

PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter



November 9, 2016

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

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

Welcomes you to our

**PHI ZETA RESEARCH DAY FORUM
November 9, 2016**

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

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



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PHI ZETA RESEARCH DAY FORUM

NOVEMBER 9, 2016 – VETERINARY EDUCATION CENTER

8:30: Opening Statement

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

8:40-11:30 MORNING Presentations - Overton Auditorium

Veterinary Students -- Moderator: Dr. Bruce Smith

- | | | |
|------|-------------------|---|
| 8:40 | Keshley Allen | Pharmacokinetics of Levetiracetam in Foals |
| 8:52 | Carol Kraneburg | Contraception of Feral Cats with Phage-based Vaccines Targeting Gonadotropin Releasing Hormone |
| 9:04 | Kaleigh Myers | Transdermal Delivery of Meloxicam to Red-Tailed Hawks in PLO Gel Pilot Studies |
| 9:16 | Jonathan Tubbs | We can't answer that: The Importance of Careful Interpretation of Swine Production Data |
| 9:28 | McKenzie George | Cyclobenzaprine as a potential pharmacological management tool for controlling stereotypical behavior |
| 9:40 | Victoria Crabtree | What Makes Tenrecs Tick? Community Conservation and Small Mammal Health in Madagascar |

Graduate Students and Residents – Moderator: Dr. Bruce Smith

- | | | |
|-------|-------------------------|---|
| 9:52 | Mariano Mora | Voriconazole Thermogel for Subconjunctival Injection in Horses: in vivo studies |
| 10:04 | Crisanta Cruz-Espindola | Validation of a cannabinoid assay in canine and equine plasma and commercial cannabinoid products |
| 10:16 | Fatma Eldemery | Infectious Bronchitis Virus Recombinant Spike Proteins Confer Protection against Challenge |
| 10:28 | Eric Fish | Normal and malignant canine mammary epithelial cells shed exosomes in vitro with differentially expressed microRNA profiles by deep-sequencing analysis |
| 10:40 | John Roberts | Characterization of Ovarian Follicular Dysplasia (OFD) in five Florida beef herds |



PROGRAM

- 10:52 Melissa Singletary Nanoparticle filtrate obtained from the olfactory epithelium elicit enhanced olfactory neuron response
- 11:04 Katherine Nash Effect of decreased platelet count on multiple electrode impedance aggregometry in dogs
- 11:16 Leah McGlinchey Ex vivo comparison of the bursting strength of surgeon's knots compared to self-locking knots for closure of ventral midline celiotomy in horses

11:28–1:00 POSTER Presentations -VEC Lobby with Refreshment

1:00-5:30 AFTERNOON Presentations - Overton Auditorium

Graduate Students and Residents (continued)

Moderator: Dr. Yaxiong Tao

- 1:00 Austin Conley Pharmacokinetics and pharmacodynamics of rifampin in dogs
- 1:12 Saiada Farjana Chicken embryonic kidney cell-adapted infectious bronchitis virus (IBV) spike protein shows reduced binding to host cells
- 1:24 Sonya Hansen Traumatic atlantoaxial subluxation in dogs: 8 cases (2009-2016)
- 1:36 Jacob Barnoski Serum theophylline after multiple dosing with transdermal gels in cats
- 1:48 Roxanne Rodriguez Galarza Ex-vivo corneal permeation of nepafenac 0.1% ophthalmic suspension in different species (porcine, canine, equine and feline)
- 2:00 Ashley Smith Survival Time and Prognostic Factors for Canine Small Intestinal Adenocarcinoma: A Retrospective Study of 29 Dogs (2006-2016)
- 2:12 Kamoltip Thungrat **(Postdoctoral Fellow)**
The factors of association of antimicrobial resistance and their prescribing practices for treatment of Escherichia coli infections in dogs and cats in the United States
- 2:24 Matthew Coleridge Comparison of lameness scores after a low 4-point nerve block to lameness scores after a low 6-point nerve block in horses with experimentally-induced pain in the metatarsophalangeal joint

2:36 -3:00 Break and Snack - VEC Lobby

Faculty – Moderator: Dr. Juming Zhong



PROGRAM

- 3:00 Ricardo Stockler A Descriptive Analyses of the Commensal Luminal and Mucosal Microbiome of the Duodenum Using a Cannulated Calf Model
- 3:15 Randolph Winter Effects of a combined endothelial colony forming cell / PEG-fibrinogen microsphere scaffold on healing rate and vascularization in distal limb wounds of horses
- 3:30 Julie Gard Assessment of a Topical Alternative Therapy for Experimentally Induced Infectious Bovine Keratoconjunctivitis
- 3:45 R. Curtis Bird Phenotype Analysis in Spontaneous Canine Models of Breast Cancer based on Estrogen Receptor- α , Progesterone Receptor and c-erbB/HER1-4 Receptor Gene Expression

4:00 KEYNOTE LECTURE-Dr. Iain Buxton

5:00 INDUCTION AND AWARDS ANNOUNCEMENT

INDUCTION of new Phi Zeta Members

AWARD presentation

5:30 RECEPTION - VEC Lobby



PHI ZETA KEYNOTE SPEAKER AND JOY GOODWIN LECTURER

“Regulation of CAP-Protein S-Nitrosation in the Origin of Human Preterm Labor”

Iain L. O. Buxton Pharm.D.

Regents Professor, Foundation Professor and Chairman
Department of Pharmacology
University of Nevada School of Medicine

Dr. Iain Buxton received his Bachelor of Arts degree from University of California-San Diego in 1973. He then earned his Doctor of Pharmacy from University of the Pacific School of Pharmacy and Pharmacology in 1978. Dr. Buxton returned to San Diego where he was a clinical resident at the Veterans Affairs Medical Center and later, Director of the Investigational Drug Studies program at the same institution. In 1981, Dr. Buxton joined the Department of Medicine as a fellow in cardiovascular pharmacology. In 1984, Dr. Buxton joined the faculty at the University of California San Diego as an Assistant Research Pharmacologist. In 1985, Dr. Buxton joined the Department of Pharmacology at the University of Nevada as an Assistant Professor. Dr. Buxton was immediately successful and promoted to a tenured Associate professor in 1989 and Full professor in 1995. In 2008, Dr. Buxton was named UNR Outstanding Researcher of the Year and in 2011 was named Regents Professor. In 2013, Dr. Buxton was named Foundation Professor. Today, Buxton is Professor and Chair at the Department of Pharmacology, jointly appointed in the Department of Obstetrics & Gynecology as Clinical Professor.



The broad interests of Dr. Buxton's laboratory are those of receptor-signal transduction in mammalian systems with modern biochemical and molecular methods that include intracellular imaging of events such as calcium release. One of his principal interests is the problem of premature delivery of babies. The signals that initiate contraction of the uterus at the time of labor are not known. They have recently described the contractile actions of adenylyl purines on the smooth muscle of guinea pig uterus and found that the receptor that mediates the contraction of the tissue changes its coupling mechanism significantly during pregnancy in a fashion consistent with a role for these compounds in human parturition. The problem of premature delivery is a devastating human problem that takes its toll both in lives and dollars. His research contributes to a better understanding of the onset of labor in order to help eliminate the problem of premature delivery.



Posters

Undergraduate Students

Jonathan Dismukes	Expression of the INK4AB/ARF tumor suppressor transcription factor MSK1 in canine breast cancer
Landon Stewart	Breast Cancer Cell-Specific Gene Regulation <i>in vitro</i> via siRNA Nanophages

Veterinary Students

Sonja Cox	Evaluating Purification Methods of Adeno-Associated Viral Gene Therapy for Treatment of GM1 Gangliosidosis
Melissa Crepps	Conditionally Replicative Adenovirus for Treatment of Canine Osteosarcoma
Joy Dillon	Cardiovascular and Toxicity Effects of the Redox Responsive MRI Contrast Agent Mn(II) H ₄ qtp2
Patrick Dittmer	Comparing OFA and PennHIP for Hip Evaluation Methods
Sarah Escaro	In Vitro Measurement of Friction of Intact Equine Articular Cartilage Against Various Surfaces
Katie Goebel	Canines as models of human disease: A strategy of reducing heterogeneity for hereditary breast cancer susceptibility gene discovery
Courtney Hawthorne	Evaluation of miRNA stability after exposure to various external conditions
Rhiannon Hedges	Adeno-Associated Viral Gene Therapy in the Tay-Sachs Sheep
Arielle Higgins	Phage-GnRH Constructs for Population Control of Feral Animals: Evaluation in Mice
Kelly Himeback	The effects of endothelial colony forming cells (ECFCs) on blood flow in equine distal limb wounds
Emily Hipp	Modeling the Effects of Bolus Fluid Administration on Canine Platelet Function: A Comparison of Different Fluid Formulations
Courtney Howard	Urethane's effect on cardiorespiratory coupling: It'll take your breath away
Freelie Mitchell	Optimization of DNA Vaccine Targeting GnRH Receptor for Animal Contraception



Carter Mobley	Optimizing and Testing a Cell Culture Model for Parkinson's disease
Samantha Morici	Evaluation of Promoter Tumor-Specificity for use in Oncolytic Virotherapy
Rachel Roberson	Endothelial colony forming cells as treatment for equine distal limb wounds

Graduate Students

Henri Alexandre Giblot Ducray	Impacts of Heat Stress Mitigated by a Yeast Fermentate Product
Gustavo Agne	Prolonged Oral Torsemide Administration in a Horse with Congestive Heart Failure and Atrial Fibrillation
Allison Biddick	Endogenous morphine concentrations in septic versus healthy dogs
Matthew Coleridge	Meta-Analysis of the Effect of Small Intestinal Resection and Anastomosis Technique on Survival and Post-Operative Ileus in Horses
Han Fang	Effect of Niacin on Nonalcoholic Fatty Liver Disease in Adiponectin Knockout Mice: A Pilot Study
Samantha Hagerty	Zinc Metal Nanoparticles in Olfactory Sensory Neuron Signal Transduction
Rochelle Jensen	An adenoviral vectored GnRH vaccine for estrous suppression in mares
Thibaud Kuca	Genomic change associated with serial infections of pregnant cattle and sheep with bovine viral diarrhea virus
Michelle LaRue	Spinal lymphoma in 18 dogs (2001-2015)
Gisela Martinez-Romero	Quantitative evaluation of Mammaglobin-A gene expression in canine mammary tumors
Daniel Newhard	Successful Transvenous Electrical Cardioversion in Dogs with Atrial Fibrillation
Abdul Mohin Sajib	Evaluation of Tumor Specific Promoters for Use in Conditionally Replicating Adenovirus Mediated Virotherapy of Canine Lymphoma
Ramon Zegpi	Kidney-cell adapted Infectious Bronchitis ArkDPI Vaccine Confers Effective Protection Against Challenge



Post-graduate/Faculty

Payal Agarwal

Oncolytic Adenoviruses for the Treatment of Canine Osteosarcoma

Randolph Winter

Effect of Heartworm Disease and Heartworm-Associated Respiratory Disease (HARD) on the Right Ventricle of Cats



Veterinary Student Poster Presentations

Pharmacokinetics of Levetiracetam in Foals

Keshley D. Allen¹, Dr. Aime K. Johnson², Dr. Dawn M. Boothe^{2,3}, Dr. Jacob A. Johnson²

¹DVM Candidate class of 2018, College of Veterinary Medicine, Auburn University, AL

²Department of Clinical Sciences, AUCVM, AL

³Department of Anatomy, Physiology & Pharmacology, AUCVM, AL

Introduction. Seizures in foals may be due to a lack of oxygen to the brain during parturition, head trauma, infections, or developmental disorders. Early treatment with control of seizure activity can lead to a good long term recovery. Current options for seizure control in horses are few and may have detrimental effects in sick foals. Levetiracetam (LEV) has been successfully used for the management of seizures in dogs and cats with little to no adverse effects. The primary purpose of this study was to demonstrate that LEV will achieve and maintain therapeutic dosing concentrations (5-45 µg/mL) for a 12 hour dosing interval in neonatal to pediatric foals following both oral and IV dosing. In addition, age-related changes in the disposition of LEV in foals that might reflect physiologic and metabolic changes accompanying maturity was evaluated.

Methods. Eight healthy foals (5 male, 3 female) were studied at three ages using a randomized cross over design for two routes at each age: intravenous (IV) (20 mg/kg) and oral (30 mg/kg) as a neonate, at one month of age, and four months of age. A four day washout period elapsed between each route. Intravenous LEV was administered via a temporary catheter in the opposing jugular vein and removed after the drug was given. The oral dose was administered via a nasogastric tube and flushed with 120 ml of water. Blood was sampled at 18 different time points over 48 hours to allow characterization of absorption and elimination. Serum LEV was quantitated using a polarized immunofluorescence assay validated for horses (CV was <10% and accuracy was 99-100%; lower limit of quantitation was 2 µg/mL). Data was subjected to noncompartmental analysis. For each foal and each age, absolute bioavailability was based on $(AUC\ PO \times Dose\ IV) / (AUC\ IV \times Dose\ PO)$.

Results. All animals tolerated all dosing with no apparent side effects, and the key pharmacokinetic parameters did not appear to differ among the age groups. The maximum serum concentration (C_{max}) for the neonates was 32 µg/mL +/- 3, with a time to C_{max} (T_{max}) of 77 min +/- 33. For one month of age, the C_{max} was 36 µg/mL +/- 4 with a T_{max} of 57 min +/- 29. The C_{max} for four to five months was 34 µg/mL +/- 2 with a T_{max} of 78 min +/- 42. The oral bioavailability ranged from 93% to 97%, depending on the age of the foal. Levetiracetam remained above the 5 µg/mL minimum for therapeutic levels for almost 24 hours. At 12 hours, the concentration averaged at 13 µg/mL for the oral drug and 8 µg/mL for the IV drug. The volume of distribution in all age groups indicated distribution to total body water, including intracellular distribution. Oral half-life was found to be 394 min +/- 124 for neonates, 348 min +/- 105 for one month, and 458 min +/- 148 for four to five months. IV half-life was found to be 420 min +/- 77 for neonates, 422 min +/- 126 for one month, and 412 min +/- 223 for four to five months.

Conclusions. The dose of levetiracetam was sufficient for both oral and intravenous administrations. The serum concentration remained well within the reference interval during the proposed 12 hour dosing interval. The data appears to support twice a day dosing. There were minimal age-related changes. Other than minor variations in bioavailability, the pharmacokinetics among the 3 ages was remarkably similar. Additional research of LEV is needed in diseased foals to determine if the disease affects absorption, distribution, and elimination of levetiracetam.

Acknowledgments. Thank you to the co-authors for performing this study and allowing student assistance in their research. And thank you to Merial and other sponsors from the Auburn College of Veterinary Medicine for offering students research opportunities.



What Makes Tenrecs Tick? Community Conservation and Small Mammal Health in Madagascar

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²School of Forestry and Wildlife Sciences, Auburn University, AL

Introduction: Madagascar is home to some of the most unique mammals on the planet, including lemurs, tenrecs, and the tufted-tailed rats. Over 90% of Madagascar's forests have been destroyed due to slash-and-burn deforestation techniques that may alter habitats in a way that make better breeding habitat for zoonotic arthropod vectors of disease, such as ticks. The life cycle and the interactions between ticks, mammals, humans, and other endemic Malagasy species remain unclear. The aims of this study were to describe tick populations and the life stages that infest endemic small mammals in the rainforests of Madagascar; and evaluate the impact that habitat loss has on tick infestation and small mammal health by comparing populations in primary and secondary community forest habitats and a third, heavily disturbed site with daily human foot traffic. Due to their small body size, and terrestrial nature, we hypothesize that the tenrecs will be more heavily infested with ticks than other small mammal species, and will primarily harbor tick larvae.

Methods: The primary forest at Torotorofotsy¹ was compared to the secondary disturbed forest at Analamazoatra² and heavily disturbed non-protected forest³. Trapping was performed at each location along three transects, one in each habitat type, for 28 days. Fifteen pitfall traps and Sherman traps were used for non-invasive capture of endemic species. During processing, body mass and condition were recorded along with morphometric measurements and tick infestation was quantified. Representative tick samples were collected in 90% Ethanol for morphological and molecular identification. Blood and fecal samples were also collected as part of a long-term collaborative study. All captured small mammals were processed and released at their site of capture without sedation. Research protocols and sample collection was approved by the Malagasy Ministry of the Environment and Forest Ecology, under permit numbers #120/16 MEF/SG/DGF/DAPT/SCBT. Research protocols were also reviewed and proved by the Auburn University Institutional Animal Care and Use Committee (IACUC ID#2016-2897).

Results: A total of 15 species were captured across the habitat gradient, belonging to seven genera. There were 36 captures at Analamazoatra/Mitsinjo, 5 of which were at the campsite, and 16 mammalian captures at Torotorofotsy. A total of 52 small mammals were captured, including 12 tenrecs, 18 endemic lemurs, 13 endemic rodents, and 9 invasive rodents. Of those, 32.7% were parasitized by ticks. 20% of all mammals captured in the most disturbed and primary forest sites were parasitized by ticks, while 41.7% of captured animals in the secondary community forest were parasitized. More endemic mammals were captured in the secondary community forest, while more invasive species were captured in the heavily disturbed site. 83.3% of the tenrecs captured were parasitized by ticks. All tick life stages: larvae, nymphs, and adult ticks were collected from captured tenrecs contrary to our hypothesis that tenrecs would primarily harbor tick larvae. In total, we recovered 11 species of ticks, including *Ixodes lunatus*, *Ixodes albignaci*, *Ixodes colasbelcouri*, *Ixodes randrianasoloi*, *Haemaephyssalis theilerae*. Six of the tick species collected have not been previously described and are currently undergoing descriptions at the US Tick Collections.

Conclusion: In this study, we examine the influence of habitat disturbance and community conservation on small mammal species diversity and health. Our results support the hypothesis that there is a greater diversity of species in community protected forests than in unprotected sites; however, contrary to our predictions, the greatest percentage of parasitized small mammals were captured in a community protected forest.

Acknowledgments: This research was funded by Merial. We thank Jordan Broadhead, Gabriel Andrle, and the forest guides at Association Mitsinjo for assistance.

¹Torotorofotsy GPS coordinates: 18°77.501 S 048°43.359 E.

²Analamazoatra/Mitsinjo GPS coordinates: 18°52.544 S 048°22.153 E.

³Disturbed habitat/campsite GPS coordinates: 18°56.278 S 048°24.835 E.



Cyclobenzaprine as a potential pharmacological management tool for controlling stereotypical behavior

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²Department of Anatomy, Physiology, and Pharmacology, Auburn University, AL

Introduction. In animals, stereotypic behaviors or repetitive, invariant behaviors with no obvious goal or function such as swaying or pacing are thought to be a result of boredom, stress, or pain. The occurrence of these behaviors is a significant concern for the management of captive bear. Cyclobenzaprine is a muscle relaxant that reduces reducing tonic somatic motor activity by influencing both gamma (γ) and alpha (α) motor systems of the central nervous system. There appears to be some similarity between the effects of structurally related tricyclic antidepressants, including reserpine antagonism, norepinephrine potentiation, potent peripheral and central anticholinergic effects, and mild sedation. In humans cyclobenzaprine is used to treat skeletal muscle conditions. Recent human studies also suggest that cyclobenzaprine may prevent recurrent thinking, a common component of post-traumatic stress disorder (PTSD). The subject of this pilot study was a nineteen-year-old male intact American Black Bear that persistently showed extensive stereotypies including swaying, chomping, head bobbing, and restlessness in addition to decreased normal foraging behavior. The hypothesis was that cyclobenzaprine has a similar effect in black bear as humans: causing a decrease in recurrent thinking which decreases outward signs of stress and decreases stereotypical behavior displayed throughout the day.

Methods. Video surveillance (up to 3 cameras) was utilized in the bear's enclosure recording all behaviors 24 hours per day to evaluate the incidence of stereotypical behavior. Due to visibility limitations of the cameras, data was only recorded during daylight hours. Data was collected for a total of 104 days: 54 days before therapy and 50 days after the addition of cyclobenzaprine. Cyclobenzaprine was administered at 0.12 mg/kg (30 mg) orally once daily with food at approximately 6 to 7 pm.

Results. Prior to therapy, the bear displayed stereotypical behaviors an average of 43.75% of the daylight hours. After beginning cyclobenzaprine therapy, the bear displayed stereotypical behaviors an average of 13.23% of the day at times usually associated with cleaning or other human interaction. This was a decrease in stereotypical behavior by 69.76%. Additionally, natural foraging behaviors occurred an average of 1.88% of the daylight hours prior to therapy. After starting cyclobenzaprine, the bear displayed natural foraging behaviors an average of 14.41% of the day. This was an increase in natural foraging behavior of 766.49%.

Conclusions. To date, Fluoxetine is the only other drug with published information as being used to decrease stereotypical behavior in bear and it had no change on natural behaviors. Stereotypical behavior can be triggered by stimuli similar to PTSD is in humans. Given the recent evidence that cyclobenzaprine can alleviate signs of PTSD in humans, the data collected in this study supports our hypothesis that cyclobenzaprine can substantially reduce stereotypical behavior in black bear and cause a substantial positive increase in natural behaviors. No negative side effects were seen at the dose administered. Considering the degree of improvement seen with this pilot data, further investigation is warranted both on the effects of this drug in different subjects and full pharmacokinetic evaluation of cyclobenzaprine in black bear.

Acknowledgments. Noah's Ark Animal Sanctuary, Allison Hedgecoth, Erin Nipper



Contraception of Feral Cats with Phage-based Vaccines Targeting Gonadotropin Releasing Hormone

Carol Kraneburg¹, Aime Johnson², Becky Jones¹, Anna Cochran-Johnson¹, Jessica Cannon¹, Carla Barstow², James Wright³, and Tatiana Samoylova^{1,3}

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Introduction. Overpopulation of feral animals, including cats, is a growing concern in the United States and in many other regions worldwide. It was estimated that there are 70 million feral cats in this country alone. Controlling animal populations with anti-fertility vaccines was recognized as one of the most promising approaches. The focus of our research is on development of anti-fertility vaccines that are composed of whole phage particles carrying peptides with contraceptive properties for use in feral animals. The vaccines are designed to stimulate potent antibody responses against gonadotropin releasing hormone (GnRH), a master reproductive hormone. Anti-GnRH antibodies inactivate endogenous GnRH that in turn causes reduced release of gonadotropic hormones leading to gonadal atrophy in adult animals or lack of development in sexually immature animals. Previously, several phage-GnRH constructs with potential contraceptive properties were generated via selection from a phage display library. When tested in mice, such phage-GnRH constructs stimulated production of anti-GnRH antibodies that resulted in suppression of serum testosterone, a major indicator of impaired fertility. The goal of the present study is to test these phage-GnRH vaccines for contraceptive potentials in cats.

Methods. Five domestic sexually mature male cats (8-9 months old) were characterized as to their reproductive parameters and then immunized with a phage-GnRH vaccine. Cat blood and semen samples as well as testicular volume data were collected during a 4-month period post-immunization. GnRH antibodies and testosterone in cat serum, testicular volume, and quality and quantity of sperm have been evaluated.

Results. In response to the vaccination with phage-GnRH construct, all cats developed anti-GnRH antibodies. In individual cats, the antibodies appeared at different time points (from 2 to 8 weeks) and their levels were different. The antibody titers continued to increase up to week 16 (latest data time point). Testosterone in the majority of serum samples collected from immunized cats over 4-month period was lower than normal testosterone for the group. Total testicular volume dropped in four cats (7-25%) compared to the pre-immunization measurements. In one cat, the testicular volume was increased. At this time, all cats continue to produce sperm; however, the number of abnormal sperm cells in ejaculates is increased and is characteristic for testes undergoing atrophy.

Conclusions. In summary, the study demonstrated that phage-GnRH vaccines could be a feasible solution for contraception of cats. To assess levels and duration of the immunization-associated changes, the experiment will continue for an additional three months.

Acknowledgments. This study was supported by Merial Summer Program for Veterinary Scholars, Auburn University Intramural Grants Program, and Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University.



Transdermal Delivery of Meloxicam to Red-Tailed Hawks in PLO Gel Pilot Studies

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

Introduction. Topical application of medication via pharmacist compounded pluronic lecithin organogels (PLO) have been embraced by the veterinary profession as an effective method of systemic drug delivery despite lack of scientific evidence of quality, efficacy, and safety of these unapproved products. Compared to canine and feline, avian stratum corneum is only a few cell layers thick, and as such, topical drug preparations may have less of a barrier for entering into systemic circulation. Meloxicam is a commonly used analgesic in avian patients, and compounding pharmacies offer it as a transdermal product. However, no transdermal pharmacokinetics or efficacy data has been reported in any avian species. Investigators hypothesized that plasma drug concentrations of meloxicam will not reach adequate therapeutic levels in raptors when administered via pluronic lecithin organogel.

Methods. A dose escalation pilot study was implemented using a parallel design (3 doses, n=6, 2 subjects per dose) in red-tailed hawks (RTHA). Subjects initially received meloxicam in a transdermal PLO preparation at 1.5 mg/kg. Whole blood was drawn from either the right or left basilic vein at specified time intervals for a 12 hour period. Subjects were monitored for 48 hours for any signs of adverse reactions at the site of application and again examined one week post-study. Serum was collected and analyzed using high performance liquid chromatography. The dose was escalated to 3 mg/kg and then 5mg/kg, and the study repeated. The quality of each gel was assessed for accuracy and precision.

Results. Meloxicam was not detected in RTHA plasma after application of the transdermal gel at 1.5mg/kg or 3mg/kg. Drug was detected at 5mg/kg but only at 45 min (78 ng/mL) and 480 min (48 ng/mL). Both accuracy and precision of all gel concentrations were considered poor; the actual concentration of the gel was consistently lower than the target concentration by $37\% \pm 15$, and the coefficient of variability ranged from 7 to 29.

Conclusions. A previous study demonstrated that 0.5 mg/kg PO achieved peak plasma meloxicam concentrations of approximately 182 ng/mL. This study demonstrated that the transdermal PLO gel did not predictably deliver meloxicam even when subjects received approximately eight times that oral dose. Additionally, the compounded product used in this study was both inaccurate and imprecise. This may be due to the drug molecule having unequal distribution throughout the gel, lack of dissolution, or degradation. These results suggest that transdermal meloxicam in a PLO gel does not effectively penetrate the skin of RTHA to achieve therapeutic concentrations. These results also demonstrate that assessing compounded product accuracy and precision in clinical trials is paramount to a successfully implemented study. Additional studies are necessary to confirm the lack of effective delivery of meloxicam PLO transdermal gel in RTHA. Limitations of this study included the single dose regimen and the small sample size. Future studies include establishing that meloxicam can be compounded into a quality PLO preparation, multiple dosing, and increased sample size.

Acknowledgments. Clinical Pharmacology Laboratory at Auburn University, Southeastern Raptor Center, Meriel Veterinary Summer Scholars Program.



We can't answer that: The Importance of Careful Interpretation of Swine Production Data

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Introduction. Disease mitigation and reproductive potential are key factors in swine production. The batch farrowing system is built around sow reproduction and mitigation of disease. In this system, farrowing occurs every 3 or 4 weeks in an all-in all-out approach. Batch farrowing allows for simultaneous down time in all farrowing rooms for cleaning, disinfection, and drying and is a tool for disease management. Some of the major swine diseases that could be mitigated by use of batch farrowing include Influenza A virus, Porcine Circovirus type 2, and Porcine Reproductive and Respiratory Syndrome virus. *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is endemic in most swine herds worldwide and is the principle cause of enzootic pneumonia. The effect of batch farrowing on prevalence and clinical disease suggestive of *M. hyopneumoniae* infection has not been investigated. Therefore, the objective of this study was to analyze diagnostic and production data from a swine production system to identify whether the prevalence of *Mycoplasma hyopneumoniae* and associated respiratory disease had changed post-conversion to batch farrowing.

Methods. The research question was developed through discussions with representatives of a swine production system of ~20,000 sows who reported a perceived increase in respiratory disease post conversion to batch farrowing of ~50% of the system. A working hypothesis and research goals were generated and a timeline established. The hypothesis was that conversion to batch farrowing increased respiratory disease associated with *M. hyopneumoniae* infection. Initial data review to summarize finisher close-outs and ELISA for *M. hyopneumoniae* (ELISA Oxoid) from finishers through descriptive statistics was conducted. Production flows in the data were classified as either batch or control (non-converted) groups with pre and post conversion data for each. Further analysis prompted a comprehensive investigation using sow farm production data divided into the same classifications.

Results. A comparison of means of key production outcomes pre and post conversion follows: Feed Efficiency - Batch 2.68 to 2.7, control 2.59 to 2.65; finish viability % - Batch 92.5 to 94.0, control 94.7 to 95.3; average daily gain (kg)– Batch 0.87 to 0.89, control 0.92 to 0.91; average wean age – Batch 20.3 to 20.2, control 19.5 to 20.2. Detailed discussion and analysis of these data revealed several confounders present in the data sets. Conversion to batch farrowing required a change in breeding timing affecting wean age by parity and extended nursery time. Confounders also included changes to the vaccination program and other health challenges. Confounders could not be accounted for in data analysis and made further statistical analyses and interpretation difficult.

Conclusion. It is noted therefore, that careful interpretation of swine production data with extensive knowledge of the system is needed by researchers and practitioners before recommendations can be made and questions can be answered. If significant confounders exist, production data analysis should be carefully performed to avoid misleading conclusions. The definition of a research question prior to data collection is ideal to obtain meaningful answers.

Acknowledgments. University of Minnesota College of Veterinary Medicine and F. Ménard Inc.



Graduate Student Platform Presentations

Serum theophylline after multiple dosing with transdermal gels in cats.

Jacob L Barnoski¹, Tekla M. Lee-Fowler¹, Dawn M. Boothe², and Ellen N. Behrend¹

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² Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Alabama 36849

Introduction: Oral bronchodilators, a component of long term management of feline asthma, are difficult to administer on a daily basis for many owners. Transdermal (TD) formulations of medications have been developed to address this issue. Our objectives were: 1) determine if therapeutic serum concentrations could be achieved using long term once daily dosing of TD theophylline 2) evaluate the difference between two TD theophylline formulations.

Methods: Healthy cats, between 1 and 10 years of age, were evaluated in a two way, randomized, double-blinded, cross-over study. Participants received TD theophylline at 15mg/kg for 21 days in either PLO or Lipoderm® formulation. On day 22, serum was collected 2, 6, 14, and 24 hours after dosing. After a 14 day washout period, serum was collected to verify non-detectible theophylline concentrations. The alternate formulation was administered for 21 days, and sampling was repeated. Serum theophylline concentrations were determined using an automated immunoassay. Formulations were compared using a paired t-test.

Results: Seven cats completed both arms of the study. Serum theophylline concentrations in the human therapeutic range were achieved for 2/7 cats in the PLO and 2/7 cats in the Lipoderm® groups. No significant difference was detected between the TD formulations at any time point. No adverse reactions were reported.

Conclusions: Once daily dosing of TD theophylline appears safe but does not reliably achieve therapeutic concentrations in all cats. Individual cats may achieve therapeutic concentrations. No significant difference was noted between PLO and Lipoderm® formulations.

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Comparison of lameness scores after a low 4-point nerve block to lameness scores after a low 6-point nerve block in horses with experimentally-induced pain in the metatarsophalangeal joint

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Introduction

This study evaluated whether a 6-point nerve block provides significantly more analgesia to the metatarsophalangeal joint than a low 4-point nerve block.

Methods

A sensor-based, motion analysis system was used to evaluate the gait of 6 horses before induction of lameness, after administration of interleukin-1 β into a metatarsophalangeal joint, after anesthesia of the medial and lateral plantar nerves and the medial and lateral plantar metatarsal nerves, and after anesthesia of the lateral and medial dorsal metatarsal nerves. The magnitude of hind limb lameness was calculated as the sum of hip drop and hip hike (HS) for all trials.

Results

Treatment of the metatarsophalangeal joint with interleukin significantly altered HS as compared to untreated controls. There was significant difference in HS between treatment with interleukin and the 4-point nerve block. No significant difference in HS between the 4-point block and the 6-point block was identified.

Conclusions

The results indicate that innervation of the metatarsophalangeal joint by the dorsal metatarsal nerves is too minimal to interfere with localizing the site of pain causing lameness to the metatarsophalangeal joint using regional analgesia.



Pharmacokinetics and pharmacodynamics of rifampin in dogs

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Introduction. *Staphylococcus pseudintermedius* is the most common pathogen associated with pyoderma in dogs. Normally is responsive to cephelexin, the first choice systemic antibiotic for its treatment, increasingly, isolates are expressing methicillin resistance. For example, in our hospital, the percent of *S. pseudintermedius* isolates expressing resistance increased from 22% in 2007 to 43% in 2012. Many of these methicillin resistant isolates are also multidrug resistant. Rifampin is an antimicrobial to which these isolates appear remain susceptible. Increasingly this drug is being used to treat infection in dogs. However, little epidemiologic data is available to guide the empirical use of this drug in the absence of culture and susceptibility data. The purpose of this study was to determine the Minimum Inhibitory Concentration (MIC) of *Staphylococcus pseudintermedius* isolates expressing resistance to methicillin; and to compare the MIC to isolates expressing susceptibility.

Methods. *S. pseudintermedius* expressing either Methicillin resistance (SPMR) or Methicillin susceptible (SPMS) were obtained from clinical patients presented to the Small Animal Veterinary Teaching Hospital at Auburn University. and determined the MIC of rifampin in both. MIC, or Minimum Inhibitory Concentration. The strains of *S. pseudintermedius* were obtained from the Bacteriology department of the Auburn University Veterinary School. A total of 100 strains (50 SP-MR and 50-SPMS) collected between 2006 to 2016 were studied. Protocols established by the Clinical Laboratory Standards Institute were followed. In order to be able to expand the concentrations tested beyond those limited for clinical use, E-test® methodology was used. E-test® strips contain the antimicrobial of interest at concentrations that range from 0.002ug/ml to 32ug/ml fold. CLAS guidelines were used to help determine an outline to follow when starting this study. Previously frozen strains were grown out using TA, Tetrazolium and Arabinose, and then used to inoculate Mueller-Hinton upon which a Rifampin E-strip was then placed. The MIC (indicated by the point of no growth) was read the following day. In the absence of canine breakpoints, human breakpoints were used: isolate were considered susceptible at \leq to 1ug/ml and resistant if the $>$ 4ug/ml. In the 50- SPMS isolates, the MIC's were 6 at 0.003UG/ml, 16 at 0.004ug/ml, 18 at 0.006ug/ml, 9 at 0.008ug/ml and 1 at 0.094ug/ml. For SP-MR isolates, 1 at 0.002ug/ml, 6 at 0.003ug/ml, 25 at 0.004ug/ml, 10 at 0.006ug/ml, 4 at 0.008ug/ml and 4 at 0.012ug/ml.

Results. The MIC₅₀, or the MIC where fifty percent of the isolates are susceptible, of the one hundred strains that were tested was found to be 0.004ug/ml and the MIC₉₀ was 0.008ug/ml.

Conclusions. Methicillin resistant isolates are not expressing resistance to rifampin; as such, it is a reasonable choice for treating methicillin-resistance *Staphylococcus pseudintermedius*. Further studies are indicated to determine how rapidly resistance emerges in treated isolates.

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Validation of a cannabinoid assay in canine and equine plasma and commercial cannabinoid products.

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Introduction. The approval of medical marijuana in many states has led to a plethora of internet cannabinoid products being marketed to pets and people. However, there is no regulatory oversight regarding product quality. Several HPLC methods have been described in the literature, but most of them failed to separate efficiently all the cannabinoids. This study describes the development of a specific reverse phase high and ultra-performance liquid chromatographic (UPLC-HPLC) with a simultaneous UV and MS detection of 5 major Cannabinoids: Cannabidiol (CBD), Cannabigerol (CBG), Cannabinol (CBN), (-) Δ^9 -THC and Cannabichromene (CBC) in oil, capsules, biscuits, plants, and horse and canine plasma.

Methods. Based on previously reported methods and in-depth study of the chemical structures, physical properties, and sample type (commercial products and plasma), two different analytical methods were evaluated for optimal chromatographic conditions necessary for separation of these structurally similar compounds. Included were 2 differing columns (C8 and C18) that varied in length and particle size, different temperatures, 2 mobile phases, 2 flow rates, and 2 detection methods: ultraviolet (UV) and mass spectrometry (MS). Optimal sample preparation also was determined such that matrices (eg, plant matter, excipients) could be removed without loss of recovery of each compound. This included identifying the most appropriate solvent for each product, and optimal methods for sample sonication, precipitation and solid phase extraction (SPE). Once optimal conditions were determined, a full validation was implemented to assure a robust, precise and accurate assay.

Results. The optimal chromatographic conditions for preparations were: separation using a C8 5 μ m, 250 x 4.6 mm column at 40 °C, using acetonitrile: water 83:17 (v/v) mobile phase, flow rate of 1.5 mL/min, detection at 210 nm. For plasma samples: separation was accomplished with a C18 1.8 μ m, 2.1 x 50 mm column, mobile phase of a gradient of 0.1% Formic Acid/ acetonitrile (1.5 min at 70% of acetonitrile, followed by 90% over 1.5 min), a flow rate of 0.5 mL/min, and MS detection. Cannabinoids were extracted from commercial products with methanol. Cannabinoids were extracted from plasma using protein precipitation (THC-D3 added as internal standard) followed by SPE using an Oasis HLB cartridge and methanol elution. The eluent containing the compounds was dried under nitrogen. The concentrated, cleaned sample was reconstituted with methanol. 50 μ L of preparation solutions and 1 μ L of plasma solution was injected into the respective instrument. The lower limit of quantification (LOQ) for the HPLC-UV method was 2 μ g/mL for CBD and 25 ng/mL for CBN, CBG, THC and CBC each. The LOQ for UPLC-MS method was 4 ng/mL in horse and canine plasma using 100 μ L sample size for all Cannabinoids. The % recovery (Accuracy) was 90% \pm 15 % with a 7 % Precision.

Conclusions. We have successfully validated a cannabinoid assay for quantitation of the 5 major therapeutically beneficial cannabinoids in plasma or preparations. This assay will support clinical trials demonstrating safety and efficacy of these promising agents. Further studies will focus on validation in human plasma.

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Infectious Bronchitis Virus Recombinant Spike Proteins Confer Protection against Challenge

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Introduction. Infectious bronchitis virus (IBV) is an important coronavirus of chickens causing tremendous economic impact worldwide. IBV undergoes an evolutionary process resulting in continuous emergence of new serotypes and lack of effective vaccination thus, new safe and effective vaccines are required. Spike (S) protein is the most variable protein of IBV and the major inducer of neutralizing antibodies. The spike S1 subunit mediates viral attachment to host cells. The spike S2 subunit, which is more conserved among IBV strains and does not contain an independent receptor-binding site, is responsible for membrane fusion and likely assists in virus attachment. Based on our results showing increased binding to chicken tissues of S1+S2 ectodomain protein compared to S1 protein, we hypothesized that S1+S2 ectodomain protein confers better protection against challenge than S1 protein. Therefore, we compared immunization with recombinant S1+S2 versus S1 proteins against IBV challenge using a prime-boost approach.

Methods. Strep-tagged soluble trimeric recombinant spike proteins, S1 and S1+S2, were produced in human embryonic kidney (HEK) 293T cells from codon-optimized constructs. The proteins were purified using Strep-Tactin® Sepharose columns. Immunizing protein emulsion was prepared using Seppic Montanide™ ISA 71 VG adjuvant. Four groups of specific-pathogen-free (SPF) chickens were used. Chickens in groups A and B were primed with 10 µg of S1 and 20 µg of S1+S2 proteins respectively, by subcutaneous injection at 12 days of age then boosted 21 days later. Additional C and D groups were unimmunized (adjuvant only) challenged and unimmunized unchallenged control groups. Groups A, B and C were challenged with IBV Ark-type strain (10^5 50% EID) 3 weeks after boost. The effect of immunization (protection) was evaluated 5 days' post-challenge by viral load in the lachrymal fluids and tracheas as determined by qRT-PCR, and tracheal histopathology.

Results. The chickens immunized with recombinant S1+S2 protein showed statistically significant reductions of viral load both in the lachrymal fluids and the tracheas compared to those immunized with recombinant S1 protein. Consistent with results of viral load, statistically significantly lower thickness of mucosa and lymphocyte infiltration, and deciliation and necrosis scores revealed that recombinant S1+S2 protein provided better protection against tracheal damage after challenge compared to recombinant S1 protein and unimmunized challenged controls.

Conclusions. Recombinant S1+S2 ectodomain protein confers better protection than recombinant S1 protein against Ark challenge in a prime-boost regime. This suggests that the S2 domain has an important role in inducing protective immunity. Thus, including the S2 domain with S1 might be promising for better viral vectored and/or subunit vaccine strategies.

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Chicken embryonic kidney (CEK) cell-adapted infectious bronchitis virus (IBV) spike protein shows reduced binding to host cells

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Introduction. Avian Infectious bronchitis virus (IBV), a gammacoronavirus is one of the most economically important pathogens of chickens. The S1 subunit of the IBV spike (S) protein mediates viral attachment and the S2 subunit is involved in fusion to host cells. IBV can replicate in CEK (chicken embryonic kidney) cells, but virus must be adapted to these cells for efficient propagation. It was observed that two amino acid changes in the S1 protein and one amino acid change in the S2 protein occurred during adaptation of an IBV Ark vaccine strain to CEK cells (Ghetas, et al., 2015). We hypothesized that these alterations in the S gene of CEK-adapted IBV allow the virus to attach more efficiently to CEK cells compared to Ark vaccine strain, thus contributing to adaptation.

Methods. Secreted strep-tagged recombinant proteins representing S1 and S proteins of Ark vaccine strain and CEK-adapted vaccine strain were produced in HEK293T cells and affinity purified using Strep-Tactin Sepharose column. Recombinant proteins containing only one or two of the three amino acid changes found in CEK-adapted vaccine spike protein were also generated. For binding assay, the S1 or S proteins complexed with streptactin-HPRO were incubated with acetone-fixed CEK cells, and bound protein detected with chromogenic substrate AEC (3-amino- 9-ethyl-carbazole).

Results. We observed very little binding to CEK cells by vaccine S1. However, markedly increased binding occurred with whole vaccine S protein. Contrary to the expected improved binding to CEK cells, no binding of CEK-adapted S protein to CEK cells was observed. Then, effects of each of the three amino acid changes in spike protein associated with adaption to CEK cells were tested individually. Each of the three amino acid changes reduced, but did not abolish, binding of spike ectodomain to CEK cells. Together they abolished binding. Taken together our results suggest that all three changes in S of CEK-adapted virus contribute to the lack of binding.

Conclusions. Each of the three changes in S protein associated with adaption to CEK cells, either alone or together, reduced or abolished rather than improved binding to CEK cells. Thus, factors other than improved attachment to CEK cells are involved in adaptation of this vaccine strain to CEK cells, and changes in the genome outside of the spike gene might contribute to this adaptation.

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Normal and malignant canine mammary epithelial cells shed exosomes in vitro with differentially expressed microRNA profiles by deep-sequencing analysis

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Introduction. Breast cancer (BC) in women and canine mammary tumor (CMT) patients share clinical, pathological, and molecular similarities that suggest dogs may be a useful translation model. Many cancers, including BC, shed exosomes that contain microRNA (miRs) into the microenvironment and circulation, and these may represent biomarkers of metastasis and tumor phenotype.

Methods. Normal canine mammary epithelial cells (CMEC) and five CMT cell lines were grown in DMEM + 2% serum-free media supplement. Exosomes were isolated by precipitation and centrifugation then analyzed by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Exosomal RNA was submitted for deep-sequencing of miRs on an Illumina HiSeq2500 sequencer.

Results. CMEC and CMT cell lines shed round, “cup-shaped” exosomes approximately 25-30 nm on TEM. DLS confirmed an average diameter of 23.7 nm. Deep-sequencing averaged ~15 million reads/sample. 290 unique miRs were detected, with 209 having 2-fold difference between one or more CMT and CMEC samples. Only 2% of miRs were universally up-regulated in CMT samples (miR-7, miR-18a, miR-19a, miR-155, miR-181a, and miR-181b); many other miRs had down-regulated or mixed expression. The universally up-regulated miRs were predicted in silico to target canine Estrogen Receptor, Progesterone Receptor, Epidermal Growth Factor Receptor and ErbB-2/HER-2.

Conclusions. CMEC and CMT cells shed exosomes in vitro that contain differentially expressed miRs. CMT exosomal RNA expresses a limited number of miRs that are universally up-regulated relative to CMEC, and these are predicted to target biologically relevant hormone receptors. These results may inform future studies of circulating exosomes and miRs as biomarkers in women and dogs.

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Traumatic atlantoaxial subluxation in dogs: 8 cases (2009-2016)

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Introduction. Atlantoaxial subluxation is due to a congenital malformation of the first two cervical vertebrae. Atlantoaxial subluxation due to trauma has not been previously reported in dogs. The objectives of this study were to identify cases with traumatic atlantoaxial subluxation and to characterize the presentation, treatment, and outcome for these cases.

Methods. Medical records (2009-2016) from an electronic database were reviewed to identify cases with a diagnosis of traumatic atlantoaxial subluxation. Patients were excluded if they had evidence of congenital atlantoaxial subluxation.

Results. Eight cases of traumatic atlantoaxial subluxation were identified. Of these cases the majority were male (6/8 [75%]). Mean age was 5.28 +/- 3.35 years; and median body weight was 4.86 kg (range 2.95-25). No single breed was overrepresented. Modified Frankel Scores were evaluated for each patient on presentation and at discharge. On presentation, 75% (6/8) of patients were classified as nonambulatory tetraparetic and the most common injury was trauma inflicted by another animal (5/8 [62.5%]). The mean Animal Trauma Triage score was 2.13 +/- 1.45. Average length of hospitalization was 12.5 +/- 4.6 days. Diagnosis was made using a variety of imaging modalities including vertebral column radiographs (6/8 [75%] of which 83.3% were diagnostic), CT (7/8 [87.5%]), and MRI (7/8 [87.5%]). Fractures were identified in 62.5% (5/8) of cases. Of those with fractures identified, 80% (4/5) had at least one cervical vertebral fracture and 60% (3/5) had concurrent skull fractures. The majority of cases underwent surgical repair (7/8 [87.5%]). Complications were seen in 87.5% (7/8) cases; the most common of which was aspiration pneumonia (3/8 [37.5%]). All eight cases survived to discharge. At the time of discharge, 4/8 (50%) were classified as ambulatory tetraparetic, including two that were nonambulatory tetraparetic on presentation. The four cases that were nonambulatory tetraparetic at discharge progressed to being ambulatory within two months of surgery.

Conclusions. Traumatic atlantoaxial subluxation is an uncommon occurrence. With surgical stabilization and appropriate aftercare, these patients can have an excellent prognosis.



Ex vivo comparison of the bursting strength of surgeon's knots compared to self-locking knots for closure of ventral midline celiotomy in horses.

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Introduction.

Knot strength, holding capacity, suture type and closure pattern play an integral role in prevention of incisional complications following closure of the equine linea alba. The objective of this study was to test the bursting strength of equine linea alba in an ex vivo model following closure of a simple continuous suture pattern with a novel self-locking knot combination. This novel self-locking knot combination has been shown *in vitro* to have a higher knot holding capacity and smaller volume compared to traditional end and start knots, properties that could lead to reduced incisional complications. Our hypotheses included the following: 1) the novel self-locking knot combination of a Forwarder start and Aberdeen end knot (F-A) would have a significantly higher bursting strength than traditional surgeon knot combination of a surgeon start and surgeon end knot (S-S); 2) failure would occur by breakage of the suture regardless of the knot combinations and 3) suture failure would occur at the knot.

Methods.

A 20 cm ventral midline celiotomy was created in 14 fresh equine cadavers. Horses were randomly assigned to celiotomy closure with a single suture strand of 3 Polyglactin 910 in a simple continuous pattern utilizing either a F-A (n=7) or S-S (n=7) knot combination. Prior to closure, a 200L polyurethane inflatable bladder was placed in the abdomen, then insufflated until failure of the celiotomy closure. Treatment group, celiotomy closure time, bursting strength (mmHg), and failure mode were compared using the Wilcoxon Rank Sum test.

Results.

The median bursting strength for closure with the S-S combination was significantly lower (290 mmHg) than the median bursting strength for closure with the F-A combination (388 mmHg) ($P=0.035$). There was no significant correlation between horse age or weight and the bursting strength of the closure (age: $p=0.10$; weight: $P=0.47$). Failure occurred most commonly at the knot when closed with the S-S combination (n=6), but the majority of F-A closures failed at the fascia adjacent to the incision (n=6). The median closure time was 10.15 minutes (IQR 9.6-12.4) and 12.26 minutes (IQR 8.3-14.3) for S-S and F-A knot combinations, respectively. There was no significant difference for closure time (minutes) between the two knot combinations ($P=0.48$).

Conclusions.

Closure of a ventral midline celiotomy with the self-locking knot combination of a Forwarder start and Aberdeen end knot results in a significantly higher bursting strength compared to the conventional knot combination of with a surgeon start and surgeon end knot. The S-S knot combination mostly failed at the knot, whereas the F-A knot combination mostly failed along the fascia adjacent to the incision.



Voriconazole Thermogel for Subconjunctival Injection in Horses: *in vivo* studies.

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Introduction: Keratomycosis is a sight-threatening disease in horses. Treatment of keratomycosis is challenging, expensive, lengthy and intensive. Subconjunctival (SC) administration of a slow-release, thermosensitive, biodegradable voriconazole-thermogel may allow for sustained delivery of therapeutic concentrations of voriconazole to the cornea. In vitro studies showed sustained release of the voriconazole in therapeutic concentrations for up to 28 days, however there is a need to demonstrate the safety and concentrations achieved in the different ocular tissues of live horses.

Methods: In phase one, 6 horses received topical voriconazole (1%) every 4 hours (group 1) for 2 days and a single SC injection of 1.5% voriconazole-thermogel (group 2) in alternate eyes in a cross-over design. Aqueous humor and tears were collected on day 2 in group 1 and on days 2, 7, 14, and 23 in group 2 for determination of voriconazole concentrations. A complete ophthalmic examination including a modified Hackett-McDonald microscopic ocular inflammatory scoring system was used to provide a single inflammatory score for each examination. In phase 2, 3 horses received SC 1.5% voriconazole-thermogel either 2 days or 2 hours before euthanasia for determination of voriconazole concentrations in ocular tissues.

Results: Physical and ophthalmic examination revealed no adverse reactions. No significant difference was found from baseline in the cumulative daily inflammatory scores throughout the entire study period in any of the horses. The mean drug concentration in tears and aqueous humor on day 2 for group 1 was 3.5 and 1.5 $\mu\text{g/ml}$ respectively. Levels in tears and aqueous humor for the horses in group 2 was detectable but not quantifiable on days 2 and 7 post injection, and non-detectable on days 14 and 23. On preliminary results for the horses in phase 2, voriconazole was detected in all of the ocular tissues with variations in the concentrations between animals.

Conclusions: SC injection of voriconazole-thermogel is safe to perform in horses. Quantifiable voriconazole concentrations in the tissues versus tears and aqueous humor is likely due to the lipophilicity of the drug and the lack of ocular inflammation in the horses. In normal horses, low aqueous humor concentrations detected can be related to intact ocular barriers.

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Effect of decreased platelet count on multiple electrode impedance aggregometry in dogs

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Introduction. The Multiplate™ analyzer is a multiple electrode impedance aggregometers (MEPA) that measures platelet aggregation in response to platelet agonists and can be used to diagnose acquired and inherited platelet disorders. The purpose of this study is to assess the effect of platelet count on platelet aggregation as measured by Multiplate™. We hypothesized that MEPA results would be directly affected by platelet count in dogs.

Methods. 24 healthy dogs with normal baseline bloodwork were enrolled. Citrated venous samples were aliquoted into multiple fractions with progressively depleted platelet counts. All manipulated samples were corrected to within 2% of native hematocrit by addition or removal of platelet poor plasma. Aggregation was initiated in each sample by addition of adenosine diphosphate (ADP). Aggregation area under the curve (AUC), velocity (V) and maximum aggregation (MA) results were analyzed using linear mixed models, controlling for hematocrit, platelet count and leukocyte count, and including a random interceptor for subject, with significance set at $P > 0.05$.

Results. Initial hematocrit, leukocyte count and platelet count did not differ significantly between gender groups. Leukocyte count was significantly positively associated with AUC, V and MA, while hematocrit and platelet count did not have a statistically significant independent effect.

Conclusions. The findings indicate that leukocyte count is positively associated with ADP induced MEPA using citrated whole blood.

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Characterization of Ovarian Follicular Dysplasia (OFD) in five Florida beef herds.

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Introduction. A slaughterhouse study commissioned by Florida Cattleman's Association in 2007, identified ovarian follicular dysplasia (OFD) as a primary cause of infertility in Florida beef cows. Ovaries with OFD have progressive bilateral development of solid clustered follicles containing multiple Call-Exner bodies that progress to bilateral Sertoli-type Granulosa theca cell tumors.

Methods. Four hundred and fifty cull cows selected by private veterinarians representing five Florida ranches received reproductive tract palpation, ultrasound examination with 5 MHz linear probes (Aloka, Ibex) and blood collection. Based on ultrasound findings, 10-16 females per ranch were followed to slaughter the following day for collection of reproductive tracts, and ovarian sampling for RNA sequencing. Ovaries with OFD were graded histologically I to IV and follicular morphometrics were recorded. Circulating levels of progesterone (P4) and Anti-Mullerian Hormone (AMH) in serum were quantified in blood samples from approximately 200 cows.

Results. Ovarian Follicular Dysplasia was identified at slaughter in 38/66 cows. Infertility from other disease was diagnosed in 8/66 and 20/66 were determined to be normal. The distribution of OFD for 38 affected females was Grade I 17/38, Grade II 15/38, Grade III 4/38 and Grade IV 2/38. Increased hyperechogenicity and decreased number of fluid filled follicles could be detected with ultrasound and were present in higher grades OFD. No relationship was identified between circulating levels of AMH and P4 and OFD. Initial RNA sequencing analysis demonstrates 628 up-regulated and 457 down-regulated genes in OFD ovarian tissue. Twenty-eight microRNAs were found to differ between Non-OFD and OFD ovarian samples. Of those 28, 23 were increased in expression in OFD and 5 were decreased in expression.

Conclusions. OFD was the leading cause of infertility identified at slaughter in the five ranches. Increased ovarian hyperechogenicity is caused by dystrophic mineralization of dysplastic follicles. Routine ultrasound can detect cows with advance OFD and these cows may serve as sentinels to identify OFD affected herds. Single analysis of progesterone and Anti-Mullerian Hormone in serum did not serve for ante mortem diagnosis of OFD. Considerable genetic variation was detected between OFD and Non-OFD ovaries and multiple targets for continued bioinformatics studies have been identified.

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***Ex-vivo* corneal permeation of nepafenac 0.1% ophthalmic suspension in different species (porcine, canine, equine and feline)**

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Introduction. Topical non-steroidal anti-inflammatory drugs are used commonly to treat intraocular inflammation (surgical and nonsurgical) in veterinary medicine. Nepafenac the only prodrug NSAID, has not been evaluated for use in dogs, cats or horses. The purpose of this investigation was to evaluate the *ex-vivo* transcorneal permeation of nepafenac 0.1% and compare its permeability profile across the porcine, canine, equine and feline corneas.

Methods. Fresh corneas were obtained from porcine, equine, canine and feline eyes free from corneal disease that were euthanized for reasons unrelated to this study. Corneal buttons (8 mm) were dissected using standard eye bank technique within 2 hours of enucleation. Corneas were mounted horizontally between the donor and the receiving compartments of an all-glass modified Franz diffusion cell (0.20cm²), which were maintained at 37°C. The donor compartment was filled with 0.1 mL of nepafenac 0.1% formulation (Nevanac, Alcon Laboratories, Inc Fort Worth, Texas, USA) n=4 per species studied. Samples (1ml phosphate buffered saline pH 7.4) were removed from the receiving compartment at set times: 0, 1, 2, 4, 6, 8, 12, 24 hours. High-performance liquid chromatography was used for nepafenac analysis concentration. The cornea and residual solutions were collected at the end of the experiment. Permeability parameters were determined and compared with ANOVA statistical analysis (P>0.05)

Results. Mean permeation rates ($\mu\text{g}/\text{cm}^2/\text{hr} \pm \text{SEM}$) were 0.752 ± 0.116 , 1.281 ± 0.247 , 0.944 ± 0.098 and 2.494 ± 0.171 for the porcine, canine, equine and feline corneas, respectively. Permeation rate of nepafenac 0.1% of feline corneas was significantly greater than other species ($p < 0.05$).

Conclusions. The results showed that 0.1% Nepafenac is able to permeate the cornea in normal porcine, canine, feline and equine eyes in an *ex-vivo* model. The data obtained demonstrated an interspecies difference with the feline cornea showing a significant increase in the permeation rate which suggests a potential alternative to treat intraocular inflammation in this species.



Nanoparticle filtrate obtained from the olfactory epithelium elicit enhanced olfactory neuron response

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Introduction: Zinc metal nanoparticles (ZnNPs) are present in human and animal blood¹. The endogenous and engineered ZnNPs were found to enhance olfactory responses to the odorant by about 3-fold as measured by whole cell patch-clamp or electroolfactogram (EOG)². Increased cAMP production through phosphodiesterase inhibition resulted in no EOG enhancement by ZnNPs indicating that the ZnNPs were functioning at the olfactory receptor level³. Physical characterization of the engineered ZnNPs revealed their peak activity at 1.2 nm in size and a non-oxidized state⁴. The endogenous existence of ZnNPs within the olfactory epithelium (OE) and respiratory epithelium (RE) tissues has not been described. This study examined the presence, and physiological properties of endogenous ZnNPs obtained from the OE and RE comparative to engineered ZnNPs.

Methods: The isolation of endogenous ZnNPs began with collecting OE and RE by microsurgery. Extra-fine extracts have been achieved through grinding and consecutively filtering 30-kDa and 3-kDa filters to produce nanoparticle isolated filtrates. Physiological testing by EOG on freshly isolated rat OE and odorant responses were evoked by the odorant mixture of ethyl butyrate, eugenol, and (+) and (–) carvone. ZnNPs were produced from bulk metal rods by an underwater high-voltage discharge method. Calculation of the concentration of endogenous nanoparticles was performed using the relative EOG peaks as a function of engineered zinc concentration for a calibration curve.

Results: The response as measured by EOG demonstrated the engineered ZnNPs, OE and RE filtrates mixed with odorant evoked higher olfactory responses compared to the response induced by odorant alone. The calculated concentrations of ZnNPs in OE and RE filtrates are $5.3 \times 10^{-3} \pm 5 \times 10^{-4}$ nM and $2.7 \times 10^{-3} \pm 5 \times 10^{-4}$ nM, respectively. The concentrations of ZnNPs in OE and RE are 10.3 ± 1.0 nM and 7.9 ± 1.5 nM, respectively.

Conclusions: The presence of ZnNPs within the body may have physiological significance. To determine the role of ZnNPs in olfaction, many questions still have to be answered. However, the presence of olfaction enhancing nanoparticles within the OE and RE suggests a physiological role in the initial events of olfaction at the receptor level. The nanoparticle action in this study on EOG is analogous to that with zinc nanoparticles described previously while the further characterization of the filtrate by transmission electron microscopy and energy-dispersive X-ray spectroscopy are needed.

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Survival Time and Prognostic Factors for Canine Small Intestinal Adenocarcinoma: A Retrospective Study of 29 Dogs (2006-2016)

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Introduction. Small intestinal adenocarcinoma is an infrequently described neoplasm in dogs. This tumor is locally invasive and exhibits a moderate metastatic rate. The current literature is limited regarding the prognosis for this disease. Previously, median survival times of 8-10 months were reported in two small populations of dogs treated with surgery alone. Regional lymph node metastasis was documented as a negative prognostic indicator and reduced survival time to 3 months. To the authors' knowledge, there are no studies investigating the role of adjuvant chemotherapy and/or non-steroidal anti-inflammatory drugs (NSAIDs) for this disease. The primary objective of the study was to compare the outcome of dogs with small intestinal adenocarcinoma treated with surgical resection to those that received adjuvant chemotherapy and/or NSAIDs. The null hypothesis was that the addition of chemotherapy and/or NSAIDs would not improve patient survival times when compared to surgical resection alone. The secondary objectives of the study were to describe the affected population and identify any potential prognostic factors.

Methods. The medical records of 29 client-owned dogs with surgically resected, histologically diagnosed, small intestinal adenocarcinoma were retrospectively reviewed. The following information was recorded for analysis: signalment, clinical signs, physical examination findings, packed cell volume, total solids, diagnostic imaging results, tumor size, tumor location, characteristics on histopathology reports (serosal extension, lymphatic invasion, surgical margins, lymph node metastasis), type of adjuvant chemotherapy, NSAID administration, and patient survival. Variables were assessed for associations with survival time via Kaplan-Meier analysis and Cox linear regression.

Results. The overall median survival time (MST) for dogs with small intestinal adenocarcinoma undergoing surgical excision was 544 days (95% CI, 369-719 days). The 1- and 2-year survival rates were 60.3% and 36.5%, respectively. On multivariate analysis, only age category was found to be an independent predictor of survival ($P=0.003$). Dogs < 8 years experienced a significantly longer survival (MST 1193 days) compared to dogs \geq 8 years (MST 488 days). The presence of lymph node metastasis, administration of adjuvant chemotherapy, usage of NSAIDs, or any other assessed variables did not statistically influence patient survival.

Conclusions. Canine small intestinal adenocarcinoma carries a fair prognosis following surgical excision even in cases with lymph node metastasis. Prospective studies are warranted to better characterize the effects of adjuvant chemotherapy and/or NSAID administration on patient survival.

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Post-graduate/Faculty Platform Presentations

Phenotype Analysis in Spontaneous Canine Models of Breast Cancer based on Estrogen Receptor- α , Progesterone Receptor and *c-erbB*/HER1-4 Receptor Gene Expression

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Introduction. Canine & human breast cancers have similar etiologies, genetics & natural history making canine breast cancers excellent intermediate models of human disease. This investigation was designed to develop validated QrtPCR (quantitative reverse transcriptase polymerase chain reaction) assays for transcripts encoding estrogen receptor alpha (ER1) & progesterone receptor (PR) as well as luminal epithelial-specific proto-oncogenes encoding *c-erbB*-1 (EGFr/epidermal growth factor receptor/HER1), *c-erbB*-2 (cHER2), *c-erbB*-3 (cHER3) & *c-erbB*-4 (cHER4) receptors. These assays will provide rapid assessment of breast cancer phenotype for canine cancers similar to that used in human disease. These transcripts were selected based on the importance of their involvement in neoplastic transformation in breast cancer. The role of relatively underexplored HER3 & HER4 receptor expression was also evaluated to assess their involvement in the development of neoplastic potential.

Methods. Well characterized p16-defective stable canine mammary cancer cell lines (CMT9, 12, 27, 28, 47 & 119), normal canine mammary epithelial cells (CMEC) and normal canine fibroblasts (NCF), were used to investigate expression of the major breast cancer-specific hormone receptors ER1 & PR as well as the proto-oncogenes encoding HER1-4 receptors. These canine-specific assays were developed based on unique sequences characteristic of each transcript carefully avoiding regions of common or shared sequence homology to ensure no cross-amplification within these gene families was detected. The role of relatively underexplored HER3-4 receptor expression was also evaluated in the development of this mRNA-phenotype analysis. Expression of each gene was validated by DNA sequencing following PCR amplification.

Results. Of 6 CMT cell lines, 3 were HER2 positive phenotype due to high levels of HER2 expression & essentially non-detectable levels of ER1 & PR expression. One cell line was designated Luminal A as it expressed low levels of HER2 & high levels of PR but no ER1. Two CMT lines were designated Luminal B as they expressed HER2 at high levels as well as lower or equal amounts of ER1 & PR. When HER1-4 receptor expression was considered, 3 CMT cell lines expressed high levels of HER3 & 1 of these CMT lines also expressed HER4.

Conclusions. Expression of ER1/PR & *c-erbB*-2/HER2 in CMT cells successfully defined distinct human-like breast cancer phenotypes for canine mammary cancers. Investigation of expression profiles of all 4 epidermal growth factor receptor family genes (HER1-4) in CMT models also provided an enriched molecular classification of canine breast cancer. This strategy identified new extended phenotypes using a precision medicine approach for diagnosing canine breast cancer subtypes. HER3 was identified as a key receptor suggesting important potential as a therapeutic target.

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Assessment of a Topical Alternative Therapy for Experimentally Induced Infectious Bovine Keratoconjunctivitis

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Introduction: Infectious bovine keratoconjunctivitis (IBK), commonly called “pink eye”, is a painful condition affecting beef and dairy cattle worldwide. The bacterium, *Moraxella bovis* is known to be responsible for this condition. Antibiotic therapy has been primarily utilized for this condition. However, there is a need to reduce the amount of antibiotics utilized in food animals.

Hence, the objective of this research was to determine if utilization of an alternative therapy (Vetericyn Plus™ Pinkeye Spray) would promote healing and aid in the reduction of pain and infection due to experimentally induced IBK.

Materials and Methods: Thirty dairy bull calves having determined to have normal ophthalmic examinations and who were culture negative for *M. bovis* were randomly assigned to three groups for a single eye block randomized blinded challenge study. Calves were housed in pairs according to their respective group in an approved isolation facility. On day zero a 0.6 mm corneal lesion was made on the center of the left corneas of Groups 1 and 2 utilizing n-heptanol. Immediately following lesion formation, 1.0×10^7 of *Moraxella bovis* (strain Epp63-300; origin: NADC) was administered topically to the left central corneas of Groups 1 and 2. The calves in Group 3 (Control group) received topical corneal administration of *M. bovis* to their left eyes but nothing further. Starting on day one, each calf in Group 1 received two mLs of Vetericyn Plus™ Pinkeye Spray topically to each cornea twice daily for 10 days. Additionally, calves in Group 2 each received two mLs of 0.9% Saline topically to each calves' cornea twice daily for 10 days.

Each animal was given a pain score twice daily (based on blepharospasm, ocular discharge and tearing). All eyes were cultured on day -7, 0, 1-5, and day 10. Digital images were taken of each eye of each calf from day 0 to day 10 of the study. The lesions were measured daily utilizing Image-J technology. Additionally, serum and plasma samples were drawn from all calves on days 0, 1, 10, 11, and 17 and evaluated for changes in sodium and chloride levels. Statistical evaluation was performed utilizing SAS® and a Kenwood-Roger correction was utilized.

Results: All calves in group 1 and 2 developed lesions in the left eye as determined by fluorescein staining. All calves in group 2 developed lesions consistent with IBK in the left eyes. Calves in group 2 only were determined to be culture positive for *M. bovis* during the study period. Between Days 1 and 2, Group 1 had significantly, $P < 0.05$, decreased pain scores when compared to controls. On average there was a reduction in pain score by 79.1% by day 2 and an 83.7% reduction in pain by day 10 when compared to controls. Group 2 had an average reduction in pain score of 18.3%, and 67.9% by day 2 and by day 10, respectively, when compared to controls. There was no significant difference in sodium and chloride levels in the plasma and serum among all three groups at any of the sampling time points ($P < 0.05$). The days to cure was significantly different between Group 1 and Group 2 ($P = 0.0161$) and there was a significant difference in the treatments when evaluating lesion width and circumference ($P = 0.0147$) and ($P = 0.0375$), respectively.

Conclusions: The results of this study indicates that an alternative therapy (Vetericyn Plus™ Pinkeye Spray) can significantly aid in the reduction of pain, infection and healing time of corneal lesions in calves experimentally infected with *M. bovis*.

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A Descriptive Analyses of the Commensal Luminal and Mucosal Microbiome of the Duodenum Using a Cannulated Calf Model.

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Introduction The gut microbiome provides important metabolic functions for the host animal. Bacterial dysbiosis as a result of bacterial, viral, and parasitic gastrointestinal infections can adversely affect the metabolism, productivity, and overall health. The objective of this study is to characterize the commensal microbiome present in the lumen and the epimural surface of the duodenum of cattle, as we hypothesize that due to metabolic processes and or host proprieties, there are differences in the natural microbiota present in the epimural surface and luminal contents of the bovine duodenum.

Methods Duodenal lumen contents (LS) and epimural surface biopsies (EPS) were collected from 6 dairy crossbred steer calves. A flexible video-endoscope was used to harvest four biopsy samples via a T shaped intestinal cannula. In order to assess as much environmental and individual calf microbiota variation as possible, each calf was sampled three times over a six week period. A total of 36 samples were collected, 18 LS samples and 18 EPS specimens. The DNA was extracted from the samples and submitted for 16S rRNA gene PGM bacterial sequencing.

Results The top five phyla present in the LS consisted of Firmicutes (52%), Bacteroidetes (32%), Proteobacteria, Spirochetes and Fibrobacteres. In contrast, in the EPS, 75% were Firmicutes and 10% of Bacteroidetes, followed by Proteobacteria, Tenericutes and Cyanobacteria ($p < 0.03$). Firmicutes and Bacteroidetes composed over 80% of the microbiome present in both sample locations. The percentages overall bacterial diversity for the phylum Firmicutes and Bacteroidetes between sample locations were also considered to be statistically different ($p < 0.03$).

Conclusion Changes in the ratio of Firmicutes to Bacteroidetes can adversely affect the ability of the gut to absorb or secrete metabolic byproducts. Characterizing the gastrointestinal microbiome in vivo is imperative. This study satisfied the hypothesis as differences in the natural microbiota of the LS and EPS were found. Further study is warranted to explore the impact of medical therapy and or environmental effects on the metabolically-active gut microbiome of ruminants.

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The factors of association of antimicrobial resistance and their prescribing practices for treatment of *Escherichia coli* infections in dogs and cats in the United States

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Introduction: Antimicrobial use is a major contributor to antimicrobial resistance, yet few published data are available regarding a pattern of antimicrobial use in companion animal practice. To effectively prevent and control resistance, medical communities need to monitor and limit antimicrobial use. The objective of this study is to demonstrate the pattern of antimicrobial prescribing for treatment of feline and canine *E. coli* infections and to identify associated factors including demographics, signalment and patient conditions.

Methods: A retrospective study was conducted during January 2008 and January 2013. *E. coli* spontaneous canine (n=610) and feline (n=213) infections (n=823) were diagnosed in veterinary practices in the US. A questionnaire was sent to veterinarians submitting samples to veterinary diagnostic microbiology laboratories which subsequently cultured *E. coli* and determined its antimicrobial susceptibility to 16 drugs. The information collected by included patient signalment (species, gender, breed, and age), history of *E. coli* infection (location, severity, duration, treatment, and response), veterinarian antimicrobial prescribing behavior (antimicrobial selection, and dosing regimens), and demographic data of clinics (number of clinicians [specialty], number of patients, and zip code). Descriptive statistical analyses were performed to assess the pattern of antimicrobial prescription. Logistic regression identified factors associated with antimicrobial prescriptions.

Results: Antimicrobials were used to treat 93.2% of animals from which *E. coli* was cultured. Antimicrobials most frequently used were amoxicillin-clavulanic acid (AMC; 24.8%), and enrofloxacin (ENR; 22.2%). Regarding appropriate antimicrobial use according to current guidelines, the recommended dosing interval of q 8 hrs for the time-dependent drug, amoxicillin with or without clavulanic acid was followed only in 4% of those patients receiving this drug for treatment of UTI. For the concentration-dependent drug enrofloxacin for which a recommended dosing regimen is 5 to 10 mg/kg q 24 hrs, the average dose for dogs was 4.7 ± 3.8 mg/kg at q12h and 6.0 ± 2.4 mg/kg q 24 hrs. None was above 10 mg/kg. The proportion of extreme-drug resistant isolates (resist to all 6 antimicrobial class) that were inappropriately treated empirically (before culture results were obtained) was 93% for ENR (13/14). 76% of patients receiving AMC had susceptible isolates (145/190). Interestingly, the *E. coli* infection resolution rate did not differ for AMC (67%) or ENR (69%).

Conclusions: Clinicians frequently prescribe antimicrobials empirically. However, this data indicates that dosing regimens are not in concert with current recommendations intended to minimize the advent of resistance. Our study suggests that improving the procedures of antimicrobial prescription in small animals may possibly lead to decrease the therapeutic failure and prevalence of emerging antimicrobial resistance.

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Effects of a combined endothelial colony forming cell / PEG-fibrinogen microsphere scaffold on healing rate and vascularization in distal limb wounds of horses

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Introduction. Endothelial colony forming cells (ECFCs) are progenitor cells which function in neovascularization and may be useful therapeutically in conditions characterized by poor blood supply, such as distal limb wounds in the horse. Combination ECFC /hydrogel scaffold injectable therapy may ensure cell survival and cell localization to improve neovascularization and healing.

Methods. Autologous ECFCs were isolated from 6 adult horses, labeled with quantum nanodots (QD), and a subset of cells encapsulated in poly(ethylene) glycol fibrinogen microspheres (PEG-Fb MS). Full-thickness dermal wounds were created on each distal limb and randomly assigned to injections with empty PEG-Fb MS, serum, ECFCs, and ECFCs encapsulated into PEG- Fb MS. Analysis included wound surface area (WSA), granulation tissue scoring (GS), thermography, and immunohistochemical staining of biopsied wounds for von Willebrand factor (VWF).

Results. Treatments were well tolerated in all horses. QD Labeled cells were identified in week 1-3 biopsies in ECFC and ECFC-MS groups. There was no significant effect of treatment on GS or thermographic imaging, but the WSA was influenced by treatment ($p=0.0002$), with ECFCs having the smallest WSA at 4 weeks. GS were greater for wounds biopsied ($p=0.0009$) and for hindlimbs ($p=<0.0001$). Thermographic analysis revealed that hindlimbs had a greater temperature than forelimbs ($p=<0.0001$). Blood vessel quantification by vWF immunostaining revealed no treatment effect.

Conclusions. Lack of treatment effects on blood flow and vessel density may be due to cell therapy or due to model variability and sensitivity of analysis. Despite variability within the model, ECFC treated wounds had a significantly positive effect on wound healing.

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Undergraduate Poster Presentations

Expression of the INK4AB/ARF tumor suppressor transcription factor MSK1 in canine breast cancer.

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

Methods.

Cell culture: Six established canine mammary tumor cell lines (CMT 9, 12, 27, 28, 47, 119) and one primary canine mammary epithelial cell culture (CMEC) were grown in Alpha-MEM supplemented with 10% FBS, 100 µg/ml penicillin, and 100 µg/ml streptomycin.

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

RT-PCR: RNA was analyzed by a reverse transcriptase-PCR protocol. The forward primer for MSK1 was 5'-GACATGACAAGGCAGTTGACTGGT-3' and the reverse primer was 5'-GAAGGAGCAACAAGGAATAGCCCT-3'. Reverse transcriptase was performed for 45 minutes at 48°C. The cDNA amplification program consisted of an initial denaturation step (94°C for 2 minutes) followed by 30 amplification cycles of denaturation (94°C for 1 minute, 65°C for 1 minute, and 68°C for 1 minute). A final extension at 72°C for 7 minutes was included. The DNA was purified using the QIAquick Gel Extraction Kit (Qiagen). Equivalent volumes of each purified PCR product DNA were resolved on a 2% agarose ethidium bromide-stained gel. In order to confirm the nucleotide sequence of the amplified products, purified PCR product DNA was sequenced (MGH Sequencing – Harvard U) and compared to mammalian MSK1 sequences in GenBank using DNA-Star software.

Results. In all CMT and CMEC cell lines, the MSK1 PCR DNA product was detected on ethidium bromide-stained agarose gels at revealing the predicted 450 bp amplicon. The specificity of the amplified product was confirmed in all CMT and CMEC cell lines by DNA sequencing. The amounts of amplicon recovered, using this semi-quantitative assay, suggest enhancement of expression in neoplastic cells.

Conclusions. MSK1 was expressed in all 6 established CMT cell lines as well as in the CMEC cell line culture. Enhanced expression of MSK1 in neoplastic cells confirmed the attempts by these cells to suppress proliferation but then fail in this effort due to defects in the p15/p16 encoding INK4AB/ARF locus. To our knowledge, there are no studies that have analyzed the expression of MSK1 in canine mammary gland cancers or to assess its value as a therapeutic target in canine breast cancer patients.

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Breast Cancer Cell-Specific Gene Regulation *in vitro* via siRNA-Nanophages

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Introduction. Short interfering RNAs, or siRNAs, are a useful molecular biology tool for controlling gene expression by acting as a molecular “on/off” switch for specific gene expression. The translation of siRNA delivery to *in vivo* applications is limited primarily because of their instability in serum and non-specific delivery. The pVIII major coat protein of the fd bacteriophage was shown previously to form stable complexes around double stranded DNA/RNA duplexes, called siRNA-Nanophages, provide protection from degradation and cell-specific delivery. It was hypothesized that siRNA-Nanophages can be used as a non-toxic system to deliver siRNAs specifically to the breast cancer cell line, MDA-MB-231, *in vitro*. To prove this hypothesis, we transformed the Luciferase gene (*Luc2* from *Photinus pyralis*) into representative pancreatic and breast cancer cell lines and used it as a model gene system to control gene/protein expression using Luciferase-specific siRNAs and cell specific targeting provided by nanophages.

Methods. Breast cancer cell-specific landscape phages, displaying the peptides DFPPSSAE and DMPGTVLP, were selected and characterized previously. The phages were propagated using standard procedures. Isolated major coat protein, pVIII, was obtained by size exclusion chromatography (SEC) of disrupted phages and eluted in 100 mM cholate/10 mM Tris-HCl, pH 8.0/0.2 mM EDTA running buffer. siRNA-Nanophages were prepared by combining gene-specific siRNA duplexes and isolated pVIII protein at a 1:40 molar ratio. Complexes were concentrated by centrifuging through a 30K MWCO filter unit and suspending in 10 mM Tris-HCL, pH 8.0/0.2 mM EDTA buffer. Luciferase-expressing cell lines were treated with complexes of *Luc2* siRNA-Nanophages or siRNA-Lipofectamine (positive control) for 24 hours. Luciferase activity was quantified after 72 hours by a luciferase assay (to measure protein expression) and RT-qPCR with luciferase-specific primers (to measure mRNA Expression).

Results. Transfecting siRNAs into either cell line with lipofectamine, produces down regulation of the Luciferase gene at both the gene and protein level in both cell lines without altering the expression of other genes in the cell. Lipofectamine is non-specific and toxic to cells, so there is some death among the cells. The pancreatic and breast cancer cells show the same percentage of down regulation irrespective of cell type. Transfecting using Nanophages produces similar down regulation of at both the gene and protein level in both cell lines. Preparations did not demonstrate significant toxicity, however viability assay will be performed in the future.

Conclusions. siRNA-Nanophages can be used to produce efficient gene silencing in a cell-specific manner *in vitro* with this model system. These particles have the advantages of being non-toxic and delivering the siRNAs to the specific cells of interest. We plan to study the effect of gene silencing by siRNA-nanophages *in vivo* using tumor xenograft models of the two luciferase-expressing cell lines.

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Veterinary Student Poster Presentations

Evaluating Purification Methods of Adeno-Associated Viral Gene Therapy for Treatment of GM1 Gangliosidosis

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Introduction. GM1 Gangliosidosis is a lysosomal storage disease that results from a genetic defect in the enzyme β -galactosidase (β -gal for short). The disease causes neurologic deterioration due to the lack of β -gal, which is the enzyme that breaks down the sphingolipid GM1 ganglioside. This compound then accumulates in the lysosome, causing pathology primarily in neurons. The feline model of GM1 gangliosidosis is ideal for study. Clinical signs are mainly neurological and include ataxia, tremors, and difficulty walking. We use AAV gene therapy to treat this disease. The virus contains the gene that encodes β -gal, the missing enzyme. The virus is injected bilaterally in the thalamus and lateral ventricles and infects the patient's cells, injecting the genetic information. The result is the cells have the missing gene and can now produce β -gal. When preparing the viral vector to be injected, two different purification methods can be used: purification via centrifugation in cesium chloride and purification via centrifugation in iodixanol. My hypothesis is that vector purification methods influence therapeutic effect of AAV vectors.

Methods. This project included two treatment groups: three cats treated with AAV vector purified in cesium chloride and three cats treated with AAV vector purified in iodixanol. Multiple methods were utilized to analyze therapeutic efficacy while studying the 6 cats this summer. I performed fluorescent assays of lysosomal activity, histochemical staining of β -gal activity, CSF analysis of cellular damage, and MRI images. I performed enzyme assays on brain, spinal cord, cerebrospinal fluid, liver, heart, skeletal muscle, spleen, kidney, and sciatic nerve on the two cats that are deceased. The major methods of evaluating both treatment groups together were enzyme assays of CSF and analysis of MRI images.

Results. When analyzing results from brain and spinal cord enzyme assays of the cesium chloride treated cats, fold normals of β -gal activity of untreated animals were significantly less than fold normals of the treated group. We saw successful restoration of β -gal enzyme activity in all brain and spinal cord sections using cesium chloride to purify vector. Unfortunately, we can't yet compare these to the iodixanol treatment group, because all three of those animals are still alive. When analyzing results from peripheral tissue enzyme assays of the cesium chloride treated cats, all of the untreated animals had 0 β -gal activity in any of their peripheral tissues. β -gal activity was successfully restored in peripheral tissues using cesium chloride purified vector. When analyzing CSF, iodixanol cats had slightly more β -gal activity restored than did cesium chloride cats. CSF analyses of cellular damage included looking at AST and LDH levels, which are leakage enzymes that are high when cellular damage is present. AST and LDH levels were higher, in general, in cesium chloride cats vs. iodixanol cats. MRI images show more severe damage in the cesium chloride cats vs. iodixanol cats.

Conclusions. While it is hard to establish statistical significance due to small numbers in each treatment group, iodixanol seems to work slightly better. This will be an ongoing project while we wait for the other four cats to reach humane endpoint, at which point we will analyze brain and peripheral tissues for β -gal activity.

Acknowledgments. Martin Lab Group, Scott Ritchey Research Center



Conditionally Replicative Adenovirus for Treatment of Canine Osteosarcoma

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Introduction. Osteosarcoma is a malignant proliferation of osteoblasts that is the most common bone tumor in dogs. Because it most often occurs in the distal limbs, current standard of care involves amputation of the limb combined with chemotherapy. Prognosis is poor because this type of cancer has early metastasis, so by the time the patient shows clinical signs the cancer has likely already spread to vital areas. Metastasis is fatal in most dogs by one year, with only 20% surviving to two years following diagnosis. A previous study used recombinant technology to modify an existing virus with the desired promotor. The previous study did not affect the survival time of osteosarcoma patients. This experiment used similar mechanisms, but with a completely synthetic conditionally replicating adenovirus (CRAD). We hypothesize that administered CRAD will both kill tumor in the short-term via cytotoxic effect and incite an antitumor immune response.

Methods. Mycoplasma PCRs were performed on all cell lines to ensure purity from infection. Any infected lines were treated in 50uL plasmacure. rtPCRs were run on all lines to ensure presence of osteocalcin, which acts as the viral promotor. A qPCR was run on the cell lines to determine quantities of osteocalcin expression. The cell line with the highest expression was used for transfection of the virus.

Results. Cell lines JD, Katey, and D17 were all found to express osteocalcin. D17 was found to express the highest quantity of osteocalcin.

Conclusions. D17 was determined to be the best cell line for viral transfection and effective amplification of virus based on its higher expression of the osteocalcin promotor. D17 will be used for serial passage of virus in order to expand virus. Lysate from infected cells will be transferred to new cells to spread infection. Efficacy of viral replication and cell lysis will be monitored. In the future we will do clinical trials with the virus containing the osteocalcin promotor and compare those results with the previous clinical trial to verify the results are comparable or improved. If the virus proves effective, it could then be customized for patient need via altering the promotor or payload based on the individual disease process.

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Cardiovascular and Toxicity Effects of the Redox Responsive MRI Contrast Agent Mn(II) H₄qtp2

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Introduction. The over-production of reactive oxygen species (ROS), known as oxidative stress, has been implicated in many mechanisms of tissue injury. The exact role of ROS remains poorly understood, which has motivated efforts to develop imaging techniques capable of differentiating normal from aberrant biological redox activity. Our group has tested a number of redox responsive MRI contrast agents that can detect hydrogen peroxide. In this study, a mononuclear Mn(II) complex with the redox-active ligand N,N'-bis(2,5-dihydroxybenzyl)-N,N'-bis(2-pyridinylmethyl) -1,2-ethanediamine (Mn(II)H₄qtp2) responsive to hydrogen peroxide has been synthesized and characterized. This focus of the present study was to determine the cardiovascular and toxicity of Mn(II)H₄qtp2.

Methods. Male Sprague Dawley rats were anesthetized with isoflurane and the jugular vein cannulated for fluid and drug infusion and the carotid artery cannulated for blood pressure and heart rate measurements. ECG electrodes were attached in lead II configuration. Mn(II) H₄qtp2 was dissolved in 200 ml DMSO/PBS and infused at 1-10 mg/kg body weight intravenously and cardiovascular parameters measure. Hearts were sectioned and fixed in either formalin for histological analysis, or quick frozen in liquid nitrogen for molecular and biochemical analysis. To determine toxicity effects of Mn(II)H₄qtp2, two-day post fertilization zebrafish were incubated with Mn(II) H₄qtp2, the ligand without complexed manganese (H₄qtp2), or their vehicle for 1, 4 and 24 hours and heart rate and survival was monitored.

Results. Doses of Mn(II) H₄qtp2 above 3mg/kg iv produced increases in arterial pressure and corresponding decreases in heart rate. No cardiovascular effects were observed at 1mg/kg iv. Hematoxylin and eosin staining in hearts showed no significant changes in cardiac structure between the control and the treated rats. In the zebrafish studies, Mn(II) H₄qtp2 (0.5-125 micromoles/liter) had no effect on heart rate or survival up to 24 hr incubation. At 250 micromolar, which corresponded to the calculated blood concentration of the highest dose given iv in rats, there was a significant decrease in heart rate and survival at 24 hr. The ligand without complexed manganese had no effect on heart rate or survival and any concentration or time point

Conclusions. Mn(II) H₄qtp2 (1mg/kg) did not produce alteration in measured cardiovascular parameters. The estimated blood concentration of this dose also did not affect heart rate or survival rate in zebrafish. Studies with the ligand without complexed manganese suggest the toxicity seen with the highest concentration of Mn(II) H₄qtp2 are due to manganese. Future cardiac MRI studies examining the ability of Mn(II) H₄qtp2 to detect oxidative stress in the heart will be carried out at 1mg/kg.

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Comparing OFA and PennHIP for Hip Evaluation Methods

Patrick Dittmer (Vet Student c/o 2019)

Introduction.

A great topic of concern in veterinary medicine is hip dysplasia amongst larger, working dog breeds. When breeding in a closed colony of high-performance dogs, it becomes increasingly important to select-breed for the best quality hips. Also, it puts selective pressure on lower quality hips to try to force them out of later generations and population. The two methods currently utilized by Auburn's Canine Performance Sciences (CPS) are Orthopedic Foundations for Animals and the PennHIP screening. This study is to compare the data from past and current Auburn CPS canines and compare OFA assessments to PennHIP distraction index (DI) values and see how they correlate.

Methods.

Every canine that goes through the Auburn CPS (born or purchased) gets both OFA and PennHIP screened and put into a database for future use in breeding information or selling to a client after training. This data was compared and analyzed to figure out what is the best screening process to predict future hip problems.

Results.

Out of 114 total joints compared, 95% (108) are normal by OFA standards but only 22% (25) are normal according to PennHIP. This gap is troubling, 73% of the OFA normal are "false negatives", meaning they are OFA normal but possess joint laxity to cause hip dysplasia. 25 hips have DI of <0.30 and all of these were rated excellent or good by OFA. On the other hand, 32 hips rated excellent by OFA had an average PennHIP of 0.36 and only 6 of them had a DI of <0.30. The CPS normal OFAs (excellent, good, fair) have a distraction index of 0.38 which is above the "normal" standard for OFA breeding.

Conclusions.

OFA normal does not mean a PennHIP normal. Breeding by OFA rating and standards allows for a huge range of joint laxity to stay in a genetic line and a breeding colony. A good PennHIP score always has a normal OFA score with it; it should be integrated into as the top method for breeding standards. This corresponds with a 2010 article that found HE radiograph does little to observe joint laxity and is a rating more of the look of a hip.

PennHIP should be integrated as the standard for rating hips when evaluating not only high performance canines but all canines. OFA has done little to improve hips since its start in 1966 for all dog breeds so its chances of improving a small, closed colony is minimal. Auburn CPS should rely primarily on PennHIP for breeding preference and use OFA for supplemental joint health.

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In Vitro Measurement of Friction of Intact Equine Articular Cartilage Against Various Surfaces

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Introduction. Articular Cartilage (AC) is one of the main components of a synovial joint that allows both static and dynamic movement to occur in a low friction environment. Specifically equine AC of both the carpus and stifle joint undergo a specific cyclical loading motion, which predisposes these joints to various pathologies. This research examined the coefficient of friction (COF) of AC of the equine carpus and stifle against an opposing AC surface, glass, and metal at various load forces using a pin on disc testing method. It was hypothesized that the coefficient of friction at a 10N force will be lower than that of the coefficient of friction at a 5N force in a cartilage-on-cartilage model due to the fluid film principle of lubrication. The cartilage-on-cartilage model was hypothesized to have a lower coefficient of friction than the other materials tested. Finally, due to biotribological differences between the carpus cartilage-on-cartilage model was predicted to have a lower coefficient of friction than the stifle cartilage-on-cartilage model.

Methods. AC surfaces from the distal radial trochlea, second carpal bone, and bilateral femoral condyles with intact subchondral bone were isolated from the surrounding soft tissue structures from 10 horses. Tests were conducted using a Bruker UMT-3, Each test was set up with the flatter, larger bone fixated to a stationary, self-leveling plate submerged in PBS and the smaller, more convex testing materials were then attached to a movable pin that applied a predetermined vertical load force. The pin slid at a previously set speed across a specific horizontal tract for five minutes. Data was analyzed using the mixed model for repeated measures analysis of variance. Tukey's method for multiple comparisons was used to compare the different materials.

Results. COF was found to be significantly different when comparing the cartilage testing surfaces at the different load forces. The carpus cartilage-on-cartilage model at the 5N load force model was lower to the 10N load force model in both left and right legs. For both left and right hind limbs, the COF was lower in the 10N load force model than in the 5N load force model. At a 10N load force, a significant increase was noted in the average COF between the cartilage and glass models as well as the cartilage and metal models. When looking at the average COF found in the left and right stifle test, a significant increase was noted between the cartilage and metal models at both a 5N and 10N load force. Finally, when comparing the results of the average COF friction in the carpus and stifle, a significant increase was noted between the carpal cartilage COF and stifle cartilage COF at a 5N load force. At the 10N load force, a significant increase was noted between the glass and metal carpal models and the glass and metal stifle models.

Conclusions. Based on the results, an increase in load force did statistically affect the COF in fore and hind limbs in the cartilage-on-cartilage model. When comparing surface materials in both front and hind limbs, the COF was found to be lower in the cartilage-on-cartilage model than in the glass or metal models.

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Canines as models of human disease: A strategy of reducing heterogeneity for hereditary breast cancer susceptibility gene discovery

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Introduction. Variants within known breast cancer susceptibility genes only explain a fraction of breast cancer cases with a suggestive family history. Thus, there is a pressing need for breast cancer susceptibility gene discovery. To date, gene discovery efforts have largely been thwarted by human genetic heterogeneity, and the most successful studies have focused on isolated/founder human populations, suggesting that the best approach involves studying homogenous cohorts. Due to generations of artificial selection, dog breeds are similar to founder/isolated human populations, but represent a simpler and more powerful model for gene discovery.

Methods. A cross-species sequence comparison of known breast cancer gene mRNA and proteins was conducted using data from the most recent canine and human genome builds, Broad CanFam3.1/canFam3 and GRCh38/hg38, respectively. Alignment and comparison tools included NCBI (National Center for Biotechnology Information) Nucleotide BLAST, ExPASy Bioinformatics Resource Portal SIM Protein Alignment Tool, and InterPro: protein sequence analysis & classification. Literature review of identified canine variants within suspected canine mammary tumor genes (known human breast cancer genes), with a focus on germline, coding variants, was also conducted. A selected cohort of canine mammary tumor affected, AKC-registered dogs (n=85, 32 breeds) was inspected for relatedness using an internet pedigree search and mapping using Cyrillic software.

Results. Human and dog sequence comparison showed remarkable similarity between known high-penetrant breast cancer genes and suspected canine mammary tumor genes and proteins. Spanning six genes and accounting for gaps, mRNA sequence matches ranged from 82-96%; protein identities were 69-100% matched between the species. Five large canine families were delineated through pedigree mapping. All twenty Golden Retriever samples were from only 2 families, with sixteen stemming from a single common sire.

Conclusions. Canines are genetic homologues to humans that can be studied separately or in parallel to enhance hereditary breast cancer disease gene discovery efforts that apply to both species. Registered purebred canine pedigrees are well-documented and predictably homogenous. Future studies should investigate the inheritance patterns, validate current canine gene and variant data, and embrace current sequencing efforts to provide a more complete picture of the genetic contributions to human and canine forms of breast cancer.

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Evaluation of miRNA stability after exposure to various external conditions

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Introduction. An important biological function of microRNAs (miRs) is to regulate expression levels of target genes. High stability in blood and their specificity in targeting genes make both freely circulating miR and exosomal miRs ideal molecular biomarkers for various diseases. However, it has not been elucidated whether various sample storage techniques and miR isolation methods affect miR stability and biological variability in dogs. It was hypothesized that exosomal miRs were more resistant to degradation when exposed to external environmental storage conditions in comparison to freely circulating plasma miRNA.

Methods. Blood samples were collected from 10 healthy dogs. Isolated plasma was aliquoted and placed in one of the following storage conditions in duplicate: -80°C for 72 hr, -20°C for 72 hr, -20°C for 72 hr with 3 freeze/thaw (F/T) cycles, -20°C for 72 hr with 6 F/T cycles, 4°C for 24 hr, or room temperature (RT) for 24 hr. miRs were isolated from each sample using either the Qiagen miRNeasy Serum/Plasma kit or the Invitrogen Exosome RNA Kit. Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) was then performed to investigate the relative expression levels of miR-24. Custom, validated primers were used with the miScript SYBR Green PCR Kits (Qiagen). The CFX 96 Thermocycler (Bio-Rad) was heated at 95°C for 15 min, followed by 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s, and a melting curve analysis was performed. Each miR expression was normalized against miR-16. All experiments were performed in triplicate. Relative expression was calculated by the comparative threshold cycle method ($\Delta\Delta C_t$ method), and the fold change relative to controls was calculated using the Student's *t*-test with a *p* value set at $p < 0.05$.

Results. Samples treated at -80°C and -20°C for 72 hr did not show a significant difference in the expression levels of miR-24 using the two different isolation techniques ($p = 0.43$, $p = 0.2055$, respectively). After subjecting samples to 3 F/T and 6 F/T cycles, plasma miR-24 demonstrated a faster degradation than exosomal miR ($p = 0.0002$, $p = 0.0314$, respectively). There was no significant difference noted in samples stored at both 4°C and RT ($p = 0.1478$, $p = 0.64$, respectively).

Conclusions. Short term storage of samples at -80°C and -20°C was comparable. The repeated F/T cycles caused miR degradation in the freely circulating plasma samples and the exosome isolated samples, but the freely circulating plasma miR isolates appear more susceptible to biological instability. Further validation and quantification of these findings needs to be performed using other molecular techniques and a larger sample size. Evaluating the effects of systemic disorders on isolated miR stability is a potential future direction for this project.

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Adeno-Associated Viral Gene Therapy in the Tay-Sachs Sheep

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Introduction. Tay-Sachs Disease is a lysosomal storage disorder that causes neurodegeneration in people, typically at a young age. It is characterized as a GM2 Gangliosidosis, which is a collective term for diseases expressing extreme accumulation of GM2 Gangliosides in the lysosomes of cells due to loss of GM2 ganglioside degradation. GM2 gangliosides are glycosphingolipids located in the plasma membranes of cells. Destruction of these compounds involve movement into lysosomes, where they are then hydrolyzed by the lysosomal enzyme, Hexosaminidase A, with the aid of the GM2 Activator Complex. Hex A is a heterodimer composed of both α - and β -subunits. In those afflicted with Tay –Sachs, a mutation occurs, which codes for the α -subunit of the Hex A enzyme. This mutation causes a nonfunctional Hex A enzyme to be produced eliminating GM2 ganglioside degradation in the cell. This loss of GM2 ganglioside breakdown capability causes accumulation of GM2 gangliosides in the lysosome causing the presence of membranous cytoplasmic bodies and the swelling of cells. Though this can occur in all cells in the body, swelling in neurons occurs more rapidly providing explanation for the manifestation of neurological signs seen with the disease.

There is no known treatment for Tay-Sachs Disease. Those afflicted must treat the clinical signs. However, an adeno-associated virus has been manufactured carrying bicistronic genes coding for both α - and β -subunits. This vector would in theory deliver the genetic material to code for the missing α -subunit of the nonfunctional Hex A enzyme, which would supply means for degradation of the accumulating GM2 Gangliosides. In our experiment we used Jacob Sheep, the only known model for Tay-Sachs, and hypothesized that these TSD sheep treated with the bicistronic vector of the adeno-associated virus would achieve good biodistribution of the enzyme Hexosaminidase A throughout the brain and result in extension of lifespan.

Methods. There were five sheep represented in the treatment group for the experiments. The procedure involved injection of the AAV vector into the lateral ventricle and bilaterally into the thalamus of all five sheep. Enzyme assays were then performed on the brain, spinal cord, and CSF of normal, untreated and AAV-gene therapy treated sheep (except for the one sheep still alive during the experiment). Levels of other lysosomal enzymes were also measured to evaluate their lysosomal health. During the analysis of the enzyme assays, lysosomal enzymes, Hex A and Hex T (total Hexosaminidase) were focused on in order to determine whether or not efficacy of the treatment was established.

Results. The results of the experiment showed a significant increase in lifespan to 15.0 +/- 3.0 months compared to the untreated 9.2 +/- 1.1 months. Enzyme assays of the brain showed an increase in Hex A activity throughout. Enzyme assays of the spinal cord showed increased Hex A activity in selected portions. CSF enzyme assays and showed no significant increase, and finally, aspartate aminotransferase and lactate dehydrogenase, measures of cell degradation, showed varying levels of improvement.

Conclusion. Overall a marked improvement was recorded in regards to increased Hex A activity of the TSD sheep treated with the AAV bicistronic vector showing promise for future testing and eventual submission into human trials.

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Phage-GnRH Constructs for Population Control of Feral Animals: Evaluation in Mice

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Introduction. Population control of feral animals is a significant problem in many parts of the world including the United States. To solve the problem, various approaches have been offered depending on the species of concern as well as site-specific needs and availability of resources. Our focus is on the development of vaccines for population control of feral cats and dogs via contraception. While surgical sterilization remains the gold standard for contraception of cats and dogs, the need for professional veterinarian involvement in the procedure dictates its relatively high cost. Non-surgical sterilization would be beneficial in this respect and also would eliminate post-operative animal care and complications.

Methods. We develop contraceptive vaccines for cats and dogs that are based on bacterial viruses, bacteriophages (phages). Phage particles can be modified to display fusion peptides with desired antigenic properties on their surfaces. For animal contraception, the gonadotropin releasing hormone (GnRH) peptide represents a major interest since anti-GnRH antibodies can disrupt the hypothalamus-pituitary-gonadal reproductive axis, leading to sterilization. Such phage-peptide fusions were identified in our previous study via selection from a phage display library using GnRH antibodies as a selection target. Here, these phage-peptide constructs were tested for stimulation of anti-GnRH humoral immune responses and suppression of testosterone in mice. Five mouse groups (n=10) were immunized with either individual phage constructs displaying GnRH-like peptides (groups 1-4) or their combination (group 5). AdjuVac was added to the phage preparations as adjuvant. The experiment continued for 13 weeks and sera collected from the immunized animals at five time points were assayed for the presence of GnRH antibodies and testosterone levels.

Results. Sequences of fusion GnRH-like peptides displayed on the phages were as follows: (1) **EHP**SYGLA, (2) **EPT**SHWSA, (3) **DGL**RPQAP, (4) **EGL**RP^{**SG**}Q, and (5) combination of 1-4. (Note: amino acid residues that are identical to those in GnRH peptide are bolded). GnRH antibodies were detected in serum samples collected from mice immunized with either one of the constructs as well as with their combination starting at week 4 (first blood collection time point). The highest antibody levels were found in mouse groups 1, 2, and 5. These immune responses peaked at week six and continued for the duration of the experiment (13 weeks). Significant testosterone suppression was found in mouse groups 1 and 5. Testosterone mean values for these groups at certain time points were significantly lower as compared to their pre-immunization group values.

Conclusions. Phage-GnRH constructs stimulated production of neutralizing GnRH antibodies in mice. Importantly, suppression of testosterone (indirect indicator of possible impaired fertility) was achieved with a single phage immunization, no boosters were given to mice at any time points. Taken together, the study demonstrated that targeting GnRH by using phage-based vaccines could be a viable option for animal contraception.

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The effects of endothelial colony forming cells (ECFCs) on blood flow in equine distal limb wounds

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Introduction: Endothelial colony forming cells (ECFCs) are a subset of endothelial progenitor cells that can be isolated from peripheral blood. ECFCs are thought to play a significant role in wound healing by relocating to sites of injury and aiding in vascular repair while promoting angiogenesis. Treatment with ECFCs may be beneficial with wound healing in horses due to their susceptibility to exuberant granulation tissue. Combination ECFCs with a biomaterial should increase viability and engraftment of cells after injection. We hypothesized that injection of distal limb wounds with ECFCs and poly(ethylene glycol)-fibrinogen (PEG-Fb) encapsulated ECFCs would decrease wound healing time via angiogenesis and vascular repair.

Methods: Baseline thermographic images were taken of each distal limb of three horses to ensure adequate blood flow prior to beginning the study. Two 6.25 cm² full dermal thickness wounds were then created on each distal limb of the three horses (eight wounds per horse). Twenty-four hours post-surgery, each wound was injected with either ECFCs, horse serum, PEG-Fb encapsulated ECFC microspheres, or PEG-Fb microspheres. All clinical investigators were blinded to treatment. Thermographic images were taken every week for four weeks. Four of the wounds (each treatment) were biopsied at baseline and then each week at the leading edge of the wound for four weeks using a 6mm punch. The remaining 4 wounds were biopsied at baseline and on week 4 (at the center and edge) using a 6mm punch. Immunohistochemical staining for von Willebrand factor (vWF) was performed on all biopsies. Slides were digitally scanned using an Aperio ScanScope, and quantification of staining was performed using Visiopharm software.

Results: Thermographic analysis revealed that blood flow was greatest above or below the wound and lowest in the center of the wound. The thermographic measurement location below the wound had a significantly greater decrease in the percent change of temperature from baseline compared to the wound center and edge ($p < 0.0001$, $p < 0.0001$); the location above the wound had a similarly significant decrease in temperature from baseline compared to the wound center or edge ($p = 0.0003$, $p = 0.0008$). Hindlimbs had a significantly greater temperature at all time points compared to forelimbs ($p < 0.0001$). Analysis of vWF content varied significantly by week ($p = 0.0105$), with the vWF content in week 4 biopsies being significantly greater than that in week 3 ($p = 0.0362$). The treatments did not have a significant effect on either the thermographic analysis or vWF content.

Conclusion: The lack of detectable effect of cell therapy on blood flow could be due to the cell therapy itself or due to the sensitivity of the model and analysis techniques.

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Modeling the Effects of Bolus Fluid Administration on Canine Platelet Function: A Comparison of Different Fluid Formulations

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Introduction. The critical patient is at significant risk for hemostasis disturbances resulting in both hemorrhage and thromboembolism. These patients are often candidates for large volume intravenous fluid replacement. The goal of this study was to evaluate the effect of blood dilution in vitro on platelet aggregation, using volumes intended to model the administration of an IV fluid bolus.

Methods. Sixteen healthy dogs between 1-12 years of age participated in the study. Fluid effects on platelet function were assessed using 0.9% isotonic saline, Plasma-lyte, Vetstarch, and 7.5% hypertonic saline. The Multiplate Analyzer® was used to assess the impact each had on platelet aggregation. ADP was used as the agonist to stimulate platelet activation. In vitro blood dilution was performed by combining 200uL of each solution with 1mL of citrated blood to simulate a 15 ml/kg bolus (16.7% volume replacement). Platelet aggregation was measured for six minutes in arbitrary aggregation units (AU) and plotted over time to obtain the area under the curve (AUC). Platelet aggregation in unaltered blood was tested for comparison and each blood solution was run as both an unactivated control, without an agonist, and after addition of ADP to trigger platelet activation.

Results. Dilution with isotonic saline was used to measure the impact of blood dilution alone and resulted in a statistically significant decrease in AUC from the unaltered blood, likely due to the decreased platelet concentration after dilution. Addition of hypertonic saline produced a dramatic decrease in measured platelet aggregation, with the mean area under the curve reduced by 71% from the control. Although Vetstarch has documented effects on blood coagulation, no significant effect on platelet aggregation was observed. Results with Plasma-lyte were inconsistent, but dilution with this fluid sometimes resulted in immediate platelet aggregation independent of the addition of an agonist.

Conclusions. Administration of a bolus of hypertonic saline may have a substantial effect on primary hemostasis in clinical patients. Trends in measured platelet aggregation suggest that some fluids have effects that cannot be explained by dilution alone, although statistical significance was not always achieved.

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Urethane's effect on cardiorespiratory coupling: It'll take your breath away.

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Introduction: Animal models are commonly used to study respiratory and cardiovascular control systems. Despite its prevalence, this research is often confounded by the unknown effects of commonly used anesthetics. A primary example of this challenge is seen when using *in vivo* urethane anesthetized rodents which demonstrate appropriate blood pressure, respiratory rate, and heart rate. While these baseline parameters are physiologically "normal", what is less apparent is the reduced or absent coordination of these homeostatic systems in the anesthetized model. In contrast, *in situ* decerebrate rodent preparations (unanesthetized) have well-coordinated cardiorespiratory coupling expressed as respiratory modulation of heart rate (respiratory sinus arrhythmia – RSA) and "blood" pressure (Traube-Hering waves). This influence of the respiratory rhythm on cardiovascular parameters can be measured after both removal of the lungs and neuromuscular blockade indicating that the coupling occurs at the neural level. Based on these observations, we hypothesize that urethane anesthesia interferes with brainstem circuitry linking respiratory and cardiovascular control networks.

Methods: Experiments (n=12) were performed using juvenile Sprague-Dawley rats (P21-35) in the *in situ* working heart brainstem preparation. Efferent phrenic, thoracic sympathetic, and vagus nerve activity was monitored using glass suction electrodes. Heart rate was derived from a bipolar silver wire electrode attached directly to the heart. Perfusion pressure was measured at the site of cannulation. A 16 channel multi-electrode array was used to monitor activity in the ventrolateral brainstem areas controlling respiratory rate and pattern in a subset of the experiments (n=7). Signals were digitized and recorded using CED Spike2 software and a Power1401 data acquisition system. The nerve activity, heart rate pattern, and vascular pressure were qualitatively assessed using waveform averaging. Baseline patterns were compared with epochs following stepwise administration of urethane (1.5 ug/ml; 0.1-4.7 ml/200 ml perfusate) anesthesia to the perfusion solution.

Results: The *in situ* rat preparation produced robust cardiorespiratory coupling in HR, tSNA, and PP. The PNA was attenuated in a dose dependent fashion with administration of urethane anesthesia as was respiratory modulation of HR, tSNA, and PP. Select populations of neurons in the respiratory brainstem were silenced with increasing administration of urethane while other populations were relatively unaffected.

Conclusions: Urethane anesthesia attenuates respiratory output. Given the loss of respiratory modulated neurons in the brainstem we conclude that urethane's effect is likely antecedent to the spinal motoneuron pool. As respiratory rate remains relatively stable prior to complete uncoupling, we conclude that urethane acts on a respiratory population downstream of the respiratory central pattern generator which relays respiratory rhythm to autonomic efferents.

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Optimization of DNA Vaccine Targeting GnRH Receptor for Animal Contraception

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Introduction. Overpopulation of feral cats and stray dogs is a serious animal welfare as well as public health problem worldwide. Non-surgical, safe, cost-effective methods to reduce the number of animals born are urgently needed as a practical solution. The focus of our research is on development of DNA-based contraceptive vaccines that target gonadotropin releasing hormone receptor (GnRHR), a crucial player in animal reproduction. Previously, such vaccines were shown to stimulate immune responses against GnRHR resulting in suppression of testosterone (indirect indicator of vaccine contraceptive value) in immunized mice. While the desired functional effects were achieved, they appeared to be transient, most likely, because amount of the expressed antigen was not high sufficiently and duration of the expression was not long enough to establish immunological memory. The goal of the present study is to enhance and extend GnRHR expression for induction of strong immunological responses leading to immunological memory and subsequent loss of fertility in immunized animals.

Methods. Two plasmids encoding ubiquitin-feline GnRHR (Ub-Fe-GnRHR) genes were constructed by standard molecular cloning techniques. To produce a plasmid with CpG-free CMV promoter, original CMV promoter in pSF-CMV-Ub-FeGnRHR construct was replaced with CpG-free CMV promoter. A plasmid with dual antigen expression was generated using pSF-CMV-CMV-Sbf1 (Oxford Genetics) plasmid with two CMV promoters.

Results. For enhanced and extended GnRHR expression, an existing Ub-FeGnRHR construct was optimized. Two new constructs were created: (1) ubiquitin feline GnRHR construct with CpG-free promoter. One of the major reasons for transgene silencing and subsequent loss of expression is CpG methylation in the promoter area. To prevent gene silencing, CMV promoter in the existing Ub-FeGnRHR construct was replaced with CMV CpG-free promoter. (2) Ub-FeGnRHR construct with dual antigen expression system. It was shown by others that a plasmid with dual antigen expression cassette (with 2 promoters/antigen encoding sequences) increases transgene expression significantly. The inserts appeared to be of correct molecular weight. Sequences of the inserts were verified and found to be accurate.

Conclusion. Two new DNA-based GnRHR-targeting constructs were created and characterized for further tests in animals.

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Optimizing and Testing a Cell Culture Model for Parkinson's disease

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Introduction. Approximately 50,000 Americans are diagnosed with Parkinson's disease (PD) annually. A hallmark of PD is dysfunctional mitochondria resulting in lower yields of ATP and increased levels of reactive oxygen species (ROS). The effects of mitochondrial dysfunction are typically the greatest within muscle and nervous tissue due to their high energy requirements. PD, in particular, results in the death of dopaminergic neurons in the midbrain, which leads to the neuromotor dysfunction associated with PD. The cause of PD is not fully understood; there is no cure and current treatment options are limited. The goal of this project is to develop a cell culture model of mitochondrial dysfunction similar to that seen in PD for testing of potential therapeutic compounds.

Methods. The cell culture model was based on the N27 Cell Line, a line of dopaminergic neurons derived from rat mesencephalon. This cell line was selected due to its similarity to the dopaminergic neurons known to be damaged by PD. The N27 cell line was maintained on 10 cm plates, incubated at 37°C in 5% CO₂. The cells were grown out and incubated on 96-well plates for conducting treatment assays. Rotenone, a commonly used pesticide, was used to induce damage in the N-27 cells similar to that seen in human PD. Rotenone produces mitochondrial damage by inhibiting Complex I, resulting in increased ROS levels within cells. The potential therapeutic compound being tested was PMX-550DBr, a butyrylcarnitine compound suspected to decrease the effects of ROS produced as a result of mitochondrial damage. PMX-550DBr is a conjugate of butyric acid, a short chain fatty acid, and L-carnitine, an essential molecule in shuttling fatty acids into mitochondria that also has antioxidant properties. The protective effects of PMX-550DBr were measured using two cell viability assays, Resazurin and XTT, and CellROX, an oxidative stress assay.

Results. A significant challenge in testing and analyzing the effects of PMX-550DBr was optimizing cell culture methods and rotenone treatment. In earlier assays, 96-well plates were coated with poly-L-lysine to improve cellular adhesion. However, cell attachment was poor—many cells were lost during normal treatment and analysis protocols. Poly-L-lysine was replaced with a combination of poly-D-lysine and laminin. Determining the appropriate concentrations and treatment duration for rotenone proved to be difficult. It was important for a successful cellular model to induce oxidative damage with minimal losses from apoptosis and necrosis. With optimization of the protocol, assays showed rotenone-induced oxidative damage while PMX-550DBr appeared to provide protection.

Conclusions. The XTT assay in particular showed a general trend toward protection of rotenone-treated N27 cells with PMX-550DBr co-treatment, but the data were not statistically significant. Although improvements were made with the N27 cell culture model, further optimization is required to validate the assays.

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Evaluation of Promoter Tumor-Specificity for use in Oncolytic Virotherapy

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Introduction.

Conditionally Replicative Adenoviruses (CRAds) are genetically modified oncolytic viruses that utilize transcriptional targeting for selective replication in tumor cells. Tumor-specificity is afforded by the promoter, a section of the viral DNA that controls the replication of the viral genome. CRAds may kill tumors through a variety of mechanisms including cytopathic effect, immune stimulation, and through the expression of additional anticancer genes carried by the virus. These genes can then be expressed and the protein product used to convert a non-toxic prodrug into a harmful, toxic, substance within the cancer cell yielding cell death. In our previous study, we evaluated the exogenous rat promoter, Progression Elevated Gene 3 (PEG3) and the canine promoters cCXCR4, cTERT, and cSurvivin for their tumor-specific and pan-tumor properties. The current study is focused on honing in on previous cSurvivin results and evaluating the degree to which cSurvivin is upregulated in tumor cells when compared to non-tumor cell lines.

Methods.

In order to explore the tumor-specific activity of PEG3, the promoter was joined with a GFP reporter gene and was evaluated using a series of cellular transfections and infections which were compared with a CMV-driven GFP control. Endogenous canine promoters CXCR4, TERT, and Survivin were examined using Reverse Transcriptase qPCR and normalized against a Beta Actin positive control. The results of this study showed evidence of cCXCR4 and cSurvivin tumor-specific and pan-tumor properties, warranting further study. In this study, the cCXCR4 promoter was double-digested using EcoR1 and Kpn1 restriction enzymes and joined with a GFP reporter gene in a PDC 311 plasmid. A similar process was completed for cTERT to complement data from previous experiments. cSurvivin has been constructed and is currently being amplified by E.Coli competent cells. Once sufficient levels of DNA have been produced, the cCXCR4, cTERT, and cSurvivin will be transfected into numerous tumor and non-tumor cell lines and compared to a CMV plasmid positive control. The transfection efficiency and level of expression will then be qualified and quantified via light microscopy and flow cytometry, respectively.

Results.

cSurvivin has been constructed but has not yet been amplified to the necessary level. Once appropriately concentrated samples have been achieved, the next phase of the experiment will begin with transfection of cancerous (CMT12, CMT28, CML10, CML7) and non-cancerous (FDK, NCF, MDCK) cell lines.

Conclusions.

Previous analysis of cCXCR4 and cSurvivin showed markedly enhanced expression in tumor cells when compared with normal tissues, warranting further study of these promoters. In this study, we have produced several plasmids to further evaluate the degree to which cSurvivin is upregulated in tumor cells versus non-tumor cell lines.

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Endothelial colony forming cells as treatment for equine distal limb wounds

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Introduction. Endothelial colony forming cells (ECFCs) are progenitor cells that function in vascular repair and neovascularization. Therefore, ECFCs may be useful in the treatment of conditions characterized by poor blood supply. An equine distal limb wound model was used to evaluate the effects of treatment with ECFCs on wound surface area and granulation tissue formation. To improve cell survival and localization upon injection, ECFCs were also encapsulated in polyethylene glycol-fibrinogen (PEG-fibrinogen).

Methods. Three horses had two 6.25 cm² dermal wounds created on each distal limb. Each wound randomly received 1 of 4 treatments by subcutaneous injection: serum, PEG-fibrinogen microspheres (MS), ECFCs, or ECFCs encapsulated in PEG-fibrinogen microspheres (ECFC-MS). Wound healing was assessed weekly using digital image wound surface area (WSA) analysis and granulation tissue scores (GS) assigned by blinded observers. Four wounds per horse (1 per treatment) were biopsied at baseline and then weekly while the other four wounds per horse (1 per treatment) were biopsied at baseline and week 4 only.

Results. The effect of treatment group on the percent change in WSA was significant ($p=0.0002$), with ECFCs alone having the smallest WSA measurements. The ECFCs alone and ECFC-MS groups had significantly smaller WSA compared to MS alone ($p=0.0005$ and $p=0.0014$, respectively). Compared to serum, ECFCs alone had a WSA decrease which trended toward significance ($p=0.0742$). GS were greater for hindlimb wounds ($p=<0.0001$) and for wounds biopsied weekly ($p=0.0039$). GS were also different due to the individual horse ($p=<0.0001$).

Conclusions. Injection with ECFCs alone or ECFC-MS significantly reduced wound surface area, which may indicate enhanced healing.

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Graduate Student Poster Presentations

Impacts of Heat Stress Mitigated by a Yeast Fermentate Product

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Introduction.

Temperature is one of the most severe stressors affecting human and animal health. Exposure to heat stress conditions can lead to acute, chronic, and even lethal illnesses. Heat stress can cause the loss of the gut barrier integrity, resulting in the increased translocation of the luminal antigens (bacteria and endotoxins) into the bloodstream. Excessive activation of inflammation due to translocation of the gut lumen content may contribute to the tissue injury, multiorgan dysfunction, and death. There are strong evidences that stress compromises the gut microbiota and as a result decreases the immune resistance of the organism. Different approaches are proposed for the mitigation of heat stress adverse effects, among which are special diets and probiotics. However, there is no data about the efficacy of prebiotics in the mitigation of stress-induced complications. The main aim of this study was to assess the efficacy of a yeast (*S. cerevisiae*) fermentate EpiCor (EH), having prebiotic activity, in the prevention of heat stress-related complications.

Methods.

To analyze of the protective effect of EH during heat stress, male Sprague–Dawley rats weighing 250–300 g were used. Animals were treated by oral gavage with EH or PBS once a day for 14 days. On the 15th day, half of the rats of each group were exposed to heat stress conditions (45° C, relative humidity 55% for 25 min), while the other half of the rats were placed at room temperature. Histological changes in the intestine (villi height, total mucosal thickness, number of goblet and Paneth cells), serum lipopolysaccharide level (LPS), as well as vesiculation of erythrocytes were analyzed and compared between groups.

Results.

There was a significant decrease in small intestinal villi height and total mucosal thickness in heat stressed rats pre-treated with PBS. Goblet and the Paneth cell count was also significantly decreased in this group of rats. Treatment of rats with EH before heat stress conditions prevented the damaging effect of heat stress on intestinal morphology. Rats pre-treated with PBS under heat stress conditions resulted in a significant increase of serum LPS levels and amount of erythrocyte vesiculation. Oral administration of EH protected animals against these adverse effects of heat stress.

Conclusions.

Pre-treatment of rats with EH was effective in preventing the heat stress-related complications, such as changes of gut morphology, increased release of LPS into circulation, and pathological impact on blood erythrocytes.

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Prolonged Oral Torsemide Administration in a Horse with Congestive Heart Failure and Atrial Fibrillation.

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Introduction: Injectable loop diuretics are commonly used in horses to treat acute pulmonary edema secondary to congestive heart failure (CHF). In the horse, diuretic therapy is restricted to injectable medications as there are no current data that support the use of oral loop diuretics such as furosemide. A previous study showed that furosemide bioavailability after oral administration was poor, erratic and variable among horses. Torsemide is a high ceiling loop diuretic that is 10 times more potent than furosemide and has consistently high bioavailability after oral administration. Torsemide has been widely used in humans and dogs and it has been associated with less diuretic resistance and potassium loss. The purpose of this study was to determine whether torsemide, a potent loop diuretic, could be used as an oral alternative for management of CHF.

Methods: In a pilot study, torsemide (Demadex®) was administered orally for a total of 7 days (6mg/kg, every 12 hours) to a horse with congestive heart failure and atrial fibrillation secondary to myxomatous mitral valve degeneration. Blood samples for measurement of plasma torsemide concentrations were obtained one hour after each drug administration. Reversed-phase high-performance liquid chromatography was employed to quantify torsemide concentrations in plasma. Pharmacodynamic effects of oral torsemide were evaluated by daily physical examination, electrocardiography, and serum biochemistry profile. At the end of the study period, the horse was euthanized due to his disease stage and poor long-term prognosis.

Results: The horse tolerated administration of oral torsemide with no adverse effects noted. At day 7, a significant decrease in the ventral edema and venous congestion was noted by subjective visualization. Torsemide plasma concentration significantly increased at day 5 with a mean plasma peak concentration of 12.04 ug/mL. No evidence of azotemia was observed throughout the study period. Electrolyte measurements revealed mild hyponatremia and hypochloremia and moderate hypokalemia. The moderate hypokalemia was observed at day 4 (1.9 mmol/L) and potassium was supplemented by oral administration of potassium chloride (KCl) at a dose of 0.1 g/kg, twice a day, with no further decrease in serum potassium levels. No electrocardiographic changes related to torsemide administration were observed.

Conclusions: Results indicate that torsemide has good oral absorption, achieved therapeutic plasma concentrations, and is safe to be administered orally in horses. Furthermore, remarkable improvement in clinical signs strongly suggests that torsemide can be utilized as an oral alternative for managing equine patients with congestive heart failure. The results, described herein as a pilot study, led to investigate pharmacokinetic and pharmacodynamics effects of oral torsemide in the horse that is currently being conducted by the authors.

Acknowledgement: Birmingham Racing Commission and Department of Clinical Science. The authors would also like to thank those who assisted with the care of this horse during this study.



Endogenous morphine concentrations in septic versus healthy dogs

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Introduction: Endogenous morphine-like compounds have been detected in the tissues of people and many different mammals, including dogs. Recently, a study was published that showed significantly increased endogenous morphine concentrations in the serum of septic people compared to people with systemic inflammatory response syndrome (SIRS). In this study, the mean morphine concentration in SIRS patients was 0.37-0.59 ng/mL, whereas the septic patients had significantly higher concentrations at approximately 1.5-3 ng/mL. To the authors' knowledge, endogenous morphine has not been measured in the blood of healthy or septic canine patients. We hypothesized that endogenous morphine concentrations would be significantly increased in septic dogs compared to healthy dogs.

Methods: Dogs with septic abdomens and healthy dogs were prospectively enrolled in the study. To be included, the septic dogs had to meet one of the following three criteria: visualization of ruptured gastrointestinal tract during surgery, presence of intracellular bacteria in an abdominal effusion sample, or positive fluid culture from an abdominal effusion sample. Septic dogs were also required to meet the criteria for SIRS. The healthy dogs presented to the hospital for elective procedures such as spays and neuters. Healthy patients were required to have a normal physical examination as well as normal bloodwork (complete blood count and biochemistry panel) prior to inclusion in the study. Approximately 3-5 ml of blood was taken from a peripheral vein from each patient and placed into a heparinized blood tube. Plasma samples were kept in a -80 C freezer until they were analyzed. An assay to measure endogenous opioid concentrations was developed. This assay used high performance liquid chromatography-mass spectrophotometry to evaluate the concentration of endogenous morphine compounds in the canine plasma samples.

Results: An MS/HPLC assay for the detection of morphine was used to measure endogenous morphine in canine plasma. Accuracy is $100 \pm 10\%$ and coefficient of variation (indicator of precision) is less than 15%. The lower limit of quantitation is 2.0 ng/mL. Blood samples from 6 healthy dogs and 11 septic dogs were analyzed. Endogenous morphine was not detected in any healthy or septic samples.

Conclusions: The assay that was used for this study does not appear to be clinically useful for the differentiation of septic vs non-septic dogs. If septic dogs do indeed release increased concentrations of endogenous morphine in comparison to healthy dogs, the concentrations of morphine may be lower than 2.0 ng/mL, making it undetectable with the assay used in this study. Alternatively, septic dogs may secrete endogenous opioids other than morphine that cannot be detected with our assay. Additional studies using assays to detect different types of endogenous opioids such as beta endorphins or enkephalins should be performed.

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Meta-Analysis of the Effect of Small Intestinal Resection and Anastomosis Technique on Survival and Post-Operative Ileus in Horses

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Introduction

Multiple surgical techniques have been described for successful resection and anastomosis of the equine small intestine (SI). Meta-analysis is a statistical, quantitative method designed to compare the diverse results of multiple studies. The objective was to determine which of 3 SI surgical techniques, specifically jejunojejunostomy (JJ), jejunoileostomy (JI) and jejunocecostomy (JC), demonstrated the highest odds for survival.

Methods

A comprehensive literature search was performed using terms specific to equine SI resection and anastomosis procedures. Of 1588 publications that met the initial search criteria, data was extracted from 21 studies determined to be complete and relevant to the objectives.

Results

The odds of horses undergoing a JJ surviving to discharge were significantly higher than those undergoing a JC (OR = 1.995; 95% CI [1.234,3.224]; P=0.005). Horses undergoing JJ had a greater odds of developing POI than horses undergoing JC (OR=1.269; 95% CI [0.174,9.801]; P=0.82). Horses in which SS-JJ were performed were more likely to survive to discharge than those in which EE-JJ were performed (OR=1.203; 95% CI [0.471,3.072]; P=0.699). Hand-sewn JC were more likely to survive to discharge than those in which stapled SS-JC were performed (OR=1.732; 95% CI [0.714,4.203]; P=0.224).

Conclusions

The results from this meta-analysis, an evidence-based analysis of surgical procedures, assist the surgeon in decision making during small intestinal surgery, and in determining an accurate prognosis for survival. The main limitation of this study was the lack of relevant data for analysis. Data within the analyzed studies was often incomplete, omitting specific numerical details. The literature also contains an innate bias towards publication of studies with positive findings, a common limitation for meta-analytical techniques.



Effect of Niacin on Nonalcoholic Fatty Liver Disease in Adiponectin Knockout Mice: A Pilot Study

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Introduction. Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive accumulation of triglycerides in hepatocytes, inflammation, and progression to nonalcoholic steatohepatitis (NASH). Studies in rodents demonstrate that adiponectin prevents development of NASH. Niacin, a drug that decreases plasma triglycerides and increases adiponectin, prevents hepatic steatosis in rodents and inhibits fat accumulation and inflammation in human hepatocytes. However, the role of adiponectin in niacin's ability to inhibit the development of NASH is not known.

Methods. Three-week-old male adiponectin knockout mice (n=6-7) were fed either a chow or high fat diet (HFD) for 20 weeks. Beginning at 6 weeks and continuing through the end of the study, niacin (360mg/kg/day) or vehicle was added to their drinking water. Their body weight and water intake was measured every four days and food intake was measured every eight days. At the end of study, mice were sacrificed and blood and tissues were collected for analysis, including liver and adipose tissue weight and liver triglyceride content.

Results. There was no difference in food or water intake due to diets or niacin treatment. As expected, HFD fed mice gained significantly more weight than chow fed mice. Surprisingly, niacin treatment significantly decreased body weight by 13% in HFD, but not chow fed mice. No significant change in liver or white adipose tissue weight was observed, but liver weight trended lower in niacin treated mice. Hepatic triglyceride content, macrovesicular steatosis, microvesicular steatosis, hepatocyte hypertrophy, and NASH score was significantly increased in HFD fed mice compared to chow fed mice, but did not change with niacin treatment. However, microvesicular steatosis, hepatocyte hypertrophy and NASH score trended down in niacin treated mice. Similar changes were also observed in average adipocyte area and crown-like structure number, in which there was a strong trend toward decrease in niacin treated mice. However, due to the limited number of mice in this pilot study, these data did not reach statistical significance.

Conclusions. Mice that were fed HFD developed NASH and niacin attempted to improve NASH in these mice. These findings were very promising and supported a larger study in the adiponectin knockout and appropriate control mice.

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Zinc Metal Nanoparticles in Olfactory Sensory Neuron Signal Transduction

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Introduction. Zinc metal nanoparticles (Zn NP) have demonstrated enhancement of olfactory responses to odorant stimulation *in vitro*. These particles increase responses by about 3-fold when presented to the olfactory epithelium (OE) in an odorant mixture, but do not stimulate an odor response when administered alone. The effects are specific to Zn NP, as copper, gold, and silver metal nanoparticles do not present the results observed for zinc. The purpose of our work is to investigate whether olfactory response enhancement by Zn NP observed *in vitro* is translated throughout the sensory circuitry to be perceived cognitively by the brain. We hypothesize that enhancement of olfactory sensory neuron (OSN) responses by Zn NP will also increase odor perception, characterized by activation of higher order olfaction-related brain regions. Our goal is to fully validate and characterize the fundamental role of Zn NP in the initial events of olfaction and the mechanisms by which they may enhance canine olfactory capabilities.

Methods. In conjunction with our *in vitro* analysis of OSN in the rodent OE, we noninvasively analyze *in vivo* cognitive effects of Zn NP on olfaction-related brain regions of fully awake dogs using functional magnetic resonance imaging (fMRI). We first demonstrate OE olfactory enhancement in rodents using electrophysiology methods including both electroolfactogram (EOG) and whole-cell patch clamp, followed by perceptive investigations in canines using fMRI. For electrophysiology, OE was surgically removed from adult Sprague-Dawley rats, and whole cell patch-clamp and electroolfactogram were evoked by a standard odorant solution (SOS) of ethyl butyrate, eugenol, and (+)/(-) carvone with or without Zn or control metals. For fMRI, both anesthetized and conscious dogs raised at the Auburn University Canine Detection Research Institute were included. The anesthetized cohort were sedated intramuscularly with xylazine (2.2 mg/kg) and lightly anesthetized with ketamine HCL (11 mg/kg). Conscious dogs were first trained by positive reinforcement to lie still before data acquisition took place. Using a customized odorant applicator for computer-controlled delivery and evacuation of stimulus, the same SOS was used either with or without zinc or control gold.

Results. We determine that Zn NP function at the OE in a reversible, specific, dose-dependent manner. A kinetic model of receptor/odorant/metal interactions describes the stoichiometry and mechanism of action. Calculations from the model estimate that one metal nanoparticle facilitates the binding of two receptor proteins to form a dimer, which is necessary to activate the GPCR olfactory transduction cascade. Canine fMRI results indicate that the addition of zinc nanoparticles results in a significant increase of olfactory-related brain excitation in response to odorants in both anesthetized and awake dogs, consistent with the increase in excitation observed in response to higher vs. lower concentration odorants at the OE.

Conclusions. From this work we conclude that Zn NP, which are endogenously present and synthetically produced, are involved in the initial events of olfaction. Coupling *in vitro* electrophysiological characterization of the relationship between odorant stimulation and olfactory response to *in vivo* exploration of the cognitive basis of canine olfaction bridges a major gap in mechanistic understanding. Next steps include designing stable & long-lived Zn NP that may be used for applications including improvement of detection dog performance in a specific, sensitive, and non-invasive way.

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An adenoviral vectored GnRH vaccine for estrous suppression in mares

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Introduction: The goal of this study was to evaluate an adenoviral vectored gonadotropin releasing hormone (GnRH) vaccine on suppression of estrous cyclicity and behaviour in adult mares. Effects were measured by antibody assay, ovarian activity, serum progesterone concentration and estrous behavior.

Methods: Normally cycling adult mares were assigned to treatment (n=five) and control (n=five) groups. Treatment mares were immunized against GnRH using an experimental adenoviral (Ad5, E1/E3 deleted) vector expressing an GnRH antigen. The mares were vaccinated intramuscularly twice, four weeks apart. Eleven months following initial vaccination, all treatment mares received a heterologous boost using subeffective dose of a GnRH-protein vaccine (Equity® Oestrus Control Vaccine, Zoetis, Australia). Two additional mares were given the GnRH-protein conjugate and served as controls on the effectiveness of the subeffective booster dose alone. Transrectal palpation and ultrasound of the reproductive tract were performed once to twice weekly for 15 months after initial vaccination. Mares were teased to a stallion for evaluation of estrous behaviour once to twice weekly, and venous blood was collected weekly for GnRH antibody titer and progesterone concentrations.

Results: Following initial vaccination, all five control mares (100%) displayed normal estrous cyclicity and behaviour. Four of the treatment mares (80%) displayed normal estrous cyclicity and behaviour, but one mare experienced two consecutive prolonged diestrus states lasting 70 and 84 days respectively, with serum progesterone concentrations maintained above 5.9 ng/ml, eliciting diestrus behaviour. By 7 weeks after the heterologous boost, all treatment mares became acyclic, with minimal ovarian activity and serum progesterone concentration maintained below 0.2 ng/ml. Estrous behaviour was erratic and inconsistent with displays of estrus, diestrus, and anestrus during each observation period. All treated mares were still in an anestrus state, with minimal ovarian activity and erratic estrous behaviour at the end of the 15 month study period. The low dose Equity control mares continued to display normal cyclicity and estrus. Antibody response to initial vaccination showed an initial peak at approximately 6 weeks which gradually waned. Following the heterologous boost, mean antibody response peaked at approximately 4 weeks and remained elevated for the remainder of the study period. Peak antibody response following heterologous boost was consistent with the cessation of ovarian activity and cyclicity.

Conclusions: This study shows that adult mares can develop GnRH antibodies following immunization using an adenoviral vectored GnRH vaccine. While an antibody response to GnRH was evoked with the adenoviral vectored vaccine, cyclicity and behavioural estrus were mostly unaffected until the mares were reimmunized with a heterologous GnRH antigen that raised the titer of neutralizing antibodies above an apparent threshold level needed to suppress estrus. Heterologous prime-boost strategies have been shown to invoke a greater immune response with other antigens and vaccines.

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Genomic change associated with serial infections of pregnant cattle and sheep with bovine viral diarrhea virus

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Introduction. Bovine viral diarrhea viruses (BVDV) circulating in cattle populations are genetically diverse. Acute infection of the pregnant dam during establishment of a persistent infection (PI) is an important source of genetic diversity in cattle. BVDV infection is also possible in many artiodactyls including sheep, goats, and swine. However, limited information exists regarding genomic changes introduced during BVDV infection of heterologous hosts. This study sought to characterize the genomic changes introduced during serial infections of pregnant cattle and sheep using a BVDV isolate of bovine origin.

Methods. Six pregnant heifers and six pregnant ewes were utilized. The first heifer and ewe were inoculated with 1×10^6 CCID₅₀ of the BVDV-1b isolate AU526. The 2nd, 3rd, 4th, 5th, and 6th heifers and ewes were inoculated with 1mL of BVDV-positive serum from the preceding heifer or ewe, respectively. Blood samples were collected for virus isolation (days 0, 5, 7 after inoculation) and virus neutralization (day 0 and every 28 days until parturition). Isolate AU526 and viruses isolated from serum and fetal tissue samples following passage in cell culture were sequenced on the Illumina platform. The genomic sequences were assembled using SeqManNGen and were edited with the SeqMan software of the Lasergene 10 package. Assembled genomic sequences were further edited using Aligner.

Results. Six PI calves and 4 PI lambs were born to infected dams. Open reading frame sequences of the isolate AU526, viruses isolated from the dams on days 5 or 7 after inoculation, and viruses isolated from PI offspring were sequenced. As compared to the original infecting isolate, 9 to 28 changes were noted in the viruses isolated from heifers. Four to 7 additional changes were noted when comparing the viruses isolated from PI calves to those from their dams. Similarly, 0 to 53 nucleotide changes occurred in the viruses isolated from ewes and 50 to 56 additional changes were noted in PI lambs. Interestingly, 5 to 13 amino acid changes, mostly located in the structural proteins, were present at the identical position in the last 5 ewes and their lambs. Conversely, only 4 to 5 changes were noted in the last 4 cows and their calves.

Conclusions. These results demonstrate that genomic changes are introduced in pregnant sheep at a higher rate than in pregnant cattle. Conserved amino acid changes that could be associated with host adaptation were identified in sheep to a larger extent than in cattle.

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Spinal lymphoma in 18 dogs (2001-2015)

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Objective: Spinal lymphoma in the dog has been infrequently reported and is mainly identified with previously diagnosed generalized involvement. The objective of this study was to describe clinical outcome of dogs with spinal lymphoma and compare different modalities of treatment.

Materials and Methods: Medical records of dogs with spinal lymphoma were reviewed. Patient signalment, weight, presenting signs, localization, diagnostics, treatment, and outcome were collected.

Results: Eighteen dogs' records were reviewed. Three dogs underwent surgery, chemotherapy, and radiation therapy (RT), four dogs underwent chemotherapy, two dogs underwent RT and surgery, three dogs underwent surgery and chemotherapy, three dogs underwent chemotherapy alone, one dog underwent RT and chemotherapy, one dog only received prednisone, and one did not receive any therapy. Chemotherapy was dependent on clinician preference. Overall, median survival time was 83 days (range 0-1932 days); for the twelve dogs that were not euthanized at the time of diagnosis, median survival time was 146 days (range 9-1942). Dogs receiving chemotherapy (n=13) had a longer median survival time (MST = 179 days) compared to those not receiving chemotherapy (including prednisone) (MST = 3 days) (p = 0.003). Dogs that received radiation therapy (n=7) also had a longer median survival time (MST = 179 days) compared to those who did not receive radiation therapy (MST = 42 days) (p = 0.038). However, based on multivariate analysis, no one treatment had an overall significant difference in treatment survival.

Conclusions: This study suggests that the use of adjuvant treatment for spinal lymphoma increases overall survival. There does not appear to be evidence for a particular treatment to be superior for this disease.

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Quantitative evaluation of Mammaglobin-A gene expression in canine mammary tumors.

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Introduction.

Mammaglobin-A (MGBA) is a member of the uteroglobin protein family that is overexpressed in breast cancer. Due to its tissue specificity, Mammaglobin-A is considered a target for cancer vaccine therapy. In our laboratory we have explored the expression profile of MGBA in canine breast cancers. The first approach to analyze the expression of Mammaglobin-A in canine tumors indicated that this gene was expressed in 6 established canine mammary tumor (CMT) cell lines and in normal canine mammary epithelial cells (CMEC). The aim of the present study was to analyze the expression of Mammaglobin-A by QrtPCR

Methods.

Cell culture: Six established canine mammary tumors cell lines (CMT9, 12, 27, 28, 47, 119) and phenotypically normal CMEC cells were grown in Alpha-MEM supplemented with 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin.

RNA extraction: Total cellular RNA was extracted from the cell lines using the High Pure RNA isolation kit according to the manufacturer's instructions. RNA was next analyzed by reverse transcriptase-PCR (QrtPCR) using the Access RT-PCR system according to the manufacturer's instructions. The forward primer for mammaglobin-A was 5'-ATGAAGCTGCTGAGAGTCCTTGTGCTG-3' and the reverse primer was 5'-TGCTGAGAGTCCTTGTGCTGGTTGCC-3. The DNA was purified using the QiaQuick PCR purification kit (Qiagen). Equivalent volumes of each purified PCR product DNA were resolved on a 2.5% agarose ethidium bromide-stained gel. In order to confirm the nucleotide sequence of the amplified products (CMT cell lines), purified PCR product DNA was sequenced (Dana-Farber Cancer Center DNA Resource Core) and compared to Genbank mammalian MGBA sequences. Mammaglobin-A expression was analyzed with qPCR in six CMT cell lines and CMEC cells.

Results. In all CMT cell lines and the CMEC cell line, the mammaglobin PCR DNA amplicon product was detected on ethidium bromide-stained agarose gels as the expected 237 bp fragment. The sequence obtained for the amplified product was identical to the known canine mammaglobin cDNA sequence. All analyzed CMT cell lines had increased expression levels of Mammaglobin-A when compared with CMEC cells.

Conclusions. The present study represents a quantitative evaluation of Mammaglobin-A gene expression in CMT cell lines and CMEC cells. Enhanced expression was observed in neoplastic cell lines comparable to expression profiles observed in human breast cancer.

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Successful Transvenous Electrical Cardioversion in Dogs with Atrial Fibrillation

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Introduction. Atrial fibrillation (AF) is the most common supraventricular tachyarrhythmia in dogs and conversion to normal sinus rhythm (NSR) is critical to improve hemodynamics and long-term prognosis. Antiarrhythmic medications and transthoracic electrical cardioversion are associated with side effects and excessive energy requirements, respectively. Transvenous electrical cardioversion (TVEC) is standard of care in humans. This case report describes successful electrical conversion of pathologic AF to NSR using TVEC in dogs.

Methods. Oral amiodarone was administered two weeks before and two weeks after TVEC. Using fluoroscopic guidance under general anesthesia, one cardioversion catheter was placed in the proximal left pulmonary artery and a second was advanced to the right atrium. Electrical energy was directly delivered to the atrial myocardium via a biphasic defibrillator until cardioversion.

Results. TVEC was successful in all three dogs at an average electrical current of 36.6 J (range: 30-50 J). Two dogs converted to NSR with one attempt. One dog required a second attempt at 50 J. No complications were noted with the procedure. NSR remained in all dogs four weeks after TVEC.

Conclusions. TVEC successfully converted AF to NSR in dogs with pathologic AF. Larger sample size and longer follow-up are needed to determine the long-term efficacy and prognostic benefit of TVEC in dogs with pathologic AF.

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Evaluation of Tumor Specific Promoters for Use in Conditionally Replicating Adenovirus Mediated Virotherapy of Canine Lymphoma

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Conditionally Replicative Adenoviruses (CRAds) are genetically modified therapeutic viruses that incorporate transcriptional targeting of replication to promote selective killing of tumor cells. Transcriptional targeting utilizes tissue/tumor-specific promoters driving the expression of genes in a tissue- or tumor-specific manner to allow replication of the virus in tumor cells while sparing normal cells. Selection of appropriate intermediate animal models is a basic-requirement for successful cancer virotherapy. The dog is an outstanding animal model of cancer and other complex human diseases. The outbred nature of the dog, the heterogeneity of their tumors, their genomic similarity to humans and similar disease causation, progression and pathology support the use of this animal model. Previous studies have shown high levels of expression of several promoters, including human telomerase reverse transcriptase (hTERT), survivin, chemokine receptor 4 (CXCR4) and progression elevated gene 3 (PEG3) in a variety of human cancers and murine models. The exogenous promoter PEG3 from rats has not only shown tumor-specificity in the human model, but has also shown pan-tumor properties with active transcription occurring in almost all tumor cells. None of these promoters have been tested for their potential as a transcriptional targeting tool for canine cancers. Non-Hodgkin lymphoma accounts for 83% of all hematopoietic cancer and 6 % of all malignancies in the dog. Resistance to current treatments has emerged as a critical challenge for lymphoma treatment due to the presence of genetic diversity among tumor cells. These studies explore tumor-specific activity of these promoters with the goal of identifying a suitable canine lymphoma specific promoter to generate transcriptionally targeted CRAds facilitating viral replication in canine lymphoma, but not in normal cells. In this regard, a GFP reporter gene driven by the rat PEG3 promoter was evaluated for activity after transfection into canine lymphoma cells as well as normal canine cells. The activity of the endogenous canine promoters CXCR4, cTERT, and cSurvivin were examined using quantitative reverse transcriptase PCR. Results showed negligible expression differences between normal and lymphoma cells for cTERT and PEG3 whereas cSurvivin and cCXCR4 showed markedly higher expression in tumor cells when compared with most normal cells and tissues. However, cCXCR4 also showed a high level of expression in normal peripheral blood mononuclear cells (PBMC) cells. In contrast, cSurvivin showed increased expression in canine lymphoma cells, along with other canine tumors, with reduced expression in normal canine cells/tissues and canine PBMCs. These findings will be used to generate a canine lymphoma specific CRAd. The contribution of reagents, advice and assistance by Dr. Richard C. Bird, Dr. Robert Judd, Dr. Erwin, Patricia, Krystyna Minc, and Richard Rathburn is gratefully acknowledged. This work is funded by AURIC fellowship as well as additional funding from Department of Pathobiology and Scott Ritchey Research Center.



Kidney-cell adapted Infectious Bronchitis ArkDPI Vaccine Confers Effective Protection Against Challenge.

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Introduction.

In the US infectious bronchitis virus (IBV) Arkansas (Ark)-type wild and vaccine-like strains have been identified in more than 50% of IBV respiratory disease cases in chickens. The high prevalence of Ark viruses occurs despite extensive vaccination with different commercial embryo-attenuated Ark vaccines. Embryo adapted ArkDPI- derived vaccines contain multiple minor viral subpopulations that become predominant in chickens after vaccination and provide a source for the emergence of virulent vaccine-like viruses. We previously investigated genetic and phenotypic changes associated with adaptation of an embryo-attenuated IBV ArkDPI-derived vaccine virus to chicken embryo kidney (CEK) cells. The virus population shifted towards homogeneity in spike (S) and nonstructural (NSP) genes during seven passages in CEK cells. Based on S gene sequencing, the changes of the predominant Ark population after CEK adaptation were not reverted after 5 back-passage in embryonated chicken eggs nor after a passage in chickens. Because of the advantages of this more stable and homogeneous CEK-adapted ArkDPI virus, this study was aimed at evaluating its ability to confer protection against homologous challenge.

Methods

Groups of 1-day-old SPF chickens (n=12-16) were vaccinated by eye-drop with 10^4 or 10^5 EID₅₀ of CEK adapted virus. Control groups included unvaccinated/challenged and unvaccinated/not challenged chickens. Birds were challenged ocularly at 20 days of age with 10^6 EID₅₀ of an Ark virulent strain (GenBank accession #JN861120). Protection was determined by clinical signs [respiratory rales (nasal and/or tracheal) were evaluated blindly], viral load in lachrymal fluids [quantitative reverse transcriptase PCR (qRT-PCR)]. In addition, IBV specific B lymphocytes were determined in the Harderian gland by ELISPOT. Values for each chicken group were compared by one-way ANOVA followed by a multiple comparisons post-test. Differences will be considered significant with *P* values of <0.05.

Results

CEK-adapted IBV ArkDPI administered at 1 day of age showed effective protection against Ark virulent challenge based both on respiratory signs and viral load in tears. A significant increase in IgA IBV specific B lymphocytes in the Harderian gland was determined by ELISPOT 7 d post-challenge in CEK-Ark vaccinated chickens compared to unvaccinated controls.

Conclusions.

CEK-adapted ArkDPI-derived vaccine after back passage in embryonated eggs confers protection against virulent Ark challenge. Ark-DPI vaccine adaptation to CEK improves the conventional ArkDPI vaccines as it reduces the emergence of vaccine-derived IBV Ark-like strains.

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Post-graduate/Faculty Poster Presentations

Oncolytic Adenoviruses for the Treatment of Canine Osteosarcoma

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Abstract: Twelve dogs were enrolled in a clinical trial utilizing a conditionally replicative variant of canine adenovirus type 2 (CAV2) targeted to osteosarcoma cells. Following amputation or limb sparing surgery, dogs were injected with the virus. Tumor cells were obtained from the primary lesion and blood samples were drawn both prior to and following virus administration for use in anti-tumor immunity assays. Survival times were assessed as were components of the humoral immune response.

Following injection, no adverse effects due to the injection or virus were observed in the dogs. One dog died due to surgical complications. A second dog was excused from the trial after histopathology determined that it did not have osteosarcoma. Viral shedding in urine and feces was not observed. Mean survival time was 195 days, while median survival was 131 days. Two dogs survived past 12 months (466 and 524 days) giving a long-term survival of 18%. The humoral and cellular immune responses were evaluated and indicated both a pre-existing antibody titer towards autologous tumor and the generation of additional antibodies towards the tumor post treatment.

Treatment with the CAV2 CRAAd did not result in an increase in longevity. However, differences in immune responses between short and long term survivors may explain the differences in longevity. Future studies of the cellular immune response in these dogs will provide a better understanding of their anti-tumor immune responses and could identify mechanisms by which these immune responses could be encouraged.



Effect of Heartworm Disease and Heartworm-Associated Respiratory Disease (HARD) on the Right Ventricle of Cats

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Introduction. Clinical consequences of mature adult *Dirofilaria immitis* infection is well-documented in the dog and cat with cats additionally developing significant clinical disease from pre-adult infections called Heartworm Associated Respiratory Disease (HARD). The RV myocardium has been evaluated in cat models of increased RV afterload (i.e. pulmonary artery banding), and increased density and content of RV collagen have been found. It is unknown if similar changes occur in the myocardium of cats infected with *D. immitis*. The purpose of this study was to evaluate the RV myocardium of cats infected with *D. immitis* and to correlate the observed myocardial disease with pulmonary parenchymal and vascular pathology.

Methods. The cardiopulmonary system of a total of 6 groups of 10 - 12 SPF cats were examined 8 or 18 months after infection with L3 *D. immitis*. For each 8 and 18 month time point, there was a group (10) with adult *D. immitis*, a group (10) with HARD lesions with no adult HWs, and a group (10) with minimal lung lesions treated with selamectin 30 days post-infection with no adult HWs. A group of SPF normal cats were utilized as negative controls (12). Lung pathology was objectively assessed for several anatomic areas, and these pathologic severity scores (0-3 range) in each category were combined into a score for the combination of pulmonary artery and pulmonary arteriole (PAA) score as well as into one Total Lung Pathology (TLP) score for each cat. The RV and left ventricles (LV) were evaluated via cardiac weight and RV/LV ratios for each at the end of observation time.

Results. Collagen content (collagen to non-collagen ratio) was assessed with ANOVA; and negative control cats had significantly greater collagen content than all other affected groups ($p=0.032$). Analysis of correlation and simple linear regression of the RV/LV ratios and collagen content revealed no significant relationship ($r=0.03$, $p=0.723$, respectively). However, collagen content had a modest, but significant (simple linear regression analysis) negative correlation with both PAA and TLP ($r=-0.25$ and $r=-0.26$, respectively: $p=0.032$ and $p=0.025$, respectively).

Conclusions. These data suggest that the RV of cats infected with HW have a decreased collagen content, concurrent with increasing lung pathology, with or without the physical presence of adult heartworms. The decrease in collagen was noted in HARDs cats 18 months after the infection and over 12-14 months after the death of immature adult HWs. Despite an improvement in TLP and PAA in HARDs cats at 18 months, as compared to 8 months, the loss of RV collagen content persisted. The physiologic response in HW and HARDs cats is opposite that seen in the RV of feline models of increased right ventricular afterload. These data help to clarify the pathophysiologic response in the RV of cats with heartworm disease, which may partially explain the relative absence of RV hypertrophy, pulmonary hypertension, and heart failure in heartworm-infected cats.

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