. However, the result that was found is that even in a college of veterinary medicine, nearly one-third of respondents indicated that they were not familiar with BSL. Of people who indicated that they were familiar with BSL, 4.21% of respondents indicated that they either slightly support or strongly support BSL. However, of people who indicated that they were not familiar, 11.76% indicated that they would either slightly support or strongly support BSL. Clearly those who are unaware of BSL are more likely to be in support of it as compared to those who are aware. Therefore, if one were to want to petition to remove BSL, the first step would be to increase awareness with regards to what BSL actually is. Furthermore, the data were likely skewed by the fact that this study was sent out to a college of veterinary medicine. The level of knowledge of animal welfare or animal rights is very likely not indicative of the general public. Though the study was not able to correlate breed ownership with race due to lack of diversity among the respondents, a different and potentially more important question was answered. If in a college of veterinary medicine, where almost the entire population works with animals in some form, 31% of respondents are unaware of BSL, it would stand to reason that the general public is even less informed. Raising awareness in the general public would be the most logical first step towards making any sort of legislative change.

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Surgical Correction and Prophylactic Surgical Correction of Third Eyelid Gland Prolapse: A Retrospective Study

Amelia G. Duer¹, Shannon D. Boveland^{1,4}, Erik H. Hofmeister¹, Mitzi K. Zarfoss², Susan Nelms³, and Philip A. Moore¹

¹College of Veterinary Medicine, Auburn University, Auburn, AL, USA

²PETS Referral Center, Berkeley, CA, USA

³Veterinary Eye Care, Bessemer, AL, USA

⁴College of Veterinary Medicine, Tuskegee University, Tuskegee, AL, USA

Introduction. Prolapse of the third eyelid gland – known colloquially as “cherry eye” – primarily affects younger dogs, with breed predilections noted in the American Cocker Spaniel, Beagle, Boston Terrier, English Bulldog, Lhasa Apso, Pekingese, and Shih Tzu.¹ If left untreated, the gland can become irritated, and the affected eye may be predisposed to develop keratoconjunctivitis sicca.² Treatment usually consists of repositioning the gland using anchoring and/or pocketing of the gland.¹ The purpose of this study is to evaluate if any one surgical procedure is more effective at preventing re-prolapse of the gland of the third eyelid than others.

Methods. Multi-center retrospective analysis was conducted on medical records of 245 dogs (397 eyes) that had surgery for third eyelid gland prolapse (TEGP) between 2008 and 2019. Data collected included age, sex, breed, eye(s) affected, prolapse duration, prophylactic treatment of the contralateral eye, occurrence of the prophylactically treated eye, medical management before surgery, duration of treatment prior to surgery, surgical procedure(s), post-surgical treatment(s), follow-up duration, re-prolapse of treated eye(s), time to re-prolapse, prolapse after prophylactic treatment, complications, and concurrent ocular conditions.

Results. Surgery was performed on 397 eyes. The Morgan pocket (MP) technique was performed in 375 eyes, orbital rim tacking in 1 eye, combined MP-orbital rim tacking in 7 eyes, and combined MP-intra-nictitans tacking in 14 eyes. Successful repositioning was attained in 374 (94%) eyes. Re-prolapse occurred in 23/397 (5.8%) eyes. Of the 397 eyes, 83 of the contralateral eyes were treated prophylactically based on surgeons’ preference. One prophylactically treated eye prolapsed. No correlation was found between surgical procedure(s) performed and re-prolapse ($p=0.11$). Re-prolapse was statistically significantly more common in castrated males than in spayed females ($p<0.007$).

Conclusions. MP technique alone or in combination with other surgical techniques was successful in correcting TEGP. No surgical procedure was significantly more effective at preventing re-prolapse. Benefits of prophylactic treatment of TEGP could not be determined. No concurrent ocular conditions correlated with TEGP.

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¹Martin CL. Ophthalmic Disease in Veterinary Medicine. London: Manson Publishing/The Veterinary Press, 2005. ²Plummer C, Källberg M, Gelatt K, et al. Intranictitans tacking for replacement of prolapsed glad of the third eyelid in dogs. Veterinary ophthalmology 2008; 11:228-233.



Characterization of the Cannabinoid Receptor – 1 Gene in Three Beagle Dogs and Six Cats

Sarah Ezell¹, Kamoltip Thungrat¹

¹Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL

Introduction. Cannabidiol oil and associated products are rapidly growing in popularity not only in human medicine, but also in veterinary medicine. Cannabidiol's described therapeutic benefits are largely anecdotal, and efficacy in animals has yet to be confirmed. The goal of this research project is to identify genetic variation within the cannabinoid receptor 1 (*CBR-1*) gene in three healthy beagle dogs and six cats (three healthy and three Tay-Sachs disease) and to understand the role of this genetic variation in various disease processes and in variation of clinical outcomes.

Methods. Canine and Feline CBR-1 genetic data was obtained from the NCBI database, and primers were designed using the NCBI Primer program. The primer walking method was used to sequence CBR-1. The primer walking method is utilized when working with a long sequence. The sequence of interest can be cut into smaller, consecutive fragments, and primers can be designed that sequence the individual fragments. The result will be multiple, small sequences that combine to form the complete sequence of interest. 240 primer pairs were designed for the canine model, and from those, 75 were selected for PCR. The forward primer and reverse primer PCR products were sequenced, and current alignment and analysis efforts are focused on the reverse complement sequence. The NCBI-BLAST program was used to check the site and accuracy of the selected primers. PCR was run, and gel electrophoresis was used to confirm the PCR products. Following gel confirmation, the PCR products were sent to MacroGen Labs for sequencing. The sequence results were aligned and analyzed for single nucleotide polymorphisms (SNPs) using Mega-X software.

Results. At this point in the research project, work is still being done to assess genetic variation within these sequences. Current findings on the reverse complement sequence include a SNP located at base pair 20,534 in the canine model. NCBI genomic data defines this location as a cytosine, but in two of the three beagle dogs, this location is defined as a thymine. Base pair 20,534 is located within the region of the X3 exon. Analysis of the canine and feline CBR-1 sequences is still in progress.

Conclusions. Conclusive results cannot be drawn from the obtained data at this time, and more work needs to be done to determine whether SNPs will have an impact on the amino acid sequence and protein product. The next step is to determine the potential for splice site variation, and therefore variation in mRNA products.

Acknowledgements. All work for this project is being conducted in Dr. Dawn M. Boothe's Clinical Pharmacology Laboratory.



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

Chloe Haynes¹, Damien Ruiz², Jonathan Marable², Anil Jaiswal², Amarjit Mishra², Amol Suryawanshi² and Maninder Sandey²

¹College of Veterinary Medicine, Auburn University, AL

²Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

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 (cNb6) binds to canine CTLA4 on the surface of transiently transfected HEK-293 T cells and canine peripheral blood mononuclear cells (PBMCs). For this study, we engineered a bispecific molecule (cNb6.Fc.OX40_{ECD}) by linking our previously identified anti-canine CTLA4 nanobody (cNb6) through the Fc region of canine IgG to the extracellular domain (ECD) of OX40 ligand. The open reading frame encoding the cNb6.Fc.OX40_{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 cNb6.cFc.OX40_{ECD} protein was expressed and purified from the ExpiCHO-S cells by affinity chromatography. The purified recombinant protein was evaluated for its ability to specifically bind canine CTLA4, FcγR1 and OX40 receptors. We also evaluated the ability of bispecific molecule to activate canine peripheral blood mononuclear cells (PBMCs).

Results. The open reading frame encoding the cNb6.Fc.OX40_{ECD} sequence was successfully synthesized from the Gene Universal and cloned into the mammalian expression vector pcDNA3.1/Hygro⁺. Recombinant plasmid was transiently transfected in ExpiCHO cells and bispecific molecule was successfully purified from supernatant using AKTA explorer by affinity chromatography. The reduced form of the bispecific molecule migrated at the predicted molecular weight of ~65kDa. These blots also revealed that CTLA4.Nb6.cFc.OX40_{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 and successful detection by anti-canine IgG Fc antibody. The purified cNb6.Fc.OX40_{ECD} successfully bind to the canine CTLA4, FcγR1 and OX40 receptors expressed on MDCK cells. The bispecific molecule induced T-cell activation and caused increased secretion of interleukin-2 (IL-2) and interferon-γ (IFN-γ) from canine PBMCs.

Conclusion. We have successfully engineered, expressed and purified a bispecific molecule, cNb6.Fc.OX40_{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, FcγR1 and OX40 receptors and induce secretion of IL-2 and IFN-γ from canine PBMCs.

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Investigation of Classification Accuracy and Clinical Management Trends of Corneal Ulcer Types Between General Practitioners and Ophthalmologists

SL Hoke¹, SD Boveland¹, and PA Moore¹

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

Introduction. Corneal ulcerations are classified by the amount of stromal loss as well as unique differences in their appearance and behavior. Diagnosis of these ulcers requires use of fluorescein stain and a good ophthalmic exam. Inaccurate diagnosis can result in the mismanagement of corneal ulcers, resulting in progression, infection, and possible perforation of the globe if stroma is lost, which can result in vision loss or loss of the eye. The purpose of this study is to evaluate if there is a significant difference in the accuracy of corneal ulcer type classification, and current management trends between veterinary general practitioners (GPs) and veterinary ophthalmologists.

Methods. A computer-based questionnaire was formulated utilizing 4 different corneal ulcer images for classification evaluation, diagnostics, medications, therapeutics, and re-check intervals for each ulcer type based on the respondents' responses. An alternative significance threshold of 0.01 was used. Fisher's exact test was used to compare classification accuracy of GPs and Ophthalmologists. Mann-Whitney test was used to examine comparisons between CE hours, last CE received, and re-check intervals between classified ulcer groups. Student's t-test with Satterthwaite correction was used to test if accuracy of classification correlated with age.

Results. 25 GPs and 122 ophthalmologists participated in the questionnaire. Overall accuracy in corneal ulcer classification from lowest to highest was anterior stromal (33%), indolent (86%), superficial (89%) and deep stromal (86%). There was a significantly higher correct percentage for ophthalmologists for both indolent (91.8% ophthalmologists versus 56% GPs) and superficial (93.44% ophthalmologists versus 68% GPs) ulcers. Accuracy for indolent and superficial corneal ulcer classification was significantly higher with increased ophthalmology-based CE hours (20-30 hours/year for ophthalmologists, 8-12 hours/year for GPs). Regarding the individual ulcer types, there was a significant difference ($p < 0.01$) between the antibiotics, anti-collagenases, and therapeutic procedures chosen between GPs and ophthalmologists.

Conclusions. Inconsistencies in the classification of corneal ulcers is common among GPs resulting in management practices that can be unfavorable to corneal health. Continued CE is essential for accurate diagnosis, and appropriate diagnostic and treatment of corneal ulcerations.

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Graduate Student Poster Presentations

Trichloroethylene (TCE) toxicity in zebrafish (*Danio rerio*): a multigenerational approach

Taylor Abernathy¹, Katharine Horzmann¹

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Trichloroethylene (TCE) is a volatile organic compound that has been used as a metal degreaser and as an industrial solvent. It is a significant legacy environmental toxicant and has been found at over half of the sites on the US EPA's National Priorities List. TCE is a known carcinogen and has been linked to reproductive dysfunction, central nervous system abnormalities, and congenital defects. TCE is thought to alter DNA methylation; however, the epigenetic effects are not well characterized. This study uses the zebrafish model (*Danio rerio*) to test the hypothesis that developmental exposure to ecologically relevant levels of TCE causes reproductive and multigenerational toxicity.

Methods. The F0 zebrafish were exposed as embryos to 0, 5, 50, or 500 parts per billion (ppb; µg/L) TCE, their F1 progeny were assessed for developmental toxicity. Reproductive toxicity was assessed in dose matched paired breedings via numbers of embryos produced and embryonic survival. Developmental toxicity was assessed in the F1 progeny at 120 hours post fertilization through a behavioral assay and morphologic measurements. Behavior was evaluated in larval zebrafish utilizing a visual motor response test.

Results. The reproduction evaluation demonstrated no significant differences in number of embryos produced nor embryonic survival. When considering the larval behavior assay, the 50 and 500 ppb progeny had a dose dependent decrease in distance moved, velocity, time spent moving, and counterclockwise turning frequency. The morphologic assessment demonstrated a decrease in the 50 ppb progeny head width and decreased head width to body length ratios in the 50 and 500 ppb progeny.

Conclusions. Although no overt reproductive toxicity was observed in the F0 generation, the behavioral and morphologic changes in F1 progeny support continued investigation of multigenerational TCE toxicity.

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Standing jejunal enterotomy for resolution of an ileal impaction

Sophie Boorman¹ and Fred Caldwell¹

¹Department of Clinical Sciences, Auburn University, AL

Introduction. In the equine patient, obstruction of the ileum by accumulation of dehydrated ingesta causes progressive distension of the small bowel proximal to the blockage resulting in increasing discomfort. These cases can represent a clinical conundrum; the presentation and clinical pathological values can be similar to those of horses afflicted with a strangulating lesion requiring surgical correction. Ileal impactions can be successfully treated medically, avoiding the expense and mortality risk of general anesthesia as well as decreasing hospital stay length, recuperation time and resulting in faster return to athletic activity. However, persistent impactions require surgical alleviation to avoid gastric or small intestinal rupture. In cases where an owner is discouraged from pursuing surgery due to apprehension of general anesthesia or due to financial limitations, a standing procedure to reduce the impaction would be of benefit. This case report describes the successful reduction of an ileal impaction via a right flank laparotomy and distal jejunal enterotomy.

Methods. A 22-year-old American Quarter Horse gelding presented with moderate signs of colic responsive to sedation. A presumptive preoperative diagnosis of ileal impaction was made based on physical examination, ultrasonography of the abdomen and clinical pathological parameters. Despite appropriate medical therapy and improvement to the clinical findings, the gelding continued to be uncomfortable. A standing right flank laparotomy with modified grid approach facilitated access to the ileum. The distal small intestine contained approximately 80 cm of dried ingesta from the ileocecal orifice extending aborally, confirming the preoperative diagnosis. The distal jejunum was exteriorized and an enterotomy performed, permitting lavage of the ingesta and resolution of the impaction. The enterotomy was closed in two layers with 2-0 polydioxanone followed by lavage and replacement into the abdomen. The abdominal wall was closed in three layers with 2 polyglactin 910, the subcutaneous tissue was closed using 2-0 polyglactin 25 and the skin was stapled. An aerosol bandage was applied, and the gelding returned to the stall.

Results. Postoperatively, the gelding received 22,000 iu/kg intravenous potassium penicillin every 6 hours, 6.6 mg/kg intravenous gentamicin every 24 hours and 1.1 mg/kg intravenous flunixin meglumine every 12 hours for 5 days. A 24-hour course of intravenous fluid therapy and lidocaine continuous rate infusion (1.3 mg/kg loading dose administered over 15 minutes followed by 0.05 mg/kg/min) was administered. The gelding was discharged on day 6 postoperative with instructions to complete a 14-day course of oral Trimethoprim sulfamethoxazole (30 mg/kg every 12 hours), staple removal in 10-14 days and 3 months of restricted athletic activity. At the time of follow up (1 month) the gelding was reportedly doing well.

Conclusions. The described surgical technique is a viable option for resolution of ileal impactions in horses non-responsive to medical management when financial constraints prohibit exploratory celiotomy under general anesthesia. Owners should be informed of the need to convert to general anesthesia if the presumptive preoperative diagnosis is not confirmed at surgery. Candidates for this procedure should be responsive to analgesia and be of reasonable temperament to facilitate standing surgery.

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**Identification of embryonic genomic imprinting pattern using RNA-seq analysis in a marsupial model *Monodelphis domestica***

Wenqi Cao¹, Kory C. Douglas², John L. VandeBerg³, Paul B. Samollow², Xu Wang^{1*} & Andrew G. Clark^{4*} (*: co-corresponding authors)

¹Auburn University & HudsonAlpha Institute for Biotechnology, ²Texas A&M University, ³University of Texas Rio Grande Valley, ⁴Cornell University, USA

Introduction. Genomic imprinting refers to a parental-specific gene expression phenomenon in diploid cells regulated by epigenetic modifications. Most imprinted genes are found to be expressed in brain and placenta and play fundamental roles in many aspects including neonatal growth and neurological process. In animals, genomic imprinting has been identified only in therian mammals. To date, there are 165 imprinted genes identified in human and 197 in mice, with 63 genes shared in both species. Among the imprinted genes identified in mouse and human, only 21 marsupial orthologs have been examined, and 8 were confirmed to be imprinted. Additional studies need to be performed to obtain more comprehensive conclusion about marsupial genomic imprinting pattern. In this research, we performed genome-wide RNA-seq analysis, together with histone modifications and DNA methylation profiling, to reveal the imprinting profile of *Monodelphis domestica*, and search for novel imprinted genes that may be specific in marsupial animals.

Methods. Reciprocal crosses and parental crosses were conducted using LL1 and LL2 strains of *Monodelphis domestica*. Fetal brain and extra-embryonic membranes (EEM) were collected. Total RNA was extracted and for RNA-seq library construction and Illumina sequencing. The RNA-seq data were analyzed to quantify gene expression level and parent-of-origin allelic expression. Sanger sequencing was used to confirm the genotypes in the parents and F1 animals. Pyrosequencing was performed to validate the imprinting status. Bisulfite-Sequencing and PyroMark assays were carried out to quantify DNA methylation at the promoter CpG sites.

Results. In addition to the known imprinted gene *Igf2r*, we found other 22 candidate imprinted genes in *Monodelphis domestica* according to the RNA-seq data, which exhibited monoallelic expression in fetal brain and/or EEM. 14 genes are verified to have informative heterozygous SNPs in both reciprocal F1 crosses, with trackable parent-of-origin-specific alleles to infer their imprinting status. Pyrosequencing confirmed the imprinted state for 10 of the 14 genes. 8 of them are novel genes found exclusively in *Monodelphis domestica*. Bisulfite-Sequencing and PyroMark assays revealed differentially methylated regions (DMRs) in promoter CpG island for the five coding genes among the candidate gene list.

Conclusions. We identified 8 marsupial-specific novel imprinted genes in fetal brain and placenta of the laboratory opossum, which were not known to be imprinted in any other species. We also discovered that the novel imprinted genes are regulated by differential promoter methylation, a similar mechanism as in eutherian imprinted genes. Our study will shed light on the evolution of imprinting and placental-fetal interaction in mammals.

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Topmouth culter (*Culter alburnus*) melanocortin-3 receptor: regulation of pharmacology by two isoforms of melanocortin receptor accessory protein 2

Ren-Lei Ji¹, Lu Huang², Yin Wang¹, Si-Yu Fan², Ting Liu¹, Min Tao^{1,2}, Shao-Jun Liu², Ya-Xiong Tao¹

¹ Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, United States

² State Key Laboratory of Developmental Biology of Freshwater Fish, College of Life Sciences, Hunan Normal University, Changsha, 410081, Hunan, PR China

Abstract

Melanocortin-3 receptor (MC3R) is a regulator in energy homeostasis, and interaction of MC3R and melanocortin receptor accessory protein 2 (MRAP2) plays a critical role in MC3R signaling of vertebrates. However, the physiological roles of MC3R in teleosts are not well understood. Herein we cloned topmouth culter *mc3r* with a 984 bp open reading frame encoding a protein of 327 amino acids. qRT-PCR indicated that *mc3r* had higher expression in the central nervous system. In the periphery, *mc3r* was expressed in liver, testis and head kidney in the male, whereas it was mainly present in skin and ovary in the female. Culter MC3R expressed in HEK293T cells could bind to five peptide agonists with a higher B_{max} than human MC3R (hMC3R). In addition, caMC3R had higher affinity to ACTH and lower affinity to D-Trp^{8-γ}-MSH compared to hMC3R. All agonists could stimulate caMC3R and increase dose dependently intracellular cAMP accumulation. Culter MC3R showed a higher constitutive activity in cAMP signaling, higher efficacies and R_{max} to α -MSH, des- α -MSH, ACTH and D-Trp^{8-γ}-MSH. Culter MRAP2a remarkably decreased cell surface expression but had no significant effect on total expression of caMC3R, whereas caMRAP2b had no effect on both cell surface and total expression of caMC3R. CaMRAP2a and caMRAP2b both remarkably decreased basal cAMP production. Furthermore, caMRAP2a significantly decreased B_{max} and R_{max} , while caMRAP2b did not affect ligand binding and agonist-stimulated cAMP. These results will promote further research on the physiological roles of MC3R in topmouth culter.

**Regulation of Melanocortin-5 Receptor Pharmacology by Two Isoforms of MRAP2 in Swamp eel (*Monopterus albus*)**

Ting Liu, Ya-Xiong Tao

Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University

Abstract

The melanocortin-5 receptor (MC5R) has been implicated in the regulation of exocrine gland secretion, immune regulation, and muscle fatty acid oxidation. However, its function in fish is not well established. Melanocortin-2 receptor accessory protein 2 (MRAP2) can modulate trafficking, ligand binding, and signaling of melanocortin receptors. Swamp eel (*Monopterus albus*) is an economically and evolutionarily important fish widely distributed in tropics and subtropics. To explore potential interaction between eel MC5R and MRAP2, herein we studied swamp eel *mc5r* and two isoforms of *mrp2*. Eel *mc5r* consists of a 1056 bp open reading frame encoding a protein of 351 amino acids. Eel *mrp2X1* consists of a 708 bp open reading frame encoding a protein of 235 amino acids, while eel *mrp2X2* consists of a 567 bp open reading frame encoding a protein of 188 amino acids. Interestingly, maMRAP2X2 lost the transmembrane domain. Phylogenetic analysis showed that maMC5R and maMRAP2s were closely related to piscine MC5Rs and MRAP2s. The maMC5R was further demonstrated to be a functional receptor and could be modulated by maMRAP2s in pharmacological studies. Three agonists, NDP-MSH, α -melanocyte stimulating hormone (α -MSH), and adrenocorticotropin (ACTH), could bind to maMC5R and induce intracellular cAMP production dose-dependently. Compared with human MC5R (hMC5R), maMC5R displayed a significantly decreased B_{max} , while higher binding affinity to α -MSH or ACTH. No significant difference in constitutive activity was observed between hMC5R and maMC5R. When stimulated with α -MSH and ACTH, maMC5R showed significantly lower EC_{50} and R_{max} than that of hMC5R. Two maMRAP2s had no effect on cell surface and total expression of maMC5R, whereas they significantly increased B_{max} . Only maMRAP2X2 significantly decreased the binding affinity of ACTH. Both maMRAP2X1 and maMRAP2X2 significantly reduced R_{max} but did not affect EC_{50} in response to α -MSH or ACTH. The availability of maMC5R pharmacological characteristics and the modulation by maMRAP2s will facilitate the investigation of its function in regulating diverse physiological processes in swamp eel.

Keywords: *Monopterus albus*, MC5R, MRAP2, Binding, Signaling

**Effects of lactocrine insufficiency from birth on the uterine transcriptome and gut microbiome in neonatal pigs on postnatal day 14**

Xiaolei Ma¹, Wenqi Cao¹, Clay A. Lents³, Carol A. Bagnell⁴⁺, Frank F. Bartol^{1,2+} and Xu Wang^{1,2,4+}

¹College of Veterinary Medicine, Auburn University, AL; ²Alabama Agricultural Experiment Station; ³USDA-ARS, US Meat Animal Research Center, Clay Center, NE; ⁴Department of Animal Sciences, Rutgers University, New Brunswick, NJ; ⁵HudsonAlpha Institute for Biotechnology, Huntsville, AL (⁺co-corresponding authors)

Introduction. Maternal effects on offspring phenotype do not end at birth. Bioactive factors are transmitted from mother to nursing offspring through colostrum (first milk) via a lactocrine mechanism. The lactocrine hypothesis posits that disruption of lactocrine signaling from birth will have both short- and long-term effects on the postnatal developmental program with lasting consequences. Previous research established that lactocrine insufficiency from birth in pigs, indicated by reduced serum immunoglobulin immunocrit (iCrit) ratios in nursing piglets on the day of birth (postnatal day 0, PND0), altered the postnatal uterine developmental program and reduced lifetime fecundity in adult, neonatally lactocrine insufficient females. Here, the objectives were to determine the effects of lactocrine insufficiency from birth on the gut microbiome and uterine transcriptome in gilts on PND14 using whole-genome shotgun metagenomic and transcriptomic sequencing approaches.

Methods. Crossbred gilts, born and maintained at the US Meat Animal Research Center (Clay Center, NE), were assigned to low (n=12) or high (n=10) iCrit groups based on iCrit ratio values determined on PND0. To characterize the influence of lactocrine input on PND14 gut microbiota, whole-genome shotgun (WGS) metagenomic sequencing was performed on gut content collected from the jejunum, ileum, and rectum on PND14. MEGAHIT was used for metagenome assembly and Kaiju for microbial gene annotation and taxonomy classification. Lactocrine effects on the PND14 uterine transcriptome in low/high iCrit gilts involved extraction of total RNA from each uterine sample using a Zymo Quick-DNA/RNA Miniprep Plus kit according to the manufacturer's instructions. RNA sequencing libraries were constructed using NEBNext Ultra II Directional RNA Library Prep Kit and sequenced on the Illumina NovaSeq Platform. After QC, high-quality reads were mapped to the swine reference genome (build Sus_scrofa11.1) using TopHat2. Gene read counts were summarized using cufflinks. Differentially expressed genes were identified using EdgeR with a significance cut-off of FDR < 0.1 and |Fold Change| > 1.5.

Results and Conclusions. The gut microbiome analysis generated 713 billion bp of microbial sequences from 24 fecal samples. 1.6M reference bacteria contigs were obtained through *de novo* metagenome assembly. 2.8M prokaryotic genes were predicted in these reference swine microbial contigs. The most abundant phyla in the adult swine fecal microbiome are *Firmicutes* and *Bacteroidetes* consistent with PND14 microbiome. Interestingly, enrichment of *Bacteroidetes* ($P=0.002$, paired Mann-Whitney U test) and reduced abundance of *Actinobacteria* ($P=0.03$, paired Mann-Whitney U test) was identified in high-iCrit as compared to low-iCrit samples. In uterine RNAseq analyses, three upregulated genes (*PRG4*, *C3*, and *KLK10*) in low iCrit groups were identified after multiple testing correction. Overall, 148 genes displayed differential expression between the two groups at a nominal P -value cutoff of 0.05, including the *FOXA2* gene, known to be downregulated in low-iCrit uterus samples. Results confirm and extend earlier observations indicating that lactocrine deficiency from birth affects neonatal uterine gene expression patterns and the gut microbiome.

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**Invasive blood pressure in anesthetized horses: does the artery site matter?**

Monica Midon^{1,2}, Diego I. Yamada¹, Darcio Zangirolami Filho¹, Walter H. Feringer Junior¹, Gabriel Conde¹, and Erik Hofmeister²

¹School of Agricultural and Veterinarian Sciences, Sao Paulo State University, SP, Brazil

²Department of Clinical Sciences, Auburn University, AL

Introduction. Invasive blood pressure (IBP) measurement is strongly recommended during general anesthesia in horses. This study evaluated the agreement between IBP values measured in three different arteries and by oscillometric device (NIBP) with the facial artery (FA).

Methods. Six horses (424.2 ± 40.7 kg) were sedated with xylazine (0.6 mg kg⁻¹, intravenous - IV), induced with ketamine (2 mg kg⁻¹ IV) and midazolam (0.1 mg kg⁻¹ IV), and maintained with isoflurane (1.2 MAC) for 90 minutes in lateral recumbency. FA, auricular artery (AA), lateral digital artery (LDA), and metatarsal artery (MA) were catheterized, an adult cuff was positioned on the tail, and IBP and NIBP values were recorded at 30, 45, 60, 75, and 90 minutes of anesthesia. Data were analyzed with Shapiro-Wilk and Repeated-Measures Bland-Altman.

Results. Values for FA (mmHg) were 85.4 ± 10.3 for SAP, 70.6 ± 9.3 for MAP, and 58.5 ± 9.5 for DAP. Mean bias and 95% Limit of agreement (LOA) for AA were -1.7 (-29.0 to 25.6), 2.2 (-18.4 to 22.9), 1.9 (-18.2 to 22.0), for LDA: 1.4 (-25.1 to 27.8), 2.9 (-19.3 to 25.0), 2.3 (-18.4 to 22.9), for MA: -3.2 (-28.3 to 22.0), 2.7 (-16.6 to 22.1), 4.2 (-13.8 to 22.2), and for NIBP: -5.7 (-28.3 to 16.9), 7.9 (-9.5 to 25.3), 17.2 (-2.4 to 36.8), for SAP, MAP and DAP respectively.

Conclusions. There is good agreement for IBP values among the arteries evaluated, specially for MAP values, however NIBP method results in inaccurate blood pressure values in horses.

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**Capnography waveform during Anesthetic Index determination in chickens (*Gallus gallus domesticus*)**

Monica Midon^{1,2}, Diego I. Yamada¹, Bruno W. Minto¹, Andre Escobar¹, and Erik Hofmeister²

¹School of Agricultural and Veterinarian Sciences, Sao Paulo State University, SP, Brazil

²Department of Clinical Sciences, Auburn University, AL

Introduction. The anesthetic index (AI) is the ratio between the expired concentration of an inhalant anesthetic required to induce apnea and its minimum anesthetic concentration. The isoflurane AI in birds is reported as 1.65 for ducks and 2.80 for chickens, however capnographic waveform changes were not reported. The aim of this abstract is to report the variation of capnography observed during the AI determination on chickens.

Methods. Six adult females healthy *Hy-line w36* chickens weighting 1.11 ± 0.06 kg were anesthetized twice with at least one week for washout. On the first anesthesia, the MAC of isoflurane was determined using the bracketing method. On the second anesthesia, the AI was determined adjusting the end-tidal isoflurane (FeISO) initially to 2.0 individual MAC followed by progressive increases of 0.5 MAC, until apnea was reached. Isoflurane was discontinued, birds received respiratory support and were allowed to recover from anesthesia. During both anesthesia episodes, heart rate, ECG, FeISO, systolic arterial pressure, cloacal temperature, end-tidal CO₂ and capnography were monitored. Physiological data were evaluated with a mixed-effect model with Greenhouse-Geisser correction, followed by Tukey's test.

Results. No complications attributable to anesthesia procedures were observed in any chicken. The isoflurane MAC, FeISO at apnea, and AI were $1.36 \pm 0.10\%$, $3.82 \pm 0.40\%$, and 2.80 ± 0.26 , respectively. HR and ETCO₂ were significantly higher during AI determination (HR = 250 ± 50 bpm, ETCO₂ 35 ± 6 mmHg) than in MAC determination (HR 320 ± 34 bpm, ETCO₂ 103 ± 11 mmHg). During MAC determination, capnography waveforms followed the normal pattern observed on mammals. During AI determination, an increasing gap between the caudal and cranial respiratory movement was observed in accordance with the isoflurane concentration increase. This reflects a change on the capnography wave pattern, starting with a shape similar a notch on the middle of phase III at 2.0 MAC, progressing for a double-wave at 2.5 MAC, and finally for a completely separation of waves for the caudal and cranial respiratory movement at 3.0 MAC.

Conclusions. There is a distinct change in the pattern of respiratory movement and consequently capnography waveform in chickens related to the increase in isoflurane end-tidal concentration. Observing any these patterns could be indicative of an excessive anesthetic depth.

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Elucidating the immunomodulatory mechanism of growth-promoting biodegradable microparticles using RNA-seq in mouse macrophages

Haolong Wang¹, Ludmilla Kaltenboeck¹, Xu Wang^{1,2+} & Bernhard Kaltenboeck¹⁺

¹Department of Pathobiology, College of Veterinary Medicine, Auburn University

²HudsonAlpha Institute for Biotechnology, Huntsville, USA

(⁺co-corresponding authors)

Introduction. The global population will reach 10 billion by 2050, and the demand for animal protein will increase by 56%. To meet this challenge, food animal production must increase by 60%. Animal growth rates and feed efficiency must be greatly improved to achieve this. Antimicrobial growth promoters (AGPs) is the common practice to combat persistent asymptomatic infections and promote growth. However, the overuse of low-dose AGPs accelerates the development of resistant bacteria. Furthermore, up to 90% of antimicrobials are excreted to the environment, threatening the environment and human health. To address this, Dr. Kaltenboeck developed and patented proprietary biodegradable microparticles as a non-antibiotic growth promotant, which is capable of saving up to 34.7% of feed and accelerating growth at an exceedingly low concentration of 0.2g per ton of feed. The mechanisms of the microparticle action remain to be elucidated.

Methods. To investigate the underlying mechanism of the microparticles, **J774A.1** macrophages from BALB/c laboratory mice were treated by the microparticles (MP), lipopolysaccharide (LPS), or both compounds (LPSMP). LPS is the major component of the outer membrane of Gram-negative bacteria triggering Toll-like receptor 4 and innate immune response. We conducted RNA-seq experiments with three biological replicates in the control and each treatment group. After quality control and adapter trimming, the RNAseq reads were aligned to mouse reference genome mm10 using TopHat. Gene read counts were summarized by HT-Seq. We used an R package edgeR for gene count normalization and differentially expressed gene (DEG) identification. Heatmap of gene expression pattern was plotted using pheatmap package. Gene ontology (GO) enrichment analysis was performed by the ClueGo App in Metascape. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted by Metascape.

Results and Conclusions. A total of 1,267 DEGs were discovered in the MP group, including 631 upregulated and 636 downregulated genes (adjusted $P < 0.05$). Gene ontology analysis of the upregulated genes identified the immune system and stress response-related function categories. We conclude that MP causes significant expression changes for hundreds of genes, fine-tuning the immune profile in macrophages. We then examined the induced genes under LPS challenge, defined by little to no expression in control samples (RPKM < 1) and extremely high fold change in the LPS group (FC > 4). The expression levels are slightly increased in the MP group, suggesting a slight increase in expression preparing for potential infection. On the other hand, the average expression fold changes in the LPSMP group is significantly lower than LPS alone, indicating the response to LPS stimulation is reduced under the presence of MP. In conclusion, our preliminary results are consistent with that the MP is able to fine-tune the immune system by slightly boosting specific immune pathways under non-infected conditions and reducing the unnecessary response when bacterial pathogens are present.

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**Phylogenomic analysis of the intracellular symbiont *Wolbachia* in arthropods reveals genome evolution and interclade recombination events**

Xiao Xiong^{1,2*}, Xiaozhu Wang^{1*}, Wenqi Cao¹, Chao Zhang², John H. Werren^{3†} and Xu Wang^{1,4,5†}

¹Pathobiology, Auburn University, ²Tongji University, ³Biology, University of Rochester, ⁴Alabama Agricultural Experiment Station, ⁵HudsonAlpha Institute for Biotechnology
(*co-first authors; †co-corresponding authors)

Introduction. *Wolbachia* are widespread obligate intracellular bacteria that mediate many important biological processes, such as male-killing, parthenogenetic induction, and cytoplasmic incompatibility. More than half of the arthropod species are infected by *Wolbachia*. Most of the previous research on recombination has focused on five MLST genes, *Wolbachia* surface protein (*wsp*), and 16S rRNA, or for a subset of genomes from the A-D and F supergroups. In this study, we performed phylogenomic analyses on 33 annotated *Wolbachia* genomes in the A, B, C, D, E and F supergroups to identify conserved core gene set and detect potential recombination events across the supergroups.

Methods. We conducted phylogenomic analysis using 33 annotated *Wolbachia* genomes. Homologous genes and ortholog clusters were determined by using OrthoFinder v1.1.8 with default settings. The interclade recombination screening method was developed to detect candidate recombination events between supergroups. The Pearson correlation coefficient of estimated evolutionary divergences with the identified core genes and the MLST gene set was calculated with Hmisc package in R.

Results. 210 single-copy ortholog groups were identified among all 33 *Wolbachia* genomes. Only 14 inter-supergroup recombination events were discovered in five genes (2.4%) among these 210 core genes. Interestingly, they have occurred not only between A and B supergroups (9 events), but also between A and E supergroups (5 events). Comparisons of strain divergence using the five genes of the MLST system show a high correlation (Pearson correlation coefficient $r = 0.98$) between MLST and whole genome divergences.

Conclusions. This finding suggests a relatively low frequency of intergroup recombination in *Wolbachia*. Maintenance of such selective transfers between supergroups suggests possible roles in *Wolbachia* infection related functions. Concordance of MLST and whole genome divergence indicates that MLST is a reliable method for identifying related strains and the identified core gene set is informative for strain identification. The interclade recombination screening method developed in our study will serve as a valuable foundation for investigation of recombination and genome evolution in *Wolbachia*.

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**An ex-vivo study of a minimally invasive technique for tenotomy of the tibial insertion of the semitendinosus muscle in horses**

Zetterström S¹, Boone L¹, Weatherall K¹, Caldwell F¹

¹Department of Clinical Sciences, Auburn University, AL

Introduction. Fibrotic myopathy is a mechanical hind limb gait abnormality that develops due to fibrosis and adhesions of the semitendinosus, semimembranosus, biceps femoris or gracilis muscles. Affected cases may require tenotomy of the tibial insertion of the semitendinosus muscle to relieve the mechanical gait defect. Traditional approaches describe a 4-10 cm long incision for isolation of the tibial insertion of the semitendinosus tendon for tenotomy. Using an ultrasound assisted minimally invasive technique for semitendinosus tenotomy may decrease soft tissue dissection, and therefore the potential decrease postoperative complications. The study objective was to describe a minimally invasive technique for semitendinosus tenotomy using ultrasound guidance and evaluate procedural complications.

Methods. Sixteen hind limbs from eight equine cadavers were included in the study. The hindlimbs were extended forward until palpation of the tibial insertion of the semitendinosus tendon on the caudomedial tibia was appreciated. A stab incision was made through the skin, subcutaneous tissue and crural fascia just distal to the distal border of the semitendinosus tendon caudal to the palpable tibial insertion. A pair of mosquito hemostatic forceps were introduced under the semitendinosus tendon in a distal to proximal direction and palpated under the skin proximal to the tendon. Ultrasonography was performed confirming that the tendon was isolated between the instrument and ultrasound probe. The forceps were removed and an arthroscopic retrograde knife was passed along the dissection plane created with the forceps. Following transection of the tendon, retraction of the tendon edges was appreciated via palpation and confirmed with ultrasound. All cadaver limbs were then dissected to observe complete transection of the tendon and identify iatrogenic trauma to the surrounding soft tissue structures. Variables were described by using median and interquartile ranges for continuous data and counts for categorical data.

Results. Tenotomy of the tibial insertion of the semitendinosus tendon was complete in all limbs. Complete transection of the tendon resulted in a palpable gap forming between the tendon edges which was confirmed with ultrasound observation of the transected tendon. Iatrogenic superficial lacerations to the underlying gracilis muscle or its fascia was apparent in 3/16 limbs.

Conclusions. This report describes a new technique utilizing ultrasonographic assistance for instrument positioning to achieve precise semitendinosus tenotomy through a minimally invasive approach. Minimally invasive surgical techniques have been shown to decrease soft tissue trauma and result in reduced postoperative pain and morbidity as well as a faster return to function. The semitendinosus tendon was easily identified and evaluated with ultrasound because of the size and superficial location of the tibial insertion. The technique was easy to use and no major iatrogenic trauma was observed supporting its consideration as an alternative technique for the surgical management of horses with fibrotic semitendinosus myopathy following in vivo studies.

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**An ex-vivo Study of Double Hemitenotomy for Lengthening of the Equine Deep Digital Flexor Tendon**

Zetterström S¹, Boone L¹, Weimar W², Farag R³, Caldwell F¹

¹Department of Clinical Sciences, Auburn University, AL

²Department of Kinesiology, Auburn University, AL

³Department of Polymer and Fiber Engineering, Auburn University, AL

Introduction. Deep digital flexor (DDF) tenotomy is performed to diminish rotational forces on the distal phalanx and associated pain in horses with refractory laminitis. The procedure results in a guarded prognosis for athletic use. Consequently, a surgical procedure for DDFT lengthening rather than complete transection may be advantageous and allow for better recovery of tendon function compared to traditional tenotomy. The objective of this study was to develop a double hemitenotomy (DHT) technique to lengthen the equine DDFT.

Methods. The study was divided into two parts using cadaveric models. Pairs of forelimbs were collected from horses euthanized for reasons other than laminitis or DDF tendonitis. One proximal and one distal hemitenotomy incorporating 50% of the tendon thickness were created using a Lichy teat knife within DDFTs just distal to the insertion of the accessory ligament of the DDFT and 3 cm further distal. In part 1, cadaveric DDFT pairs (n=30) were utilized. Two hemitenotomy incisions were created in one tendon while the other served as an intact control. Monotonic tensile load was applied to a maximum of 500 kg using an Instron Universal Testing Machine. Tendon lengthening, load reduction with hemitenotomy and load at failure were recorded by the Instron. In part 2, cadaveric forelimb pairs (n=16) were subjected to DHT followed by complete tenotomy (CT) under monotonic compressive load applied by the Instron. Independent and paired sample t-tests as well as repeated measures ANOVA were used to investigate differences between tensile strength, tendon lengthening and load reduction of DHT and CT tendons. Significance was set at $p < 0.05$.

Results. DHT resulted in significant DDFT lengthening and load reduction in both isolated tendons and in the complete limbs. Less lengthening was achieved with DHT compared to CT ($p < .001$). No difference in load reduction between DHT and CT was observed ($p = 0.65$). The first hemitenotomy resulted in a smaller load reduction compared to the second hemitenotomy. In part 1, 12/15 (80%) of the isolated hemitenotomized tendons failed at a mean \pm SD tensile load of 173.8 ± 127.6 kg compared with none of the intact tendons ($p = 0.003$). None of the tendons failed in part 2.

Discussion. Findings of the cadaveric study support reduction in load of the DDFT following double hemitenotomy comparable to complete tenotomy. Significant increases in tendon length and decreases in load of the DDFT were only achieved after the second hemitenotomy, supporting use of a DHT technique. DHT reduced tensile strength of the DDFT, but load at failure did not exceed reported DDFT load at stance. Failure of the DDFT after DHT was not observed in the intact limbs, likely due to re-distribution of load onto the remaining tendons and ligaments within the limb. Therefore, results of this study support further investigation into the use of double hemitenotomy and its effects on tendon lengthening, load reduction and healing in the clinical setting. Limitations of the study include use of models that were modified to accommodate the purpose of the study and the ex-vivo nature of the study not necessarily reflecting dynamic changes during in vivo conditions.

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Whole-genome shotgun metagenomic analysis revealed gut microbiome changes in beef cattle under endophyte-infected tall fescue toxicosis

Yihang Zhou^{1,2*}, Gaston Alfaro^{3*}, Wenqi Cao¹, Soren P. Rodning³, Russell B. Muntiferung³, Wilmer J. Pacheco⁴, Chao Zhang², Sonia Moisés^{3†} & Xu Wang^{1†}

¹Pathobiology, Auburn University & HudsonAlpha Institute, ²Tongji University, ³Animal Sciences, Auburn University, ⁴Poultry Science, Auburn University.
(*co-first authors; †co-corresponding authors)

Introduction. Gut microbiome correlates strongly to hosts' health in livestock. In cattle, the rumen and the gut microbiome are critical to digestion and assimilation, affecting the meat production rate. Tall fescue (*Lolium arundinaceum*), a perennial grass commonly infected with an endophyte (*Epichloë coenophiala*), is grown on over 14 million hectares in the Southeastern US. The fungal endophyte results in high concentrations of ergot alkaloids (i.e., ergovaline), inducing fescue toxicosis. The economic losses due to fescue toxicity to the forage-based livestock industry are estimated to be close to 3.5 billion dollars. Gut microbiota is directly related to animal nutrition and health, affecting every aspect of animal physiology, including metabolism, immunity, inflammation, digestion, and behavior. Although the cattle rumen microbiome has been studied extensively, the fecal microbiota and its response to ergovaline are not well understood. Here, we performed the first comprehensive study of the gut microbiome in the context and beef cattle toxicosis.

Methods. A total of 16 fecal samples were collected from 3 cows and 5 heifers at the beginning and the end of a 30-day tall fescue seed feeding experiments. These cattle were genotyped at *XKR4* and *DRD2* genes, known loci to affect tall fescue toxicosis susceptibility. Four animals were susceptible, and four were resistant. We made whole-genome shotgun metagenomic sequencing libraries and sequenced them on an Illumina NovaSeq6000 machine. We applied a standard pipeline for data preprocessing, including read filtering and trimming, *de novo* metagenome assembly, taxonomy annotation, abundance quantification. LEfSe was used to determine the differential taxonomy composition before and after treatment. The microbial genetic pathways were analyzed with HUMANN2. Our gut microbiome profiles were also compared with the rumen metagenomes in the public domain.

Results and Conclusions. In this study, we generated a total of 157 Gbp of metagenomic sequences, with 65.4 million high-quality reads per sample. Host and viral sequence contaminations were removed before performing *de novo* assembly of the microbial contigs. The reference assembly contains 16,580,560 microbial contigs with a total metagenome length of 13.1 Gbp. Gene annotation analysis identified a total of 21,950,894 bacterial genes in the gut microbiome after redundancy removal using CDHIT. We aligned the microbial reads from all 16 individual samples to our reference gut microbiome metagenome assembly, and the average mapping rate is 91.7%. The most abundant phyla are *Firmicutes* and *Bacteroidetes*. We discovered a 7.5% enrichment of *Firmicutes* ($P=0.013$) and an 18.1% reduction of *Proteobacteria* after treatment ($P=0.006$, paired Mann-Whitney U test). As the only decreased phyla, the overrepresentation in *Firmicutes* is primarily driven by a single species, *Ruminococcaceae bacterium P7*, with a 15.8% increase after treatment. Interestingly, this species is from one of the seven core rumen microbiome genera *Ruminococcaceae*. When the microbial reads were aligned to the rumen reference microbiome, the mapping increased 17% after treatment, suggesting a significant enrichment of rumen microbes. Taken together, our results indicate potential damage to the rumen microbiota in fescue toxicosis.

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