

PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter



October 30, 2013

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE



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

welcomes you to our

**PHI ZETA RESEARCH DAY FORUM
October 30, 2013**

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

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

zoetis

FOR ANIMALS. FOR HEALTH. FOR YOU.





PHI ZETA RESEARCH DAY FORUM

OCTOBER 30, 2013 - JOY GOODWIN RUDD STUDENT CENTER

8:30: BREAKFAST Buffet - Goodwin Center Lobby

9-11: MORNING Presentations - Overton Auditorium

Graduate Students

- 9:00 Marta Barba Experimental inoculation of house flies, *Musca domestica* L., with *Corynebacterium pseudotuberculosis* serovar *equi*
- 9:15 Fernanda Cesar Efficacy of ketamine hydrochloride administered as a basilar sesamoid nerve block in alleviating foot pain in horses caused by natural disease
- 9:30 Margaret M. Salter Characterization of Late Outgrowth Endothelial Colony Forming Cells in Adult Horses
- 9:45 Patricia M. Beadlescomb Bony Abnormalities in Cats with GM2 Gangliosidosis
- 10:00 Manuel F. Chamorro Assessment of vaccine efficacy in early weaned beef calves challenged with bovine viral diarrhea virus (BVDV)
- 10:15 Kh. Shamsur Rahman Defining monospecific functional immunodominant B-cell epitopes of the nine *Chlamydia* species
- 10:30 E. U. Chowdhury Synthetic Peptide Antigens for Molecular Serology of Bovine Infections with *Chlamydia pecorum*
- 10:45 Eileen K. Jenkins The effects of oral metronidazole administration on olfaction and detection capabilities of explosive detection dogs
- 11:00 Jack J. Kottwitz A Survey-based Investigation of the use of NSAIDs in Captive Elephants

11-12: POSTER Presentations- Goodwin Center Lobby

11:00 - 12:00 Poster Session – Presenters are present from 11:00 – 12:00

11:30-12:30: LUNCH Buffet - Goodwin Center Lobby



12:30-6: AFTERNOON Presentations - Overton Auditorium

- 12:30 Farruk M. Lutful Kabir miRNA Expression Profile and Regulation in Spontaneous Canine Mammary Tumor Models with INK4 Tumor Suppressor Defects
- 12:45 Kamoltip Thungrat Characterization of Virulence of Uropathogenic Canine *Escherichia coli*
- 1:00 Marike Visser Identification of possible drug interactions between levetiracetam and other ant-epileptic drugs in epileptic canine patients

Veterinary Students

- 1:15 Brittany Ball Cardiovascular and Respiratory Effects of Dexmedetomidine in Isoflurane Anesthetized Raptors
- 1:30 Jillian Costello Serum and Cerebrospinal Fluid Concentrations of Generic Levetiracetam after Multiple Oral Dosing in Healthy Adult Horses
- 1:45 Charles N. Hewett Pharmacokinetics of Levetiracetam in Foals

2:00–2:30: BREAK Buffet - Goodwin Center Lobby

- 2:30 Amy Ramos Hematological changes, cytological effects and microbiological growth secondary to 3-12 days of indwelling intrathecal catheter use in horses
- 2:45 Amy Sanders Evaluation of the digital cushion and weight-bearing surface of the bovine foot in response to modifications in housing and rearing practices of calves
- 3:00 Fallon M. Segarra Effects of Detomidine and Butorphanol on Intra-abdominal and Abdominal Perfusion Pressures in Normal Horses

Post-graduate/Faculty

- 3:15 J. Gard Comparison of the Ability of a Novel Umbilical dip, Super7+™ Navel Dip, verses that of 7% Tincture of Iodine to Desiccate the Umbilical Remnant in Neonatal Holstein Dairy Calves
- 3:30 H. Gray-Edwards Amelioration of neurologic disease after AAV-gene therapy in a Sheep Model of Tay-Sachs Disease



4:00: KEYNOTE LECTURE

Host-bacterial interactions in the gastrointestinal tract:
A cross species approach

Kenneth Simpson, BVM&S, PhD, DACVIM, DECVIM
Professor of Small Animal Medicine, Cornell University



Dr. **Kenneth Simpson**, is a Professor of Small Animal Medicine at Cornell University, College of Veterinary Medicine. Dr. Simpson earned his BVM&S from the University of Edinburgh, Scotland in 1984. In 1988 he received his PhD degree at the University of Leicester in England and completed a rotating internship in Small Animal Medicine and Surgery at the University of Pennsylvania School of Veterinary Medicine in 1989. Dr. Simpson completed his residency in Small Animal Internal Medicine at Ohio State University in 1991 and is board certified in small animal internal medicine. He is a Past President of the Comparative Gastroenterology Society and a recipient of the Pfizer Award for Research Excellence, the BSAVA Bourgelat Award for outstanding contributions to the field of small animal practice, and the AVMF/AKC Career Achievement Award in Canine Research.

Dr. Simpson's overall goal is to understand the interplay between enteric bacteria and the host that leads to inflammatory bowel disease (IBD), and to effectively translate laboratory based studies to improve detection, therapy and ultimately prophylaxis. IBD is a major cause of morbidity in the United States. Methodologically, he applies contemporary culture independent and molecular microbiological approaches to study host bacterial interactions in animals, humans and *in vitro* systems to achieve these goals. His laboratory has discovered that Adherent and invasive *E. coli* (AIEC) are associated with IBD across species (people, dogs and mice). Current studies are focused on determining what genes make an AIEC an AIEC, defining the selection pressures in the enteric microenvironment that drive the proliferation of AIEC, and elucidating bacterial and host attributes that enable adherence, invasion and intracellular survival.



PROGRAM

PLEASE JOIN US FOR THE INDUCTION AND AWARDS BANQUET

Everyone is invited! Inductees, presenters and their (1) guest are guests of Phi Zeta. Tickets for everyone else are \$40/person – Tickets available ahead of time or at the door. Deposit check for ticket with Dr. Eleanor Josephson, 109 Greene Hall, or at the banquet in the **Auburn Hotel & Conference Center**.

6:00 **BANQUET at the AU Hotel & Conference Center, Ballroom B**

6:30 **COCKTAILS at cash bar**

7:00 **DINNER**

7:30 **INDUCTION of new Phi Zeta Members**

AWARD CEREMONY to honor winners of the Platform and Poster Competitions



Posters

Veterinary Students

Cori Blair	Evaluation of the mucin-like macroglycopeptide region encoded by the feline GPIIb gene
Marta Barba	Transmission of <i>Corynebacterium pseudotuberculosis</i> in horses by house flies: serologic response
Matthew Boothe	Non-Steroidal Anti-Inflammatory (NSAID) Use in Megavertebrates: A Survey
Kathleen E. Burris	Defining the Lactocrine-Sensitive Neonatal Porcine Uterine Transcriptome Using RNAseq
Maggie E. Canning	Elastic Modulus, Fluid Load Fraction Characterization of Equine Articular Cartilage
Peter F. Canning	Histopathologic and Enzymatic Characterization of GM2 Gangliosidosis of Jacob Sheep
Blake B. DeWitt	<i>In vitro</i> vascular tube formation and uptake of acetylated low density lipoprotein diminish as passage number increases in equine endothelial colony forming cells
Alison R. Emmert	Role of the HCA ₂ Receptor during the Physiological Response to Fasting in Mice
Glennie Ferniany	Stearidonic Acid Enhances the Chemosensitivity of Canine B-Cell Lymphoma Cells by Downregulating the Activity of Multidrug Transporters
Leah N. Guidry	Predictive modeling of the equine heel
D. Andrew Hestad	Effect of Nucleophilic Thiols Penicillamine and N-acetylcysteine on Przewalski (<i>Equus ferus przewalskii</i>) and Domestic (<i>Equus caballus</i>) Horse Sperm Motility
Nicole D. McAdams	Susceptibility of Canine Mast Cell Tumors to Adenoviral Vector Delivery
Emily Mysinger	Pilot Study: Attempted Immunoneutralization of Peripheral Kisspeptin and Effects on Female Rat Fecundity
Carissa J. Norquest	Isolation and Evaluation of Four Distinct Dendritic Cell Populations from Circulating Canine Blood
Olga C. Norris	Characterization of the HCA ₂ Receptor in Cats
Justin Padgett	Age-Related Influence on Ocular Live Attenuated IBV Vaccine Induced Immune Response and Immune Protection



Veterinary Students (continued)

Amy Sanders	Evaluation of the digital cushion and weight-bearing surface of the bovine foot in response to modifications in housing and rearing practices of calves
Ashley Sharpe	Activation of the transcription factor NF-κB by the dietary polyphenol curcumin
Kelcie M. Theis	The Effect of Freezing on the Elastic Modulus and Fluid Load Fraction of Equine Articular Cartilage
Caitlin H. Trebelhorn	The plant-based Omega-3 stearidonic acid (SDA) enhances antitumor activity of doxorubicin (Dox) in human prostate cancer cell lines
Jacqueline A. Vacca	The Effects of Anemia on Thromboelastography
Kayla Waler	The Epididymis as a Target for Environmental and Hormonally-Active Chemicals

Graduate Students

Brett Augsburger	Development of a Proteoliposome Nanocarrier for Mitochondrial Gene Delivery
J. E. Bayne	Evaluation of behavioral changes in cattle using three-dimensional accelerometers during experimental infection with bovine viral diarrhea virus
Noelle S. Bergman	Local toxicity and efficacy of marginal excision combined with intralesional cisplatin bead placement for treatment of soft tissue sarcomas
Allison M. Bradbury	Therapeutic Enzyme Distribution After AAV-mediated Gene Therapy in Normal and GM2 Gangliosidosis Cats
M. Wesley Campbell	Pharmacokinetics of Cyclophosphamide in Horses
Patrick Flannery	Diindolymethane activates pregnane xenobiotic receptor-mediated CYP3A4 gene expression
Natalie S. Fraser	Effects of Chlorhexidine Hydrochloride Intrauterine Suspension Administration in Normal Mares
Rebecca George	Survival of Dogs with Nasal Lymphoma Treated with Various Radiation Protocols: 13 Cases
Heather D. Gossett	Comparative effects of phenobarbital and zonisamide on clinical patients being treated for epilepsy



Graduate Students (continued)

Madhukar Lohani	Elucidate the neuroprotective mechanisms of <i>Scutellaria lateriflora</i>
Farruk M. Lutful Kabir	Differential Expression Pattern and Recurrent Defects in p16 Tumor Suppressor Gene Locus (p16/INK4A/B) in Spontaneous Canine Mammary Tumor and Melanoma Models
Victoria J. McCurdy	Adeno-associated virus-mediated gene therapy provides long-term stabilization of neurologic disease
Vicky J. Payne	Comparison of the Images of the Canine Middle and Inner Ear using High Field and Ultra High Field MRI
James F. Shirley	<i>Salmonella</i> Enteritidis Bovine Isolate Characterization and Bacteriophage Cocktail Selection
Jameson Sofge	Pharmacokinetics and pharmacodynamics of Leflunomide and its metabolite, teriflunomide (A77-1726), in dogs

Undergraduate Students

Katharyn Brennan	Effects of Medroxyprogesterone Acetate on Uterine Development in the Dog
Sarah A. Hashimi	Branching Characteristics of Late Outgrowth Endothelial Colony Forming Cells in Adult Horses

Post-graduate/Faculty

S. H. Duran	Efficacy of Various Topical Formulations against <i>Tritrichomonas foetus</i>
J. Gard	Comparison of the Ability of a Novel Umbilical dip, Super7+™ Navel Dip, verses that of 7% Tincture of Iodine to Desiccate the Umbilical Remnant in Neonatal Holstein Dairy Calves
Stephen L. Gulley	Mucosal and Systemic Immune Responses Induced after Ocular Avian Coronavirus Vaccination are evaded by a Field Strain
S. R. Kitchens	Bacteriophage Resistant Mutant of <i>Salmonella</i> Newport and Disease in Experimentally Infected Calves



Graduate Student Platform Presentations

Experimental inoculation of house flies, *Musca domestica* L., with *Corynebacterium pseudotuberculosis* serovar *equi*

Marta Barba¹, Allison J. Stewart^{1*}, Thomas Passler¹, Terri Hathcock², Anne A. Wooldridge¹, Manuel Chamorro¹, Russell Cattley², Jerome A. Hogsette³ and Xing Ping Hu⁴.

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

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

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⁴ Department of Entomology and Plant Pathology, College of Agriculture, Auburn University, Auburn, AL

*Corresponding author

Introduction: *Corynebacterium pseudotuberculosis* infection in horses causes three different disease syndromes: external abscesses, internal infection and ulcerative lymphangitis. The route of infection in horses remains undetermined, but transmission by insect vectors is suspected. The purpose of the present study was to investigate the role of the house fly (*Musca domestica* L.) as a vector of this disease, by optimizing the inoculation of the house fly with *C. pseudotuberculosis* biovar *equi* and determining its post-exposure survival time in the fly.

Methods: To determine the optimal method for inoculation, sterile house flies were exposed to *C. pseudotuberculosis* using 3 different preparations of blood agar supplemented with dextrose for 10 different periods of exposure. The post-exposure survival time of *C. pseudotuberculosis* was investigated following 30 minutes of exposure of the flies to a blood agar plate colonized with the bacteria and moistened with 10% dextrose.

Results: Heavy growth of *C. pseudotuberculosis* was recovered from fly homogenates after exposure of 10-minutes to 24 hours. *Corynebacterium pseudotuberculosis* was isolated from homogenates of house flies up to 24 hours after a 30 minute exposure.

Conclusions: *Corynebacterium pseudotuberculosis* was readily transmitted to house flies. The protocol established can be used in experimental models to demonstrate the role of house flies as mechanical vectors in *C. pseudotuberculosis* infection in horses. The duration of the bacterial survival in the house fly is suggestive of the duration of the transmission period.

Acknowledgments: Boehringer Ingelheim Vetmedica, Inc. and Auburn University College of Veterinary Medicine Animal Health and Disease Research Funds.



Efficacy of ketamine hydrochloride administered as a basilar sesamoid nerve block in alleviating foot pain in horses caused by natural disease

Fernanda Cesar¹, John Schumacher¹, Fred DeGraves², and Sue Duran¹

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²Department of Agriculture, Western Kentucky University, KY

Introduction. Regional anesthesia is a valuable diagnostic aid used to localize lameness in horses. A local anesthetic agent capable of resolving lameness for a short period of time after being administered perineurally would be helpful because rapid return of lameness would allow for other analgesic techniques to be performed within a short time. In 2 studies ketamine HCL administered as a basilar sesamoid nerve block prevented pain caused by heat lamp stimulation of skin in the pastern region for 15 minutes. We hypothesized that when administered perineurally, ketamine hydrochloride (HCl) would quickly resolve naturally occurring lameness for a short time.

Methods. Seven horses chronically lame on a thoracic limb were selected. A wireless, inertial, sensor-based, motion analysis system was used to evaluate lameness before and after administration of 2% lidocaine and later, before and after administration of 3% ketamine over the palmar digital nerves at the base of the proximal sesamoid bones (a basilar sesamoid nerve block) at 5-minute intervals for 30 minutes. Lameness scores obtained before and after administration of lidocaine and ketamine HCl were statistically modeled using repeated measures analysis.

Results. Gait after basilar sesamoid nerve blocks using lidocaine significantly improved, but gait after performing the same nerve block using ketamine HCl did not significantly improve.

Conclusions. Ketamine administered perineurally for regional anesthesia of the digit does not desensitize the digit to the same extent as does lidocaine. 3% ketamine HCl appears to have no value as a local anesthetic agent for diagnostic regional anesthesia.

Acknowledgments. The authors thank Drs. Barba, Oyarzun and AULATH technicians for assistance.



Characterization of Late Outgrowth Endothelial Colony Forming Cells in Adult Horses

Margaret M. Salter¹, Wen J. Seeto², Blake B. DeWitt¹, Elizabeth A. Lipke² and Anne A. Wooldridge¹

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

²Department of Chemical Engineering, Samuel Ginn College of Engineering, Auburn, AL

Introduction. Endothelial progenitor cells (EPCs) circulate in peripheral blood, function in vascular homeostasis and repair, and are derived from bone marrow stem cells. The number of colonies of EPCs in peripheral blood is an emerging biomarker for metabolic and cardiovascular disease in humans. EPCs are used therapeutically for revascularization of ischemic tissues and play a key role in vascularization of engineered tissues. EPCs have not been characterized or isolated in horses. The purpose of this study was to culture a type of EPCs, late outgrowth endothelial colony forming cells (ECFCs) from healthy horses. Cultured equine cells were characterized using functional assays of vascular tube formation in Matrigel[®], uptake of Dil-labeled acetylated low density lipoprotein (Dil-Ac-LDL), and expression of the markers Von Willebrand Factor (vWF), CD34, CD14, and CD105.

Methods. A 5 mL heparinized blood sample was collected from 24 adult horses. Samples were directly plated on collagen-coated flasks in endothelial growth medium and evaluated for colony formation. Colonies were counted, harvested, expanded and used for characterization assays. All assays were performed at passage 4 or lower. Vascular tube formation in Matrigel[™] was assessed after 5, 24, and 48 hours. Cells were incubated with Dil-Ac-LDL solution for 6 hours, counterstained with 4',6-diamidino-2-phenylindole (DAPI), and imaged with a fluorescent microscope. Human ECFCs served as a positive control for both assays. Specific cell markers of vWF, CD34, CD105 and CD14 were evaluated using indirect immunofluorescence. Equine ECFCs were analyzed via flow cytometry staining for CD105. Equine carotid endothelial cells served as a positive control for all marker analysis. Descriptive statistics were performed on all results.

Results. Three of 24 horses produced colonies at 12 ± 2.5 days from peripheral blood samples with a mean of 3.5 ± 1.5 colonies per 5 mL of blood. Cells demonstrated characteristic single layer cobblestone morphology and significant outgrowth upon expansion. Equine ECFCs formed vascular tubes in Matrigel[™] with 39.3 ± 29.2 branch points per 1.04 cm^2 . Equine ECFCs took up Dil-Ac-LDL, (85% positive cells). Equine ECFCs showed positive staining for endothelial markers vWF, CD34, and CD105 by indirect immunofluorescence and were negative for the hematopoietic marker CD14. $84.9 \pm 13.6\%$ of equine ECFCs were positive for CD105 by flow cytometry.

Conclusions. ECFCs can be isolated and cultured from peripheral blood samples of healthy horses and share functional and cell marker characteristics of ECFCs characterized in other species. Information from this study will aid in future research investigating additional sampling methods to increase yield and characterization methods. Equine ECFCs have potential therapeutic use in diseases associated with ischemia or delayed vascularization in the horse.

Acknowledgments. This project is funded through the Animal Health and Disease Research grants program.



Bony Abnormalities in Cats with GM2 Gangliosidosis

Patricia M. Beadlescomb^{1,2}, Adrien-Maxence Hespel³, D. Ray Wilhite¹, Ashley N. Randle², Heather Gray-Edwards², Aime K. Johnson³, Jacob A. Johnson³, Judith A. Hudson³, Diane U. Wilson², Ronald D. Montgomery³, John T. Hathcock³, Nancy R. Cox², and Douglas R. Martin^{1,2}

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Introduction. GM2 gangliosidosis (GM2) is a rare lysosomal storage disorder caused by a deficiency of the enzyme β -hexosaminidase (Hex) that prevents the lysosomal glycolipid catabolism in all tissues. Glycolipid storage results in neurologic disease that is typically fatal in humans by 5 years of age. While there is currently no cure in humans, adeno-associated virus (AAV) gene therapy has demonstrated remarkable results with a >four-fold increase in lifespan and marked attenuation of neurologic signs. Increased survival has led to the emergence of severe peripheral disease, such as skeletal abnormalities, that are otherwise subclinical in untreated cats. The current report summarizes skeletal abnormalities in untreated and AAV-treated GM2 cats.

Methods. Monocistronic AAVrh8 vectors expressing feline Hex (8×10^{12} g.c. total) were injected bilaterally into the thalami (Thal) and lateral ventricle (intracerebroventricle, ICV) in GM2 cats at 1 month of age (GM2+AAV). Computed tomography (CT) scans were performed on untreated GM2 cats at a humane endpoint (HE; ~ 4.5 months, $n=3$), GM2+AAV cats close to HE (15.7, 17, and 30 months, $n=3$) and aged and sex-matched normal controls ($n=3$ for untreated GM2 control group and $n=2$ for AAV treated control group). CT scans were analyzed using three-dimensional (3D) imaging software, Mimics (Materialise, Plymouth, MI). Skeletons were reconstructed and sectioned into axial and appendicular components for analysis. Cross-sectional length and width of the intervertebral foramen were measured using Mimics software, and femur lengths were measured from 3D printed bone reconstructions.

Results. Bony abnormalities in GM2 cats include overgrowth of cervical vertebrae and malformation of long bones. Untreated GM2 cats at HE exhibit malformed vertebral bodies with early signs of inappropriate bony growth. GM2+AAV cats have excessive cervical bony proliferation with lateral bridging of the vertebral bodies resulting in spinal cord compression and fusion of C1-C7. Subjectively, untreated cats have reduced femoral length and AAV-treated cats exhibit narrowing of the distal femoral shaft. Cross-sectional length and width of cervical vertebrae in untreated GM2 cats were also analyzed.

Conclusions. Skeletal abnormalities are a significant cause of morbidity in AAV-treated GM2 cats, resulting in spinal cord compression and reduced mobility. Cervical vertebral bony proliferation is abnormal in untreated GM2 cats, but is most pronounced in AAV-treated GM2 cats with fusion of C1-C7. Femoral changes are present in untreated GM2 cats but increase in severity in AAV treated GM2 cats.

Acknowledgments. We would like to thank the Department of Clinical Sciences for expertise and advice. This research was funded by the Natl Inst Neurol Dis Stroke (NIH grant # 1U01NS064096-01A10) and the Scott Ritchey Research Center.



Assessment of vaccine efficacy in early weaned beef calves challenged with bovine viral diarrhea virus (BVDV)

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³Department of Animal Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction: Early weaning of beef calves is a management strategy that beef cattle producers can implement to reduce their forage needs and maintain the cow herd during severe drought. To ensure economic success of early weaning, adequate vaccination protocols must be implemented. Vaccination of young calves against BVDV is a key management strategy to prevent economic losses associated with disease caused by BVDV in beef herds. The purpose of this study was to evaluate the effectiveness of different BVDV vaccines in protecting calves against challenge with virulent BVDV 45 days after vaccination

Methods: Forty eight beef calves were early weaned at approximately 72 days of age. At weaning, calves were assigned to 1 of 4 different BVDV vaccination groups. Group A (n=12) received phosphate saline (control), group B (n=12) received BRD-Shield®, group C (n=12) received Express5®, and group D (n=12) received Bovi-shield Gold 5®. Following treatment, calves were separated in biosecure pastures. Forty five days after vaccination, all calves were challenged with virulent BVDV 2 strain 1373. After challenge, calves were examined daily. Body weights were recorded before and after viral challenge. Serum samples for BVDV virus neutralization were collected before vaccination, before challenge, and after challenge. Serum, nasal secretion, and whole blood samples were obtained in all calves after challenge for BVDV VI and CBCs. Statistical analysis was performed by repeated measures ANOVA as implemented in Proc-GLM.

Results: only one calf in the control group developed severe clinical disease; however, the control group and D group had a higher proportion of calves with respiratory and fecal scores of 2 or higher throughout the study period. Mean rectal temperatures and total WBC were similar between groups. Groups A and D had a greater proportion of calves with viremia after challenge. In Groups B and C normal antibody decay curves were abrogated after vaccination and had significantly greater ($p < 0.05$) BVDV 1 and BVDV 2 antibody titers before and after challenge. Mean individual weights at the end of the study were significantly greater ($p < 0.05$) in calves from groups B and C.

Conclusions: colostrum-derived BVDV antibodies prevented mortality but did not prevent viremia and decreased weight gain as observed in group A. Vaccination with BRD-Shield® and Express5® abrogated normal antibody decay indicating humoral response to vaccination. Higher levels of BVDV antibodies before challenge prevented viremia and increased overall weight gain in calves vaccinated with BRD-Shield® and Express5®. Lower response to vaccination in group D could be related to maternal interference or differences in cell mediated vs. humoral response after vaccination.

Acknowledgments: Alabama agricultural experimental station for funding this project and the people from animal health research at Sugg laboratory for the support.



Defining monospecific functional immunodominant B-cell epitopes of the nine *Chlamydia* species

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Introduction: The obligate intracellular bacterial genus *Chlamydia* includes nine human and animal pathogenic species. Serological diagnosis of chlamydial infection such as complement fixation or microimmunofluorescence test has low sensitivity and is technically challenging, difficult to standardize, requires cumbersome production of antigens, and demands skilled technicians. Moreover, all chlamydial species are closely related, and serological assays use antigens such as highly cross-reactive whole chlamydial organisms, highly conserved proteins, or the genus-specific LPS. This high degree of serological cross-reactivity prevents conclusive species-specific serology. Thus, simple ELISA tests that differentiate the species reactivity of antichlamydial antibodies with high specificity and sensitivity are urgently needed.

Methods: To identify species-specific B cell epitopes for such assays, we first catalogued immunodominant chlamydial proteins as reported in the literature. Potential B-cell epitopes in regions of these proteins that are polymorphic among all nine *Chlamydia* species were ranked by predictive algorithms. High-scoring peptides of such potential epitopes were synthesized with an N-terminal biotin followed by a serine-glycine-serine-glycine spacer. Monospecific mouse hyperimmune sera against each *Chlamydia* species were generated by three intranasal inoculations of cell culture- or chicken embryo-propagated elementary bodies. Biotinylated peptides were immobilized onto streptavidin-coated microtiter plates and tested with these murine sera in chemiluminescent ELISAs.

Results: Antibody-reactive species-specific epitopes were found on the chlamydial immunodominant proteins OmpA, Omp2, PmpD, IncA, IncG, CT442, IncCT529, IncCT618, and TarP. Currently used B-cell epitope prediction algorithms were inaccurate and frequently failed to correctly predict immunodominant epitopes. In contrast, an algorithm searching for intrinsically disordered, relatively surface exposed regions with undefined secondary structure proved optimal for prediction of B-cell epitopes. For each of the nine *Chlamydia* species, a total of 5-15 peptides were identified on these proteins.

Conclusions: These peptide antigens produced high and absolutely species-specific signals in a robust ELISA format. Pooled species-specific peptides were used in different host species to identify the specificity of *Chlamydia*-reactive antisera. Different combinations of such monospecific peptides can also be used to identify reactivity of an unknown serum against serovars of a single chlamydial species, or against all chlamydial species. We anticipate that these peptide ELISAs will vastly improve chlamydial serology.

Acknowledgments: Thanks for Dongya Gao for helping in laboratory works.



Synthetic Peptide Antigens for Molecular Serology of Bovine Infections with *Chlamydia pecorum*

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Introduction: We have recently developed *enzyme-linked immunosorbent assays* (ELISAs) for molecular serology of the nine species of *Chlamydia* using synthetic immunodominant peptide antigens. We hypothesize that this methodology can differentiate and quantify species and serovar-specificity of the antibody response to chlamydial infection, and tested this hypothesis using plasma samples from endemic bovine infections with *C. pecorum*.

Methods: Immunoglobulin M (IgM) and IgG antibody concentrations were determined using peptide antigens as well as total elementary body lysate (EB lysate) antigens of *Chlamydia* spp.

Results: The bovine plasmas were highly reactive with *C. pecorum* peptides, but non-reactive with peptides of any of the remaining 8 chlamydial species. In contrast, EB lysate antigens of all chlamydial species tested (*C. pecorum*, *C. abortus*, *C. trachomatis*, *C. pneumoniae*) showed high reactivity, indicating extensive cross-reactivity among chlamydial species that has historically made species-specific serology of animal chlamydial infections impossible. These results demonstrated unambiguously the validity of species-specific serology of animal chlamydial infections by use of peptide antigens derived from immunodominant B cell epitopes of each chlamydial species. Concentrations of antibodies against EB lysate of *C. pecorum* strain E58 and *C. pecorum* peptides correlated with each other, and with *C. pecorum* infection intensity as determined by quantitative PCR detection of *C. pecorum* DNA in conjunctival and vaginal swab specimens. Pools of *C. pecorum* peptides correlated better with EB lysate reactivity than single peptides, indicating the need for multi-epitope antigens in quantification of the anti-*C. pecorum* humoral immune response. The most immunodominant but highly variable, *C. pecorum* serovar-derived OmpA peptides were suitable for tracing *C. pecorum* serovar reactivity, while peptides from proteins with less intraspecies variability such as InCA, IncCT529, IncCT442 and Inc618 were suitable for species-specific antibody detection.

Conclusions: Collectively, these results establish novel species- and serovar-specific serology for detection, differentiation, and quantification of bovine chlamydial infections.



The effects of oral metronidazole administration on olfaction and detection capabilities of explosive detection dogs.

Eileen K. Jenkins¹, Tekla M. Lee-Fowler¹, T. Craig Angle², and Ellen N. Behrend¹

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

²Department of Animal Health and Performance, College of Veterinary Medicine, Auburn University, AL

Introduction. Metronidazole, an antibiotic commonly used for treatment of diarrhea and *Giardia* infection in working dogs, causes olfactory dysfunction in humans. Controlled clinical studies have not been conducted in veterinary medicine. Canine olfactory abilities are currently employed by law enforcement and military forces for tracking, trailing, and identification; to detect explosives, accelerants, and drugs; and for search and rescue. Olfactory dysfunction in working dogs can result in loss of working ability or could result in catastrophic loss of life or infrastructure in the case of explosives detection dogs. The purpose of this study was to determine if metronidazole alters olfaction ability in explosives detection dogs.

Methods. Metronidazole (25 mg/kg BID per os) was administered for five days to 16 trained explosives detection dogs. Odor detection threshold was measured on days 0 (prior to drug administration), 4 and 5, using a standard scent wheel configuration and three explosive scents: ammonium nitrate (AN), trinitrotoluene (TNT) and smokeless powder (SP). Dogs were tested using 19 different odor weights, ranging from 20 grams to 1 milligram, with the lowest repeatable measure recorded as the detection threshold. The detection threshold for each dog was determined each day of testing on each of the three scents, and then compared. Data was analyzed using a mixed model repeated measure ANOVA, Dunnett's Test to determine the effects of the medication on individual performance, and Tukey's test to determine the effect of day or odor on performance. Significance was set at the $p < 0.05$ level.

Results. Two of sixteen dogs exhibited a degradation in odor detection (5-10 mg) after five days of metronidazole, and 2/16 dogs exhibited an improvement in odor detection (2-50 mg) after five days of metronidazole. Performance on Day 4 and 5 compared to Day 0 revealed no significant differences in dogs' detection of SP, AN, or TNT ($P = 1.000$ for all).

Conclusions. Although 4 dogs exhibited changes in olfaction capability after five days of metronidazole therapy, no changes were statistically significant, and therefore unlikely to be clinically significant. There were several confounding factors that made interpretation of data difficult to analyze: dogs were not all on the same diet or physical conditioning program, and one dog experienced estrus during the study period.

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A Survey-based Investigation of the use of NSAIDs in Captive Elephants

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Introduction: Elephants in captivity often suffer from osteoarthritis and other orthopedic conditions necessitating analgesic and anti-inflammatory medication. Treatment commonly involves non-steroidal anti-inflammatory (NSAID) drugs. Wild animals in captivity often mask commonly recognized signs of pain and discomfort, complicating analgesic administration. Few evidence based drug doses are listed in the online elephant drug formulary. The purpose of this study was to examine current NSAID dosing strategies used in captive elephants. This data is intended to ultimately serve as pilot information for establishment of an easily accessible online drug database for zoo veterinarians for all megavertebrate species, including elephant, rhinoceros, hippopotamus and giraffe.

Materials: This retrospective internet based survey (Survey Monkey) examined the use of NSAIDs in elephants located in zoo and wildlife facilities worldwide. Responding veterinarians from 5 continents were all members of the American Association of Zoo Veterinarians (AAZV). Survey data was collected from September of 2012 through March of 2013. Data was compiled into a Microsoft Access file and relevant and related outcome measures were combined to focus on the following outcome measures: signalment (genus, species, facility), drugs (choices being flunixin meglumine, phenylbutazone, ketoprofen, etodolac, carprofen, meloxicam, ibuprofen, and "other"), dosing regimens (indication, route, vehicle, dose in mg/kg, interval, duration), efficacy (subjectively scored by the institution as 1 = poor or no effect, 2 = fair, 3 = good, and 4 = excellent; scores were not provided for all drugs or all facilities), ease of administration, and if applicable, adverse events (subjectively scored by the authors as 1 = mild, 2 = moderate, and 3 = severe).

Results: Of the 60 facilities that completed the survey, 38 exhibited either Asian (*Elephas maximus*) or African (*Loxodonta africana*) elephants. The most commonly used drugs and doses were phenylbutazone (n=27/38; 0.25 to 5.5 mg/kg) and flunixin meglumine (n=26/38; 0.28 to 1.5 mg/kg) followed by ibuprofen (n=22/38; 1 to 8.5 mg/kg), and ketoprofen (n=7/38; 0.5 to 2). Efficacy scores for phenylbutazone (n=25) were 4 (n=6), 3 (n=18), and 2 (n=1) and for flunixin meglumine (n=25) 4 (n=7), 3 (n=15), 2 (n=2) and 1 (n=1). Efficacy scores for Ibuprofen (N=20) were 4 (n=1), 3 (n=16), 2 (n=2) and 1 (n=2) and for ketoprofen (n=8), 4 (n=3) and 3 (n=5). A total of six adverse events were reported with NSAID use in elephants: flunixin meglumine (2 of 25 facilities using the drug); phenylbutazone (1 of 25), and ketoprofen (1 of 8). The remaining 2 were reported for carprofen with 2 of the 3 facilities using this drug reporting adverse events.

Conclusions: As with other megavertebrates species (see sister abstract), phenylbutazone and flunixin meglumine were the most commonly used NSAIDs in elephants. Reported doses for all drugs were markedly varied, varying 3 to almost 9 fold within and between institutions. While drug choices were often similar among facilities, the diversity in dosing regimens across zoological institutions has been clearly demonstrated with this first of its kind data, thus emphasizing the need for standardizing dosing regimens utilizing evidence based clinical trials.

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miRNA Expression Profile and Regulation in Spontaneous Canine Mammary Tumor Models with INK4 Tumor Suppressor Defects

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Introduction. miRNAs are evolutionarily conserved and endogenous small noncoding RNAs (~22 nucleotides) that post-transcriptionally suppress gene expression in a sequence specific manner. Expression of these small RNAs is tightly regulated during development and in normal tissues and is frequently altered in cancers. The cyclin dependent kinase inhibitors (CKIs) act as powerful cell cycle regulators and endogenous tumor suppressors. p16 is one of the critical members of INK4 CKI tumor suppressors (including p14ARF, p15, p18 and p19) defects in which have been associated with a number of cancers including human and canine breast cancers. The full-length sequence of p16 and the regulation of p16/INK4A/B gene locus (encoding alternatively spliced p16 and p14 transcripts) in canine cancers have been largely unknown. Members of the INK4 tumor suppressors are differentially expressed in canine mammary tumor (CMT) models posing the hypothesis that these genes might be post-transcriptionally regulated by miRNAs in CMTs. The goal of this study is to evaluate the genetic defects and regulation of INK4 gene expression by profiling miRNA expression in spontaneous canine mammary tumors.

Methods. Gene expression profiles and sequencing of INK4 genes have been evaluated by RT-PCR, rapid amplification of cDNA ends (RACE)-PCR, touchdown-PCR assays as well as subsequent cloning experiments. The p16 sequence was analyzed by Vector NTI and bioinformatics tools. The entire miRNA profile (dog miRNome) have been evaluated by miRNA QPCR arrays.

Results. INK4 tumor suppressors are differentially expressed while genes of the p16/INK4A/B locus (p16, p14 and p15) have been found most frequently defective in a panel of six CMT cell lines and canine primary tumors. A novel frameshift mutation has been discovered in p16 exon1a resulting in altered mRNA and protein expression in the CMT28 cell line. Additionally for the first time, the 277 most abundantly expressed and highly characterized miRNAs in the canine genome have been evaluated in the CMT27 model that has been characterized for leading INK4 gene defects.

Conclusions. This is a novel approach for evaluating the entire miRNA profile in canine breast cancer model. Several miRNAs and miRNA families such as miR-200a/b/c, miR-429, miR-141 and miR-9 that are significantly upregulated in the CMT27 cell line could potentially target INK4 genes predicted by computational analysis. A number of miRNAs (miR-143/145, miR-34a and miR-199) are also found significantly downregulated in the CMT27 model. These altered miRNAs also correlate to orthologous miRNAs identified in human breast cancer. Therefore these expression profiles of miRNAs and INK4 genes may represent critical regulatory features common to both species and will be evaluated as therapeutic targets in the intermediated canine cancer models.

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Characterization of Virulence of Uropathogenic Canine *Escherichia coli*

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Introduction. *Escherichia coli* (*E. coli*) is commonly associated with canine urinary tract infections (UTI). Dogs with UTI express varying severities of clinical signs, ranging from absent (asymptomatic bacteriuria; ABU) to severe. Severity is based, in part, on the presence of virulence factors (VF) which facilitate isolate survival in the urinary bladder. In humans, ABU is not an indication for antimicrobial therapy; treatment may increase antimicrobial resistance, and removal may allow infection with more pathogenic organisms. The aim of this study was to characterize *E. coli* from canine UTIs associated with differing severities of clinical signs, with the specific goal of describing VF in ABU and to evaluate VF role in the severity of canine UTIs.

Principal Components (PC) Analysis is a method that reduces data dimensionality by performing orthogonal transformation to convert a set of possibly correlated observational variables into a new set of linearly uncorrelated variables or PC. As such, it is suitable for data sets in multiple dimensions, such as gene expression.

Methods. *E. coli* (n=68) cultured from dogs with UTIs were classified as to severity: absent (ABU; n=15), mild (n=18), moderate (n=15), and severe (n=20). A second category, non-ABU (n=53) included mild, moderate and severe combined. The level of RNA expression of adhesins (*papG*, *papC*, *fimH* and *focA*), toxins (*hlyD*, and *cnf1*) and siderophores (*ireA*) was determined by reverse transcriptase PCR (qRT-PCR) using a LightCycler® 480 SYBR Green I master in the Roche® Light-Cycler 480. The relative expression of each gene was standardized to the housekeeping *gapA* gene and then normalized to that of *E. coli* ATCC 25922. Overexpression of any gene was defined as ≥ 2 -fold expression compared to that of *E. coli* 25922. SAS software 9.2 was used generate and analyze data. Proportions of VF expressed in isolates were determined for each level of severity, and in ABU vs non-ABU. Levels of VF gene expression were computed to PC and linear discriminant function analysis.

Results. The proportions of overexpression of *focA* and *papC* of non-ABU isolates were higher than those in ABU isolates. The overexpression of *papG* gene was detected in all ABU isolates, but not for non-ABU isolates ($P < 0.05$). The expression levels of 7 VF genes were computed by PC analysis. The first 4 PCs were selected to further analysis of the linear discrimination function. The linear discriminant analyses were able to classify severity of UTI into two group: ABU and non-ABU. The linear discriminant function estimated the severity of UTI with a 9.3% error rate and 0% false negative rate.

Conclusions. Virulence profiles of clinical canine UPEC could not accurately predict difference between ABU and non-ABU but PC analysis suggests that further clustering analysis of VF may identify a VF profile that discriminates between ABU and non-ABU prior to treatment. Our goal is to promote a “no-antimicrobial” option for animals with ABU by confirming such isolates are not pathogenic. Further, such isolates might be developed as a therapeutic alternative to antimicrobial therapy. Both goals will support de-escalation of antimicrobial use and thus antimicrobial resistance.

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Identification of possible drug interactions between levetiracetam and other antiepileptic drugs in epileptic canine patients.

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Introduction. Epilepsy is a common neurologic ailment encountered in canine patient, estimated to range from 0.5% to 5% in the canine population. While most of the patients can be managed on one antiepileptic drug (AED), 20-30% require two or more AED to decrease the frequency and duration of seizures. Levetiracetam (LEV), more commonly known by its brand name Keppra®, is an AED which has been shown to be effective in the control of epilepsy in a variety of species. Although more commonly used as an add-on AED, it also is used as the sole therapeutic intervention. Combination therapy is most commonly with as Phenobarbital (PB) or zonisamide (ZON). PB is a recognized inducer of hepatic drug metabolizing enzymes and drugs used in epileptic patients receiving PB are often characterized by a shortened elimination half-life. Among the difficulties encountered with successful control with LEV is its short elimination half-life. However, because LEV is renally excreted, interactions with PB are not expected. To our knowledge, no studies comparing the half-life of LEV compared to the half-life of LEV when used in combination with another AED in the clinical patient have been conducted. We hypothesized that the half-life of LEV would be decreased with the addition of PB, but there would be no impact on the half-life of LEV when given with ZON.

Methods. The Therapeutic Drug Monitoring Service at Auburn University includes a data base of epileptic canine and feline patients receiving differing combinations of AED drugs. This retrospective analysis of clinical samples submitted to the AUCVM Clinical Pharmacology Laboratory examined the half-life of LEV (n =51), LEV and ZON (LevZon) (n=17) or LEV and PB (LevPB) (n=45) from 2010 to 2013 in canines. Half-life was calculated ($t_{1/2} = 0.693/k_{el}$ where $k_{el} = \ln(C_1/C_2) / (t_2 - t_1)$) from peak (C_1 at 2 hours after a dose; t_1) and trough (C_2 just prior to second dose; t_2) serum concentrations. Criteria for inclusion were three times daily dosing with LEV and receiving LEV for at least 3 days to ensure steady state. For the half-life of LevPB, the patients were included who had been receiving PB for at least 28 days to assure maximal induction. If multiple samples had been submitted across time for any one patient, the most recent sample was included. A comparison of the means was performed using one - way ANOVA ($P \leq 0.05$) and Tukey's Studentized Range ($P \leq 0.05$).

Results. The results of the ANOVA demonstrated the half-life of LevPB ($3.78 \pm 2.29\text{hr}$) < LEV ($3.96 \pm 1.93\text{hr}$) < LevZon ($6.53 \pm 2.03\text{hr}$) ($P = 0.0007$). A Tukey's Studentized Range showed a statistical significance between the half-life of Lev and LevZon as well as LevPB and LevZon ($P \leq 0.05$).

Conclusions. The LEV half-life was not associated with significant changes when combined with PB. That elimination half-life was prolonged in patients receiving LevZon was a surprise since ZON, although particularly cleared by hepatic metabolism, has not been associated with drug interactions. Further studies are indicated to identify the site of these interactions. Although changes may not be clinically relevant in this small sample population, the impact on the individual patient may contribute to a decrease in breakthrough seizures due to low LEV plasma concentrations.

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Veterinary Student Platform Presentations

Cardiovascular and Respiratory Effects of Dexmedetomidine in Isoflurane Anesthetized Raptors

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Treatment of nociception in avian surgery is difficult because analgesics typically used to control peri-operative pain in mammals, such as opioids, do not provide consistent anti-nociceptive results in birds. Dexmedetomidine, a highly selective alpha-2 adrenergic receptor agonist, has demonstrated efficacy in providing analgesia in mammals but also causes significant cardiovascular changes. This study investigated the cardiovascular effects of dexmedetomidine in isoflurane anesthetized Red Tailed Hawks (*Buteo jamaicensis*). Baseline cardiovascular and respiratory parameters were recorded and then the birds received dexmedetomidine intramuscularly. Physiologic parameters were recorded every 5 minutes for 60 minutes. After 60 minutes, the birds were given atipamezole, an alpha-2 adrenergic receptor antagonist, intramuscularly to reverse the effects of the dexmedetomidine. The parameters after administration of dexmedetomidine and after administration of atipamezole were compared to the baseline data. We expect that raptors will show a similar increase in blood pressure and decrease in heart rate like their mammalian counterpart, but that the effects will be fully reversible.

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Serum and Cerebrospinal Fluid Concentrations of Generic Levetiracetam after Multiple Oral Dosing in Healthy Adult Horses

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Introduction. Levetiracetam (LEV) is a novel anti-epileptic drug that prevents neurotransmitter release by interacting with synaptic vesicle proteins. LEV is an adjunct therapy for epilepsy in humans, and experimentally mitigates damage in traumatic brain injuries. Seizure treatments in horses have historically been limited to first generation anticonvulsants. The purpose of this study was to determine the pharmacokinetic profile of generic levetiracetam in horses.

Methods. Six healthy adult horses (geldings), were studied following multiple (9 days) oral dosing (30 mg/kg every 12 hours) of a generic LEV. Serum and CSF samples were collected at predetermined intervals and analyzed via an immunoassay validated in horses.

Results. C_{max} in serum after the first dose (mean, $\mu\text{g/mL}$) was 17.04 ± 4.16 $\mu\text{g/mL}$ and T_{max} (hr) was 6.2 ± 2.49 . Concentrations were still rising in plasma and CSF at 12 hours, with CSF concentrations being 10.06 ± 3.29 $\mu\text{g/mL}$. Peak and trough LEV on day 9 in serum was 44.0 ± 12.7 and 28.1 ± 11.1 $\mu\text{g/mL}$, respectively, and for CSF, 38.6 ± 10.4 and 36.4 ± 10.6 $\mu\text{g/mL}$, respectively. The CSF to serum ratios based on mean peak and trough concentrations were 1.2 and 1.3, respectively.

Conclusions. At steady-state, LEV reaches therapeutic concentrations of 5-45 $\mu\text{g/mL}$ in serum and CSF. Therapeutic concentrations were maintained throughout the dosing interval in CSF. Further, CSF concentrations paralleled, and exceeded serum concentrations after repeated dosing. Efficacy studies are indicated, but LEV CSF pharmacokinetics when administered as a generic LEV are favorable for treatment of seizures.

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Pharmacokinetics of Levetiracetam in Foals

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Introduction. Levetiracetam (LEV) is an anticonvulsant that has been determined to be efficacious in companion animals with minimal adverse effects. The pharmacokinetics in adult horses have been reported but are likely to be different in the neonatal horse. This study was conducted to determine the pharmacokinetics of LEV in foals.

Methods. Three foals between two and six days of age were used. LEV at 20 mg/kg was administered intravenously. Blood samples were collected via a jugular catheter 5, 10, 20, 30, 45, 60, 90 minutes and 2, 3, 4, 6, 8, 12, 18, 24, 36, and 48 hours after LEV administration. LEV serum concentrations were determined by immunoassay and non-compartmental pharmacokinetic analysis of the data was performed.

Results. The mean \pm standard deviation for maximal serum concentrations of LEV was 33.7 ± 3.9 $\mu\text{g/mL}$. The area under the curve was 276.1 ± 54.5 $\mu\text{g/mL}\cdot\text{hr}$. The elimination half-life and mean residence time were 7.0 ± 1.1 hours and 9.5 ± 1.6 hours, respectively. The rate of drug clearance was 19.8 ± 0.8 mL/kg/min and the volume of distribution was 11.9 ± 1.6 L/kg .

Conclusions. As compared to adult horses, foals have a similar area under the curve but appear to have a larger volume of distribution. However, because clearance is greater, mean residence time and elimination half-life also appear to be similar. Despite the larger volume of distribution seen in foals versus adult horses, the length of time that serum concentrations of LEV were within the therapeutic window of 5-45 $\mu\text{g/mL}$ was similar to adult horses. As such, the intravenous treatment regimen used in adults may be effective in foals as well. More animal numbers are needed to prove the statistical significance of these results. Further investigation is needed to determine whether these results hold true at other preadult ages and with oral administration.

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Hematological changes, cytological effects and microbiological growth secondary to 3-12 days of indwelling intrathecal catheter use in horses.

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Introduction. Intrathecal catheters allow for experimental measurement of cerebrospinal fluid (CSF) drug concentrations and clinical administration of analgesics, antimicrobials and chemotherapeutics. Studies have been performed in sheep but published reports on their utilization in horses is sparse. This study aimed to document the effects of indwelling intrathecal catheters in horses as evidenced by daily physical examination findings, CBCs, CSF analysis with cytology and bacterial cultures.

Methods. Six healthy adult horses had catheters placed into their lumbosacral space for a period of up to twelve days on 2 occasions. CSF and blood samples were analyzed on day one, at the midpoint, and in conjunction with a CSF culture at the end of each catheterization.

Results. 9/11 catheterization periods were uneventful. All horses had a mild to marked pleocytosis at some point with or without the presence of extracellular bacteria. Bacterial cultures detected nonpathogenic contaminants. One horse developed acute fever, tachypnea, anorexia and lethargy. Systemic neutrophilia, leukocytosis and CSF neutrophilic pleocytosis prompted immediate catheter removal and treatment with trimethoprim-sulfonamide for 5 days. Recovery was rapid. In another horse, inadvertent aspiration of air resulted in severe neck pain and tachypnea, despite marked neutrophilic inflammation in the CSF, no fever or systemic CBC abnormalities were detected. Pneumocephalus was suspected and the horse recovered without catheter removal or treatment

Conclusions. Intrathecal catheters in horses may be a valuable research and clinical technique, but careful monitoring of all clinicopathologic findings should be performed. CSF neutrophilia may occur due to irritation from the catheter, bacterial contamination or pneumocephalus.

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Evaluation of the digital cushion and weight-bearing surface of the bovine foot in response to modifications in housing and rearing practices of calves

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Introduction. Lameness causes severe economic losses to the cattle industry and is especially disconcerting due to animal welfare concerns. The creation of management protocols focusing on replacement rearing and development of production animals more adept at withstanding the rigors of the industry are desperately needed. The focus of this study is to establish management protocols that will lead to enhancements in animal welfare and productive life through prevention of lameness. Thus, the study proposed herein will analyze the anatomic development of the structures of the bovine foot following implementation of two different rearing practices.

Methods. The study utilized 16 Holstein heifer calves, with random assignment of eight in the control group and eight in the treated group. Eight calves (four from each group) in the study had pedometers placed to record distance covered for a weeks' time. The control group with ages ranging from 6 to 9 months was reared in accordance with the current accepted standard practices of the dairy industry. The treated calves will were reared in accordance with the current accepted practices of the dairy industry until weaning. Ages ranging from 6 months to 9 month old treated calves were allowed free access to a half mile lane where they walked a total of at least 2 miles a day on rough terrain encouraged by placement of food and water. Both treated and control groups had their digital cushion and deep digital flexor measured utilizing an ultrasound machine with a 6 MHz transducer. The left fore medial and lateral claws, the left rear medial and lateral claws and the right rear medial and lateral claws were measured with calipers for their width and length. The ultrasound measured each claw's deep digital flexor and the digital cushion. It was determined if there is a correlation with the size of the width and length of the claw with the size of the digital cushion between the control and treated groups. The findings were further evaluated and determined to be attributed to be the institution of changes in housing and rearing protocols and these changes are not considered to be normal variance within the population of calves.

Results. Currently, there is only a minute difference between control and treated groups which may be attributed to normal variation among animals and from growth. There was difference, however, in the health of the feet of the calves. The control group had cases of epiphysitis, and dermatitis due to wet pasture. Only one pedometer from each group had results. Control group had traveled 5.1 miles total or an average of 0.73 miles a day, while the Treatment group traveled 13.1 miles total or on average 1.9 miles per day.

Conclusions. The ultrasound method employed seems to be a viable tool for monitoring of the digital cushion of cattle. We believe the heifers that had been raised on the rocky terrain pasture didn't have hoof pathology because the environment was drier and the movement of the calves helped in the prevention of standing in wet areas. Additional time is necessary to allow enough time for remodeling of tissues so recheck will be instituted.

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Effects of Detomidine and Butorphanol on Intra-abdominal and Abdominal Perfusion Pressures in Normal Horses

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Introduction. Intra-abdominal pressure (IAP) is defined as the pressure within the abdominal cavity. It is dependent on factors that include abdominal wall compliance and intra-abdominal volume. Abdominal perfusion pressure (APP) is the difference between the mean arterial pressure (MAP) and IAP, and is considered a better prognostic indicator than IAP to predict a patient's risk for intra-abdominal hypertension (IAH). IAH is a condition caused by increased IAP that decreases APP and reduces organ perfusion. Although humans with IAH have the option of heavy sedation and ventilation to lower IAP, horses can only undergo standing sedation.

Methods. This study evaluated the effects of 2 sedation protocols (10µg/kg detomidine hydrochloride [D] and 10µg/kg detomidine hydrochloride with 10µg/kg butorphanol tartrate [DB]) compared to a control (1 mL saline [C]) on IAP and APP in seven healthy adult horses. We hypothesized that sedation would decrease IAP. Using a crossover design, each protocol was tested in each horse, with a one week washout. For IAP measurements, a Codman Microsensor Catheter was placed in the peritoneal space, and an oscillometric tail cuff was used measure the indirect MAP. After a 40 minute equilibration period, the medication was given, and IAP was measured once a minute, while MAP, heart rate, and respiratory rate were obtained every five minutes over 90 minutes.

Results. Our results noted no effect of saline on any parameter. The D protocol did not affect IAP, but decreased MAP, and therefore APP, significantly after minute 40. The DB protocol was shown to significantly increase IAP after minute 20, but did not affect MAP or APP.

Conclusions. This study indicates that sedation decreases APP and increases IAP in normal horses, which is contrary to its effects in humans. Further evaluation of D and DB in horses with IAH would be indicated to determine their effects on tissue perfusion.

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Post-graduate/Faculty Platform Presentations

Comparison of the Ability of a Novel Umbilical dip, Super7+™ Navel Dip, verses that of 7% Tincture of Iodine to Desiccate the Umbilical Remnant in Neonatal Holstein Dairy Calves

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Introduction. Reduction of naval infections through appropriate management including naval dipping is beneficial to the calf and the producer and is reported to decrease naval infection rates from 20-28% to 5-14%. Obviously, naval dipping is of great importance but obtaining tincture of iodine has become problematic. Therefore, the aim of this study was to evaluate an alternative to 7% tincture of iodine, Super7+™ Navel Dip.

Methods. A total of a 100 neonatal Holstein heifers were utilized in this study. Fifty calves were dipped with Super7+™ Navel Dip immediately following calving and 50 were dipped with 7% tincture of Iodine. The umbilicus and the umbilical remnant of all calves were evaluated 48 hours following dipping of and a 1 inch segment of umbilical remnant was removed and placed in a labeled airtight container. All samples were analyzed and then again in 12 hour increments until the samples contained less than 10% moisture. A serum sample was collected from each calf within 48 hours of birth and tested for total protein and specific gravity and IgG levels.

Results. Upon evaluation, the umbilical remnants of all calves were completely desiccated by 60 hours following calving. Of the remnants dipped with 7% Tincture of Iodine 58% and 42% were desiccated by 48 hours and 60 hours, respectively. Of the remnants dipped with Super7+™ Navel Dip 88% and 12% were desiccated within 48 hours and 60 hours, respectively. Dipping with Super7+™ Navel Dip increased the percentage of calves having a desiccated umbilicus by 30% over that of tincture of iodine. Also, the umbilical remnants of all calves utilized in this study had no evidence of infection in the calves having a low total protein (less than 5.0 g/dL. The range of total protein was 4.5 to 7.2 g/dL and the range of specific gravity was 1.032 to 1.048. Additionally, there was no evidence of dermal irritation around the skin surrounding the umbilicus or any other area that may have contacted Super7+™ Navel Dip. There was a strong association between treatment A, Super7+™ Navel Dip, and drying at 48 hours. ($P = 0.0008$, Mantel-Haenszel Chi-Square). Odds Ratio = 5.31, odds of drying out at 48 hours were 5.31 times higher with treatment A compared to treatment B (tincture of iodine). Total Protein was not significantly different between treatments ($P = 0.415$, Mixed Linear Models). IgG was not significantly different between treatments ($P = 0.439$, Mixed Linear Models). Specific gravity was not significantly different between treatments ($P = 0.300$, Mixed Linear Models).

Conclusions. Super7+™ Navel Dip appears to be superior to tincture of iodine in its ability to more quickly desiccate the umbilical remnant. Hence, Super7+™ Navel Dip appears to function competently as a navel dip and is a viable alternative to 7% tincture of iodine.

Acknowledgments. Innovacyn Rioalto, CA

**Amelioration of neurologic disease after AAV-gene therapy in a Sheep Model of Tay-Sachs Disease**

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Introduction. Tay-Sachs Disease (TSD) is caused by a deficiency of lysosomal β -N-acetylhexosaminidase (Hex, EC 3.2.1.52), which consists of 2 subunits that dimerize to form different isozymes: HexA ($\alpha\beta$) and HexB ($\beta\beta$). A naturally occurring mutation of the α subunit has been documented in a line of Jacob sheep resulting in aggressive neurologic disease and death. These sheep are the only GM2 gangliosidosis model with a clinically relevant α subunit mutation, and are therefore a true representation of human Tay-Sachs disease.

Methods. Affected sheep were treated by intracranial injection at 3-4 months of age with AAV vector expressing the α subunit alone, monocistronic AAV vectors expressing the α and β subunits separately or a bicistronic AAV vector expressing both α and β subunits from the same construct. Two sheep from each cohort were humanely euthanized at a predetermined endpoint (4 months post surgery; short term) while two sheep were followed to humane endpoint (long term). Untreated affected TSD sheep and normal controls (n=4 per cohort) were also included. 3 Tesla MRI and MR spectroscopy were performed. Brain and spinal cord analyzed for Hex specific activity and corresponding 40 μ m sections stained to reveal Hex enzyme distribution.

Results T2-weighted MRI in untreated TSD sheep brain at humane endpoint show iso-intensity of gray and white matter and areas of complete inversion of gray/white matter intensities (white matter hyperintense to gray matter). The normal and $\alpha\beta$ bicistronic TSD sheep brain at 11 months of age were almost indistinguishable, with white matter hypointense to gray matter. MR spectra in the untreated TSD sheep show decreased N-acetyl aspartate (NAA) /Creatine (Cr) and increased Myoinositol (MI)/Cr which are indicative of neuronal loss and gliosis, respectively. After treatment with the bicistronic AAV vector levels of NAA/Cr and MI/Cr were partially normalized. Post-mortem analysis revealed Hex activity in untreated TSD sheep ranged from 0-0.1 fold normal in the cortex and cerebellum. After treatment with the α -subunit alone Hex activity was near normal levels in the cerebrum (0.5 - 1.5 fold normal) and cerebellum (1.3-2.3 fold normal). Treatment with the $\alpha\beta$ bicistronic vector resulted in Hex activity levels consistent with the hypothesized therapeutic threshold (~10% over untreated TSD sheep) in the cerebrum (0.2-0.5 fold normal) and cerebellum (0.3-0.1 fold normal). $\alpha+\beta$ monocistronic treated TSD sheep had dramatically increased Hex enzyme levels in the cerebrum (21.0 - 51.1 fold normal) and cerebellum (78.8-164.6 fold normal). Naphthol staining revealed widespread enzymatic distribution with the strongest staining located at the injection site (thalamus).

Discussion AAV-gene therapy in TSD sheep results in normalization of brain architecture and metabolites as measured by MRI and MRS. All treatment groups reached or exceeded the hypothesized therapeutic threshold of 10% over untreated TSD sheep, and full dose $\alpha+\beta$ monocistronic treated sheep had the highest Hex enzyme levels.

Acknowledgments. Funding for these studies was provided by the Cure Tay-Sachs Foundation, National Tay-Sachs and Allied Diseases Foundation and the Scott Ritchey Research Center.



Veterinary Student Poster Presentations

Evaluation of the mucin-like macroglycopeptide region encoded by the feline GPIba gene

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Introduction. Hemostasis at sites of vascular injury is initiated when platelets adhere to exposed subendothelium, a process that requires binding of platelet membrane Glycoprotein Ib-IX-V complex (GPIb) to von Willebrand Factor (vWf) on exposed subendothelial collagen. The vWf-binding domain is located at the amino terminus of the GPIb alpha (GPIba) subunit, separated from the platelet plasma membrane by a mucin-like macroglycopeptide region. Studies have identified polymorphic variants of human GPIba resulting from a variable number of tandem repeats (VNTR) encoding the macroglycopeptide region. Added repeats elongate the macroglycopeptide stalk, projecting the vWf-binding domain farther into the bloodstream, potentially exposing it to greater shear forces. This may lower the threshold for shear-induced interaction with vWf, increasing the likelihood of GPIba-vWf binding and subsequent platelet adhesion, leading to shear-induced platelet aggregation. This is thought to play a role in arterial thrombosis. The goal of this study was to sequence the macroglycopeptide region encoded by the feline GPIba gene to determine if similar VNTR polymorphisms exist.

Methods. Genomic DNA isolated from domestic and exotic cats was subjected to PCR using primers designed to flank the GPIba VNTR region. PCR products were separated via electrophoresis on agarose gels and extracted products were submitted for sequencing. Sequences were compared to predicted feline sequence and published human GenBank sequence.

Results. VNTR polymorphisms were present in the area encoding the macroglycopeptide region of feline GPIba in all cats studied.

Conclusions. VNTR polymorphisms encoding the macroglycopeptide region suggest the existence of polymorphic length variants of the GPIba platelet receptor among cat populations.

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Non-Steroidal Anti-Inflammatory (NSAID) Use in Megavertebrates: A Survey

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Introduction. Megavertebrates in captivity often suffer from osteoarthritis and other medical conditions necessitating both analgesic and anti-inflammatory medication. Treatment most commonly involves non-steroidal anti-inflammatory (NSAID) drugs. Often times, wild animals in captivity will mask commonly recognized signs of pain and discomfort. Compounding this problem is the absence of evidence drug formularies. The purpose of this study was to examine current NSAID dosing strategies used in megavertebrates. With this data, we intended to compile the first collective information involving the use of NSAID drugs in megavertebrates in zoological institutions. Further, the data is intended to serve as pilot information for establishment of an easily accessible megavertebrate database for wildlife veterinarians.

Materials. This retrospective internet based survey (Survey Monkey) examined the use of NSAIDs in the hippopotamus, rhinoceros, and giraffe in 60 zoo and wildlife facilities located throughout the world. Responding veterinarians were all members of the American Association of Zoo Veterinarians (AAZV). Survey data was collected from September 2012 through March 2013. Data was compiled into a Microsoft Access file and relevant outcome measures were combined to formulate a database that focused on the following outcome measures: signalment (genus, species, facility), drugs (choices being flunixin meglumine, phenylbutazone, ketoprofen, etodolac, carprofen, meloxicam, ibuprofen, and "other"), dosing regimens (indication, route, vehicle, mg/kg, interval, duration), efficacy (subjectively scored by the institution as 1 = poor or no effect, 2 = fair, 3 = good, and 4 = excellent), ease of administration, and if applicable, adverse events (subjectively scored by the author as 1 = mild, 2 = moderate, and 3 = severe).

Results. The most commonly used drugs and dosing regimens for each genus were as follows: Hippopotamus (19 facilities reporting): phenylbutazone (12/19; 2 to 6.8 mg/kg) and flunixin meglumine (11/19; 0.3 to 1.3 mg/kg). Efficacy for phenylbutazone was ranked as 4 (n=2), 3 (n=8), 2 (n=1), and 1 (n=1). Efficacy for flunixin meglumine was 4 (n=3), 3 (n=5), 2 (n=1), and 1 (n=1). For rhinoceros (33 facilities reporting): phenylbutazone (26/33; 0.5-10 mg/kg) and flunixin meglumine (25/33; 0.2-1.6 mg/kg). Efficacy of phenylbutazone was 4 (n=5), 3 (n=17), 2 (n=4), and 1 (n=1). Efficacy for flunixin meglumine was 4 (n=6), 3 (n=15), and 2 (n=4). For giraffe (45 facilities reporting): phenylbutazone (40/45; 0.75-20 mg/kg); flunixin meglumine (31/45; 0.23-2 mg/kg); and ketoprofen (10 /45; 0.5-3 mg/kg). Efficacy for phenylbutazone was 4 (n=9), 3 (n=25), and 2 (n=7). Efficacy for flunixin meglumine was 4 (n=7), 3 (n=16), 2 (n=3), and 1 (n=1). Efficacy for ketoprofen was 3 (n=4), and 2 (n=4). A total of eight adverse events, occurring among seven facilities, were reported among the three genera: 4 for phenylbutazone (3 giraffe and 1 hippopotamus), 2 for flunixin meglumine (2 giraffe), one for ketoprofen (giraffe), and one for firocoxib (hippopotamus).

Conclusions. Flunixin meglumine and phenylbutazone were the most common NSAIDs used in these megavertebrates. Doses markedly varied (up to 30 fold) both within and among facilities, which may contribute to the variable efficacy reported among facilities. The diversity in dosing regimens across institutions has been clearly demonstrated, thus emphasizing the need for standardizing dosing regimens based on evidence based clinical trials. Despite limitations of its collection, this data, which is the first of its kind in all megavertebrates, supports the need for scientific studies as a basis for dosing regimens and pharmacodynamic studies to support such clinical trials.

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Defining the Lactocrine-Sensitive Neonatal Porcine Uterine Transcriptome Using RNAseq

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Introduction. The lactocrine hypothesis for maternal programming of neonatal development was proposed to describe a mechanism through which milk-borne bioactive factors, delivered from mother to offspring as a consequence of nursing, affect the development of somatic tissues, including the uterus. Here, the objective was to employ RNAseq analysis to determine effects of age and nursing for 48h from birth (postnatal day = PND 0) on the uterine transcriptome at PND 2.

Methods. Animals and Tissue Collection: Crossbred gilts (*Sus scrofa domestica*)(n=4/group) were assigned randomly at birth: (1) to have uteri collected within 1h of birth; or to be (2) nursed *ad libitum* for 48h, or (3) gavage-fed commercial pig milk-replacer (Advance Baby Pig Liqui-Wean, Dundee, IL) for 48h (30ml/2h/kg BW). Individual uteri obtained from nursed and replacer-fed (lactocrine-null) gilts at birth and on PND 2 (50h) were trimmed of connective tissue, weighed, frozen immediately in liquid nitrogen and stored in RNAiater (Life Technologies, NY) at -80C until total RNA could be extracted and processed for RNAseq analyses.

RNAseq: For each uterus (n=4 per group), total RNA was extracted using the miRNA-easy kit (Qiagen Inc., Valencia, California, USA) according to manufacturer's protocol. The concentration and integrity of extracted total RNA was estimated using a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, California, USA), and an Agilent 2100 Bioanalyzer (Applied Biosystems, Carlsbad, CA, USA). All RNAseq procedures were performed according to protocols established at the HudsonAlpha Institute for Biotechnology. RNAseq results were validating using quantitative PCR.

Results. Effects of both age and imposition of the lactocrine-null state for 48h from birth on the uterine transcriptome at PND 2 were identified. Between birth and PND 2 for nursed gilts, 3283 genes were differentially expressed. By contrast, 4662 differential gene expression events were identified between birth and PND 2 for replacer-fed gilts. On PND 2, 896 uterine genes were differentially expressed in nursed as compared to replacer-fed gilts. Tag cloud and Ingenuity pathway analyses (www.ingenuity.com) revealed both developmentally and lactocrine-sensitive uterine organizational processes.

Conclusions. Imposition of the Lactocrine null state for two days from birth induced global changes in uterine gene expression patterns by PND 2 that were unique with respect to those seen in nursed gilts. Results support the lactocrine hypothesis.

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Elastic Modulus, Fluid Load Fraction Characterization of Equine Articular Cartilage

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Introduction: Healthy articular cartilage is vital for normal joint function. Diseases of articular cartilage can ultimately interfere with cartilage maintenance, leading to joint failure. Because articular cartilage is aneural, avascular, and alymphatic, regeneration may not correct damage. Therefore, an understanding of biomechanical properties of cartilage will lead to more physiologic artificial joints and the prevention and treatment of joint diseases. Our lab has investigated and reported thickness and roughness profiles of equine articular cartilage as an initial step to characterize biomechanical properties of articular cartilage. Further characterization of articular cartilage is needed to better expound the mechanical properties of equine articular cartilage as they relate to load and elastic modulus. We hypothesized that joints will differ in their abilities to withstand indentation forces and therefore will differ in fluid load fraction and elastic modulus properties.

Methods: Twelve cartilage surface samples taken from the weight bearing aspects of the proximal radial carpal bone of the carpus, the medial femoral condyle of the femorotibial joint of the stifle, and the distal metacarpal III surface of the fetlock joint were studied with a Bruker CETR-UMT 3 tribometer. An indentation probe applied strain onto a cartilage surface. The fluid component of the cartilage supports the initial load and is defined as fluid load fraction. The sustained load is supported by the solid matrix and is referred to as initial aggregate modulus of the solid (E_s). The fluid load fraction and E_s were compared across all four joint surfaces using a mixed model ANOVA and Scheffe's test for multiple comparisons.

Results: The three joints of interest differed significantly in E_s and fluid load fractions. Medial and lateral fetlock condyles did not differ significantly from each other in any property. Carpus and fetlock E_s values were significantly greater than the stifle's E_s . However, carpi and fetlocks did not differ significantly from one another in E_s . The stifle has the smallest fluid load fraction while the fetlock has the largest fluid load fraction.

Conclusions: The material properties were statistically different among the joints suggesting that equine joints can bear different loads, possibly due to differing functions, joint lubricating mechanisms, or fluid components of cartilage. The fetlock, carpus, and stifle model the human knuckles, wrist, and knee, respectively. Artificial joints should be designed to mimic their natural counterparts' properties. Therefore, it is plausible to predict that the artificial knee should differ from the wrist or knuckles in its E_s and fluid load fraction. The stifle's unique E_s suggests it differs in how its cartilage's solid matrix carries loads. This knowledge will assist investigators in understanding material properties of joints with different functions, motions, and loads, thus providing groundwork for a better artificial joint. It also offers insight into preventing and treating injuries due to cartilage failure.

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Histopathologic and Enzymatic Characterization of GM2 Gangliosidosis of Jacob Sheep

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Introduction: Tay-Sachs disease (TSD) is a rare autosomal recessive disorder which is characterized by the progressive accumulation of GM2-gangliosides within nerve cells. These gangliosides are components of the normal nerve cell's lipid membrane and in normal animals are broken down by lysosomal enzymes, chiefly beta N acetylhexosaminidase (Hex A). TSD symptoms begin in infancy, usually before two years of age and results in death in three to five years. To better understand and treat this disease, an accurate animal model is needed. Jacob sheep have recently emerged as a model due to the natural occurrence of the disease in their populations. Based on prior research from cat models, the sheep are subjects for intracranial enzyme delivery therapy.

Methods: The sheep are treated with Adeno-associated virus vectors encoding wild type enzymes. The current protocol is intracranial injections in the lateral ventricle and in the thalamus, injecting a vector of the alpha subunit only, monocistronic vectors with alpha and beta, or a bicistronic vector of alpha and beta together. The sheep, upon reaching a humane endpoint, are euthanized and their CNS and peripheral tissues are analyzed by 4-MU assay, using substrates Hex A, total Hex, β -Galactosidase, and Mannosidase. Additional tissue samples were stained by hematoxylin and eosin for histopathology and studied under the microscope.

Results: On histological profile, the affected sheep are characterized by swollen neurons with distended cytoplasm containing fine, clear to pale eosinophilic vacuoles which occasionally displace the nucleus peripherally. In treated sheep, neurons present with large clear vacuoles with eosinophilic material at the center. Affected on enzyme assays are generally characterized by low Hex A levels, high total Hex levels, and high levels of Mannosidase and β -Galactosidase. The injected Hex A enzyme has high activity in treated sheep across all the regions of the brain but low levels in the spine and peripheral tissue.

Conclusions: The vacuole clusters which eosinophilic cores present in treated sheep are hypothesized to be the injected enzyme surrounded by the accumulated gangliosides. They are seen in the highest abundance in regions with the most increased activity levels of Hex A. Based on the enzyme assay results of CNS tissue, the intracranial delivery of the treatment vector is adequately distributed to all regions of the brain but not to the spinal cord. Furthermore, it is not clear which treatment vector is most effective in providing therapeutic levels of enzyme activity to the peripheral tissues. The knowledge gained from this study will add to the overall understanding of the Jacob sheep model and will aid in the development of a treatment for Tay-Sachs Disease.

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***In vitro* vascular tube formation and uptake of acetylated low density lipoprotein diminish as passage number increases in equine endothelial colony forming cells**

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Introduction. Equine endothelial progenitor cells (EPCs) have become a new area of interest in regenerative medicine because of their ability to form new blood vessels and to promote endothelialization. The number and function of a subset of EPCs, endothelial colony forming cells (ECFCs), may be altered in diseases of the horse that affect the vasculature. There is the potential for ECFCs to be used therapeutically for sepsis, thrombosis, fracture healing, chronic laminitis, and chronic wounds. If these cells are to be used therapeutically, key information has to be gained on how many passages the cells can go through and still maintain their phenotype.

Methods. ECFCs were cultured from the peripheral blood of three adult horses beginning at passage 3. Cells were grown to 80-90% confluency, trypsinized, and reseeded in 75 cm collagen coated tissue culture flasks or used for assays. Cell morphology and functional characteristics of vascular tube formation in Matrigel® and uptake of low-density lipoprotein (LDL) were assessed at every passage. Cell morphology was observed at each passage, noting the change in the number of cells grown and the increase in vacuoles.

Results. ECFCs from 2/3 horses lost the ability to form tubes at Passage 10, while 1/3 stopped forming tubes at Passage 12, with an overall loss of tube formation at passage 10.6 ± 1.2 . Qualitatively, tube formation was less extensive and took longer to develop beginning at passage 7.5. No ECFCs at any passage lost the ability to uptake LDL; however, a significant ($p < 0.0001$) decrease in the percentage of cells positive for LDL uptake was identified ($89.7 \pm 6.4\%$ positive in passage 4 versus $29.3 \pm 4\%$ in passage 10). There was no difference in LDL uptake between passages 4 and 6, but passage 8 had significantly fewer LDL positive cells than passage 4 or 6. As passage increased, cell growth slowed (3 days to reach confluency at passage 10 versus 1.5 days at passage 4) and there was an increase in the number of vacuoles in the cytoplasm of each cell.

Conclusions. In conclusion, equine ECFCs completely lost their functional capability of forming tubes at an average passage number of 10.6, they decreased their uptake of LDL with each increasing passage, and cell numbers declined as the passage number increased. Based on these results, ECFCs for experimental or clinical use should be lower than passage 6.

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Role of the HCA₂ Receptor during the Physiological Response to Fasting in Mice

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Introduction: Hydroxy-carboxylic acid (HCA) receptors, primarily found in adipocytes, are a family of G-protein coupled receptors capable of sensing and responding to changes in nutrient availability. The endogenous ligands for HCA₁ and HCA₂ are lactate and beta-hydroxybutyrate (β -OHB), respectively, both of which are hydroxycarboxylic acids and intermediates of metabolism. Intermittent fasting and ketogenic diets have been used clinically to treat seizure disorders and obesity, presumably due to their ability to increase plasma ketones. However, the role of the HCA₂ receptor in the response to fasting in mice is not known.

Methods: Male and female wild type (WT) and HCA₂^{-/-} mice were subjected to a 36 hour fast. Mice were weighed and blood was drawn at 0, 5, 12, 24, and 36 hours. Blood samples were used to evaluate β -OHB, glucose, and serum adiponectin concentration. White adipose tissue was collected at 36 hours to isolate mRNA for HCA₁ and HCA₂ receptor expression analysis. Blood glucose and blood β -OHB were measured using blood glucometer and blood ketone meter, respectively. Serum adiponectin concentration was measured with a mouse adiponectin ELISA kit.

Results: HCA₂^{-/-} mice (male and female) lost less weight during fasting compared to WT mice. There was no significant change in blood glucose between the WT and HCA₂^{-/-} mice during fasting. However, blood β -OHB was significantly lower in the male HCA₂^{-/-} mice after 24 and 36 hrs of fasting compared to the male WT mice. This was also the case in the female mice, but the effect was only significant at 24 hrs. There was a significant difference in serum adiponectin in fed WT and fasted WT males, but no significant change was noted in male HCA₂^{-/-} mice. There was no significant difference in HCA₁ and HCA₂ receptor expression between fasted or fed mice in either experimental group.

Conclusions: Lack of the HCA₂ receptor may dampen the ketogenic response, protect against weight loss during prolonged fasting and may affect serum adiponectin concentration.

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Stearidonic Acid Enhances the Chemosensitivity of Canine B-Cell Lymphoma Cells by Downregulating the Activity of Multidrug Transporters.

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Introduction. B-cell lymphoma is the most common hematopoietic cancer in dogs. It is usually treated with multiple chemotherapy drugs, including vincristine and doxorubicin. While curable with chemotherapy drugs, relapses are seen and chemoresistance is a significant concern in cases of relapses. Treatment with higher concentrations of chemotherapy drugs would elicit untoward effects. Alternative and combinatorial therapies are therefore needed to targeting chemoresistant lymphoma. In this study, we sought to determine the chemosensitizing activities of stearidonic acid (SDA), a plant-based omega-3 fatty acid, in canine B-cell lymphoid tumor cells.

Methods. Cell viability was measured using ATP-based CellTiter-Glo luminescent cell viability assays. The function of multidrug transporters was studied using intracellular substrate accumulation assays.

Results. Combinatorial treatments with minimally cytotoxic concentrations of SDA, potentiated the antitumor activity of vincristine and/or doxorubicin, suggesting that SDA chemosensitizes the B-cell lymphoid tumor cells. Furthermore, SDA at its chemosensitizing concentration increased the intracellular accumulation of the substrates of multidrug transporters, indicating that stearidonic acid inhibits the efflux activity of multidrug transporters.

Conclusions. These results are consistent with the conclusion that SDA not only inhibits the growth of canine B-Cell lymphoma cells but also chemosensitizes them by downregulating the function of multidrug transporters.

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Predictive modeling of the equine heel

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Introduction. Traditionally, equine foot lameness has been attributed to pathology of the bones, synovial structures, tendons and ligaments, and the lamina of the foot. Historically, little attention has been given to the significance of the health of the equine heel soft tissue structures, which are uniquely positioned so as to provide support and protection to the navicular apparatus. The goal of this study was to determine whether the volume of the soft tissue structures of the equine heel could be predicted by the inexpensive and non-invasive methods of physical, radiographic, and ultrasonographic examination.

Methods. Thirteen left front feet were collected from Thoroughbred horse cadavers. The feet were imaged using computed tomography and magnetic resonance imaging. Using Mimics®, three-dimensional images were constructed and volumes were determined of the following anatomical structures: digital cushion, collateral cartilages, and the middle phalanx. Physical, radiographic, and ultrasonographic examinations were performed on the same feet. The relationships between clinical exam measurements and Mimics® values were explored using simple linear regression.

Results. Using the parameters designed for this study, each foot was ranked based on predicted volume. The feet were then ranked based on the following: physical exam volume with and without frog, digital cushion volume, total heel volume, coronary band circumference, digital cushion thickness, and calculated volume. Through statistical evaluation it was found that coronary band circumference was predictive of THV. No other direct correlations existed between clinical examination findings and volumes from three-dimensional reconstructions; however, there was a trend towards being able to categorize feet into high, medium, and low THV and DCV.

Conclusions. Coronary band circumference was the only parameter evaluated that predicted DCV and THV with accuracy. However a trend was noted whereby several other parameters accurately categorized the hooves into low, medium, and high THV and DCV. Physical and clinical examination parameters may be useful to later categorize hooves into low, medium and high volume for clinical purposes. Data from this study serves as a very preliminary screening for clinical examination measures that may serve as predictor variables for anatomical characteristics of the equine heel.

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Effect of Nucleophilic Thiols Penicillamine and N-acetylcysteine on Przewalski (*Equus ferus przewalskii*) and Domestic (*Equus caballus*) Horse Sperm Motility

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Introduction: In recent years, assisted reproductive technologies have become paramount in reinvigorating the population of Przewalski's Horses in North American zoos. Protecting this endangered species could be greatly aided with the establishment of frozen sperm repositories. This would serve as insurance against unforeseen catastrophes, in addition to facilitating the transport and long term storage of the male genome. However, cryopreservation of stallion sperm decreases its quality, which in turn may decrease fertility characteristics. Our current research aims to improve cryopreservation of Przewalski stallion semen by focusing on oxidative stress, one of the main processes effecting sperm function. Recent research by Aitken, *et al* (2012) reported that the addition of nucleophilic thiols – either penicillamine or N-acetylcysteine – to sperm suspension incubated at 37°C preserved motility over time.

Methods: In this study, we hypothesized that either penicillamine or N-acetylcysteine, when added to sperm suspension prior to cryopreservation, may also preserve motility after thawing. Our objectives were four-fold: 1) replicate the original research under clinical settings; 2) determine if there is a dose-response in preservation of motility with the addition of one of two nucleophilic thiols; 3) assess the ability of penicillamine and N-acetylcysteine to preserve sperm motility post-thaw; and 4) compare the effectiveness of these compounds between the Przewalski's horse stallion (n = 3 stallions; one ejaculate/stallion) and the domestic horse stallion (n = 4 stallions; two ejaculates/stallion). Each ejaculate was divided into five treatment groups (0.25 mM and 1.0 mM each of penicillamine and N-acetylcysteine, and control). Sperm motility was evaluated using a Computer Assisted Sperm Motility Analyzer immediately after the addition of the nucleophilic thiols and following freeze-thawing.

Results: There was no effect of nucleophilic thiols on sperm motility under the conditions tested. When assessing the effectiveness of these compounds between the Przewalski's horse and the domestic horse, it was noted that motility characteristics were similar between both species. There was low variability in motility parameters among domestic stallions. In contrast, ejaculates from the Przewalski's stallions exhibited high variability in motility parameters evaluated.

Conclusions: Since there was no treatment effect on sperm motility, we conclude that the nucleophilic thiols penicillamine and N-acetylcysteine exert no beneficial effects during sperm cryopreservation. However, the similarity in spermatozoal characteristics between Przewalski's and domestic stallions will allow us to apply sperm processing techniques developed for the domestic stallions to the Przewalski's horse.

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Susceptibility of Canine Mast Cell Tumors to Adenoviral Vector Delivery

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Introduction. Mast cell tumors are one of the most frequent neoplasms seen in the dermis of dogs. They frequently present with multiple tumors and metastasize through the reticuloendothelial system resulting in systemic disease. The purpose of this study was to determine if an adenoviral vector (Ad5) can be used to deliver the purine nucleoside phosphorylase (PNP) gene to canine mast cell tumor cells and to determine if the PNP gene in combination with the drug 6-methylpurine-2'-deoxyribosid (MeP-dR) could cause tumor cell death.

Methods. 2 mast cell tumor cell lines (BR, MPT-1) were used for all experiments along with a canine histiocyte/macrophage (DH82) for a control. The lines were tested for mycoplasma presence with a Takara PCR Mycoplasma Detection set and if positive, were treated until they tested negative. BR and MPT-1 were tested to confirm they were of canine origin. They were confirmed to be mast cell tumor cell lines by using RT-PCR for tryptase expression as well as toluidine blue staining of slides. The cell lines were infected with an Ad5 vector that incorporated a green fluorescence protein (GFP) gene and infection was determined using flow cytometry. The cells were then infected with Ad5-PNP vector, incubated with MeP-dR and assayed to determine both PNP expression and cell proliferation.

Results. The DH82 and MPT-1 cell lines were negative for mycoplasma. The BR cell line was initially positive for mycoplasma contamination but after treatment with Plasmocure or Erythromycin tested negative. Both BR and MPT-1 were confirmed to be canine in origin and both showed characteristics of being mast cell tumors. Neither BR nor MPT-1 showed Ad5-GFP infection at low MOI of virus while BR showed infection at higher MOIs. Both BR and MPT-1 showed minimal decreases in cell proliferation when high MOIs of Ad5-PNP virus and long exposure times to MeP-dR were combined.

Conclusions. Adenoviral vectors are not capable of transducing canine mast cell tumor cells effectively enough to be used for development of gene therapy applications for treatment of these neoplasms. Although there was some decrease in cell proliferation for cells treated with the Ad5-PNP, MeP-dR combination, it was not as much as seen in other types of cancer previously tested and alternative gene delivery vectors should be considered for mast cell tumors.

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Pilot Study: Attempted Immunoneutralization of Peripheral Kisspeptin and Effects on Female Rat Fecundity

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Introduction: According to the American Humane Association, approximately eight million unwanted animals are received by shelters annually. Surgical sterilization is expensive and invasive. Therefore, nonsurgical contraceptive methods for wild and domestic animals are in high demand. In the past, injectable sterilization has been temporary or unreliable. Kisspeptin (KP, formerly known as metastin) is a potent stimulator of central release of gonadotropin releasing hormone. Hypothalamic expression of KP has been implicated onset of puberty and control of ovulation. More recently, KP has been shown to increase dramatically in peripheral blood in pregnant human and rodents. The increased KP levels in the circulation are thought to be placental in origin, specifically in the trophoblastic cells, which are involved in placental invasion into maternal tissue. The purpose of this experiment is to investigate the effects of inhibiting kisspeptin via immunoneutralization in rats (pre or post copulation) to determine if implantation and/or placentation would be altered.

Methods: Forty young adult female Long Evans rats were vaginally lavaged daily for 14 days to verify cyclicity. In cohort 1, twenty cyclic rats were injected with either anti-kisspeptin or control serum during diestrus, then mated on the night of proestrus. All twenty rats were then sacrificed on gestational day (GD) 20. In cohort 2, twenty cyclic rats were mated on the night of proestrus, then injected with either anti-KP or control serum on GD 6. Estrous cycle status, body weight of dam and offspring, uterine weight, sex of offspring, number of offspring and evidence of fetal resorptions were recorded.

Results: In neither cohort 1 or 2 did KP antiserum treatment have any measurable effect. No treatment effect was observed when considering litter size, pup weight and uterine weight/litter size.

Conclusions: This was a pilot study in which no treatment effect of anti-KP injections were observed. Current work is focusing on validation of detection methods of circulating KP levels. In the future, multiple injections of anti-KP could be used to ensure anti-serum is present at time of implantation. Further work is needed to determine the effect of KP on implantation and fetal development of rodents.

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Isolation and Evaluation of Four Distinct Dendritic Cell Populations from Circulating Canine Blood

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Introduction. Dendritic cells are responsible for antigen presentation and recognition in the body. The presentation of antigen to leukocytes efficiently directs and defines the immune response in the host, which is critical in the regulation of the immune system against non-host pathogens and abnormal host tissue. Dendritic cell studies have enhanced our knowledge of their various functions. These functions can vary depending on the cell population and tissue location. Three dendritic cell populations have been previously identified in circulating blood, but preliminary evidence indicates the potential of a fourth distinct population in canine blood. The purpose of this experiment was to isolate the four populations and evaluate relative gene expression for a panel of dendritic cell related genes. Detailed knowledge of these dendritic cell subsets could play a major role in our ability to manipulate the host immune system in our favor.

Methods. Peripheral blood mononuclear cells (PBMCs) were extracted from whole canine blood and antibody labeled for CD11c, CD4 and CD8 expression. The labeled PBMCs underwent a four stream, unsterile sort via a MoFlo flow cytometer with 488 and 635 nm lasers. Four populations were obtained from the sort: 1) CD11c+/CD4-/CD8-, 2) CD11c+/CD4+/CD8-, 3) CD11c+/CD4-/CD8+, 4) CD11c++/CD4+/CD8+. Subsequent RNA extraction was performed via phase separation with the use of TriReagent and quality was evaluated with a NanoDrop 1000. RNA was converted to cDNA for the use of qRT-PCR to evaluate gene expression. Primers for the genes of interest were verified with whole PBMC cDNA. Quantitative RT-PCR (qRT-PCR) reactions for CD11c, CD14, CD22, CD40, CD80, CD86, CD205, CD209, MHCII, L37, and EEF2 were validated using RNA from unsorted PBMCs

Results. The qRT-PCR reactions were all validated and shown to work. Late amplification was seen for the control gene L37 in all sorted subpopulations, but no other gene amplification was observed. Due to lack of amplification in the subpopulations and the delayed threshold cycle for L37 (compared to whole PBMC qRT-PCR trials), the RNA quality was re-evaluated. Sorted cells had consistently lower RNA values than non-sorted cells. Several manipulations were attempted to improve yields with little success. Larger sort yields were obtained after an additional Ficoll separation and incubation overnight.

Conclusions. Further work into the sorting techniques and subsequent RNA extraction techniques will need to be performed to identify the source of poor yields and enhance subpopulation RNA quality to allow for further qRT-PCR.

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**Characterization of the HCA₂ Receptor in Cats**

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The hydroxy-carboxylic acid (HCA) receptors are a family of G protein-coupled receptors that recognize endogenous intermediates of metabolism and are critical for sensing nutrients. The receptor HCA₂ binds the ketone body beta-hydroxybutyrate. Work from our lab demonstrated that in addition to inhibiting lipolysis, agonists of the HCA₂ receptor increase serum adiponectin concentration which has insulin sensitizing and anti-atherogenic properties. As individuals become obese, there are marked decreases in serum adiponectin concentrations and gene expression of the HCA₂ receptor in adipose tissue, indicating possible loss of regulation of both lipolysis and adiponectin production or secretion. Cats are a well-established model of human obesity and a naturally occurring model of type 2 diabetes mellitus. Cats also exhibit similar changes in adipokine dysregulation associated with obesity. In order to characterize HCA₂ expression in cats, RNA was isolated from nine different tissues (abdominal fat, inguinal fat, heart, spleen, kidney, lymph node, liver, jejunum, and pancreas) of six lean, healthy, female cats. Tissue distribution and abundance was evaluated by quantitative RT-PCR. The product was then transfected into a plasmid and amplified. The resulting product was sequenced and compared to human, murine, and bovine sequences. HCA₂ mRNA was most abundant in spleen, inguinal fat, and abdominal fat, respectively. Pancreas and lymph node showed moderate abundance; whereas jejunum, kidney, liver, and heart demonstrated low abundance. Feline HCA₂ sequence exhibited a 91% homology to human and bovine and an 85% homology to murine sequences, respectively. In summary, the HCA₂ receptor is predominately expressed in adipose tissue and immune cells (macrophages and monocytes) in cats and relative gene expression pattern is similar to that seen in mice and people.

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Age-Related Influence on Ocular Live Attenuated IBV Vaccine Induced Immune Response and Immune Protection

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Introduction: Infectious bronchitis virus (IBV) is endemic and currently one of the most important causes of economic losses for the poultry industry. Delfra funded research in UK performed in 2000 and converted to 2008 prices estimated that the loss to the poultry industry due to IBV infection is around \$48 million a year. Furthermore, there is an estimated 50% vaccine failure for Arkansas (Ark) serotype of IBV (M.W. Jackwood, 2009), the most prevalent vaccine serotype used in the USA. In broilers in the US, live vaccines are typically given at 1 day of age at the hatchery and 2 weeks of age in the field. Increasing evidence suggests that this early immunization for IBV may be an important contributing factor for persistence of IBV Ark vaccines in flocks. We hypothesize that **IBV vaccination within the first week after hatching is detrimental for induction of mucosal and systemic immunity to IBV**. This study will provide an understanding of how the age of chicken vaccination affects the induction of IBV-specific immune responses and will provide valuable information how to optimize the delivery of IBV vaccines in the poultry industry.

Methods: SPF chickens were vaccinated with 3×10^5 50% embryo infectious doses (EID₅₀) of live attenuated ArkDPI IBV vaccine. Chickens were vaccinated 1 day, 1, 2, 3 or 4 weeks post hatch. Antibody responses were measured in plasma 7, 14 and 21 days and in tears 14 days after vaccination using an IBV-specific ELISA. Plates were coated with 5 µg/ml of heat-killed IBV and for detection of IBV-specific antibodies in tears and plasma, a biotinylated mouse-anti-chicken IgG monoclonal antibody combined with streptavidin-horseradish peroxidase and TMB substrate were used. Longitudinal 5 µm sections of trachea were made 4 days after IBV challenge with $\sim 10^6$ EID₅₀ of AL/4614/98 IBV field strain. These tissue sections were H&E stained and analyzed for mucosal thickness and lymphocyte density using Aperio Scan Scope, Image J, and Visiopharm DP software.

Results: Analyses of the IgG antibody response in tears 14 days after challenge demonstrated an age dependent increase in IBV-specific antibodies when vaccinated later in life. Furthermore, day 1 vaccinated chickens had significantly lower IgG antibodies in plasma 14 and 21 days post vaccination when compared to 28 days old vaccinated chickens. Four days after AL/4614/98 IBV field strain challenge of vaccinated bird the trachea demonstrated a decrease in mucosal thickness and lymphocyte density with age, consistent with a decrease in inflammation when vaccinated later in life.

Conclusions: The data indicate that early immunization with live attenuated IBV vaccines in chickens results in decreased mucosal and systemic humoral immune responses, which resulted in decreased mucosal immune protection after vaccination as was assessed by the inflammatory immune response in trachea after challenge. Thus, an increase in inflammation after IBV challenge in the trachea and a decrease in humoral immunity for the day 1 vaccination group was observed, when compared to IBV vaccination later in life. The data support our hypothesis and therefore it would benefit the Poultry industry to change their practice of early IBV vaccination.

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Evaluation of the digital cushion and weight-bearing surface of the bovine foot in response to modifications in housing and rearing practices of calves

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Introduction. Lameness causes severe economic losses to the cattle industry and is especially disconcerting due to animal welfare concerns. The creation of management protocols focusing on replacement rearing and development of production animals more adept at withstanding the rigors of the industry are desperately needed. The focus of this study is to establish management protocols that will lead to enhancements in animal welfare and productive life through prevention of lameness. Thus, the study proposed herein will analyze the anatomic development of the structures of the bovine foot following implementation of two different rearing practices.

Methods. The study utilized 16 Holstein heifer calves, with random assignment of eight in the control group and eight in the treated group. Eight calves (four from each group) in the study had pedometers placed to record distance covered for a weeks' time. The control group with ages ranging from 6 to 9 months was reared in accordance with the current accepted standard practices of the dairy industry. The treated calves will were reared in accordance with the current accepted practices of the dairy industry until weaning. Ages ranging from 6 months to 9 month old treated calves were allowed free access to a half mile lane where they walked a total of at least 2 miles a day on rough terrain encouraged by placement of food and water. Both treated and control groups had their digital cushion and deep digital flexor measured utilizing an ultrasound machine with a 6 MHz transducer. The left fore medial and lateral claws, the left rear medial and lateral claws and the right rear medial and lateral claws were measured with calipers for their width and length. The ultrasound measured each claw's deep digital flexor and the digital cushion. It was determined if there is a correlation with the size of the width and length of the claw with the size of the digital cushion between the control and treated groups. The findings were further evaluated and determined to be attributed to be the institution of changes in housing and rearing protocols and these changes are not considered to be normal variance within the population of calves.

Results. Currently, there is only a minute difference between control and treated groups which may be attributed to normal variation among animals and from growth. There was difference, however, in the health of the feet of the calves. The control group had cases of epiphysitis, and dermatitis due to wet pasture. Only one pedometer from each group had results. Control group had traveled 5.1 miles total or an average of 0.73 miles a day, while the Treatment group traveled 13.1 miles total or on average 1.9 miles per day.

Conclusions. The ultrasound method employed seems to be a viable tool for monitoring of the digital cushion of cattle. We believe the heifers that had been raised on the rocky terrain pasture didn't have hoof pathology because the environment was drier and the movement of the calves helped in the prevention of standing in wet areas. Additional time is necessary to allow enough time for remodeling of tissues so recheck will be instituted.

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Activation of the transcription factor NF- κ B by the dietary polyphenol curcumin

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Introduction. The incidence of obesity and diabetes mellitus are rapidly rising to epidemic levels in the United States and worldwide. Evidence suggests that excess superoxide generation leading to oxidative stress and/or the reduced capacity of organisms to regulate oxidative/redox environments play a major role in the initiation and progression of these obesity related diseases. Cells contain many pathways to combat these free radicals in order to reduce the destructive effects of oxidative stress. One approach to combat the effects of oxidative stress in the progression of disease is to use the cell's inherent ability to increase expression of various stress-related proteins. Naturally occurring dietary polyphenols such as curcumin are reported to induce expression of genes that demonstrate antioxidant and anti-inflammatory properties. The objective of this study was to determine whether curcumin activates the transcription factors NF- κ B and Nrf2 in cardiomyocytes to exert protective effects.

Methods. Rat cardiac H9c2 cells and mouse HL-1 cells were used for this study. Superoxide scavenging properties of curcumin were determined using a xanthine-xanthine oxidase reaction coupled to lucigenin-enhanced chemiluminescence. Gene expression for antioxidant enzymes were measured using real time PCR. Nuclear translocation of the transcription factors NF- κ B and Nrf2 were determined by measuring protein expression in the cytoplasmic and nuclear fractions after curcumin exposure. Knock down of NF- κ B expression was performed using RNA interference followed by immunoblotting to determine protein expression.

Results. Curcumin dose dependently scavenged superoxide anion with an $IC_{50} = 0.09$ micromoles/liter suggesting that the compound has inherent superoxide quenching properties. In rat cardiac H9c2 cells, curcumin caused a time dependent increase in the gene expression of the cardiac protectant protein heme oxygenase-1 (HO-1). Curcumin (10 micromolar) caused the translocation of NF- κ B and Nrf2 from the cytoplasmic to the nuclear fraction 15 min after exposure. In an attempt to knock down NF- κ B expression, mouse HL-1 and rat H9c2 cells were exposed to control and gene specific siRNAs for NF- κ B for 20 and 48 hours. 48 hour exposure to siRNA for NF- κ B resulted in a 48% decrease in expression of NF- κ B compared to nonsense control.

Conclusions. Curcumin is a naturally occurring phenolic compounds isolated as a yellow pigment from turmeric. It is reported to have antioxidant, anticancer and anti-inflammatory properties. In the present study we show that curcumin has potent superoxide scavenging properties in a cell free assay and increases the expression of the cardioprotective enzyme heme oxygenase-1 in cardiac myocytes. Curcumin also activates the transcription factors NF- κ B and Nrf2 that are reported to induce expression of a number of antioxidant genes. Successful knock down of NF- κ B will allow future studies to examine the effect of this transcription factor on curcumin-induced expression of protective genes in cardiac myocytes.

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The Effect of Freezing on the Elastic Modulus and Fluid Load Fraction of Equine Articular Cartilage

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Introduction: Articular cartilage is a hydrated tissue, which provides smooth, frictionless joint motion. When describing cartilage using a biphasic model, there is a fluid phase and solid matrix, which support the forces cartilage withstands during joint motion. The fluid supports initial loading of the joint but the collagen matrix supports sustained loading, which can be described by the fluid load fraction and elastic modulus. Due to difficulty in obtaining fresh equine cartilage, most research is performed on frozen samples. Although convenient, using cartilage in this manner disregards any structural damage caused by the freezing process. A more thorough understanding of biomechanical properties of cartilage will lead to more physiologic artificial joints and the prevention and treatment of joint diseases. We hypothesized that when testing frozen cartilage samples there would be differences in the elastic modulus and fluid load fraction as compared to fresh samples obtained from the same horse.

Methods: Eleven sets of joints were collected from equine cadavers. The weight bearing portion of the distal third metacarpal surface in the front fetlock, the proximal radial carpal bone in the carpus, and the medial femoral condyle in the stifle were isolated. Random selection was used to determine which side joints would be fresh or frozen. Each frozen sample was maintained at 6.9 °F for at least seven days and then thawed before testing. Samples underwent indentation testing on a Bruker CETR UMT 3 tribometer on a single point. The data from the tribometer was fit to the Ogden model for hyperelastic materials and the initial aggregate modulus of the solid matrix and fluid load fraction values were extracted. Analysis was performed using a mixed model ANOVA, which included Scheffe's Test for Multiple Comparisons to compare fresh and frozen value points.

Results: There was no significant difference found between fresh and frozen values in the initial aggregate modulus of the solid matrix (E_s) ($p = 0.9418$). There was also no significant difference found between fresh and frozen values for the fluid load fraction ($p = 0.9313$).

Conclusions: Ultimately our hypothesis that the fluid load fraction and elastic modulus would change significantly in the frozen samples was proven incorrect. However, there were differences in the average initial aggregate modulus of the solid and fluid load fraction although not significant. This leads to several points of concern for future studies in cartilage. Our project only involved one freeze-thaw cycle with a relatively short freezing duration. Multiple freeze-thaw cycles would allow the same samples to be tested several times; however reusing the same samples may expose the cartilage to damage from each of the tests as was seen in post indentation samples during this study. Further investigation into freezing times is also necessary to determine if longer freezing duration would alter properties of cartilage.

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**Plant-based Omega-3 stearidonic acid (SDA) enhances antitumor activity of doxorubicin (Dox) in human prostate cancer cell lines**

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Abstract

Introduction: Stearidonic acid [SDA; 18:4 (n-3)], a plant derived omega-3 fatty acid, has antitumor properties and protects against heart disease. Doxorubicin (Dox) is an anthracycline chemotherapeutic drug effective in the treatment of several cancers but has a cumulative cardiac toxicity. The objectives of this study were to determine the combined anti-androgenic properties of SDA and Dox and the mechanism of SDA action in human prostate cancer cell lines.

Methods: Cell viability of androgen-dependent LNCaP and androgen-independent PC-3 and DU-145 human prostatic cell lines was determined using trypan blue dye exclusion. The effect of drug treatment on cell proliferation was determined using MTT cell proliferation assays. RT-PCR and immunocytochemistry were used for quantification of androgen receptor (AR) and peroxisome proliferator-activated receptor (PPAR γ) gene expression. Functional receptor activity of PPAR γ and AR in response to SDA and Dox was measured by luciferase reporter assays using HepG2 cells transfected with human PPAR γ plasmid and LNCaP transfected with tumor necrosis factor alpha (NF- κ B), respectively.

Results: Assay by MTT showed that SDA in combination with Dox inhibited cell proliferation in all three cell lines to a greater degree than did treatment with Dox alone. Immunocytochemical assays showed down regulation of AR in LNCaP following treatment with SDA and Dox. In luciferase assay, SDA decreased TNF α -induced NF- κ B production more significantly than the marine-based omega-3 polyunsaturated fatty acid eicosapentaenoic acid [EPA; 20:5 (n-3)] and docosahexaenoic acid [DHA; 22:6 (n-3)]. In PC-3, treatment with SDA and Dox increased nuclear localization of the PPAR γ while, in HepG2 cells, SDA and Dox treatment inhibited rosiglitazone and troglitazone-induced transactivation of PPAR γ in a concentration-dependent manner.

Conclusions: Our results suggest that SDA and Dox have synergistic antitumor effects that could be important in clinical translation. SDA could increase the antitumor activity of Dox through abrogation of NF- κ B activation and down regulation of AR and PPAR γ activity. Further studies are necessary to identify concurrent cardioprotective benefits of SDA in prostate cancer treatment protocols that involve Dox.



The Effects of Anemia on Thromboelastography

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Introduction: Thromboelastography (TEG) is a global test that exceeds the capabilities of standard coagulation tests because it includes the cellular components of blood along with plasma coagulation factors and analyzes whole blood from the time of initial clot formation throughout fibrinolysis. Sick dogs are often hypercoagulable and many are also anemic. It has been suggested that anemia has an independent effect causing hypercoagulability when blood is evaluated by TEG. The goal of these studies was to investigate the effect of anemia on TEG and to determine if the magnitude of that effect was predictable based on the severity of anemia.

Methods: Two studies were performed using blood samples from healthy dogs (20 for the first study and 17 for the second study). Dogs were determined healthy based on the history, complete blood count (CBC), chemistry panel and standard coagulation tests. In both studies, blood was diluted in vitro with autologous plasma to a calculated hematocrit and TEG was run. Study 1: To assess the effect of anemia, TEG results from the undiluted blood were compared to results from blood that was diluted to 15%, 20% or 25% hematocrit with platelet-poor plasma. Study 2: To determine if there was an effect on TEG caused by dilution of platelet numbers TEG was compared between blood samples diluted to 20% hematocrit using preparations of platelet-poor plasma or platelet-rich plasma.

Results: Study 1: The diluted blood was hypercoagulable compared to the undiluted blood, but there was no predictable change in any measured parameter with decreasing hematocrit.

Study 2: No significant difference was seen when blood was diluted with platelet-rich plasmas vs. platelet-poor plasma, although platelet counts were significantly different between the two groups.

Conclusions: Dilution of whole blood with plasma results in hypercoagulability based on evaluation of coagulation by TEG, thus it is important to consider the effects of anemia when evaluating TEG results from sick dogs. The magnitude of change seen in TEG results cannot be directly predicted from the severity of anemia, and other factors are likely contributing to hypercoagulability in sick dogs. No significant difference in TEG results was seen following dilution of blood with platelet-rich and platelet-poor plasma, suggesting that variation in platelet numbers was not a significant factor in the samples that were evaluated.

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The Epididymis as a Target for Environmental and Hormonally-Active Chemicals

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Introduction. Early in embryonic development, both Müllerian and Wolffian ducts are present at the bipotential stage. In males, the Müllerian ducts degenerate under the influence of the peptide antimüllerian hormone (AMH) secreted by testicular Sertoli cells during a process that occurs prior to differentiation of the Wolffian ducts (WD) into the epididymides, vas deferentia, and seminal vesicles. WD differentiation is known to be androgen-dependent and estrogen-sensitive. Therefore, environmental chemicals that interfere with the endocrine axis (endocrine disruptors or EDs) can adversely affect development of the male reproduction system possibly through cellular mechanisms or indirectly by altered AMH secretion and function. However, little is known about hormonal regulation of sexual differentiation early in development. The lack of information impacts our ability to investigate the effects of EDs on gonadal and epididymal development. The present study was designed to describe temporal changes in expression of transcription factors that regulate rat epididymal development.

Methods. Male Long-Evans rats were sacrificed at 21, 35, and 90 days of age to collect epididymal samples, which were then homogenized and prepared for western blotting and densitometric analysis to measure protein levels.

Results. Androgen receptor (AR), estrogen receptor 1 (ESR1) and Wnt4 protein levels increased ($P < 0.05$) from 21 days of age to greatest expression levels at 35 days and then declined into adulthood. Protein expression of estrogen receptor 2 (ESR2) was greater ($P < 0.05$) and similar at 35 and 90 days of age compared to 21 days. β -catenin protein levels were greater at 21 and 35 days of age although not statistically significantly different from levels at 90 days ($P > 0.05$).

Conclusions. Together, our results showed that several transcription factors showed the greatest expression levels at 35 days compared to 21 and 90 days of age. These observations suggest that the prepubertal period is a critical period for epididymal development presumably in anticipation of the approach of puberty. AMH secretion by Sertoli cells is androgen-dependent, and the Wnt4/ β -catenin signaling pathway is subject to regulation by estrogen and ESR agonists. Thus, our results imply that exposures to EDs during development may adversely affect epididymal development. It is likely that derangements of epididymal development contribute to the increasing incidence of disorders of sexual development in the population.

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Graduate Student Poster Presentations

Development of a Proteoliposome Nanocarrier for Mitochondrial Gene Delivery

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Introduction. Although the vast majority of mitochondrial proteins are nuclear-encoded, 13 polypeptides are encoded by the ~16.5 kb closed circular mitochondrial genome. Mutations occur at higher frequency in mtDNA than in the nuclear genome, and affect not only protein genes but also mitochondrial tRNAs and rRNAs. Deleterious mutations in mtDNA generally depress cellular ATP production and manifest preferentially in tissues with high bioenergetic requirements, such as nerve, muscle, heart, liver, kidney and endocrine. The gradual accumulation of somatic mtDNA mutations has been implicated in the overall aging phenotype and many diseases, including Alzheimer's and Parkinson's. In theory, an effective gene therapy for diseases associated with virtually any mutation in the mtDNA would require technology to deliver complete, healthy mitochondrial genomes to the mitochondrial matrix in order to shift the level of heteroplasmy below the 'threshold level' (the mutant load at which the disease phenotype is expressed).

The challenges of passing through the plasma membrane and fusion with the inner mitochondrial membrane (IMM) can only be meaningfully addressed with a vehicle that has first been shown to effectively fuse with the outer mitochondrial membrane (OMM). We propose the development of a synthetic proteoliposome nanocarrier with a composition closely replicating the OMM and containing the mitochondrial membrane protein Mitofusin 2 (Mfn2) to achieve fusion with mitochondria.

Methods. Mfn2 protein will be produced via recombinant technology in *E. coli*. Liposomes will be prepared using thin-film rehydration and sized via extrusion through a polycarbonate filter. The integration of the Mfn2 protein into liposomes is accomplished by a method involving ultracentrifugation through a sucrose gradient. Split complementary luciferase proteins will be utilized to detect fusion of mitochondria with the Mfn2-proteoliposome construct through dimerization of the two halves of the enzyme via a leucine zipper.

Results. Although this project is still at an early stage, significant progress has been made related to production of recombinant Mfn2 protein. Optimization of protein purification and validation of activity will follow. Production of liposomes mimicking OMM composition is also well underway.

Conclusions. An injectable, systemic therapy for altering mtDNA heteroplasmy below threshold requires a nanocarrier that can cross the plasma membrane of most cell types followed by sequential fusion with the OMM and the IMM to deliver a therapeutic mtDNA payload. Our ultimate goal is to exploit the natural mitochondrial fusion pathway by engineering a synthetic biomimicking delivery vehicle for introducing genetic material into mitochondria *in vivo*. This project specifically addresses the first challenge in this process: design and development of a proteoliposome nanocarrier that will recognize and fuse specifically with the OMM of viable mitochondria.

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Transmission of *Corynebacterium pseudotuberculosis* in horses by house flies: serologic response

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Introduction: *Corynebacterium pseudotuberculosis* infection in horses causes three different disease syndromes: external abscesses, internal infection, and ulcerative lymphangitis. The route of infection in horses remains undetermined, but transmission by insect vectors is suspected. The purpose of the present study was to investigate the role of the house fly (*Musca domestica* L.) as a vector *C. pseudotuberculosis* by exposing ponies to house flies previously inoculated with the bacteria.

Methods: Randomized, controlled, blinded experimental study. Eight ponies were housed in individual isolation stalls. Ten wounds were created with a skin biopsy punch in the pectoral region. Cages for flies were sutured over the wound sites. Three ponies were directly inoculated with *C. pseudotuberculosis* by swabbing the wounds (positive control group). Four ponies were exposed to 20 *C. pseudotuberculosis* exposed flies inside the fly cages over the pectoral wounds for 24 hours. One pony was exposed to uncontaminated flies inside the fly cage over the pectoral wounds as a negative control. Ponies were examined daily for swelling, heat, pain and drainage from the inoculation site. Ultrasound examination was performed once weekly. Serum was collected twice weekly for synergistic hemolysis inhibition (SHI) titers. The log₂ titer was compared between groups over time using linear regression analysis, and p<0.05 was considered significant.

Results: Clinical signs of local infection were observed in the 7/7 ponies exposed to *C. pseudotuberculosis*. No clinical signs of infection were observed in the negative control. The peak serologic titer was obtained between 17 and 21 days after infection in the directly inoculated and contaminated fly exposed groups. The maximum peak titer in the directly inoculated group was 1:2048 and in the contaminated fly-exposed group was 1:512. No seroconversion was observed in the negative control. There was no significant difference in the linear increase in titers between the contaminated fly-exposed group and the directly inoculated group. The titers increases were significantly greater in both exposed groups compared to the negative control group (p=0.0002).

Conclusions: House flies are confirmed mechanical vectors of *C. pseudotuberculosis* and can transmit the bacteria to naïve ponies, demonstrated by development of clinical signs of local infection and increase in serologic titers.

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Evaluation of behavioral changes in cattle using three-dimensional accelerometers during experimental infection with bovine viral diarrhea virus

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Introduction. Bovine respiratory disease complex (BRDC) is a leading cause of morbidity and mortality in cattle. Bovine viral diarrhea virus (BVDV) contributes to BRDC development. The need for remote, objective measurement of sickness behavior in cattle has prompted the development of technologies capable of real-time collection of various physiological parameters in cattle. Using three-dimensional accelerometers, the objectives of the present study were to evaluate sickness behavior in cattle experimentally inoculated with a low-virulent strain of BVDV as a subclinical disease model. Behavior responses were evaluated in conjunction with traditional markers of inflammation.

Methods. 20 seronegative beef calves were randomly assigned to BVDV (n = 10) or control (n = 10) groups and housed separately in 2 biosecure pastures. Each calf was fitted with an accelerometer to continuously monitor behavior including time spent lying, standing, and walking throughout the 28-day trial. Baseline behavior data were collected for 7 days prior to challenge. BVDV calves were intranasally inoculated with BVDV on day 0. On days -7, 0, 7, 14, and 21, physical examinations, differential blood cell counts, and serum biochemistries were performed. Haptoglobin concentrations were determined on days 0 and 7. Clinical illness scores (CIS; 1 = normal - 5 = moribund) were assigned once daily and behavioral data were retrieved every 7 days to minimize the impact of human observation. Clinical laboratory data were analyzed using mixed models procedures as implemented in SAS® PROC GLIMMIX. Accelerometer data were converted to proportions and analyzed using a random effects logistic regression approach.

Results. Mild clinical illness (CIS 2) was observed in 4/10 BVDV calves on days 8 – 12, and in 1, 3, and 2 BVDV calves on days 14, 17, and 23 days, respectively. In the control group, CISs of 2 were observed on days 15 and 16 in 2 calves and in 1 calf, respectively. Total WBCs and neutrophil counts were significantly lower in BVDV calves on day 7 and 14 ($p \leq 0.035$). A significant decrease in lymphocyte counts was observed in principal group calves on day 7 ($p = 0.012$). Haptoglobin, fibrinogen, and serum iron concentrations were not significantly different between groups following virus inoculation. BVDV calves spent more time lying throughout the study. On day 8 of the study, BVDV calves spent significantly more time lying down than controls, and this trend was also observed on days 9 and 10. While BVDV calves spent significantly ($p \leq 0.045$) more time walking prior to BVDV inoculation and after day 10, this difference was not observed from day 0 - 9, indicating reduced walking behavior during BVDV infection.

Conclusions. In this study subjectively assigned clinical scores did not reliably differentiate principal from control calves. In contrast, three-dimensional accelerometers remotely detected behavioral differences between principal and control calves that indicated sickness following BVDV inoculation.

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Local toxicity and efficacy of marginal excision combined with intralesional cisplatin bead placement for treatment of soft tissue sarcomas

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Introduction: Adequate local tumor control of soft tissue sarcomas (STS) is often challenging with marginal excision due to infiltrative behavior. Radiation therapy following marginal excision has improved survival time and disease free interval (DFI) for canine patients with STS. Studies investigating intralesional cisplatin therapies in combination with surgery for treatment of STS have shown unacceptably high toxicity. Cisplatin beads have been used in equine medicine with success. The objectives of this study were to evaluate the local toxicity and efficacy of cisplatin beads following marginal excision for the treatment of canine STS.

Methods: Records from Pittsburgh Veterinary Specialty and Emergency Center were reviewed for canine patients who received cisplatin beads between January 2009 and September 2012. Sixty-four dogs received cisplatin beads during the study period, of which 53 tumor sites were evaluated for local toxicity and 53 were evaluated for local recurrence.

Results: Signs of local toxicity were not reported in 51%. Toxicity was graded as mild (22.6%), moderate (22.6%), and severe (3.8%). Overall, 17% of tumors recurred. The median DFI was not reached for grade 1 and 2 STS, whereas the median DFI for grade 3 STS was 148 days. The percent of grade 1 and 2 tumors that were disease free at 1, 2, and 3 years following tumor resection and cisplatin bead placement were 88.7%, 76.2%, and 64.9% respectively.

Conclusion: Intralesional cisplatin beads are well tolerated and effective at preventing/delaying recurrence of marginally excised grade 1 and 2 STS.



Therapeutic Enzyme Distribution After AAV-mediated Gene Therapy in Normal and GM2 Gangliosidosis Cats

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Introduction. The goal of gene therapy is to deliver nucleic acids that encode a therapeutic protein without producing toxicity. This requires a delicate balancing act of maximizing therapeutic protein expression while minimizing vector dosage. AAV-mediated gene therapy has emerged as the most promising treatment for lysosomal storage diseases such as GM2 gangliosidosis (GM2). GM2 is caused by a deficiency of a hydrolytic enzyme, Hexosaminidase (Hex), which leads to accumulation of gangliosides within neurons. The feline model of this fatal disease has allowed for extensive safety and efficacy testing prior to initiating human clinical trials.

Methods. Safety studies were conducted on six normal cats treated by bilateral thalamic and deep cerebellar nuclei (DCN) injections of AAVrh8 encoding the therapeutic enzyme (AAV-Hex) at a dosage of 5×10^{13} ge/kg of brain weight. Efficacy studies were conducted on three GM2 cats treated by bilateral thalamic injections and nine GM2 cats treated by bilateral thalamic and DCN injections. H&E staining was used for evaluation of brain pathology. Global Hex activity was measured in frozen sections with various synthetic substrates and localized expression of Hex was determined by immunofluorescence (IF) on paraffin-embedded tissue sections.

Results. There were no clinical signs of toxicity in the six normal cats treated with AAV-Hex despite extraordinary Hex activity (53-fold above normal) at the injection site. H&E sections from the lateral thalamus and dorsal hippocampus revealed notably eosinophilic neurons, suggesting high-level expression of the therapeutic protein that was confirmed by IF. Treating GM2 cats by bilateral thalamic injections proved efficacious, significantly prolonging survival ($p=0.0064$) compared to untreated cats, and restoring Hex activity to an average of 8.6-fold above normal ($n=3$, $sd=1.5$) at the injection site. Again, expression of the therapeutic enzyme in neurons of the lateral thalamus and dorsal hippocampus was verified by IF. In two of three cats treated in the thalamus only, numerous Purkinje cells expressed Hex in the cerebellum. Treating GM2 cats by bilateral thalamic and DCN injections has proven even more successful at prolonging survival ($p<.0001$) and is significantly better than treating the thalamus alone ($p=0.0287$). This is likely due to astounding levels of therapeutic enzyme achieved at the cerebellar injection site, reaching 52-fold above normal ($n=7$, average=19.2, $sd=19.4$). Exceedingly eosinophilic neurons within the treated DCN were confirmed by IF to have high expression levels of Hex.

Conclusions. AAV-mediated gene therapy achieved remarkably high expression levels of Hex without leading to clinical signs of toxicity. The therapy has proven to significantly improve lifespan, and is most effective by treating both the thalamus and cerebellum. Immunofluorescence elucidated the cellular localization of Hex and will allow for further optimization and targeting of the therapy.

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Pharmacokinetics of Cyclophosphamide in Horses

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Introduction. Cyclophosphamide (CP), a pro-drug, is an alkylating chemotherapeutic agent. CP is administered intravenously (IV) or per os (PO), for the treatment of immune-mediated disease and a variety of malignancies in horses. The current usage and dosing schemes in horses involve extra-label use of CP at doses extrapolated from human literature and other veterinary species. The objective of this study was to evaluate the oral absorption of CP and the bioavailability and adequacy of the currently used 200 mg/m² dosage of CP when administered IV and PO.

Methods. Plasma samples were collected at 17 time points and analyzed for the parent compound and the active metabolite (4-OHCP) using an established HPLC assay.

Results. Plasma samples were collected at 17 time points and analyzed for the parent compound and the active metabolite (4-OHCP) using an established HPLC assay. Blood collected following IV and PO CP at the published dose of 200 mg/m² revealed minimal plasma concentrations of the parent compound making pharmacokinetic analysis impossible. Further, drug exposure measured by area under the curve (AUC) and maximum concentration (C_{max}), following administration of 400 mg/m² IV and 600 mg/m² PO did not approach values comparable to current canine literature.

Conclusions. Understanding the pharmacokinetics of CP in horses will pave the way for accurate therapeutic, maximally tolerated dose escalating studies in horses. Accurate therapeutic doses will have implications for the future treatment of equine malignancies.

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Diindolymethane activates pregnane xenobiotic receptor-mediated CYP3A4 gene expression

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Introduction: Cytochrome P450 3A4 (CYP3A4) is the most prevalent drug-metabolizing enzyme in human liver and intestine. Pregnane xenobiotic receptor (PXR), which is predominantly expressed in liver and intestine, plays a central role in activating the expression of CYP3A4. Activation of PXR and, subsequently its target gene CYP3A4, not only plays a substantial role in xenobiotic detoxification, but also causes undesired drug-drug interactions. Recently, an increased awareness has been given to therapeutic compounds for potential induction of drug-drug interactions through activation of PXR. Here, we studied, whether Diindolymethane (DIM), a naturally-occurring anticancer compound, could induce PXR-mediated expression of CYP3A4 in HepG2 human liver carcinoma cells and LS174T human intestinal epithelial cells.

Methods: PXR transactivation assays were performed to study PXR-mediated CYP3A4 promoter activity in HepG2 and LS174T cells. The CellTiter-Glo luminescent cell viability assays were used to assess cell viability. Quantitative RT-PCR assays were conducted to examine CYP3A4 gene expression in LS174T cells.

Results: DIM significantly enhanced PXR-mediated CYP3A4 promoter activity in a concentration-dependent manner in both HepG2 and LS174T cells. In addition, DIM apparently increased CYP3A4 gene expression LS174T cells. DIM was found to be not toxic to HepG2 and LS174T cells in our experimental conditions.

Conclusions: These preliminary results suggest that DIM activates PXR-mediated CYP3A4 gene expression. Our future studies will determine the mechanisms of PXR activation by DIM.

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**Effects of Chlorhexidine Hydrochloride Intrauterine Suspension Administration in Normal Mares**

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Introduction Endometritis is a common clinical condition affecting broodmares, and is associated with a variety of microbial agents. Treatment of infection is of paramount importance to preserve uterine health. One goal of treatment is to eliminate the infection while not causing further damage to the endometrium. However, there are treatment agents that may cause permanent damage to the uterine endometrium when infused improperly. In 1979, a report in the *Veterinary Record* (Jackson PS, et al) stated that chlorhexidine gluconate was very irritating to the mare's endometrium and the investigators advised against using this antiseptic for treatment of endometritis. Since that time, an FDA approved commercial product containing the active ingredient chlorhexidine hydrochloride has become available for use in mares but subsequently has not gained popular acceptance by practitioners. We evaluated the use of chlorhexidine hydrochloride suspension in the uterus of normal mares to determine what, if any, adverse effects were noted.

Methods Twelve healthy, adult light breed mares were used for this study. All procedures were approved by the Auburn University Institutional Animal Care and Use Committee. All mares were determined to be reproductively normal prior to inclusion in the study by procurement of the following endometrial samples: biopsy for histopathological evaluation, cytology, and bacterial culture. Mares with evidence of endometritis on any of the three samples were excluded. Mares were then randomly assigned to the treatment group (n=6) and control group (n=6). All Mares were examined by transrectal palpation and ultrasonography by one of the authors (AKJ or RRW); when estrus was detected and the presence of a pre-ovulatory follicle was noted (30mm or greater with uterine edema present), each mare was treated with 28 mLs of chlorhexidine hydrochloride suspension (treatment group) or an equal volume of Lactated Ringer's solution (control group) once a day for 3 consecutive days. Biopsy and cytology samples were taken 3, 7, and 14 days after completion of treatment. Cytology and biopsy samples were read by a board certified pathologist (LN) blinded to the treatments, and biopsy samples were graded using a standardized Kenney score.

Results The data was analyzed using Pearson's Chi-Squared tests for pairwise analysis. There was no significant difference with respect to Kenney grade biopsy score, degree of endometrial fibrosis, or presence of cytologic inflammation between control and treatment group (p=0.54, 0.49, 0.06, respectively). Subjectively, mares treated with chlorhexidine hydrochloride exhibited a transient, but profound, increase in uterine edema post treatment. The chlorhexidine hydrochloride suspension was grossly visible in the uterine lumen when mares were examined with transrectal ultrasonography for up to 4 days after treatment.

Conclusions Based on analysis of endometrial biopsy scores, degree of fibrosis, and presence or absence of inflammation, there was no difference between mares treated with chlorhexidine hydrochloride and control group mares. This treatment does not appear to have a deleterious effect on short term uterine endometrial health.

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Survival of Dogs with Nasal Lymphoma Treated with Various Radiation Protocols: 13 Cases

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Introduction: Tumors of the nasal cavity comprise approximately 1% of all neoplasms in dogs. Canine nasal lymphoma is rare. Reports evaluating the outcome of treatment are lacking. The goal of this study was to evaluate the overall median survival times (MST) in dogs with nasal lymphoma that were treated with irradiation, and/or chemotherapy.

Methods: A multi-institutional retrospective study of canine nasal lymphoma was performed. Thirteen cases of nasal lymphoma that received radiation therapy and two cases that received chemotherapy alone were identified. The date of diagnosis, histopathological features, treatment received (radiation and/or chemotherapy protocols) and date of death were reviewed. Kaplan-Meier Survival Analysis was performed to generate overall MST and log-rank tests compared palliative (< 45 Gray) versus definitive irradiation (> 45 Gray). Survival times for patients treated with or without chemotherapy were also evaluated.

Results: The overall MST for dogs receiving radiation for nasal lymphoma was 550 days. Four of 13 cases were censored because they were lost to follow up. Cases treated with definitive irradiation (10/13) had a MST of 550 days, those treated with palliative radiation (3/13) had a MST of 33 days, and those treated with chemotherapy alone (n=2) had a MST of 157 days. Cases that received additional treatment with chemotherapy in addition to irradiation had a MST of 550 days, and those without chemotherapy had a MST of 244 days.

Conclusions: Results of this study suggest that radiation therapy is indicated in treatment of nasal lymphoma in dogs.

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Comparative effects of phenobarbital and zonisamide on clinical patients being treated for epilepsy

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Introduction. Epilepsy is a common neurologic finding in the canine, and most are managed medically through treatment with traditional anti-epileptic drugs (AEDs) such as phenobarbital and potassium bromide. Approximately 20-30% of the dogs treated for epilepsy become refractory to the traditional AEDs and veterinarians are forced to turn to newly developed medications for seizure control. Zonisamide, which undergoes both hepatic and renal metabolism, suppresses epileptic discharges through blockade of sodium and calcium channels as well as GABA potentiation. In epileptic patients receiving combination therapy, autoinduction of hepatic enzymes (cytochrome p450) by phenobarbital may reduce the elimination half-life of zonisamide which also undergoes hepatic metabolism.

Methods. The Therapeutic Drug Monitoring Service at Auburn University's Clinical Pharmacology Laboratory includes a data base of epileptic canine and feline patients receiving differing combinations of AED drugs. Samples received from canine epileptics receiving zonisamide (ZON) (2003-2013) or ZON with phenobarbital (Zon PB) or bromide (ZonBR; control) were studied. Zonisamide was detected in canine serum using the ARK Diagnostic (Sunnyvale, CA) Zonisamide® Immunoassay on a Siemens (New York, NY) Dimension Xpand Plus® general chemistry analyzer validated for canine serum. ZON half-life was calculated ($t_{1/2} = 0.693/k_{el}$ where $k_{el} = \ln(C_1/C_2)/(t_2 - t_1)$ from peak (C_1 at 2 hours after a dose; t_1) and trough (C_2 just prior to second dose; t_2) serum concentrations. Inclusion criteria included: no other drugs and treatment for at least 27 days. Half-lives among groups were compared by one way ANOVA.

Results. A total of 164 canine patients were included in this study: ZON (102), ZonPB (51), and ZonBr (11). The mean half-life (h) for the treatment groups were: ZON (49 ± 22) and ZonBr (45 ± 21) > ZonPB (39 ± 21) ($p = 0.04$).

Conclusions. Despite the small sample size, this study suggests that PB induction of drug metabolizing enzymes shortens ZNS elimination half-life. This has implications in the epileptic patient when PB is begun (therapeutic failure) or discontinued (accumulation of ZON and toxicity). The impact of this finding on control versus not control of epilepsy in patients receiving ZON as an AED has yet to be determined.

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Elucidate the neuroprotective mechanisms of *Scutellaria lateriflora*

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Introduction. *Scutellaria lateriflora* (American skullcap), a native plant of North America, has been used by Americans and Europeans as a nerve tonic and an anxiolytic agents. GABA and glutamate are the major neurotransmitters associated with hyperarousal and excitotoxicity. Bioactive compounds (phytochemicals) present in the selected medicinal plants affect the neurotransmission and also can have neuroprotective properties. The neuroprotective effects of *Scutellaria lateriflora* (alcoholic extract) are not fully elucidated. Therefore, the objective of the current study is to investigate the neuroprotective mechanisms of *Scutellaria lateriflora*.

Methods. Neuroprotective effects were evaluated against hydrogen peroxide induced cytotoxicity using cell viability assay. The antioxidant and anti-apoptotic potential of *Scutellaria lateriflora* was determined in differentiated hippocampal (H19-7) and pheochromocytoma (PC-12) cells. Furthermore, the effect on glutamatergic receptor expression was also studied. One way ANOVA and Dunnet post hoc test was used for finding statistically significant differences between each mean value at $P \leq 0.05$.

Results. *Scutellaria lateriflora* suppressed caspase-3 expression and scavenged the reactive oxygen species significantly. Decreased reactive oxygen species ($P < 0.05$) and caspase-3 activity ($P < 0.05$) strongly correlated with the increased cell viability. Additionally, *Scutellaria lateriflora* increased phosphorylated *cAMP response element-binding* protein (pCREB) and brain-derived neurotrophic factor (BDNF) expressions in H19-7 cells. However, N-methyl-D-aspartate (NR2A and NR2B) receptors expression was not affected by *Scutellaria lateriflora*.

Conclusions. Therefore, the findings of the current research indicate that *Scutellaria lateriflora* exhibit neuroprotection by affecting the nerve growth factors and exhibiting antioxidant and anti-apoptotic action. These findings provide scientific basis for the nerve tonic actions of this botanical therapy against various neurodegenerative diseases.



Differential Expression Pattern and Recurrent Defects in p16 Tumor Suppressor Gene Locus (p16/INK4A/B) in Spontaneous Canine Mammary Tumor and Melanoma Models

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Introduction. In normal epithelium, cell proliferation is tightly controlled by several regulators of the cell cycle check points such as cyclin dependent kinase inhibitors (CKIs) that act as powerful cell cycle regulators as well as endogenous tumor suppressors. The p16/INK4A/B locus encodes three related genes including p16, p14ARF and p15 of which p16 and p14 are alternatively spliced products with different first exons. p16 has been attributed as the most potent tumor suppressor member of the locus, defects in which are strongly associated with tumor susceptibility and its lesions are second in frequency only to p53 for human malignancies. The full-length sequence of p16 and the regulation of p16/INK4A/B gene locus in canine cancers have been largely unknown due to missing coding sequence from the canine genome and presence of extremely GC rich content with repetitive sequence. The objective of this study is to elucidate the regulation of p16/INK4A/B gene locus by determining p16/INK4 gene expression, sequence defects in spontaneous canine mammary tumor (CMT) and melanoma (CML) models.

Methods. Six established CMT cell lines, five malignant CML cell lines, normal canine mammary epithelial cells (CMEC), normal canine fibroblasts (NCF) and several primary tumors from dogs were cultured and used for RNA extraction. Gene expression profiles and sequencing of INK4 genes have been evaluated by optimized RT-PCR, rapid amplification of cDNA ends (RACE)-PCR, touchdown-PCR assays as well as subsequent cloning experiments. The p16 mRNA and predicted protein sequences were analyzed by Vector NTI and bioinformatics tools.

Results. INK4 tumor suppressors are differentially expressed while genes of the p16/INK4A/B locus (p16, p14 and p15) have been found frequently defective in all CMT and CML cell lines. p16 expression defect is more prominent in both canine tumor models. The complete p16 coding sequence from CMT28/CMEC has been identified. A novel frameshift mutation identified in p16 exon 1 α of CMT28 mutation causes altered mRNA and protein expression changing the p16 reading frame which shifts to that of p14ARF. Moreover, the mutation can impose more effects on the predicted protein structure disrupting the native folding of p16 protein and thus abolishing function and stability. Using the same experimental approaches, a large deletion mutation in p16 exon 1- α from a CML cell line was also discovered.

Conclusions. This is the first evidence of the full-length sequence of the p16 transcript obtained from CMT and CMEC/NCF cell lines as well as the discovery of a novel frameshift mutation in p16 exon 1- α from a CMT model. Differential expression of the INK4 genes in our CMT and CML cell lines suggested that defects in these tumor suppressors frequently promote cell transformation in these cancers. The p16 gene defects mostly accumulated in exon 1 α in both canine tumor models and this mapping strongly correlates to p16 mutations in human breast cancers. This study identifies p16 as a critical regulator and a potential therapeutic target in canine and human cancers.

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Adeno-associated virus-mediated gene therapy provides long-term stabilization of neurologic disease

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Introduction. Lysosomal storage diseases (LSDs) have an incidence of 1 in 7700 births and are caused by mutations in genes encoding lysosomal proteins required for degradation of cellular substrates. GM1 and GM2 gangliosidosis (GM1 and GM2) are LSDs caused by deficiency of the ganglioside catabolizing enzymes β -galactosidase (β gal) and Hexosaminidase (Hex), respectively. The resulting accumulation of ganglioside inside lysosomes of the central nervous system (CNS) causes progressive neurodegeneration and death, often in infancy. Adeno-associated virus (AAV)-mediated gene therapy has the potential to provide a permanent source of deficient enzyme and has proven successful in gangliosidosis mouse models. Prior to initiation of clinical trials, gene therapy must be tested in large animal models with a size more closely resembling humans.

Methods. AAV vectors were constructed to encode functional β gal or Hex cDNA. The therapeutic vectors were injected bilaterally into the thalamus and deep cerebellar nuclei of pre-symptomatic GM1 and GM2 cats. Short-term cats were euthanized 16 weeks post-treatment for biochemical analysis of therapeutic effect (GM1, n = 7; GM2, n = 3). Long-term cats were euthanized at humane endpoint defined by the inability to stand on 2 consecutive days (GM1, n = 12; GM2, n = 9).

Results. Sixteen weeks post-treatment enzyme activity against synthetic chromogenic and fluorogenic substrates was restored to near or above normal levels throughout the entire CNS and pathological storage material was substantially reduced in all areas. In long-term therapeutic experiments, 9 AAV- β gal treated GM1 cats currently range in age from 25.9 to 49.3 months and most show only mild or no clinical disease symptoms (untreated survival, 8.0 ± 0.6 months). Quality of life of AAV-Hex treated GM2 cats was also substantially improved with survival up to 35.7 months (untreated survival, 4.4 ± 0.6 months).

Conclusion. No effective treatment exists for human gangliosidosis with palliative measures being the current standard of care. AAV-mediated gene therapy has proven the most promising experimental treatment to date in feline gangliosidosis models. Treatment resulted in widespread enzyme correction and subsequent reduction of pathological storage material throughout the central nervous system leading to significant improvements in quality of life and survival.

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Comparison of the Images of the Canine Middle and Inner Ear using High Field and Ultra High Field MRI

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Introduction. The aims of this study are to evaluate the ability of high field and ultra-high field MRI to provide anatomical detail of the middle and inner ear and to determine the presence of detrimental artifacts. Susceptibility artifacts occur as the result of small variations in the magnetic field strength near the interfaces of substances of different magnetic susceptibility, such as air-bone interfaces. Using higher strength magnets causes increased susceptibility artifact near air filled structures. The relatively large air filled tympanic bulla next to the bone structures of the middle and inner ear could then be a potential cause of susceptibility artifact. The gain in spatial resolution when imaging with increasing strength magnets will be assessed by subjective comparison and evaluated for any increase in susceptibility artifact.

Methods. Cadaver canine heads will be obtained. At least one head from each of the conformation types will be imaged (dolichocephalic, mesaticephalic, brachycephalic), to allow for different volumes of air that may be present within the tympanic bulla and anatomic variation that may contribute to the possible formation of artifact. Images of the head will be performed on a Phillips 1.5 Tesla system, a Siemens 3Tesla system, and Siemens 7Tesla Magnetom system. Each cadaver head will be imaged using a 2-D T2 weighted Turbo Spin Echo sequence and a 3-D T1 weighted gradient echo sequence in transverse, sagittal and dorsal planes.

Results. This is an ongoing study, with only 2 heads currently scanned on the 3 and 7 Tesla systems and one on the 1.5 T system. Comparison exams of images at this time indicate significant improvement of detail of structures, with more confident identification of specific structures. Minimal interference from susceptibility artifact was noted on these images.

Conclusions. Preliminary data and images show increased spatial resolution and anatomic detail with no interference from increase in susceptibility artifact in the higher magnet strength systems. Additional scans to confirm these conclusions are planned within the next 2 months.

Acknowledgments. I would like to thank Dr. Thomas Denny and Dr. Ronald Byers at the Auburn University MRI Research Center, for assistance in the use of the 3T and 7T systems. Dr. John Hathcock, Clinical Sciences Radiology Dept., for assistance in the use of the 1.5 T system at the College of Veterinary Medicine, Auburn University. The funding for this project is provided by the Research and Graduate Studies Committee of Auburn University.



***Salmonella* Enteritidis Bovine Isolate Characterization and Bacteriophage Cocktail Selection**

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Introduction. In 2012, the Center for Disease Control and Prevention investigated a human outbreak of *Salmonella* Enteritidis (S.E.) linked to ground beef products from a single supplier. We hypothesize that S.E. disseminates from the bovine gut and invades peripheral lymph nodes, which are not removed at slaughter, and thus are a potential source of ground beef contamination. In preparation for this work, S.E. isolates from a range of hosts were characterized. Additionally, a set of bacteriophages that target S.E. was selected as a potential alternative treatment to antibiotics for pathogen reduction in beef production.

Methods. Growth rates in Brain-Heart Infusion Broth and Davis Minimal Broth supplemented with dextrose of S.E. isolates from a bovine and chicken yolk-sac source were determined from optical density and CFU/mL growth curves. From a library of 60 *Salmonella*-targeted bacteriophages, ten that showed strong lytic activity against the bovine S.E. strain were selected for inclusion in the cocktail and characterized using transmission electron photomicroscopy. *Salmonella* serotype host range and efficiency of plating were determined for each bacteriophage chosen in the initial selection. Growth/killing curves of a few S.E. strains were conducted in the presence of bacteriophage.

Results. Growth curve results suggest *in vitro* growth differences between the bovine and yolk sack S.E. strains. Six of the ten S.E.-targeted bacteriophages were shown morphologically to resemble members of the Myoviridae bacteriophage family, two resemble the Siphoviridae, and two resemble the Podoviridae. Bacteriophages with strong lytic activity on agar lawns against the S.E. bovine strain also demonstrated lytic activity against several other S.E. isolates from a range of hosts, several *Salmonella* Serovars in our panel, as well as other Enterobacteriaceae, including *Escherichia coli* O157:H7. Growth/killing curves of several of the bacteriophages confirmed their lytic activity against multiple S.E. isolates.

Conclusions. *In vitro* growth differences between the bovine and yolk sack S.E. isolates could suggest physiological differences in S.E. strains due to host adaptation. Further work with these strains in an experimental calf model of infection is warranted. Once this experimental calf infection model is developed, experiments with bacteriophage treatment of S.E.-infected animals will be initiated.

Acknowledgments. We thank Heather Worley and Austin Conley for technical assistance. This work is supported by the Animal Health and Disease Research Program of the College of Veterinary Medicine.



Pharmacokinetics and pharmacodynamics of Leflunomide and its metabolite, teriflunomide (A77-1726), in dogs.

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Introduction. Leflunomide is a human approved immunomodulatory drug that is commonly used in dogs for a variety of immune-mediated diseases. Leflunomide is a prodrug with its activity solely dependent on rapid and complete conversion to the active metabolite, teriflunomide (A77-1726). No studies appear to have described the pharmacokinetics of leflunomide or its metabolite in dogs. The purpose of this study was to describe the disposition of teriflunomide in dogs after single oral dosing (pharmacokinetics) and then to determine a therapeutic range for teriflunomide in dogs with spontaneous immune-mediated disease (pharmacodynamics).

Methods. Apparently normal, healthy dogs (n=4) were orally dosed with 4 mg/kg of leflunomide. Blood samples were intermittently collected between 0 minutes and 60 hours. Leflunomide and teriflunomide were quantified using HPLC. Clinical samples (n=192) were monitored as part of the Auburn University Clinical Pharmacology Laboratory Therapeutic Drug Monitoring (TDM) service. Samples included were received between January 2012 and October 2013. Concentrations were compared among animals whose disease was considered controlled versus not controlled using ANOVA.

Results.

Pharmacokinetics: No leflunomide was detected in any of the samples. Mean pharmacokinetic values for teriflunomide were: $C_{max}=18.9\pm 8.5$ mcg/ml, $T_{max}=1.38\pm 0.25$ hr, $AUC=804\pm 285$ hr*mcg/ml, Half-life= 25 ± 10 hr, $Vd/F=23\pm 32$ L/kg, $Cl/F=0.49\pm 0.6$ L/hr/kg, and $MRT=175\pm 234$ hr. There was also no leflunomide detected in any of the clinical samples.

Pharmacodynamics: Out of 192 clinical samples, 125 were controlled, 23 were not controlled, and 44 were unspecified (based on clinician assessment). The most common indication was granulomatous meningoencephalitis (GME) at approximately 20%. In controlled animals, using a 95% confidence interval, the recommended therapeutic range of teriflunomide is 23.1-30.2 mcg/ml (20-30 mcg/ml) with sample collection any time during the dosing interval. The mean dose of leflunomide used to achieve control was 2.5 mg/kg once daily (CI95% of 2.3-2.8 mg/kg). The correlation (r) between leflunomide dose and teriflunomide plasma concentrations was 0.01.

Conclusions. The pharmacokinetic study indicates a 24 hr dosing interval is appropriate based on a half-life of 25 hr. Based on a 25 hr half-life in the single dose study, and an anticipated accumulation of drug concentrations of two, a dose of 3.5 (3-4 mg/kg) once daily initially should achieve concentrations within the therapeutic range at steady-state (3-5 days). However, the lack of relationship between dose and teriflunomide plasma concentrations supports the importance of TDM to adjust doses for chronic therapy. Timing of sample collection can be throughout a 24 hour dosing interval.

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Undergraduate Poster Presentations

Effects of Medroxyprogesterone Acetate on Uterine Development in the Dog

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Introduction: The uterine gland knockout (UGKO) phenotype was produced in both sheep and mice by strategic administration of progestins to neonates from birth (Postnatal Day = PND 0). Adult UGKO animals lack endometrial glands and cannot support pregnancy. Induction of the UGKO phenotype in dogs would provide a means of inducing sterility non-surgically. In the dog, uterine gland development (adenogenesis) begins during the first week of neonatal life and progesterone receptors are present in uterine tissue at this time. Medroxyprogesterone acetate (MPA) acts as a bioactive progestin in the dog. Here, objectives were to determine effects of neonatal MPA treatment on endometrial gland development and cell proliferation in the canine uterus.

Methods: Mixed breed puppies were given either MPA (10mg/kg body weight, i.m.) or an equal volume of sterile saline beginning at PND 5. Injections were repeated 14 days later and again once birth weight had tripled. Serum MPA levels were determined on PND 5 (baseline prior to treatment) and then 14, 28 and 42 days post-treatment. Puppies were spayed at seven weeks of age. Seven puppies from three litters were used (3 MPA and 4 controls). Uterine tissues were stained with hematoxylin and cross-sections were imaged using the Aperio system. Gland penetration depth was determined from four quadrants, beginning at the mesometrium. Two sets of measurements were taken per quadrant with fourteen total sets of measurements obtained per animal. Additionally, uterine cross sections were stained with POPO-1 to visualize cell nuclei, and immunohistochemically stained for cytokeratin 8 (CK8), an epithelial marker, and proliferating cell nuclear antigen (PCNA), a marker of cell proliferation. Primary antibodies were labeled using unique fluorescent dyes with specific emission wavelengths: A594 (PCNA), A568 (CK8), A490 (POPO-1). Images were obtained using the Nuance FX multispectral imaging system. Spectrally unmixed images were analyzed using Cell Profiler and Cell Profiler Analyst (cellprofiler.org). Quantitative data were subjected to analyses of variance.

Results: No effects of treatment on uterine gland penetration depth were identified. However, MPA levels were elevated at the first time point post-treatment and thereafter. Consistently, a treatment by cell-compartment interaction was detected ($P < 0.01$) for PCNA labeling index, indicating compartment-specific reductions in cell proliferation associated with MPA exposure.

Conclusion: Establishing non-surgical methods of sterilization for companion animals has obvious benefits. Here, potentially anti-adenogenic levels of MPA were present in the circulation within 24 hours of initial treatment and were sustained thereafter. Exposure to MPA did reduce cell proliferation in some cell compartments more than others. Results indicate that induction of the UGKO phenotype in dogs should be possible with identification of appropriate anti-adenogenic conditions.

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Functional characterization of equine endothelial colony forming cells directly cultured from bone marrow and analysis of vascular tube formation with the ImageJ angiogenesis analysis program

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Introduction: Endothelial colony forming cells (ECFCs) can be cultured from peripheral blood or directly from bone marrow and are thought to play a part in adult neovascularization, vascular homeostasis, and repair. Investigation and characterization of ECFCs is important for the possibilities of therapeutic revascularization of ischemic tissues. Standard functional assays describing ECFCs include vascular tube formation in Matrigel® and uptake of acetylated low density lipoprotein (LDL). The purpose of this investigation was to optimize the Image J angiogenesis analysis program in order to objectively describe vascular tube formation in Matrigel® by equine ECFCs. Vascular tube formation and LDL uptake were also evaluated in ECFCs directly cultured from bone marrow of two adult horses in order to compare their characteristics to ECFCs cultured from peripheral blood.

Methods: Two-dimensional Matrigel tube formation images at 24 hours from increasing passages (P3, P4, P6, P8, P10) of peripheral blood ECFCs from 3 adult horse cells were analyzed with Image J using the angiogenesis macro program. Images were opened in TIFF or JPEG format then analyzed using the human umbilical vein endothelial cell (HUVEC) phase contrast option in the program. Two images were analyzed per horse, per passage. Program analysis data was entered into a spreadsheet that included the number of branches, number of segments, number of nodes, total branch length, and total segment length. Data were analyzed with an ANOVA to test for significant differences between horses and passage number. Frozen ECFCs cultured from sternal bone marrow samples from 2 adult horses were thawed and propagated in endothelial growth media. Once they reached 80-90% confluency, the cells were seeded onto Matrigel® in a 96 well culture plate at a density of 10,000 cells/well and 7500 cells/well or seeded into a 24 well plate and incubated with Dil-Ac-LDL for 6 hours and counterstained with DAPI. The cells in Matrigel® were imaged at 0, 5, 24, 48, 72, and 96 hours for tubule formation, and the cells incubated with LDL were evaluated at 6 hours with fluorescent microscopy for Di-Ac-LDL uptake.

Results: Based on the statistical data from the Image J angiogenesis analysis, further modification of the program would be necessary in order to obtain quantifiable results. Whole image analysis is inaccurate in regards to eliminating background and picture quality discrepancies. Current analysis of the bone marrow derived ECFCs is underway in order to characterize the cells using the Matrigel® and LDL assays.

Conclusions: According to the results of this study, future investigation could be directed at deriving a qualitative scale to describe the branching characteristics of the cells, and to optimize a method for measuring branching length and counting the branch points manually. Furthermore, the image taking protocol should be carefully noted and modified to optimize the images in order to obtain better data from the available program options.



Post-graduate/Faculty Poster Presentations

Efficacy of Various Topical Formulations against *Tritrichomonas foetus*

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Introduction. *Tritrichomonas foetus* is a protozoon that causes venereal disease in cattle resulting in significant economic loss. In cows, it causes reproductive tract infection which results in infertility, embryonic death and abortion. Bulls are silent carriers and thus primarily responsible for the transmission of the disease. Because there is no legal treatment for this disease in the United States, infected bulls must be culled. The purpose of this study was to formulate safe and legal topical treatments for *T. foetus* infection in bulls and determine the efficacy of the formulations *in vitro*.

Methods. Six formulations consisting of ponazuril, albendazole or oxibendazole, each with and without the addition of a topical enhancer, as well as the enhancer alone, were compared to a control to test for efficacy *in vitro*. After establishing an effective concentration of 0.5 mL, efficacy was evaluated by adding 0.5 mL of a formulation to 1 mL of *T. foetus* cultured in Diamond's media. The change in concentration of *T. foetus* over time was calculated by counting organisms/mL using a hemacytometer.

Results. The results of all treatments, including the topical enhancer alone, indicated a significant reduction in the concentration of *T. foetus*. Oxibendazole and oxibendazole with enhancer had the largest effect, reducing the concentration to 0 organisms/mL within 2-4 hours.

Conclusions. *In vitro* tests indicate that the combined formulas have the same, or better, efficacy as the drug alone. Oxibendazole had the greatest effect reducing the concentration of *T. foetus* to 0 within 2-4 hours. With evidence of effective topical treatments *in vitro*, further tests should be conducted to determine if these formulations are effective *in vivo* and applicable for treatment of infected bulls.

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Comparison of the Ability of a Novel Umbilical dip, Super7+™ Navel Dip, verses that of 7% Tincture of Iodine to Desiccate the Umbilical Remnant in Neonatal Holstein Dairy Calves

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Introduction. Reduction of naval infections through appropriate management including naval dipping is beneficial to the calf and the producer and is reported to decrease naval infection rates from 20-28% to 5-14%. Obviously, naval dipping is of great importance but obtaining tincture of iodine has become problematic. Therefore, the aim of this study was to evaluate an alternative to 7% tincture of iodine, Super7+™ Navel Dip.

Methods. A total of a 100 neonatal Holstein heifers were utilized in this study. Fifty calves were dipped with Super7+™ Navel Dip immediately following calving and 50 were dipped with 7% tincture of Iodine. The umbilicus and the umbilical remnant of all calves were evaluated 48 hours following dipping of and a 1 inch segment of umbilical remnant was removed and placed in a labeled airtight container. All samples were analyzed and then again in 12 hour increments until the samples contained less than 10% moisture. A serum sample was collected from each calf within 48 hours of birth and tested for total protein and specific gravity and IgG levels.

Results. Upon evaluation, the umbilical remnants of all calves were completely desiccated by 60 hours following calving. Of the remnants dipped with 7% Tincture of Iodine 58% and 42% were desiccated by 48 hours and 60 hours, respectively. Of the remnants dipped with Super7+™ Navel Dip 88% and 12% were desiccated within 48 hours and 60 hours, respectively. Dipping with Super7+™ Navel Dip increased the percentage of calves having a desiccated umbilicus by 30% over that of tincture of iodine. Also, the umbilical remnants of all calves utilized in this study had no evidence of infection in the calves having a low total protein (less than 5.0 g/dL. The range of total protein was 4.5 to 7.2 g/dL and the range of specific gravity was 1.032 to 1.048. Additionally, there was no evidence of dermal irritation around the skin surrounding the umbilicus or any other area that may have contacted Super7+™ Navel Dip. There was a strong association between treatment A, Super7+™ Navel Dip, and drying at 48 hours. ($P = 0.0008$, Mantel-Haenszel Chi-Square). Odds Ratio = 5.31, odds of drying out at 48 hours were 5.31 times higher with treatment A compared to treatment B (tincture of iodine). Total Protein was not significantly different between treatments ($P = 0.415$, Mixed Linear Models). IgG was not significantly different between treatments ($P = 0.439$, Mixed Linear Models). Specific gravity was not significantly different between treatments ($P = 0.300$, Mixed Linear Models).

Conclusions. Super7+™ Navel Dip appears to be superior to tincture of iodine in its ability to more quickly desiccate the umbilical remnant. Hence, Super7+™ Navel Dip appears to function competently as a navel dip and is a viable alternative to 7% tincture of iodine.

Acknowledgments. Innovacyn Rioalto, CA



Mucosal and Systemic Immune Responses Induced after Ocular Avian Coronavirus Vaccination are Evaded by a Field Strain

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Introduction: Respiratory viruses in humans, including coronaviruses, have a close association with ocular diseases and use the eye as a portal of entry into the host, which also functions as a primary site of replication. Similar observations have been made for the avian coronavirus, i.e., infectious bronchitis virus (IBV), which first replicates in the Harderian gland, an eye-associated lymphoid tissue, and subsequently invades other tissues such as the trachea, kidneys and oviducts. IBV tropism to host cells is mediated by spike (S) proteins which form trimers and are cleaved into an amino-terminal S1 fragment, which mediates host attachment and is the target for neutralizing antibodies, and a carboxy-terminal S2 fragment which anchors the protein into the membrane and mediates fusion with the host cell. To generate protective immunity to IBV live attenuated IBV vaccines are delivered via spray or drinking water to induce T and B cell responses in lymphoid tissues such as the head-associated lymphoid tissues (HALT), i.e., Harderian glands and conjunctiva-associated lymphoid tissue, and spleen. **The goal of this study is to analyze the mucosal and systemic immune response induced to a live-attenuated Arkansas serotype IBV vaccine strain in chickens and measure its ability to recognize the S1 sequence of an IBV field strain.**

Methods: To measure antibody responses in plasma and tears an IBV-specific ELISA was developed using either purified, heat-killed IBV as coating antigen or 10 amino acids overlapping 17-mer peptides covering the S1 sequence. Expression of IFN- γ was analyzed by quantitative reverse transcription PCR (qRT-PCR) using qScript One-Step SYBR green qRt-PCR kit. The housekeeping gene β -actin was used for normalization. The fold increase was calculated using the cycle threshold (Ct) $\Delta\Delta$ Ct analysis. Primers were based on previous publication (Kaiser et al.,2000). Melting curves were run for each sample. The hemagglutination inhibition (HI) assay was performed as previously described (D.J. King, 1988).

Results: The IFN- γ response to IBV is biphasic, consisting of an earlier response at 5-6 days post vaccination and a second response around 10 days post vaccination. The secondary IBV IFN- γ response is restricted to the spleen rather than mucosa-associated lymphoid tissues. IBV vaccination increases IBV-specific IgA levels in plasma and tears 3-4 days earlier than IgG levels in the primary response. IgA antibodies prevail in the primary response while IgG antibodies dominated the memory response. To address whether mutations in the IBV S1 protein affect antibody recognition, an overlapping peptide array was used. Distinct B cell epitopes are recognized which are altered in a field IBV strain, which decreases recognition.

Conclusions: The IgA response to IBV occurs 3-4 days earlier than the IgG antibody response in the primary humoral response while the secondary response is dominated by IgG antibodies. The IFN- γ response to IBV is biphasic, consisting of an earlier response at the beginning of the expansion phase and a second response coinciding with the effector phase. The secondary IBV IFN- γ response is restricted to the spleen rather than mucosa-associated lymphoid tissues, indicating the generating of a central memory T cell response. Distinct B cell epitopes are recognized which are altered in an IBV field strain contributing to immune escape by diminishing the recognition by antibodies of linear B cell epitopes and decreasing the HI titer. These data indicate a reduction in neutralizing antibodies and distinct roles for the mucosal and systemic immune compartments in the IBV response.

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Bacteriophage Resistant Mutant of *Salmonella* Newport and Disease in Experimentally Infected Calves

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Introduction. *Salmonella* Newport is an important pathogen of cattle that is transmitted through the food chain to humans. Acquisition of multiple drug resistance by *S. Newport* has complicated treatment and necessitated the search for novel approaches to pathogen reduction. One strategy being evaluated by our research group is bacteriophage treatment for the reduction of *S. Newport* in dairy calves. One challenge to bacteriophage treatment is the rapid development in *Salmonella* of bacteriophage resistance. However, little work has been performed to determine if these bacteriophage resistant *Salmonella* mutants can cause disease in cattle. We hypothesize that bacteriophage resistant *S. Newport* would not be able to colonize cattle and therefore would not be a major concern in bacteriophage treatment protocols.

Methods. From a library of 60 *Salmonella*-targeted bacteriophages, five that were active against *S. Newport* were characterized using transmission electron photomicroscopy. One of these bacteriophages, S50, was added to a growing culture of the parent strain of *S. Newport* to generate a bacteriophage resistant mutant of *S. Newport*. The phage resistant mutant was administered orally at a dose of 10^9 colony forming units (CFU's) to two calves to determine if the mutant alone could colonize. After 11 days, the calves were challenged with the phage sensitive parent strain of *S. Newport* to determine if the mutant provided protection from infection of the parent *S. Newport*. A second pair of calves were challenged with a total dose of 1.96×10^{10} CFU's composed of a 1:1.3 ratio of parent:mutant in a competition experiment designed to determine how well the mutant competed against the parent strain *in vivo*.

Results. Three of the five *S. Newport*-targeted bacteriophages were shown morphologically to resemble members of the Myoviridae bacteriophage family, two resemble the Siphoviridae, and one resembles the Podoviridae. The bacteriophage resistant mutant of *S. Newport* selected using bacteriophage S50 was resistant to phages S11, S40, S41, and S50. In the first pair of calves given the mutant *S. Newport* alone, fecal shedding of the mutant by one calf ceased by day three post-inoculation and by the other calf by post-inoculation day four. Neither calf inoculated with the bacteriophage resistant mutant *S. Newport* strain showed any disease signs. When given the parent phage sensitive strain, the calves were negative for fecal shedding of *Salmonella* by 6 days post-inoculation and showed no disease signs. The calves involved in the competition experiment fecally shed high numbers of both mutant and parent *S. Newport*, with clinical symptoms from day 3 to day 6 for one calf and from day 2 to day 4 for the second calf. The length of fecal shedding was also extended, out to day 24 for both calves.

Conclusions. The generation of a multi-phage resistant *S. Newport* mutant by exposure to only one of these bacteriophages indicates that four of our five cocktail phages may recognize the same receptor on their *Salmonella* host. The conflicting calf fecal shedding results with the bacteriophage resistant mutant suggests that the mutation(s) may have reduced its ability to colonize it's host.

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