Abstracts

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ORAL PRESENTATIONS

Research Session I – Interns and Residents

Effect of Sample Storage Time and Temperature on Rate of Serum Glucose Concentration Decline in Four Species
Nancy Collicutt, Bridget Garner, Melinda Camus, and Kelsey Hart

Although delays between blood sample collection and analysis are common in veterinary medicine, the effect of prolonged serum-clot contact time on serum glucose concentration is not well established. In this study, the effect of storage time and temperature on serum glucose concentration was explored in dogs, horses, alpaca, and sturgeon. Whole blood specimens were divided into 7 no-additive tubes and serum separated from one sample within one hour to serve as the reference sample. The remaining samples were stored at 4°C and 25°C, then centrifuged and serum glucose measured by automated analysis at 2, 4, and 8 hours post-collection. Data was compared within species across time points and temperatures using repeated measures ANOVA. There were no significant interactions between serum glucose decline and packed cell volume in all species at both storage temperatures. The decline in serum glucose concentration for all samples stored at 4°C was not significant, except for the 8 hour samples from sturgeon and dogs. At 25°C, serum glucose concentration was comparable to reference values at 2 hours in the horse, sturgeon, and alpaca, but significantly lower at 4 and 8 hours in those species and at all time points in dogs. The rate of glucose decline was fastest in dogs and slowest in horses. Therefore, storage at 4°C limits serum glucose decline for at least 4 hours in all species tested and up to 8 hours in horses and alpacas. At 25°C, serum-clot contact time should not exceed 2 hours in dogs and 4 hours in horses, alpacas, and sturgeon.
Effect of Packed Cell Volume on Glucose Concentrations Obtained by a Veterinary Glucometer  
S. Lane, A. Koenig, B. Brainard

Point-of-care (POC) glucometers have been reported to provide inaccurate measurements for anemic and hemoconcentrated samples. The purpose of this study was to determine the effect of a range of packed cell volumes on a veterinary-marketed POC glucometer and to develop and validate a formula to correct the POC reading given a known packed cell volume.

Sixty mLs of heparinized blood were obtained from 6 healthy dogs; each was processed into packed red blood cells and plasma. Packed red cells were resuspended using varying quantities of plasma to achieve packed cell volumes (PCV) from 0-94%. Duplicate readings were obtained using the POC glucometer (POCgluc); samples were then centrifuged, decanted, and frozen. Plasma samples were batch analyzed on a clinical laboratory biochemical analyzer (CPgluc).

Mean PCV and mean POCgluc readings were calculated for each dilution. POC failed to read four samples with PCV >80%. A repeated measures ANOVA was used to test for differences between POCgluc and CPgluc. Delta glucose (Δgluc) was calculated by subtracting POCgluc from CPgluc. A linear regression model was used to describe the relation between Δgluc versus PCV from which a correction formula for POCgluc (CorrPOCgluc) was developed. The formula was clinically evaluated using POCgluc, PCV and CPgluc measurements of 30 dogs admitted to the intensive care unit. Clarke and Consensus error grid analyses were examined to assess clinical risk associated with using uncorrected and corrected glucose measurements.

There was a significant difference between POCgluc and CPgluc (p<0.0001) for both research and clinical samples. For research samples, mean Δgluc was 41 mg/dl (maximum difference 99 mg/dl) while the mean difference between CorrPOCgluc and CPgluc was 5.5 mg/dl (maximum difference 19 mg/dl). For the clinical samples, mean Δgluc was 29.1 mg/dL (max difference 180 mg/dL) while mean difference between CorrPOCgluc and CPgluc was 5.5 mg/dL (maximum difference of 181 mg/dl) (p<0.0001). Clarke (p=0.0004) and Consensus (p=0.0008) error grid analyses differed significantly between POCgluc and CorrPOCgluc for both experimental and clinical populations with more of the CorrPOCgluc measurements falling within the zone of minimal risk (Zone A).

PCV has a relevant impact on the POCgluc measurements in dogs. In hemodilute samples, POC provides falsely increased results while in hemoconcentrated samples, POC results are falsely decreased when compared to the reference standard. Use of a correction formula more closely approximates actual plasma glucose concentrations in patients with abnormal PCVs, which may reduce the risk of therapeutic decision making.
Outcome of Positive-Pressure Ventilation in Dogs and Cats with Congestive Heart Failure: 16 Cases (1992-2012)

Thomas H. Edwards, Amanda Erickson Coleman, Benjamin M. Brainard, Teresa C. DeFrancesco, Bernard D. Hansen, Bruce W. Keene and Amie Koenig.

Congestive heart failure (CHF) can lead to life threatening pulmonary edema. In severe CHF, positive pressure ventilation (PPV) can support oxygenation and ventilation, allowing time for resolution of edema by pharmacologic therapy. The objective of this study was to determine the duration of ventilation, complications and clinical course of small animals undergoing PPV for treatment of CHF.

Medical records were searched at two university teaching hospitals to identify patients who received PPV for treatment of CHF. Six cats and ten dogs were included. Patient signalment, PPV duration, underlying cardiac disease, arterial or venous blood gas values, pharmacologic therapy before, during and after PPV, drugs used to provide anesthesia, complications and outcome were recorded.

Overall survival to discharge was 62.5% (10/16). Mean (± SD) duration of PPV was 30.8 ± 21.3 hours and average time from presentation to initiation of PPV was 5.9 ± 6.4 hours. Azotemia at the time of initiation of ventilation and use of pentobarbital were negatively associated with survival to discharge (P=0.011 and P=0.036 respectively). The survival to discharge rate was 77% (10/13) for patients treated after 2005 or those not receiving pentobarbital. No significant association existed between signalment, nature of heart disease, furosemide dose, length of ventilation, first time CHF events, or lactate levels with survival to discharge.

Small animals requiring PPV for treatment of CHF have a good overall prognosis for hospital discharge and require relatively short duration of PPV. Azotemia and the use of pentobarbital are negatively associated with outcome.
Effect of Red Blood Cell Product Age on Occurrence of Acute Canine Transfusion Reactions

Maglaras C, Koenig A, Bedard D, Brainard BM

The age of red blood cell (RBC) products has been shown to correlate with increased risk of transfusion reactions in humans; however, no similar data exists in veterinary literature. The objective of this study was to determine if RBC product age affects morbidity and mortality in dogs. The hypothesis was that both would increase with age of product.

Medical records (2010–2012) were reviewed for dogs receiving RBC products. Patient signalment, reason for transfusion, source of product, dose, rate of administration, use of multiple transfusions, underlying disease, occurrence and type of reactions, various hematologic parameters, and survival were recorded. Data was analyzed for association between potential risk factors and occurrence of transfusion reactions as well as between transfusion reactions and survival.

Of 333 transfusion events in 211 patients, 84 transfusion reactions occurred. Fever was most common (41/84), followed by hemolysis (21/84). The odds of hemolysis (but not fever) significantly increased with age of product (p<0.0001). For every additional day of product age, odds of hemolysis increased by 1.11x. Occurrence of transfusion reactions was associated with higher dose of product, longer duration of administration, and immune mediated disease but not with source of product or reason for anemia. Administration rate was significantly slower in patients with febrile transfusion reactions (p <0.0001). Product age and occurrence of reactions were not associated with increased mortality.

Age of stored red blood cell products is associated with increased risk of hemolytic transfusion reactions. Adjustment of recommended storage duration may be warranted.
Chronic kidney disease (CKD) is the most common metabolic disease in domesticated cats where it is characterized by tubulointerstitial fibrosis. We have developed a model of transient unilateral renal ischemia, which results in histologic evidence of chronic fibrosis that has morphologic similarities to spontaneous feline CKD. Previous studies have demonstrated that smooth muscle actin (SMA) accumulation is directly associated with renal fibrosis in cats with spontaneous CKD. We hypothesized that SMA content would be greater in kidneys after ischemia, compared to contralateral kidneys, and that the SMA content would be sustained for 6 weeks. Kidneys were collected at 3, 6, 12, 21, and 42 days after transient in vivo ischemia (1 hour; n=3/day). Histologic sections were prepared and stained with anti-SMA antibodies. Histomorphometric analysis of SMA was used to quantify interstitial fibrosis using commercially available image analysis software. For each section, 3 measurements were made in the cortex, corticomedullary junction, and medulla to determine the percentage of tissue staining positive for SMA in a 100x photomicrograph. Our study demonstrated that the SMA content was increased in the post-ischemic kidneys, and this increase was sustained for 6 weeks after the initial insult. The renal tubules in the corticomedullary junction are highly susceptible to ischemic injury consistent with our finding of increased SMA positive staining at this location in the post-ischemic tissues. These results provide quantitative evidence that a single ischemic episode can result in a ‘profibrotic’ environment within the feline kidney that is chronically sustained. Further, transient renal ischemia resulted in a consistent increase in SMA compared to controls and provided an objective outcome measure for future studies. This model provides a unique opportunity to study the mechanism of renal fibrosis in feline CKD and to evaluate the efficacy of therapies intended to slow this progressive disease.
Attenuation of the Pressor Response to Exogenous Angiotensin by Enalapril and Telmisartan in Healthy Dogs

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Overstimulation of the renin-angiotensin-aldosterone system is central to many clinical syndromes affecting canine patients. This study evaluated the ability of an oral angiotensin receptor blocker, telmisartan (TEL), to blunt the rise in blood pressure (BP) induced by exogenous angiotensin I (Ang I) as compared to both placebo (PL) and the angiotensin-converting enzyme inhibitor enalapril (EN). Telemetric BP transducers were surgically placed in the femoral arteries of six purpose-bred beagles. Following one week of oral therapy with EN (0.5 mg/kg q 12h), TEL (1.0 mg/kg q 24h) or PL, each dog was anesthetized 90 min post-dose for challenge with exogenous Ang I. In addition, following one week of oral therapy with either EN or TEL, dogs were anesthetized 12 or 24 hours post-dose (i.e., at trough drug activity), respectively, for challenge with exogenous Ang I. Continuous, direct arterial BP responses were recorded during escalating bolus doses of intravenous Ang I. A blinded observer evaluated BP responses for changes in systolic BP (ΔSBP) and diastolic BP (ΔDBP). Repeated-measures ANOVA was used to compare responses between groups. P < 0.05 was considered significant.

At 90 minutes post-dose (EN and TEL), 12 hours post-dose (EN) and 24 hours post-dose (TEL), both EN and TEL produced significant attenuation of ΔSBP and ΔDBP when compared to placebo. ΔSBP and ΔDBP were significantly lower with TEL than EN at Ang I doses ≥ 100 ng/kg at each time point post-dose. At 90 minutes post-dose and at all doses of Ang I, TEL resulted in complete extinction of the pressor response (ΔSBP and ΔDBP = 0 mmHg) in 5/6 dogs and near-complete extinction of the pressor response (ΔSBP and ΔDBP ≤ 3 mmHg) in 1 dog.

These results suggest that at 90 min post-dose, TEL (1 mg/kg q 24h) more effectively attenuates the pressor response to Ang I than either EN or PL and that this effect persists at the time of each drugs’ trough activity. Based on these findings, TEL may prove useful for the treatment of cardiovascular and renal diseases in dogs.

Research Support: Endowed Research Funds of the College of Veterinary Medicine at University of Georgia

Student Support: Grant from Merial Ltd, the Veterinary Medical Experimental Station (VMES) and the College of Veterinary Medicine at UGA.
Immunologic Effects of Low-Dose Hydrocortisone in Adult Horses

Emily McManus and PI Dr. Kelsey A Hart

Low-dose hydrocortisone (LDHC) therapy modulates inflammatory responses and improves outcomes in people with critical illness-related cortisol insufficiency (CIRCI). LDHC decreases pro-inflammatory cytokine expression without impairing innate immune responses in neonatal horses, and may help foals with CIRCI. CIRCI also occurs in critically ill horses, but the immunologic effects of LDHC therapy have not been characterized in this population. We hypothesized that, as in foals, LDHC would dampen pro-inflammatory responses without impairment of neutrophil function in adult horses. Hydrocortisone (0.429 mg/kg/day IV) and saline were administered to 8 adult horses and tapered over 3.5 days in a randomized cross-over design. Peripheral blood leukocytes were collected before, during, and after treatment for measurement of: 1) endotoxin-induced pro-inflammatory cytokine gene expression using real-time quantitative RT-PCR; 2) phagocytosis of labeled, killed Staphylococcus aureus or Escherichia coli via flow cytometry; and 3) toll-like-receptor(TLR)-2- and TLR-4-mediated and overall reactive-oxygen species (ROS) production via a fluorometric assay. Phagocytic function and TLR-mediated ROS production were unchanged with LDHC treatment, though overall ROS production capacity was significantly decreased in LDHC-treated horses. A general trend towards an increase in phagocytic activity in LDHC-treated horses was observed, with a significant increase in phagocytosis of S. aureus. Endotoxin-induced pro-inflammatory cytokine gene expression was decreased and anti-inflammatory cytokine gene expression was increased in LDHC-treated horses, though specific effects were different than those previously reported in foals. This LDHC regimen has anti-inflammatory effects without impairing of neutrophil function in adult horses, though further study is needed to determine its efficacy in horses with CIRCI.

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Student Support: Merial Ltd, VMES, & UGA College of Veterinary Medicine
Dietary phytochemicals reduce hepatic lipogenesis and lipotoxicity in the post-menopausal rat model

Tucker Avra, Colette Miller, Suresh Ambati, Erica Bass, Natalie Hohos, Emily England, Diane Hartzell, MaryAnne Della-Fera, Srujana Rayalam, Clifton Baile

Females have reduced risk of steatosis compared to males, which reverses with age due to reductions in 17β-estradiol (E2). E2 reduces hepatic steatosis through downregulating lipogenic signaling. Phytochemicals bind to E2 receptors with weaker affinity, but show efficacy in animal models to decrease hepatic steatosis through downregulation of lipogenic genes. As phytochemicals have synergistic properties, the purpose of this study was to investigate the differences in hepatic lipogenesis after prolonged feeding of a phytochemical blend. Aged rats (n=36) underwent a sham surgery or were ovariectomized (OVX). For 16 weeks rats were fed either a control diet or ones containing varying doses of phytochemicals with high vitamin D (diet 2: 1000 mg/kg Genistein (G); diet 3: 500 mg/kg G, 200 mg/kg Resveratrol (R), and 1000 mg/kg Quercetin (Q); diet 4: 1000 mg/kg G, 400 mg/kg R, and 2000 mg/kg Q). After sixteen weeks there was no difference in body weight change between the groups. There was an increase in hepatic lipid mass following OVX (0.27±0.01 v 0.32±0.01 mg/g liver tissue; p=0.03). OVX resulted in increased expression of lipogenic genes ACC1, FASN, and SCD1; inflammatory gene SOCS3; apoptotic genes BBC3, CASP2, ANXA5, and MADH1; fibrotic genes SERPINH1, BGN, and SPARC; and the angiogenic gene VEGF as compared to non-OVX. OVX + high dose phytochemicals suppressed these OVX-induced increases in gene expression. These findings support the hypothesis that a dietary phytochemical blend minimizes the effects on the liver of OVX in rats, an animal model for human menopause. These data provide support for the anti-lipogenic and lipotoxic effects of synergistic phytochemical combinations. This finding may suggest efficacy of phytochemicals in reducing fatty liver disease in women.
Research Session III – Graduate Students (DVM/PhD)

Defining the Role of *Rhodococcus equi* Virulence Associated Protein A (VapA) in Aberrant Phagosomal Maturation

L.M. Wright, V.J. Starai, and M.K. Hondalus

*Rhodococcus equi* is a Gram-positive bacterium that causes pneumonia in foals less than six months in age. When inhaled, *R. equi* is phagocytosed by alveolar macrophages and, if the bacterium contains a VapA-type virulence-associated plasmid, is able to resist killing and replicate intracellularly. Intracellular survival of *R. equi* has been shown to be associated with abnormal maturation of the bacteria-containing phagosome. Specifically, acidification of the phagolysosome is known to be inhibited when the bacterium has a functional *vapA* gene. The purpose of this study is to determine VapA’s function and mechanism of action during macrophage infection.

To simplify the study of phagosomal maturation, the model organism *Saccharomyces cerevisiae* is being exploited. The budding yeast is easy to work with, genetically tractable, and the protein machineries involved in eukaryotic intracellular membrane dynamics are well characterized and highly conserved between yeast and higher eukaryotes. Expression of *vapA* in yeast produces an abnormal vacuolar morphology. As the yeast vacuole is the functional equivalent of mammalian lysosomes, it is likely that VapA alters eukaryotic endolysosomal traffic. To address this activity, pull down assays using recombinant GST-VapA and yeast extracts are currently underway to identify yeast protein(s) that interact with VapA. Through identification of interacting yeast partner protein(s), it is hypothesized that insight into the mechanism VapA during *R. equi* infection will be gained.
Network-based Vaccination Improves Prospects for Disease Control in Wild Chimpanzees

Julie Rushmore*, Damien Caillaudbc, Richard J. Halla, Rebecca M. Stumpfd, Lauren Ancel Meyersc, and Sonia Altizera

Many endangered wildlife populations are vulnerable to infectious diseases for which vaccines exist; yet, pragmatic considerations often preclude large-scale vaccination efforts. These barriers could be reduced by focusing on individuals with the highest contact rates. However, the question then becomes whether targeted vaccination is sufficient to prevent large outbreaks. To evaluate the efficacy of targeted wildlife vaccinations, we simulate pathogen transmission and control on monthly association networks informed by behavioral data from a wild chimpanzee community (Kanyawara, Kibale National Park, Uganda). Despite considerable variation across monthly networks, we find that targeting the most connected individuals can prevent large outbreaks with 35% fewer vaccines than random vaccination. Transmission heterogeneities might be attributed to biological differences among individuals (e.g., sex, age, dominance, and family size). Thus, we also evaluate the effectiveness of a trait-based vaccination strategy, as trait data are often easier to collect than data on individual positions in a social network. Our simulations indicate that a trait-based strategy can prevent large outbreaks with up to 18% fewer vaccines than random vaccination, demonstrating that individual traits can serve as effective estimates of connectivity. Overall, these results suggest that fine-scale behavioral data can help optimize pathogen control efforts for endangered wildlife.
Resistance, Replacement, and Fitness of UGA Sheep Gastrointestinal Nematodes: A Farm Tail
Melissa M. Miller, Adriano F. Vatta, Sue B. Howell, Bob E. Storey, Adam Michalak, Ray M. Kaplan

The UGA sheep flock experienced serious clinical parasitism as a result of a severe infection of Haemonchus contortus (H. contortus), a blood-sucking gastrointestinal nematode that causes anemia and even death. This population of H. contortus was highly resistant to benzimidazole, ivermectin, and moxidectin and borderline resistant to levamisole in vitro. Thus, treatment with these anthelmintics could not effectively control the infection. This flock realized several deaths post-treatment as a result of haemonchosis. The purpose of this study was to replace the current multiple-resistant worm population with susceptible worms and to study the molecular evolution of resistance to benzimidazole anthelmintics over time. The flock was moved to concrete pens on August 1, 2011 and treated with two combination treatments of albendazole and levamisole for 3d, resulting in 98.7% fecal egg count reduction (FECR). Each animal was inoculated with 5000 infective larvae from a known benzimidazole-susceptible lab isolate with the goal of replacing the resistant worms with susceptible worms. DrenchRite Larval development assays (LDA) and fecal egg count reduction tests (FECRT) were completed at various time points throughout the study to determine in vitro and in vivo resistance status, respectively. After establishment of the susceptible isolate, the flock was moved to a new farm where livestock were not previously grazed, thus establishing a new susceptible population. Initially post-replacement, albendazole treatment showed 97.0% FECR and LDA showed full reversion to susceptibility status, confirming successful replacement of the resistant worm population. FECs were monitored over time and eggs were isolated for further analysis to determine allele frequency of single nucleotide polymorphisms that are associated with resistance to benzimidazoles. In April 2013, as part of normal management practices the flock was selectively treated based on FAMACHA and a FECRT was completed along with a LDA. FECR was 41.4%, 98.7%, 1.2%, and 60.4% for albendazole, levamisole, ivermectin, and moxidectin, respectively, and the LDA showed reversion to resistance for all four anthelmintics. These data indicate that we successfully replaced a resistant worm population with a susceptible population, but lab isolates may lack the fitness to compete with field isolates. Thus, the susceptible lab isolate may have died more readily, leaving behind a population of predominantly resistant worms.

<table>
<thead>
<tr>
<th>Anthelmintic</th>
<th>Pre-Replacement</th>
<th>Susc. Isolate</th>
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<th>April 2013</th>
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<td>Benzimidazole*</td>
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<td>Moxidectin†</td>
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<td>2.925 (S)</td>
<td>7.8 (S)</td>
<td>93.75 (R)</td>
</tr>
</tbody>
</table>

* Effective concentration 50s for 3 anthelmintics and †effective concentration 95 at 4 different time points of study. R: Resistant, S: Susceptible, BDL R: Borderline Resistant
Diagnostic Quality of Percutaneous Hepatic Fine Needle Aspiration Versus Laparoscopic Biopsy in Rabbits
L.M. Proenca; J. Mayer; M.S. Camus; N.M. Nemeth; A. Sharma; D. Stelmach; S.J. Divers.

ABSTRACT

Definitive diagnosis of liver pathology generally relies on demonstration of a host response, which is often dependent on tissue sampling. The study objective was to compare diagnostic quality of hepatic percutaneous ultrasound-guided fine needle (22-gauge) aspirates (FNA) with laparoscopic hepatic biopsies in seven healthy adult rabbits. Biopsies were obtained with either 1.7-mm or 3.0-mm biopsy forceps. Cytology was assessed on a scale of 0 to 3 (unusable to optimal) regarding cellularity, cell distribution, and morphology, and 0 to 3 (none to abundant) for blood contamination and cell disruption. Biopsies were evaluated on a scale of 0 to 5 for artifactual changes (ranging from <10% to >75% of tissue affected). Portal triads (PT) and central veins (CV) were quantified.

Aspirates were moderately to highly cellular (2.54) with good cell distribution (2.56), minimal cell breakage (0.8), and moderate blood contamination (1.96). The 1.7-mm biopsies averaged 1.3 for artifactual changes, and contained an average of 0.6 PT and 1.0 CV. The 3-mm biopsies averaged 2.7 for artifactual changes, with an average of 4.0 PT and 4.14 CV. Overall, 3.0-mm biopsies provided more continuous tissue and associated architectural landmarks for evaluation than two 1.7-mm biopsies. All but one 3.0-mm biopsy had at least one PT suitable for evaluation, and all had one or more CV. In contrast, half of the 1.7-mm biopsies had no discernible PT, and many also lacked CV for evaluation. When investigating liver disease in rabbits, the superiority of 3.0-mm laparoscopic biopsies over 1.7-mm biopsies and FNA should be considered to maximize diagnostic potential.

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Pharmacokinetics of Levetiracetam in Hispanolan Amazon Parrots following Oral Administration of a Single Dose
Rodney Schnellbacher, Hugues Beaufrère, Robert D. Arnold, Thomas N. Tully, Jr., Joerg Mayer, Stephen J. Divers

Abstract
Avian long-term anticonvulsive treatments have been poorly described and few pharmacokinetics studies have been performed, with mixed results. Levetiracetam, a new anticonvulsive drug, has shown good efficacy for monotherapy or adjunctive treatment of seizures in both human and veterinary medicine. Pharmacokinetic studies in domestic animals have shown it to have a favorable profile with rapid oral absorption, absence of hepatic metabolism, and a high safety margin.

The aim of this study was to determine the pharmacokinetics of oral levetiracetam in Hispanolan amazon parrots, *Amazona ventralis*. Twenty healthy individuals were divided into two treatment groups and were administered either a 50 or a 100 mg/kg dose. Blood samples were collected at baseline and intermittently for 16 hrs. No animals showed any adverse behavioral effects to either dosing. Levetiracetam was quantitated in serum using an ARK Diagnostic Levetiracetam® Immunoassay (Sunnyvale, CA) on a Siemens Dimension Xpand (New York, NY) general chemistry analyzer.

Mean pharmacokinetic parameters were estimated using a non-compartmental analysis. The concentration time profiles resembled characteristic absorption, with maximum plasma concentrations (C<sub>max</sub>) of 56.5 and 93.9 mg/L (T<sub>max</sub>) at 30 to 60 min; terminal half-lives (t<sub>1/2</sub>) at 2.38 and 2.37 hrs; volumes of distribution (V<sub>d</sub>) at 0.807 and 0.773 L/kg; areas under the curve (AUC) of 14,125 and 28,182 mg•min/L, and clearance rates (Cl) at 3.65 and 3.60 mL/min/kg for 50 mg/kg and 100 mg/kg respectively. Plasma concentrations were greater than 5 mg/L for up to 9.4 and 12 hrs suggesting an 8hr and 12hr oral dosing at 50 and 100 mg/kg respectively would be sufficient to maintain systemic drug levels at or above what has been found to be clinically therapeutic in humans.
Assessment of the Immune Function of Urbanized White Ibis through a Bacterial Killing Assay

Karen Christ, Sonia Hernandez, Shannon Curry, Catie Welch, and Kristen Navara.

White Ibis (Eudocimus albus) are wading birds native to southern Florida, and are regarded as an indicator of wetland ecosystem health. As a result of decades of anthropogenic draining of the Everglades, White Ibis have adapted to foraging in a variety of human developed habitats, including recreational parks and ponds, progressively habituating to the presence of people. With the Everglades restoration project underway, return of White Ibis to the area may serve as an important marker of restoration success. Increased utilization of urban habitats has altered their behavior and shifted their natural diet to lesser quality foods and water, including human handouts. We hypothesize that this shift from natural diet, in conjunction with other stressors including increased contact with people and other unfamiliar species, will result in reduced immune function among urbanized birds. Blood samples were collected in March and December 2012 from White Ibis across six recreational parks in Palm Beach County, Florida where people have been observed feeding wild birds. To assess the function of the complement arm of the immune system, we utilized a bacterial killing assay in which plasma was mixed with a known quantity of E. coli, streaked on agar plates, and incubated for 16 hours at 37°C, after which, we quantified bacterial colonies. Simultaneously, we incubated control plates (no plasma), to compare the number of colony producing units. We found that White Ibis in urban areas of Palm Beach County, FL are highly variable in their ability to kill bacteria via the complement system. Further work is needed to compare results against truly wild White Ibis.

Student Support: Grant from Merial Ltd, the Veterinary Medical Experimental Station (VMES) and the College of Veterinary Medicine at UGA

Research Support: Grant from the American Association of Zoo Veterinarians Research Grant Program
Malignant Epithelial Neoplasm in a Wild Black Rat Snake (*Elaphe obsoleta obsoleta*): A Case Study
Jessica Comolli and Haley Olsen, Rodney Schnellbacher, Mauricio Seguel, Kaori Sakamoto, Stephen Divers

Retrospective studies of zoological collections across the nation document rates of neoplasia in captive snakes varying from 2.9% to 23.1%. These studies represent the majority of data for the prevalence of neoplasia in snakes with the rate of neoplasia in wild snakes representing an area of especially scant documentation and research. This is a case of an adult black rat snake (*Elaphe obsoleta obsoleta*) that presented to the Wildlife Treatment Center at the University of Georgia, College of Veterinary Medicine. The snake presented with a 3cm by 3cm right submandibular mass. The mass was lanced and purulent exudate noted. Radiographs revealed that the lesion was a soft tissue mass with amorphous, intra-lesional, mineral inclusion. Preliminary biopsy results deemed the mass to be neoplastic in origin and likely malignant. Based on these results, it was determined that rehabilitation and release were unlikely to be successful and the decision was made to euthanize. Additional histopathology revealed an infiltrative mass of epithelial origin containing scant dentin-like material with a mitotic rate average of 2 per 40X high power fields (HPF). While a mass of this nature does not appear to have been previously described in wild snakes, the histologic characteristics of the mass suggest that this neoplastic epithelium could be odontoblastic in origin based upon comparison to cases of ameloblastic carcinomas in humans and chinook salmon. Further diagnostics based on immunohistochemistry (IHC) and electron microscopy (EM) are pending.
Prevalence of Ophthalmic Disease in Blue-Eyed Horses

BE Bergstrom, AL Labelle, KE Myrna, RE Hamor

There exists amongst referring veterinarians and the lay public a perception that blue-eyed horses have increased frequency of ocular disease. The purpose of this study is to assess the prevalence of ocular disease in horses with blue or heterochromic eyes relative to those with brown eyes.

Medical records of horses presenting to either the Comparative Ophthalmology services or Equine Medicine/Surgery services at 2 institutions were reviewed. Signalment, ocular and non-ocular diagnoses were recorded. Ocular disease was divided into four categories: adnexa, cornea, intraocular/orbit and squamous cell carcinoma (SCC). Owners were contacted by telephone to confirm iris color. Chi-square analysis was used to compare group proportions.

A total of 165 eyes of horses with ocular disease and 212 eyes of horses without ocular disease were included. Blue eyes were equally common in the ocular disease and non-ocular disease groups (p=0.265). There was no significant difference in the proportion of blue and brown eyed horses when comparing the adnexal and corneal (p=0.548), corneal and intraocular/orbit (p=0.379), and adnexal and intraocular/orbit (p=0.843) categories. A significant difference was detected in the proportion of blue-eyed horses between the adnexal (p=0.000), corneal (p=0.033), intraocular/orbital (p=0.000) and the SCC categories, with a higher proportion of blue-eyed horses in the SCC than other groups.

Horses with blue or heterochromic irides are more likely to have ocular squamous cell carcinoma than horses with brown irides, but are not more likely to have adnexal, corneal or intraocular/orbital disease.
Production of Parathyroid Hormone Related Protein in Equine Ophthalmic and Periocular Squamous Cell Carcinoma

Dustin S Major, Elizabeth W. Howerth, Sheryl Coutermarsh-Ott, Katherin E. Myrna

The purpose of this study was to characterize the expression of parathyroid hormone related protein (PTHrP) by equine ocular squamous cell carcinoma (SCC).

Records from horses biopsied for periocular SCC at the University of Georgia VTH from January 2008 to March 2013 were reviewed. Biopsies from 21 horses with confirmed SCC were immunohistochemically stained with a commercially available goat anti-PTHrP antibody using equine parathyroid as a positive control. Slides were independently graded by two authors using an Allred score combining stain intensity scale (0-3) and proportion of staining (0-5). One sample was “pre-cancerous” with moderate lymphoid follicular hyperplasia, which is reported separately. Records were reviewed for signalment, lesion location, and serum calcium.

The mean age of horses was 13.8 years (2-28 years). Breeds included Paint Horse (11/21), Tennessee Walking Horse (4/21), Quarter Horse (3/21), one Thoroughbred, and one Percheron. Lesion locations were conjunctiva (10), limbus (2), cornea (9), eyelid (4), third eyelid (4) and periocular skin (1). Allred scores were 6 (1/21), 7 (14/21) and 8 (5/21). The “pre-cancerous” lesion was on the third eyelid of a Paint Horse and received an Allred score of 6. Bony invasion was present in two horses, but no definitive evidence of metastasis or recurrence was noted in any patient. Total serum calcium (tCa) was available for 12 horses and ionized calcium (iCa) for 3 horses. Values were overall normal with three being very mildly hypocalcemic and one very mildly hypercalcemic.

Ophthalmic SCC in horses expresses PTHrP. A “pre-cancerous” lesion demonstrated mild expression. Serum calcium levels were not significantly elevated, indicating that PTHrP expression by the tumor was not sufficient to cause a clinically detectable increase in serum calcium levels.
In-vitro mechanical evaluation of a ZipFix™ implant for prosthetic laryngoplasty in horses.
Harry Markwell, P.O. (Eric) Mueller
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Inadequate abduction, partial or complete implant failure of prosthetic laryngoplasty in horses is a frequently reported complication in the first six weeks after surgery. Recently, a ZipFix™ implant has been developed for use in soft porous bone or cartilage in human sternal closure. The objective of this study was to evaluate the resistance to implant failure of the sternal ZipFix™ implant for use prosthetic laryngoplasty in horses, evaluated using an in-vitro model.

Twelve (12) cadaveric larynges were collected from horses (5-23yo), for disease not associated with the upper respiratory tract. Larynges were stored in saline at -20 deg Celsius. Individual arytenoid and cricoid cartilages were dissected from soft tissues and mounted on an Instron (Model 3365, Instron, Norwood, MA) for testing. In the arytenoid cartilages, a single ZipFix™ implant (n = 12) was compared to a single strand of #5 Ticron (n = 12). In the cricoid cartilages, a single ZipFix™ implant (n = 6) was tested and compared to a single strand of #5 Ticron (n = 6). The ZipFix™ implant was placed in the arytenoid cartilage, approximately 15 mm from the apex of the muscular process in dorsoventral direction. A single strand of #5 Ticron was placed in a conventional caudomedial to craniolateral direction. In the cricoid cartilages, implants were placed 10 mm lateral to the dorsal spine of the cricoid cartilage exiting 10 mm rostral. The same investigator placed all implants. Cartilages were tested for single cycle to failure at a distraction rate of 0.83 mm/s. Peak load [N] at complete failure, stiffness [N/mm], and distraction distance from 25N to peak load [N] was recorded. Statistical significance was established at P≤0.05.

No significant effect of age or the side of arytenoid was identified. In the arytenoid cartilages, peak load at the time of failure for the ZipFix™ was significantly higher at 359 ± 46 N than the Ticron 159 ± 19 N at (p<0.0001). In the arytenoid cartilage, the ZipFix™ 31 ± 3.5 N/mm had a significantly higher stiffness of compared to Ticron 13 ± 1.9 N/mm (p<0.0001). In the cricoid cartilages, when tested for peak load at failure, no significant difference was observed in the ZipFix™ 266 ± 31 N compared to Ticron 231 ± 40 N (p<0.05). In the cricoid cartilage, the ZipFix™ 21 ± 3 N/mm was significantly stiffer than the Ticron 14 ± 4N/mm. Three (3) arytenoid cartilages fractured on passing of the ZipFix™ and were removed from the study.

The ZipFix™ failed at a significantly higher peak load in arytenoid cartilages and had a significantly steeper slope indicating greater stiffness. Within the cricoid cartilages, the ZipFix™ had a significantly steeper slope indicating greater stiffness. In the cricoid cartilage, no significant difference in peak load was found between the ZipFix™ and the #5 Ticron. The ZipFix™ has potential to provide a greater resistance to implant failure within cartilage if used for prosthetic laryngoplasty. This study provides justification for further in-vitro or in-vivo evaluation of the ZipFix™ implant for use in prosthetic laryngoplasty.
Pharmacodynamic Evaluation of Oral Rivaroxaban in Healthy Adult Cats
A. Dixon-Jimenez, G. Rapoport, MB. Brooks, JC. Abrams, BM. Brainard

Cats with primary cardiomyopathy (CM) have a high incidence of arterial thromboembolism (ATE). A safe and effective oral anticoagulant for cats with CM has yet to be identified. Rivaroxaban (RVX) is an orally administered direct inhibitor of activated factor X (FXa). In people, RVX is used for prevention of deep vein thrombosis and ATE. Prothrombin time (PT), dilute prothrombin time (dPT) and activated partial thromboplastin time (aPTT) become linearly prolonged in people with increasing plasma RVX levels. We hypothesize that oral administration of RVX to cats will result in predictable plasma drug concentrations within the target range reported for efficacy in people and will result in dose-dependent prolongation of dPT, aPTT and anti-factor Xa activity (aXa). We also hypothesize short and long-term oral administration of RVX will be well-tolerated and safe for use in cats.

RVX tablets (10mg Xarelto®, Bayer Healthcare) were divided into halves and quarters. Tablets were weighed to estimate actual milligrams (mg) of drug, with the assumption that the active ingredient was evenly distributed. The 1.25mg tablets were prepared as tablet triturates from the commercially available tablets. A single oral dose of RVX was administered to 6 cats at 1.25mg (G1) and 2.5mg (G2.5) and to 3 cats at 5mg (G5). Blood samples were obtained at baseline and then at 3, 8, and 24 hours following the dose, except for G2 which was evaluated at 1, 3, and 12 hours. Citrated plasma was frozen at -80°C until batch analysis at the Cornell University Comparative Coagulation Laboratory. At each time point, aXa and dPT were evaluated, and aPTT was evaluated at each time point for G2.5 and G5. After a one-month washout period, once daily 1.25mg RVX was given to 6 cats for 30 days (G30). Citrated blood samples were analyzed for aXa, dPT, and aPTT at baseline, and on days 7, 14, 21, 28 and 42.

RVX administration, at all dosages studied, was well tolerated in healthy cats and resulted in dose-dependent prolongation of coagulation times. Peak anticoagulant effects were seen at the 3 hour time point for all assays. Oral RVX demonstrated dose-dependent aFXa activity in cats. A standard dose of 1.25mg resulted in a maximum mean aXa of 2.25 +/- 1.3 U/mL. In the higher dose groups (G2.5, G5), most coagulation effects returned to baseline by 24 hours. In the 1.25mg dose group (G1), coagulation effects were normal at the 12 hour time point. The duration of action of the 1.25mg dose are similar to those seen in studies that have shown positive outcomes in a number of different thrombogenic conditions in people. The higher doses, especially in smaller cats, may result in high aXa values, which may predispose to hemorrhage. Further studies to investigate the pharmacokinetics and pharmacodynamics of RVX in cats with heart disease are necessary.
Dematiaceous, or melanized, fungi cause phaeohyphomycosis in increasing numbers of invertebrates, poikilothermic vertebrates and endothermic vertebrate species, including humans. These include both novel pathogens and records of known pathogens broadening their host range. Infections by six *Exophiala* spp. are among the most common causes of phaeohyphomycosis in fish, including several recently described and emerging species. Diagnostic records from over 1300 fish cases submitted to the Aquatic Pathology Service, University of Georgia from public aquaria were reviewed to evaluate the incidence of piscine exophialosis. Phaeohyphomycosis was diagnosed in 25 fish, including one elasmobranch, two freshwater teleosts, and 22 marine teleosts, including seven seahorses and a seadragon. Macroscopic changes varied from none to the extensive presence of black pigmented nodules. Histologically, lesions were typified by necrosis, granulomatous inflammation and angioinvasion, involving light brown, septate hyphae, with thin non-parallel walls. Lesion distribution varied from locally extensive to widely disseminated in most organs. While formalin fixation precluded culture in many cases, when attempted on fresh necropsy specimens, four isolates were identified morphologically as *Exophiala* sp. These four isolates were identified to species by DNA sequencing, two as *Exophiala pisciphila*, one as *Exophiala xenobiotica* and one as *Exophiala lecanii-corni*. Many *Exophialia* spp. are ubiquitous in water and soil and are often considered opportunistic invaders. Similar to humans, immunosuppression, as well as preexisting infectious and traumatic conditions may act as risk factors. While the majority of cases reported here have been isolated occurrences, epizootics of disease with high mortalities have been reported in cultured fish. Further assessment of disease trends, improved diagnostic methods, and increased awareness will enhance our understanding and ability to mitigate diseases in cultured fish.
Research Session VI – Veterinary & Undergraduate Students

Determination of host factors required for infection of the domestic cat with the human parasite, \textit{Brugia malayi}

Zachary T. Moore, Erica J. Burkman, Bridget Garner, Kaori Sakamoto, and Andrew R. Moorhead.

Lymphatic filariasis presents a major problem to human health worldwide. Infection with filarioid parasitic worms, such as \textit{Brugia malayi} or \textit{Wuchereria bancrofti}, can result in severe sequelae, such as elephantiasis and hydrocoele. Like other filarial worms, \textit{B. malayi} requires both an arthropod intermediate and a specific vertebrate definitive host for survival. Maintaining the life cycle of these parasites has been shown to be difficult in a laboratory setting. Previous research has demonstrated that only 10-20\% of experimentally infected domestic felines (\textit{Felis catus}), which are a suitable, non-primate, definitive host, develop a patent infection. We hypothesized that the difference between cats that develop a patent \textit{B. malayi} infection, versus those that do not, is due to a Th1 versus Th2 immune bias. To explore this, 10 cats were infected with third-stage larvae of \textit{B. malayi} isolated from mosquitoes. Blood was extracted via venipuncture, biweekly for a total of 8 months, which is when patency (microfilaremia) occurs. For each 2-week time point, concentrations of TNF-alpha, a primarily acute Th1 cytokine, were measured by enzyme-linked immunosorbent assay. After 19 weeks, 5 cats presented with microfilaria in their blood. Between the 2 populations of microfilaria-positive and negative cats, there was no significant difference in the TNF-alpha levels. However, concentrations of IL-4 showed that the absence of an IL-4 spike could indicate a possible \textit{B. malayi} infection. Kappa analysis of complete blood counts showed that concentrations of neutrophils, lymphocytes, eosinophils, basophils or monocytes could not be used at predicting a patent infection. Elucidation of the specific role that immune bias plays in \textit{B. malayi} infections may be an important factor in optimizing productive infection in cats, leading to a better understanding of lymphatic filariasis overall.
Backyard Chickens and Salmonella: Prevalence and Public Health
Anna Jeffers, Taylor Winkleman, Margie Lee, Karen Hilyard, John Maurer, Caroline McNichols, Tiffany Kwan

There is a lot of information and misinformation on the internet about Salmonella and raising backyard chickens (and the benefits and dangers inherent in the practice) but despite the rising popularity of raising chickens in urban, suburban, and rural areas, virtually no scientific data on the subject exists. This pilot study is a two-pronged qualitative and quantitative approach to address the need for scientific data and determine the direction of future studies. A prevalence survey was done on backyard flocks via fecal sampling. Concurrently, a series of interviews was conducted to analyze how knowledgeable the general public is about Salmonella risk, common practices used by backyard flock owners, and any common practice that may put the public at greater risk of contracting Salmonella from their chickens.

Fecal samples were obtained from volunteer flocks and were analyzed via culture in a lab at the Poultry Diagnostic Research Center at UGA. Volunteers participated in oral interviews that were recorded, transcribed, and are still being analyzed for content by the College of Public Health at UGA. Prevalence in volunteer flocks was determined to be less than five percent. While the interviews are still being transcribed, preliminary results demonstrate that there is a wide variety of practices and many levels of knowledge about Salmonella and other risks commonly associated with backyard flocks. The results of this study will help define the need for and direct the focus of future studies in backyard flocks and Salmonella. This study will also help define the general risk to the public and need for good, reliable public health information about Salmonella and backyard chickens.
Fat to Protein Ratios and Serum Nonesterified Fatty Acids: Predictors of Milk Yield

Jana Powell, Michael Overton, and Emmanuel Rollin

This study used herd data acquired from Michigan, USA to investigate the relationships between fat to protein ratios (FPR) and serum nonesterified fatty acids (NEFA) as well as the relationship between each of these variables and mastitis, days open, first heat, and culling. Receiver operator characteristic (ROC) curves were used to determine a cutpoint for this herd that would determine lactation success based on 305ME (305 day mature equivalent milk yield). FPRs were determined to have no significant correlation with any of the other variables. NEFAs, conversely, appear to have a correlation with 305ME (305 day mature equivalent milk yield), days to first heat, and days open. ROC curves were then used to determine a cutoff point for FPRs as a predictor of milk yield (305ME), NEFAs as a predictor for FPR, and NEFAs as a predictor of milk yield (305ME). Results showed that when looking at all the lactation groups combined, the FPR cutoff point was established at 1.3, which means that an FPR greater than 1.3 is associated with a decrease in the average milk yield for each lactation group. However, the area under the curve being a 0.5 makes this test just as effective as flipping a coin. When looking at the cutoff points by lactation group, the sensitivity increased, but the specificity decreased. The area under the curve for each of the lactation groups was also very close to 0.5. NEFAs were found to be a good predictor of FPR. For the third lactation group, the area under the curve was 0.63, which makes it better than a 50% chance test. The established cutpoint for this group was 0.06mEq/L. Overall, NEFAs above or below this cutpoint may be useful, in this herd, to determine FPR, but this is not really needed considering that FPRs had no correlation with any of the studied variables and very little predictive value for determining milk production. Based on the third ROC analysis, it was determined that NEFAs could be a useful predictor of 305ME in the second and third lactation groups. The area under the curve was determined to be close to 0.7 for both of these categories. A NEFA above 0.09 mEq/L in the second lactation group is more likely to have a decrease in milk production. The sensitivity for this test was determined to be 56% and the specificity was determined to be 76%. A NEFA above 0.07 mEq/L in the third lactation group is also more likely to have a decrease in milk production. The sensitivity for this test is 78% and the specificity is 61%. Overall, within this particular herd, neither the NEFAs nor the FPRs are overwhelmingly predictive of milk production. In conclusion, this dataset does not indicate FPR as a good predictor for lactation success. Conversely, NEFAs appear to be better predictors of early lactation performance.
Analysis of Mitochondrial Targeting Sequences Using GFP Vector in Tetrahymena thermophila

Munhofen, J, Delco, M, Clark, T, and Cassidy-Hanley, D.

Understanding host pathogen interactions for the control of infectious disease is vital for the progression of biomedical research. Through this understanding, novel treatments can be developed to aid in the control of diseases. Tetrahymena thermophila, a non-pathogenic ciliate closely related to Ichthyophthirius multifilis, a pathogenic protist that causes white spot disease in fish, provides a good model for both host-pathogen interactions and control of infectious diseases. Previous discoveries demonstrated that these single cell eukaryotes, when under conditions of stress, could jettison whole, intact mitochondria without cell lysis. The transfer of materials outside cells is not uncommon, such as seen in mammalian granulocytes ejecting mitochondrial proteins and DNA into the extracellular space; however, whole organelles have not been shown outside the cell membrane in any system other than Tetrahymena. Mitochondria have been imaged outside the cell membrane in fixed Tetrahymena cells using transmission electron microscopy, but it has not yet been demonstrated in live cells.

In order to view the real time extrusion process, our goal was to identify mitochondrial leader sequences that would traffic green fluorescent protein (GFP) to the mitochondria in order to distinguish it from other organelles. A construct of the leader sequences was synthetically created with restriction sites that matched the restriction sites of a specific plasmid. These leader sequences were then isolated and inserted in frame with a GFP reporter. This vector was propagated in bacteria, isolated, and digested with restriction enzymes. The linearalized vector carrying the leader sequences and GFP was transformed using biolystic transformation into Tetrahymena. To determine the effectiveness of the leader sequence’s ability to traffic GFP to the mitochondria, an inducible promoter was initiated using cadmium.

Our results showed that we successfully cloned and identified leader sequences that target GFP to mitochondria in Tetrahymena. Future work will aim to capture mitochondrial extrusion in real time. This technology will help us further examine mitochondrial behavior in living cells to better understand mitochondrial biology and the extrusion phenomenon. This project was completed with research preformed as part of Cornell Veterinary Leadership Program under the direction of Dr. Ted Clark and Dr. Donna Cassidy-Hanley.
Cellular Expression and Antimicrobial Activity of a Novel Protein, NCAMP-1
Rebekah Packer, Liliana Jaso-Friedmann, John H. Leary III, Donald L. Evans, Mary K. Hondalus

Cationic antimicrobial peptides (CAP) have been widely described as effectors of innate immunity in both plants and animals. NCAMP-1 is a novel histone-like CAP originally isolated from fish macrophages, and the gene encoding this protein is conserved across various vertebrate species. In the present study we determined NCAMP-1 expression in porcine and equine leukocytes using flow cytometry and investigated the antimicrobial effects of this protein against certain porcine and equine pathogens. Anti-NCAMP-1 monoclonal 9C9 and rabbit polyclonal antibodies identified NCAMP-1 in the cytosol and cell membranes of porcine macrophages and equine leukocytes. Similar to its bactericidal effects on fish pathogens, NCAMP-1 displayed antimicrobial properties against the porcine and equine pathogens tested. These results suggest that NCAMP-1 may play an in vivo role in innate immune responses of pigs and horses and could prove valuable in the development of novel therapeutics to improve animal health.
Effect of Firocoxib on COX2, mPGES1, and cPLA2 Gene Expression in Equine Mononuclear Cells
Joshua Edward Darden, Sarah Breidling, Londa Berghaus, Michel Vandenplas, Natalie Norton, Michelle Henry Barton

Overexpression of cyclooxygenase 2 (COX2) is involved in the pathogenesis of inflammatory reactions. The short-term objective of this study is to test the efficacy of the selective COX2 inhibitor, firocoxib, to downregulate lipopolysaccharide (LPS) induced gene expression of COX2, as well as other genes involved in eicosanoid metabolism, including cytosolic phospholipase A2 (cPLA2) and microsomal prostaglandin E2 synthase 1 (mPGES1), in equine peripheral blood mononuclear cells in an in vitro model. Our hypothesis was firocoxib will down regulate LPS-induced gene expression of COX2, cPLA2, and mPGES1.

Blood was collected from eight horses by jugular venipuncture. Mononuclear cells were separated by and incubated for 4 hours as follows: Media (control), O55:B5 LPS at final concentration of 1 µg/mL, LPS 1 ng/mL, 100 ng/mL firocoxib (Merial, Duluth, GA), LPS 1 µg/mL and 100 ng/mL firocoxib, and LPS 1 ng/mL and 100 ng/mL firocoxib. After four hours, the supernatants were collected and stored at -80°C for determination of PGE2 concentration. Cells were lysed and stored at -80°C. Gene expression was determined by qRT-PCR wherein changes in gene expression were calculated by relative quantification against 18S rRNA using the $2^{\Delta\Delta CT}$ method. PCR efficiency for target genes and equivalent amplification efficiencies of the target gene and 18S rRNA were also determined. PGE2 and PCR data were compared by ANOVA and Wilcoxin Matched Pair test, respectively. The COX2 primer set has been previously validated in our laboratory. Each primer pair for target genes cPLA2 and mPGES1 yielded single products on dissociation curve analyses. mPGES1 and cPLA2 PCR efficiencies were 100% and 102%, respectively. The slope of the plot of $\Delta C_T$ (target – 18S rRNA) versus log cDNA was less than 0.1 for both cPLA2 and mPGES1, indicating equivalent amplification efficiencies. Addition of firocoxib to LPS-induced cells significantly decreased PGE2 concentrations to levels indistinguishable from control values. Compared to LPS alone, firocoxib lowered high and low dose LPS-induced gene expression for COX2 and mPGES1 by 50%, 43%, 46%, and 41%, respectively. The decrease in COX2 and mPGES1 gene expression was significant for high dose LPS. Lipopolysaccharide did not increase cPLA2 gene expression above control levels.

The primer sets used herein for mPGES1 and cPLA2 were successfully validated for use in equine mononuclear cells. As expected, firocoxib significantly reduced LPS-induced PGE2 concentrations. Firocoxib down regulated COX2 gene expression when high doses of LPS were used to induce mononuclear cells. Firocoxib may be a useful adjunct treatment in horses with diseases in which COX gene expression is upregulated.
Research Session VII – Graduate Students

Disposition of Ampicillin Trihydrate in Plasma, Uterine tissue, and Lochial Fluid of Post-Partum Dairy Cattle

Brent C. Credille; Steeve Giguère; Thomas W. Vickroy; Heidi J. Fishman; A. Lee Jones; Maren E. Mason; Rachel O. DiPietro; Douglas T. Ensley

The objective of this study was to determine the disposition of ampicillin in plasma, uterine tissue, lochial fluid, and milk of post-partum dairy cattle.

Ampicillin trihydrate was administered intramuscularly (IM) at a dose of 11 mg/kg of body weight every 24 h (n=6, total of 3 doses) or every 12 h (n=6, total of 5 doses) for 3 days. Concentrations of ampicillin were measured in plasma, uterine tissue, lochial fluid, and milk using High Performance Liquid Chromotography (HPLC) with ultraviolet absorption.

Quantifiable ampicillin concentrations were found in plasma, milk, and lochial fluid of all cattle within 30 min, 4 h, and 4 h of administration of ampicillin trihydrate, respectively. There was no significant effect of dosing interval (every 12 versus every 24 h) and no significant interactions between dosing interval and sampling site on the pharmacokinetic variable measured or calculated. Median peak ampicillin concentration at steady state was significantly higher in lochial fluid (5.27 µg/mL after q 24 h dosing) than in other sample types and significantly higher in plasma (3.11 µg/mL) than in milk (0.49 µg/mL) or endometrial tissue (1.55 µg/mL).

Ampicillin trihydrate administered once daily by the IM route at the label dose of 11 mg/kg of body weight achieves therapeutic concentrations in the milk, lochial fluid, and endometrial tissue of healthy post-partum dairy cattle. Twice daily administration does not provide any advantages over once daily dosing.
Fluoroquinolone-induced Tendinosis in the Chicken – a Proposed In Vivo Chemical Model
S.E. Quattlebaum, U. Blas-Machado, J.T. Halper

Degenerative tendon disease (tendinosis) is characterized by focal tenderness and pain (both activity-related and upon palpation) in the tendon, and tissue changes made evident by abnormal cellular and matrix arrangement on histopathology (Dirks & Warden, 2011). Fluoroquinolone-induced tendinosis remains a widely accepted association in both human and veterinary medicine, but a lack of accepted animal models coupled with very few publications mapping the pathogenesis in vivo led to the development of this experiment. The purpose of this study is to investigate the chicken as an in vivo chemical model of fluoroquinolone-induced tendon degeneration, and further characterize the pathogenesis of the disease in the gastrocnemius tendon.

Sixty, one-day-old, Avian reovirus-free, White Leghorn chickens were used in the study. Forty-eight chickens were exposed to enrofloxacin through drinking water or subcutaneous injection (both methods were tested at low and high dosages in different test groups) for seven days. The remaining twelve chickens served as controls: six chickens were exposed to enrofloxacin-free drinking water with no injections, and six chickens were injected with physiological saline solution. Chickens were observed clinically and necropsied at experimental days 0, 14, and 42 to identify and evaluate possible changes exhibited by the gastrocnemius tendon. Immunohistochemistry for decorin, procollagen I, and collagen III was evaluated to profile enrofloxacin-induced gastrocnemius tendon injury.

Unlike the control subjects, treated animals necropsied on days 14 and 42 exhibited microscopic lesions characteristic of tendon degeneration regardless of necropsy date, dose, or treatment route. Among the pathologic observations were: increased vacuolation of fibrochondrocytes, prominent blood vessels, steatosis, and apoptotic necrosis. Injured areas previously rich in fibrocollagen were observed to be replaced with fibrocartilage, and these areas displayed diminished immunohistochemical detection of decorin, procollagen I, and collagen III.

This experimental use of enrofloxacin treatment resulted in gastrocnemius tendon degeneration in treated subjects supporting the use of the chicken as an in vivo chemical model for fluoroquinolone-associated degenerative tendon disease.
Chelonid Fibropapilloma-Associated Herpesvirus Infection in Symptomatic and Asymptomatic Infected Green Sea Turtles
Annie Page-Karjian, Terry M. Norton, Julia Zhang, Corrie C. Brown, Nicole L. Gottdenker

Chelonid fibropapilloma-associated herpesvirus (CFPHV) is an alphaherpesvirus commonly associated with fibropapillomatosis (FP), the transmissible neoplastic disease that mainly affects green sea turtles (*Chelonia mydas*). The purposes of this study are to 1) investigate alternative and previously unidentified cell types that support CFPHV life stages of replication and latency in infected green sea turtles; and 2) identify alternate route(s) of CFPHV transmission in addition to cutaneous viral shedding. This is an ongoing comparative study of 120 rehabilitating green sea turtles in GA and FL, USA. Turtles are retrospectively assigned to one of 3 study groups based on identification of CFPHV presence/absence in samples using PCR screening: symptomatically infected (tumors, CFPHV+), asymptotically infected (no tumors, CFPHV+), and uninfected (no tumors, CFPHV-). Viral DNA and RNA are quantified in various biological sample types using pathogen-specific molecular assays (laser capture microdissection, conventional and quantitative PCR (qPCR), electron microscopy). Novel qPCR assays are employed to differentiate and compare the expression of two viral gene targets: DNA polymerase, a key enzyme in DNA synthesis during replication, and latency-associated nuclear antigen (LANA), required for maintenance of episomal viral DNA during latency. A comparison of expressed quantities of DNA polymerase and LANA, and the turtle host health status, could provide important information about viral life cycle stages within the host tissues of symptomatic, asymptomatic and latently infected turtles. These diagnostic assays are also implemented to identify cryptic sources of transmissible viral particles in specific sample and/or cell types involved in symptomatic and asymptomatic CFPHV infection. As an inexpensive, rapid screening tool for identifying subclinical CFPHV carriers, these assays help to determine quarantine status and avoid transmission opportunities among rehabilitating turtles, and allow us to better establish infection baselines within free ranging marine turtle populations. Identification of anatomic sites of viral localization in symptomatic, asymptomatic and latently infected turtles, and identifying alternate routes of viral transmission, may help elucidate heretofore under-explored aspects of the CFPHV life cycle. This in turn will benefit future studies that examine external factors- ecologic, immune-mediated, or microbial- that may influence FP development in CFPHV-infected turtles.
All Newcastle disease viruses (NDVs) are part of a single serotype; however, current vaccine strains display between 15 and 18% amino acid differences at the F and HN protein compared with current virulent viruses. Previous studies have shown that increased amino acid similarity between NDV vaccines and field viruses is important to decrease virus shedding after challenge. In the present study, two lentogenic recombinant viruses were generated by replacing the F and HN genes from virulent NDV of genotypes VIIId (from South Africa) and XIII (from Pakistan), into the LaSota vaccine backbone (genotype II). The pathogenicity of the recombinant viruses was attenuated by changing the fusion protein cleavage site. Intracerebral pathogenicity index, clinical signs, and virus shedding were also evaluated to determine if the vaccine viruses were capable of replicating or causing disease in chickens. One day old SPF chicks were vaccinated with live virus and 14 days after vaccination were challenged with their respective homologous virulent virus from South Africa or Pakistan to test their performance in comparison with the LaSota vaccine strain. Results from these experiments demonstrate that these experimental vaccines replicate in birds and do not cause disease; even more, this vaccines conferred 100% survival, prevented clinical signs, and decreased oropharyngeal virus shedding compared with the LaSota strain. In conclusion, the F and HN genes homologous to the circulating virulent NDV are sufficient to decrease virus shedding of the challenge virus, even more than the LaSota vaccine strain. Also, these recombinant viruses seem to be good candidates to be used as live vaccines.
VacSIM for Influenza - New Delivery Method Increases Vaccine Efficacy

E. Farah Samli, Lisa M. Shollenberger and Donald A. Harn

VacSIM is a patent-pending method of delivery that increases vaccine efficacy. VacSIM is an inert biopolymer possessing unique biophysical properties. It can be stored at 4-22°C, as a viscous-liquid and is flexible enough to incorporate various antigens, adjuvants or organisms for vaccine delivery. Upon injection VacSIM undergoes a phase shift to a semi-solid gel, forming a temporary depot of vaccine components. Formation of this depot may enable time-released delivery of vaccine components and increased activation of antigen presenting cells, as they specifically home to the concentrated antigen. VacSIM has been shown to enhance vaccine-specific immune responses to two different *Burkholderia* candidate vaccine antigens and the rHepBsAg vaccine in mice. Currently, VacSIM is being used in three different schistosome vaccine trials, in China and the Philippines, targeting water buffalo. Thus, VacSIM appears to enhance immunogenicity and efficacy of a wide variety of vaccines.

Therefore we were interested in testing VacSIM to enhance influenza vaccines. Influenza is a highly infectious virus with a global impact. Symptoms of infection are generally mild but can be life-threatening in immune compromised individuals. Annual vaccination is the ideal method of protection. The flu vaccine is typically 70-90% effective in healthy adults, but considerably less effective in higher-risk populations. Our goal is to increase vaccine efficacy in order to improve protection for all populations. Mice vaccinated with influenza A/Puerto Rico/08/34 (WIV) or with conserved subunit (rNP) using VacSIM, showed increased humoral response (flu-specific, functional and cross-reactive antibodies), greater protection from challenge and improved viral clearance compared to controls.
Helminth parasites bias the host immune system towards T helper (Th) 2-type and often induce immune suppression. Our lab and others have shown that helminth infection inhibits Th1 vaccine-specific responses. Previous studies have shown the complex mixture of molecules that comprise saline soluble egg antigens (SEA) from the helminth parasite *Schistosoma mansoni* function to induce Th2-biasing in naïve individuals. SEA has been used experimentally as a Th2-type adjuvant for vaccine antigens. In preliminary studies, we asked if co-administration of SEA with a *Listeria* vector HIV-1 gag vaccine, would suppress host cytotoxic T lymphocyte (CTL) and Th1 responses to the HIV-1 gag epitopes. As expected, co-administration of SEA biased the host immune system towards Th2-type. Yet unexpectedly, we observed that co-administration of SEA with the *Listeria* vector HIV-1 gag vaccine significantly increased the frequency of IFN-γ producing gag-specific Th1 and CTL responses compared to mice that received only the *Listeria* vector HIV-1 gag vaccine. This result suggests that there are components in SEA that are potent inducers of Th1-type responses, which, if identified, could be utilized as adjuvants to promote Th1-type vaccine-specific immune responses for HIV-1 and other vaccines. We are continuing to examine the adjuvant properties of SEA and determine which class(es) of molecules in SEA promote Th1-type immune responses.

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Differential Utilization of Tpl2 by Toll Like Receptors
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Toll-like receptors (TLRs) are germline encoded pattern recognition receptors (PRRs) involved in the early detection of pathogens, and stimulation of PRRs activates various intracellular signaling cascades important in innate immune responses. Tumor progression locus 2 (Tpl2), also designated Cot or MAP3K8 is a serine-threonine MAP kinase involved in many signaling pathways with critical functions in regulating innate and adaptive immune responses. Endogenous Tpl2 is expressed as 52KDa and 58KDa isoforms and associates stoichiometrically with NF-kB1/p105 in unstimulated cells. Upon activation, Tpl2 dissociates from p105 and is transiently free to activate its substrates until it undergoes rapid proteosomal degradation. Tpl2 is activated by LPS, TNFa and IL-1b and is essential for signal transduction, including activation of the MAP kinase ERK, in response to diverse TLR ligands. Tpl2 also regulates cytokine secretion and response to various cytokines in a cell type- and stimulus-specific manner. In this study, utilization of Tpl2 by TLRs in transducing ERK activation signals was investigated. Contrary to previous studies, we demonstrate restricted requirements for Tpl2 in transducing signals by a subset of TLRs. Specifically, TLR4 and 7 stimulation in bone marrow derived macrophages (BMDMs) led to Tpl2 degradation and ERK activation. In response to LPS stimulation, Tpl2 degradation occurred independently of the MyD88 adapter protein, but required the TLR4 co-receptor CD14. In contrast, Tpl2 degradation was not observed upon TLR3 and 9 stimulation, even though ERK activation was still evident in these samples. Furthermore, ERK activation was significantly impaired in Tpl2 deficient cells in response to all ligands tested, including TLR3 and 9. Experiments using the protein synthesis inhibitor cycloheximide revealed that TLR3- and 9-mediated activation of ERK required de novo protein synthesis. Neutralizing antibodies against TNFa and IL-1b demonstrated that a cytokine feedback loop indirectly induced ERK activation in response to TLR3 and 9 ligands. Collectively, these findings reveal a differential requirement for Tpl2 in transducing signals by TLRs. Furthermore, these findings could be exploited to selectively modulate TLR signaling pathways and may provide important insights into adjuvant activities of diverse TLR ligands.
CD14 via the TRIF Pathway Regulates Macrophage Polarization and Th2 Immune Responses

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Macrophage plasticity plays a key role in regulating inflammation during infection and metabolic diseases. Macrophages adopt M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotypes depending upon their exposure to pathogen products and/or host cytokines. Pathogen products like LPS and/or IFNg, promote an M1 phenotype, whereas the Th2 cytokines IL-4/IL-13 or helminth products drive an M2 phenotype. M2 macrophages help in suppression of inflammation during infection with pathogens/parasites and help regulate inflammation during autoimmune, cardiovascular and metabolic disease. How macrophage polarization is regulated at the signaling level is not well defined. We initiated our studies by testing the ability of macrophages to polarize when simultaneously exposed to M1 activating agent, LPS or poly I:C and M2 activating agent, IL-4. We found that wild-type (WT) macrophages completely failed to polarize towards M2 when co-incubated with IL-4 and LPS or PolyI:C. The inhibition or negative regulation of alternative activation of WT macrophages was CD14 dependent as CD14 deficient macrophages did polarize towards alternative activation in response to IL-4 with or without LPS stimulation. We then demonstrated that the CD14/TRIF/STAT1 pathway, in addition to mediating macrophage classical activation (M1), negatively regulates IL-4Ra-STAT6 mediated M2 polarization. Examining mice infected with the helminth parasite S. mansoni, we observed that CD14 plays a crucial role in regulation of infection induced Th2 responses and alternative activation of macrophages (M2). Infection of Cd14−/− mice resulted in enhanced production of CD4+ specific IL-4, IL-5 and IL-13 cytokines associated with increased regulatory T-cells (CD4+Foxp3+). Livers from infected CD14 deficient mice showed significant pathological changes including higher recruitment of AAMφs and an increase in schistosome egg granuloma size and collagen deposition compared to infected WT mice. Finally, we were able to rescue the phenotype in Cd14−/− mice by adoptively transferring WT macrophages. This confirmed that macrophages are indeed responsible for the CD14 mediated negative regulation of Th2 and alternative activation in vivo in response to schistosome eggs. Over all, we have shown that CD14 regulates macrophage M1/M2 plasticity by controlling TLRs/TRIF/STAT1 and IL-4Rα-STAT6 contrasting pathways, which influences overall Th1/Th2 immune outcome during infection.
POSTER PRESENTATIONS
Veterinary & Undergraduate Students

Pathogenesis of Low Virulence Newcastle Disease Virus Infection in Mallards
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Waterfowl are reservoirs of low virulence Newcastle disease viruses (loNDV). We investigated the viral shedding patterns, serology, pathology and viral antigen distribution of loNDV in experimentally infected Mallards. One-month-old birds were intranasally inoculated with $10^6$ EID$_{50}$/0.1ml of loNDV isolated from a naturally infected Mallard. Oropharyngeal (OP) and cloacal (CL) swabs were collected post inoculation and tested by real-time reverse transcription polymerase chain reaction (rRT-PCR). Blood was collected at 7 and 14 days post inoculation (dpi) and the sera were tested by hemagglutination inhibition test. Two birds were euthanized and necropsied daily from 1 to 3 dpi. Infected birds lacked overt clinical signs and were serologically positive for NDV on 7 and 14 dpi. OP viral shedding was intermittently detected between 1 to 3 dpi, while CL shedding was detected from 3 to 9 dpi. Some birds had enlarged spleens with lymphoid hyperplasia from 1 to 3 dpi. Distended, fluid-filled ceca and lymphoplasmacytic colitis were seen on 3 dpi. Immunohistochemistry revealed NDV nucleoprotein antigen in epithelial cells of the trachea and lamina propria macrophages of the respiratory and intestinal tracts during the first 3 dpi. In conclusion, Mallards intranasally infected with loNDV have early OP shedding and later CL shedding as detected by rRT-PCR. The presence of NDV antigen in the respiratory tract correlated with the detection of loNDV in OP swabs between 1 and 3 dpi.
Activity in Nodose Ganglia Neurons after Treatment with CP 55,940 and Lipopolysaccharide

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Previous work has shown that endocannabinoids have an anti-inflammatory action during challenge with lipopolysaccharide (LPS). Endocannabinoid receptors are located on cell bodies of vagal afferent nerves in the nodose ganglia. An interaction between signaling pathways involving LPS and endocannabinoids has been suggested. The purpose of these studies is to explore the effect of pretreatment with a cannabinoid agonist, CP 55,940, on nodose neuron activation by LPS. To determine the effect of CP 55,940 and LPS on neuron activation, rats were anesthetized and nodose ganglia were extirpated. The neurons were dissociated and plated. The cells were treated with media, CP 55,940, LPS, CP 55,940 followed by LPS, or AM 251, a CB1 receptor antagonist, and CP 55,940 followed by LPS. Immunohistochemistry was performed to stain the cells for cFos as a measure of cell activation. Neurons were identified using neurofilament immunoreactivity. The neurons on each slip were counted using fluorescence imaging, and the number of neurons that were cFos positive was counted in order to calculate the percentage of activated neurons per coverslip. Pretreatment with CP 55,940 decreased the percentage of neurons expressing cFos-immunoreactivity in response to LPS. This observation suggests that endocannabinoids inhibit LPS activation of nodose ganglion neurons.
Pathology of *Haemonchus contortus* in New World Camelids in Georgia: A Retrospective Study

E.E. Edwards, L.H. Williamson, B. Garner, B. Storey, and K. Sakamoto,

Most small ruminant farms in temperate climates are plagued by *Haemonchus contortus*, a hematophagous abomasal parasite that causes anemia, hypoproteinemia, weight loss, and often death. *H. contortus* is becoming a major issue in new world camelids as well, namely llamas and alpacas (*Llama glama*, *Vicugna pacos*), yet little research has been done regarding its pathology in these species. Here we present a retrospective study of new world camelids that presented to the University of Georgia Veterinary Teaching Hospital and Athens Diagnostic Laboratory from April 2003 to April 2013. Antemortem fecal egg count (FEC) estimates performed on 30 alpacas that presented to the Teaching Hospital were negatively correlated with hematocrit, hemoglobin, and red blood cell count. Total protein was not significantly correlated with FEC. On post mortem examination, 50 of 235 adult camelids (21.3%) were infected with *H. contortus* with varying numbers of adult worms present in the third gastric compartment (C3). In 29 of these cases, this parasite was the major cause of death (12.3%). Common gross lesions were abdominal, peritoneal, and pericardial effusions, visceral pallor, subcutaneous edema, serous atrophy of fat, and thin blood. Nematodes were often visible histologically. Histopathological lesions included centrilobular necrosis of the liver, hepatic atrophy, lymphoplasmacytic inflammation of C3, and extramedullary hematopoiesis in both the liver and spleen. In conclusion, the pathologic and hematologic changes of infected camelids are similar to those of small ruminants. This study emphasizes the importance of parasite control and herd monitoring in new world camelids using the FAMACHA© system and FEC estimates.
Effects of Multimin® on the Risk of Respiratory Disease and Growth in Preweaned Group Housed Holstein Heifers

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Abstract

Despite the continuous introduction of new vaccines and vaccination strategies, dairy farms continue to struggle with respiratory disease in replacement animals. The NAHMS Dairy 2007 Heifer Calf Health and Management Practices on U.S. Dairy Operations, 2007 lists that 12.7% of pre-weaned dairy heifers suffer from respiratory disease and 93.4% of those diagnosed with respiratory disease were treated with an antibiotic. Respiratory disease causes great loss in replacement dairy heifers. Estimates by Kaneene in 1990 place losses in excess of $14.00 per calf-year. Trace mineral deficiencies, particularly selenium, may be linked to an increased incidence of disease. Supplementation of trace minerals may decrease the risk and impact of respiratory disease by enhancing the calves’ immune system. A trial was conducted on a dairy in east Georgia to test the effects of supplementing injectable trace minerals on the risk of pre-weaning respiratory disease in group-housed Holstein replacement heifers and on commonly measured growth characteristics. A total of 350 calves were randomly assigned to either the treatment (n = 170) or control (n = 180) groups. Treatment groups received an injection containing 5 mg selenium, 60 ng zinc, 10 mg manganese, and 15 mg copper (Multimin 90®, Multimin USA) at day 1 and day 14 of life. A calving score, birth weight and hip heights were recorded at birth for all calves. All calves were also tested for Bovine Viral Diarrhea Virus (BVDV) and serum total protein was recorded between two and six days of age. Calves were regularly monitored for signs of respiratory disease (BRD). At the day of weaning, all animals were weighed and hip heights were recorded. Upon analysis of the data, there was no difference between the groups with respect to birth weight, birth height, or serum total protein. There was also no difference between groups with respect to dam parity distribution, the percentage of births that required assistance during calving, the percentage of twin births, or the percentage of calves with failure of passive transfer. This study found that there was not a significant difference between treatment groups with respect to either ADG or weaning height. There was also no difference between treated and untreated groups with respect to the proportion of calves that were treated for BRD, or the proportion of calves that died prior to weaning. The data from this experiment suggests that there is no correlation between supplementing injectable trace minerals and the occurrence of BRD or growth in replacement dairy heifers.
A stroke is an onset of focal neurological deficits due to a disturbance of blood supply to the brain. There are many possible origins, but pre-disposing factors in human and veterinary medicine include old age, high blood pressure, endocrinopathies and high cholesterol. A stroke may result in either ischemic infarction or hemorrhage affecting the brain. Due to multiplanar capabilities and high-resolution parenchymal images, MRI is now the gold standard for the diagnosis and evaluation of acute ischemic stroke. The size and structure of pig brains are similar to that of humans, especially when compared to mice, and so has prompted interest in their use as a model for ischemic infarction.

We hypothesized that T2 FLAIR hyper-intensity measurements associated with the ischemic infarction at 24 hours could be utilized as a quantitative measurement tool for swelling and provide predictive measurements of lesion size at 90 day post-ischemic stroke. Our aim was to determine whether or not the size of the 24-hour ischemic lesion measured using T2 FLAIR was predictive of the size of the 90-day affected hemisphere.

A stroke was induced in 6 adult mini-pigs following cauterization of the middle cerebral artery. The resulting ischemic infarction was assessed using MRI at 24 hours and 90-day post-operative. Each patient’s MRI was evaluated on transverse (axial) T2 FLAIR images using OsiriX software. Ischemic lesion area was measured at 24 hours from each pathologic 3mm slice; additionally each affected hemisphere was measured in total at both 24 hours and 90 days. Normal brain hemisphere measurements were made separately from the corresponding affected ones for comparison. The average area of the hemisphere affected by stroke was divided by the area of the normal hemisphere resulting in a size ratio after the initial injury as well after 90 days. Statistical analysis was performed using Pearson’s correlation method to test for correlations of ratio of ischemic tissue to normal hemisphere size at 24 hours to 90-day hemisphere size; and between the ratio of the affected hemisphere area at 24 hours and 90 days. The ischemic hemisphere at 24 hours ranged from 11.27cm$^2$ to 12.83cm$^2$ (mean- 11.84 cm$^2$), which was larger than the normal hemisphere (8.08cm$^2$ to 10.33cm$^2$; mean- 9.59cm$^2$). At 90 days, the affected hemisphere ranged in area from 6.30cm$^2$ to 10.26cm$^2$ (mean- 8.3cm$^2$) which was smaller than the unaffected hemisphere which ranged from 9.29cm$^2$ to 11.31cm$^2$ (mean- 10.32cm$^2$). There was not a statistically significant correlation between the stroke size on T2 FLAIR at 24 hours (p=0.5064) or affected hemisphere size ratio and the atrophy noted after 90 days (p=0.4632). Using this model, we are unable to solely rely on this tool for prediction of lesion size after ischemic infarction.

In conclusion, this model provides a reliable assessment of brain tissue swelling and atrophy in response to an ischemic infarction, yet T2 FLAIR images cannot be used to predict final tissue damage based on this pilot study.
Comparison of Abdominal Ultrasonography and Radiography in the Investigation of Feline Abdominal Disease
Wade Won, A. Sharma

Selecting the appropriate test for evaluating a specific clinical question is essential for good patient management. The investigation of feline abdominal disease commonly uses radiography and ultrasonography. Determination of an appropriate diagnostic test should be based on the accuracy and confidence in test results for answering the clinical question, providing a diagnosis, or guiding the next step in patient care. Performing a test with low diagnostic yield is inefficient and can result in increased cost to the client, increased duration of hospital stay, and strains on hospital resources, including but not limited to personnel and time. Several academic institutional policies require abdominal radiography prior to ultrasonography when performing diagnostic imaging for abdominal disease. The purpose of this retrospective study was to compare the clinical diagnostic benefit between abdominal radiography and ultrasonography in the investigation of feline abdominal disease.

Ninety-six cases were selected based on the criteria that both orthogonal abdominal radiography and ultrasonography examinations were completed during the same admission period and for the investigation of the same presenting complaint. The presenting complaint had to be consistent with intra-abdominal disease. The cases were selected over a one year period from 2011-2012. Cases were excluded if abdominal imaging was performed as a recheck for previously diagnosed disease. Other cases were excluded if abdominal imaging was performed as part of a staging process for non-abominal disease (e.g. pelvic limb paresis prior to MRI).

Preliminary results showed that ultrasonography provided a tentative or definitive diagnosis in 65% of cases compared to 27% based on radiography. Out of the selected cases, 25% were diagnosed both by ultrasonography and radiography. Thirty three percent of cases were neither diagnosed by ultrasonography or radiography. These cases were diagnosed by other means such as blood results, MRI, necropsy, or other tests (e.g. endoscopy, cytology, biopsy). Preliminary statistics showed that ultrasonography gave additional clinically relevant information 78% of the time (with a 95% confidence interval 68.53%-85.92%), demonstrating a significant difference.

Ultrasonography provides more clinically relevant diagnostic information when compared to radiography in the evaluation of feline abdominal disease. The implications of of these preliminary finding suggest that diagnostic imaging test selection can be modified when investigating certain feline abdominal diseases without compromising accuracy and confidence in test results.
EFFECTS OF AGE, BREED, AND PARTURITION INDUCTION ON SERUM FREE CORTISOL FRACTION IN NEONATAL FOALS

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Neonatal foals have dramatically increased free (biologically-active) cortisol as compared to adult horses. This increases cortisol clearance in foals, and could contribute to total cortisol insufficiency in ill foals. The age at which free cortisol decreases to adult levels is not known in foals, nor is the effect of breed or parturition induction on free cortisol. The objective of this study was to assess free cortisol in healthy horse and pony foals after induced and spontaneous parturition during the first 12 weeks of life. Blood was collected from QH foals and pony foals after induced or spontaneous parturition at birth, 12-24 hours, 36-48 hours, 5-7 days, and 2, 4, 8, and 12 weeks of age, and from healthy adult horses in April. Serum total cortisol concentration (TCC) and free cortisol fraction (FCF) were measured with chemiluminescent and ultrafiltration assays respectively. Data were compared with ANOVAs, with statistical significance set at P<0.05. TCC and FCF were not different between horse and pony foals. There was no significant effect of parturition induction on FCF, though there was a tendency towards higher FCF in induced foals (P=0.054). Foals had significantly lower TCC than adult horses at all ages, while FCF was comparable to adults by 2 weeks of age. These findings suggest that while total circulating cortisol in foals remains lower than adult horses during the first 3 months of life, free, biologically-active, cortisol in foals is higher than adults at birth and decreases to adult levels by 2 weeks of age.
Next-generation sequencing of MHCI and MHCII gene targets from the endangered Florida puma

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The Major Histocompatibility Complex (MHC) is a component of the adaptive immune system involved in self/non-self recognition and disease susceptibility. Some endangered species maintain high genetic diversity at the MHC despite repeated population bottlenecks. Florida pumas, an endangered population of *Puma concolor* (approx. 150 individuals) in southern Florida, underwent a bottleneck in the 1980s with as few as three breeding individuals over two generations. To reverse inbreeding depression, in 1995 the population was introgressed with five female Texas cougars. We hypothesize that Florida puma MHC diversity increased as a result of this introgression event.

Because of a longitudinal conservation study, we have over 200 Florida puma samples from 1980–present, including the five Texas females. Additionally, we have samples from puma populations in Brazil (n=5), Canada (n=9), Costa Rica (n=2), and the western United States (n=26). Functionally important antigen-binding sites from the MHCI and MHCII loci were amplified with a touchdown PCR using high-fidelity polymerase. Additionally, piroplasm prevalence was detected using nested PCR.

Preliminary Sanger sequencing for both MHCI and MHCII DRB loci revealed numerous polymorphic bases and ~85% similarity to the cheetah, *Acinonyx jubatus*. High prevalence of piroplasms was detected (61% had a *Babesia* sp. and 16% had *Cytauxzoon felis*) in pumas from Texas and Florida.

Using next-generation sequencing (Illumina MiSeq), we are determining allelic diversity of the MHC I and II. We expect that introgressed individuals will exhibit higher MHC diversity corresponding with increased fitness and survival. Future studies with these data will address parasite- and sexual-mediated selection of MHC genotypes. Because most felid species are threatened or endangered, these data could have important implications for wild felid conservation.

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Bisphenol-A (BPA) is a common chemical that has been used in plastic and resin production since the 1960s, and its ubiquity in our environment has raised public health concerns. Currently, there are few robust in vitro models in which to study mechanisms of developmental toxicity. Human neural progenitor cells (hNPs), a derivative of human embryonic stem cells, show characteristics of developing neurons suggesting they may offer a novel model for investigating neural developmental toxicity. To determine the effects of BPA on fetal neural development, metabolomics-based profiling of hNP cells was performed. Our objective was to utilize GC/MS-based metabolomics to evaluate the changes in metabolite profiles after exposure of hNP cells to 0, 0.1, 1, 10, or 100 µM BPA for 48 hours. After BPA exposure, extra- and intra-cellular metabolites were extracted and derivatized prior to GC/MS analysis. Principle component analysis (PCA) was performed to determine the changes in metabolite profiles. Analysis showed that control and lower doses of BPA were separated from the high dose of BPA (100 µM) over principle component one. A PLS-DA scores plot of extracellular metabolites demonstrated elevated glucose and sugar derivative excretion and a decrease in amino acid excretion in the BPA treated groups. Based on our preliminary work, the metabolomic profiles of hNP cells change in response to BPA only at high doses which also result in cell death.
Staphylococcus spp. Antibiotic Resistance On The Rise; Georgia’s Perspective
Michelle Keeling & Susan Sanchez

The global rise of *Staphylococcal* infection (*Staph*) poses a veterinary challenge as increases in Meticillin resistant *staphylococcus* (MRS), indicating general beta-lactam resistance, often corresponds with resistance from other drug classes making treatment prolonged and/or difficult. We characterized the *Staph* infections submitted to the University of Georgia’s (UGA) Athens Diagnostic Laboratory (ADL) from clinically ill patients over the years 02, 05, 08 and 11 from UGA’s small and large animal hospitals and those submitted from private clinics. We hypothesized that the prevalence of MRS increased annually as has the prevalence of multi-drug resistance (MDR) within them. We aimed to identify differences in drug resistance between primary and tertiary care centers and hypothesized a higher prevalence of MRS in tertiary care settings who often perform surgical interventions, and take referrals from failed primary care treatment posing higher infection risks. Over the study, 3406 of the phenotypically identified *Staph* infections had antimicrobial sensitivity testing. Oxacillin was used as a proxy for Meticillin resistance and Tetracycline, Trimethoprin-sulfa and Fluoroquinolones as indicators for MDR. *S.pseudintermedius* comprised 66% (2235/3406) of the total infections & 50% (293/580) of MRS infections. Undifferentiated coagulase (-) infections comprised 21% (727/3406) of all infections & 33% (191/580) of MRS. *S.aureus* made up 9% (305/3406) of total infections and 11% of MRS (64/580). Undifferentiated coagulase (+) infections comprised the rest; the relative proportion of *staph* subtypes remained consistent over the years. Year tested was associated with MRS (p<.0001); the prevalence of MRS increased from 6% (45/722) in 2002, 11% (98/857) in 2005, 19% (181/977) in 2008 and 32% (256/794) in 2011. The prevalence of MDR *Staph* infections also increased linearly and was associated with year (p<.0001). In 2002, 4% (2/45) of all MRS were resistant to all three drug classes used to indicate MDR compared to 36% (91/256) in 2011. Conversely, in 2002, 36% (16/45) of all MRS cases were sensitive to the three drug classes; this dropped to 19% (48/256) by 2011. Care center was associated with MRS (p<.0001). Primary care contributed 79% (2091/2639) of the cases and had an overall MRS prevalence of 18% (367/2091) while tertiary care centers contributed 21% (548/2639) of cases but had a MRS prevalence of 31% (168/548); 767 cases did not specify location and were removed from this analysis. Companion animals (dog, cat, horse, ferret, gerbil) comprised 97% (3301/3406) of all clinically ill cases which is concerning for practitioners in this population. Treatment and prevention options must be carefully evaluated as resistance increases; this is paramount within tertiary care settings.
Effects of a 5 degree head-up incline on arterial oxygenation in anesthetized horses
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While under the influence of anesthetics, equine dissolved oxygen concentration (PaO₂) decreases over time. The abdominal organs may be a primary cause of decreased ventilation, exacerbating absorption atelectasis. This creates areas of the lung receiving venous blood but no air for gas exchange and results in a decreased PaO₂. The thoracic pressure is hypothesized to be reduced if the horse’s head is tilted up. This would result in less atelectasis, improving PaO₂. The purpose of the experiment is to determine the effects of a 5 degree head-up table tilt on arterial oxygenation in anesthetized horses. The criteria for horses selected for the study included: greater than 12 months, over 200kg, and free of cardiopulmonary disease. Client consent was obtained and horses were randomized to be tilted or not during surgery. An arterial blood gas was taken immediately after placement of the arterial catheter and before the horse was weaned onto spontaneous ventilation. The change in PaO₂ was taken as the difference between the first and last blood gas measurement and was compared between the two groups using a 2-way t-test, with significance set at α <0.05. Nineteen out of a goal of 54 horses have had data collected so far. The average change in PaO₂ for tilted horses was 134 mmHg and for non-tilted horses was 81 mmHg (P=0.19). Although this is not statistically significant, with a larger sample size, there may be a clinically relevant difference revealed. A 5 degree head up tilt may improve oxygen concentration over time in anesthetized horses.

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Characterizing Equine Synovial Explants to Investigate Mesenchymal Stem Cell Interactions in Osteoarthritis

Katy Mayhew, Jennifer Mumaw, Merrilee Thoresen, Lindsey Boone, and John Peroni.

Osteoarthritis (OA) is a chronic, inflammatory disease affecting the joints of millions of people and animals. Current treatments aimed at symptom relief do not effectively prevent deterioration of the articular cartilage, subchondral bone, and synovial membrane. Inflammation exerts a potent effect on progression of the disease and impacts each joint structure. Current \textit{ex-vivo} synoviocyte culture systems fall short of replicating \textit{in-vivo} conditions, limiting understanding of the role of synovium in OA and investigation of disease-modifying therapies. We hypothesized a novel culture system utilizing punch biopsies of synovial membrane and joint capsule would allow viable explant maintenance for 6 days. We quantified the presence of viable cells by confocal microscopy using live/dead staining. Cell viability of 92-96\% was maintained throughout the experiment indicating the effectiveness of this system.

Current treatment of OA includes use of corticosteroids to combat chronic inflammation, however does little to delay disease progression. Mesenchymal stem cells (MSCs) are proposed to modulate inflammation and slow OA progression. Concurrent use of these therapies necessitates understanding steroid effects on MSCs. In our experiments, MSCs treated with steroid concentrations similar to clinical intra-articular applications had significantly reduced viability. Moreover, the application of MSCs into an intra-articular environment predisposes cells to endogenous glucocorticoids. We observed an initial decrease in cell viability in cortisol concentrations mimicking physiological levels of healthy and sick equine, however this effect was not observed at higher doses encouraging further exploration to determine the thresholds at which MSC and corticosteroid therapy may be used in a therapeutic setting.
Effects of Age on the Bioactivity of Equine ACTH in an Ex Vivo Model

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Critical illness-related corticosteroid insufficiency (CIRCI) occurs in 30-40% of septic neonatal foals, and is associated with decreased survival. The pathogenesis of CIRCI in foals is poorly understood, though recent evidence suggests that despite higher plasma adrenocorticotropic hormone (ACTH) concentrations in neonatal foals, cortisol responses to ACTH are impaired. This could be explained by release of less bioactive ACTH isoforms in neonates as compared to adult horses, as in other species.

The objectives of this study were to compare ACTH bioactivity in foals and horses and to investigate effects of foal age on ACTH bioactivity using an ex vivo model. Blood was collected from 8 healthy adult horses once and from 8 healthy foals at birth, 12-24 hours, 36-48 hours, 5-7 days, and 2, 4, 8, and 12 weeks of age. Plasma ACTH concentration was measured with a chemiluminescent immunoassay. Adrenal tissue was collected from a healthy horse and adrenocortical explants exposed to plasma from horses and foals for 90 minutes. Supernatant cortisol concentrations were measured via ELISA and expressed as cortisol:protein:ACTH ratios. Data were compared among groups with repeated-measures ANOVA and unpaired t tests, with statistical significance set at P<0.05.

A significant effect of foal age was found, with the lowest ACTH bioactivity seen in foals at birth. Cortisol:protein:ACTH ratios were significantly lower in foals than in adult horses at all ages except 12-24 hours, 36-48 hours, and 4 weeks of age. These data suggest that increased ACTH concentrations seen in neonatal foals might be partly composed of less bioactive ACTH isoforms.
Graduate Students

Generation of a Unique RNAi Oocyte-Conditional Pericentrin Knockdown Mouse Model

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An abnormal chromosome number (aneuploidy) in developing embryos is the leading genetic cause of congenital birth defects and pregnancy loss, with most aneuploidies attributed to chromosome segregation errors during meiotic division in oocytes. Accurate chromosome segregation is crucially dependent on meiotic spindle assembly and the establishment of stable chromosome-microtubule attachments. Notably, spindle formation in oocytes differs from mitotic cells and is regulated by unique microtubule organizing centers (MTOCs) that lack centrioles. Yet, the molecular composition of MTOCs and control of spindle assembly in mammalian oocytes is poorly understood. In this study we developed a unique genetic mouse model to test the function of a key MTOC-associated protein, pericentrin, considered to be critical for the recruitment and anchoring of pericentriolar matrix (PCM) proteins at MTOCs. We previously demonstrated that pericentrin depletion in mouse oocytes, in vitro, disrupts meiotic spindle stability. To test pericentrin function in vivo we generated an oocyte-conditional transgenic RNAi knockdown mouse model. Post-transcriptional degradation of pericentrin (Pcnt) mRNA levels in oocytes was induced using an established RNAi vector (pZP3-intron-EGFP-gene specific inverted repeat). A two step cloning procedure was used to introduce a Pcnt inverted repeat, such that the oocyte-specific Zona Pellucida 3 (ZP3) promoter drives the synthesis of double stranded hairpin RNA against the pericentrin gene. The construct was microinjected into pronuclear stage embryos. A total of 33 pups were born, from which 11 were confirmed by PCR to carry the transgene and 9 survived. Transgenic founders were mated to CF1 wild type mice, and analysis of the F1 progeny confirmed effective transmission of the transgene to their offspring. To establish transgenic lines, F1 transgenic males were used for backcrossing with CF1 females. Oocytes were collected from wild-type (WT) control and transgenic female offspring from 3 established mouse lines (A, E and F), to assess pericentrin mRNA and protein expression using quantitative-PCR (Q-PCR) and immunofluorescence analysis, respectively. Efficient and significant knockdown of Pcnt transcript levels in oocytes collected from transgenic females was detected by Q-PCR. Moreover, the loss of pericentrin protein expression was confirmed by immunofluorescence. MTOCs in all prophase-I arrested (GV-stage) and metaphase-II stage oocytes from control females were brightly labeled with anti-pericentrin. In direct contrast, oocytes from transgenic females expressed no pericentrin, while surrounding granulosa cells exhibited bright pericentrin labeling at MTOCs. These results confirm the oocyte-specific ablation of pericentrin in transgenic mice. This unique genetic model will provide critical new insight into the molecular mechanisms that regulate MTOC function in mammalian oocytes and the formation of a stable meiotic spindle, essential for accurate chromosome segregation. *Funded by NIH (HD 0713330) to MMV.
Tumor Progression Locus 2 (Tpl2) inhibits T regulatory cell development and immunosuppressive function

Xin Li, Nicole Acuff, Angela Peeks, Rebecca Kirkland and Wendy Watford

T regulatory cells (Tregs) are a specialized subset of T cells with immunosuppressive properties. They can arise naturally in the thymus (nTregs) or are induced from naïve T cells in the periphery (iTregs) and function to maintain peripheral tolerance by preventing immune responses to self-antigens. Treatment with antigen-specific immunosuppressive Tregs is now being evaluated for therapeutic potential with autoimmune diseases like type-1 diabetes and graft-versus host disease. However, clinicians face significant obstacles in obtaining enough highly purified Tregs for clinical use. For these reasons, it is important to learn more about Treg development and functions. In this regard, defects in some TCR signaling components have been shown to regulate TCR signal strength and augment T regulatory cell lineage commitment. We previously demonstrated that the serine-threonine kinase, Tpl2, regulates TCR signaling and inflammatory cytokine secretion in CD4+ T cells. Herein, we demonstrate that Tpl2 is preferentially expressed by Tregs compared to naïve CD4+ T cells and that Tpl2 regulates Treg development and function. Tpl2−/− mice possessed increased proportions of peripheral Tregs in vivo. The enhanced Treg development was not due to T cell-extrinsic factors, since Treg development from naïve T cells cultured in vitro was similarly enhanced. RT-PCR, ELISA and biochemical analyses showed that Tpl2-deficient T cells secrete increased levels of IL-2, which can potentiate iTreg differentiation. Furthermore, Tpl2 inhibits Treg expression of characteristic immunosuppressive cytokines, IL-10 and IL-35. Accordingly, tpl2−/− Tregs were fully suppressive in vivo in a T cell transfer model of colitis. Collectively, these results demonstrate that Tpl2 has an important, non-redundant physiological role in limiting Foxp3 expression and Treg development and function. The implication of this work is that a Tpl2 inhibitor could deviate pathological immune responses in a variety of autoimmune diseases towards tolerogenic responses through Treg induction. Furthermore, Tpl2 inhibition might also provide a means of enhancing Treg expansion in vitro for use in clinical applications. Future studies will address whether currently available Tpl2 inhibitors preferentially promote Treg development.
Liposome Encapsulated Gentamicin for the Treatment of *Rhodococcus equi* in a Mouse Infection Model


*Rhodococcus equi*, a facultative intracellular pathogen and an important cause of pneumonia in foals, is highly susceptible to killing by gentamicin *in vitro*. However, gentamicin does not appear to be effective *in vivo*, due to its poor cellular penetration. Encapsulation of drugs in liposomes enhances cellular uptake. The objective of this study was to compare the efficacy of two different formulations of liposomal gentamicin to that of free gentamicin and of other antimicrobials, for the treatment of *R. equi* in a mouse infection model.

Athymic nude mice were infected intravenously with $5 \times 10^6$ CFU of virulent *R. equi*. On day 4 after infection mice were treated intravenously with 2 different formulations of liposomal gentamicin (with and without polyethylene glycol (PEG) coating), empty liposomes, free gentamicin, subcutaneous rifampin and clarithromycin, or saline (5 mice per group). Mice were subjected to euthanasia 8 days post-infection. Effects of drug on CFU in spleen and liver were assessed using an ANOVA.

Mice euthanized 8 days post-infection and treated with PEG-coated liposomal gentamicin had significantly ($P = 0.005$) lower CFUs of *R. equi* in the spleen and in the liver compared to control mice or mice treated with free gentamicin. Compared to treatment with clarithromycin-rifampin, treatment with PEG-coated liposomal gentamicin resulted in a significantly ($P = 0.036$) greater reduction in the numbers of *R. equi* CFU in the liver relative to untreated controls.

These results underscore the potential of liposomal gentamicin as a new treatment for infections caused by *R. equi*. 
Histopathologic characterization of *Mycobacterium chelonae* infection in captive Syngnathids

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A retrospective study evaluating animals in the family Syngnathidae, which includes weedy seadragons, leafy seadragons, seahorses, and pipefish was performed to characterize histopathologic lesions associated with mycobacterial infections. From November 2006-March 2013, a total of 211 syngnathids (seahorses n=116, weedy seadragons n=51, pipefish n=32, leafy seadragons n=12) were evaluated through the University of Georgia Aquatic Pathology Service. Seventy-seven (seahorses n=38, weedy seadragons n=19, pipefish n=13, leafy seadragons n=7) out of the 211 animals submitted were diagnosed with mycobacteriosis by histopathology aided by special stains, culture, or PCR identification. Twenty five animals were cultured and the most prevalent bacterial species (n=20/25) isolated was *Mycobacterium chelonae*. Of the animals cultured, only one pipefish had consistent histologic lesions but did not culture positive for *Mycobacterium* spp. Lesions were identified in swim bladder, gill, aorta, heart, skeletal muscle, liver, intestine, ovary, mesentery, and skin. Lesions are regularly predominated by coagulative necrosis of the affected tissue, with large nodular aggregates or sheets of macrophages that have cytoplasm laden with abundant acid-fast bacterial rods. Additionally, free bacteria were also identified in tissues including vessels in the gills where large colonies of bacteria produced aneurysm of the vessel wall. Lesions in the aforementioned syngnathid species differ significantly from mycobacterial infections in other teleost species that typically respond to low numbers of acid fast bacteria with granuloma formation. It is unclear whether the difference in response is due to virulence factors of the bacterial strains involved or the variability in host response. Further investigation into the pathogenesis of mycobacteriosis in syngnathids is warranted to elucidate these differences.
Rhodococcus equi is a Gram-positive soil saprophytic bacterium that is found ubiquitously in nature and may cause pyogranulomatous pneumonia if inhaled by susceptible foals. It is also an occasional pathogen of swine, which likely acquire infection through ingestion and may develop clinical submaxillary lymphadenitis. The ability of R. equi to replicate within murine and equine macrophages and cause disease in foals is dependent on the presence of an extrachromosomal virulence plasmid. This plasmid houses a pathogenicity island (PAI) region that contains a novel family of genes of unknown function referred to as the virulence associated protein, or vap family. Relative to equine isolates of R. equi, swine isolates are largely unstudied. It is known, however, that the PAI region of swine plasmids differs in vap gene composition compared to that of plasmids derived from foal clinical isolates. Although all vapS have sequence similarity, vap gene composition is distinct among equine and swine strains. Plasmids of equine isolates are always of the pVAPA type, so named because they contain vapA. Plasmids from swine strains are most often of the pVAPB type and contain vapB. The discovery that distinct plasmid types, pVAPA and pVAPB, are generally restricted to equine isolates and swine isolates respectively, gives rise to the hypothesis that differences in vap gene possession are responsible for host species specificity and more specifically raises the question whether host species tropism is plasmid dependent and expressed at the level of intramacrophage replication. To begin addressing the hypothesis, bacterial conjugation was utilized in order to transfer the pVAPB plasmid into a plasmid-cured R. equi isolate originally obtained from an infected foal. The growth capabilities of the equine isolate possessing a swine pVAPB plasmid will be examined in various host macrophages in order to determine if plasmid type possession results in host species tropism.
Elevated Synovial Fluid Adenosine Triphosphate in Dogs with Osteoarthritis or Sodium Urate-Induced Inflammation
Bryan Torres, Megan Hansen, Steven Budsberg

Currently, the association between osteoarthritis (OA) and joint pain is unpredictable and is not linear. Thus, there is a need for improved understanding and to establish a novel outcome measure that correlates with a decrease in the actual joint pain pathway. Both objective and subjective outcome measures have been utilized with varying degrees of correlation between them. Therefore, establishing a novel outcome measure that could be correlated with a decrease in the actual joint pain pathway could be highly beneficial to researchers and clinicians to aid in accurately assessing the efficacy of purported therapeutic agents, especially if it can be correlated to either of the current indirect outcome measurement systems used. Studies of OA pain involving biomarkers are limited. There is some encouraging early data with one study demonstrating a correlation between synovial fluid ATP concentrations and OA knee pain in humans. To the author’s knowledge there are currently no reports describing the presence or role of SF ATP concentrations in naturally occurring canine OA or in a urate-induced synovitis (UIS) model of inflammation. Our hypothesis is that elevated SF ATP concentrations are present in the stifle joints of dogs with either naturally occurring OA or sodium urate-induced inflammation (UIS) as compared to the SF in normal control stifle joints. Stifle SF was collected 26 normal dogs, 25 dogs UIS, and 32 dogs with naturally occurring OA. ATP concentration was determined with a luciferase assay. Comparison of the 3 groups was performed with the Kruskal-Wallis test and multiple comparisons were performed with Dunn’s multiple comparisons test. Evaluation of the effect of cruciate or meniscal pathology on SF ATP levels in OA dogs were performed with an ANOVA All hypothesis tests were 2-sided and the significance level was α=0.05. SF ATP from normal dogs was significantly lower than the SF from UIS (p<0.0001) or OA (p<0.0001). There was no difference between UIS and OA (p=0.639). Cruciate ligament status (p=0.279) and meniscal status (p=0.760) were found to have no effect on SF ATP concentration in the OA group. These data support our hypothesis and support ATP as a potential biomarker that can be directly related to OA. Furthermore, these data reveal SF ATP concentrations equivalently elevated in the UIS joints as in the naturally occurring OA stifles. Future studies will focus on the correlation of SF ATP as a biomarker for joint pain and dysfunction in OA as well as for tracking clinical improvement in dogs with OA undergoing therapeutic treatment.
Placental malaria (PM) is a clinical manifestation of *Plasmodium falciparum* infection that results in low birth weight and contributes to 363,000 infant deaths in Africa per year. Clinical cases of placental malaria exhibit significant increases in fibrin deposition in infected placentae relative to those that are uninfected, and widespread placental hemorrhaging and disruption, significant fibrin deposition, and upregulated tissue factor (TF) expression drive fetal loss in association with *P. chabaudi* AS infection in our murine model of PM. Following these observations, our lab is assessing the effect of coagulation in *P. chabaudi* AS infection during pregnancy using C57BL/6J (B6) mice with either a null mutation in TF that are transgenic for human TF expressed at 1% of the normal level (LTF-/-) or that are heterozygous for human TF (LTF+/+-). Preliminary data suggests that infected pregnant (IP) LTF-/- mice follow a slightly divergent course of infection with *P. chabaudi* AS to that seen in IP B6 mice. IP LTF-/- mice drop below their pre-infection weight and become severely anemic much earlier than IP B6 mice. IP LTF+/+- also show an altered course of infection, presenting with an intermediate phenotype with weight loss and anemia falling between that seen in LTF-/- and B6 IP mice. No significant difference in course of parasitemia existed between these three groups. IP LTF-/- and IP LTF+/+- mice showed more severe pathology that IP B6 mice, with more intrauterine hemorrhaging and smaller pups, but, like wild-type IP B6 mice, abort between D10-12. Interestingly, IP LTF-/- mice die between D11.5 and D12, though the exact cause of death has not been determined. These data indicate TF plays a role in the maintenance of pregnancy during *P. chabaudi* AS infection and may affect the progression of this disease.
The serine-threonine kinase, Tpl2, promotes colitis in a T cell transfer model
Nicole Acuff, Xin Li, Rebecca Kirkland and Wendy Watford

Inflammatory bowel diseases (IBD) are among the most prevalent gastrointestinal diseases of the US. IBD describes chronic inflammation of the intestines and includes Crohn’s disease (CD) and ulcerative colitis (UC). One model for studying IBD is the adoptive transfer of CD4⁺CD45RBhi cells into Rag-deficient mice, which induces rapid inflammation of both the small and large intestines, similar to Crohn’s disease. CD is driven by a combined Th1/Th17 response and production of inflammatory cytokines IFNγ and IL-17A. One molecule gaining interest as a therapeutic target for treating chronic autoimmune diseases is the serine-threonine kinase, Tpl2. Initial characterization of Tpl2-deficient mice showed major defects in the induction of proinflammatory cytokines, particularly TNFα, and therefore, Tpl2 is now being investigated as a treatment for TNF-mediated autoimmune diseases. Current treatments for CD include immunomodulation, such as anti-TNF treatment, with a goal of patients entering clinical remission with mucosal healing of the intestines. Our data indicate that Tpl2 promotes T cell-mediated inflammation through increased levels of the inflammatory cytokines TNF and IFNγ, but not IL-17A, with more widespread inflammation of the colon.
Targeting the Plasmodium Kinome with a Novel Class of Peptide-based Antimalarials
Briana Flaherty, Yuxiao Wang, Teinhuei Grace Ho, Eileen Kennedy, and David Peterson

With *Plasmodium* drug resistance on the rise and few drugs in the pipeline, future malaria control efforts rely heavily on the development of new antimalarials that act on alternative targets or utilize novel mechanisms of action. Phylogenetic analyses demonstrate unique homologues of human protein kinases in the *P. falciparum* genome. Our research seeks to target such *Plasmodium* kinases using a new class of inhibitors – chemically stabilized peptides – that inhibit protein-protein interactions. Stabilized peptides targeting two kinases were developed and tested on *P. falciparum*-infected red blood cells in vitro. Peptides were analyzed for cell-specific permeability, parasite growth inhibition, and intracellular location. Results demonstrate peptides to be specifically permeable to infected, but not uninfected, red blood cells. Both peptides demonstrate clear evidence of inhibition of parasite growth and localize within the parasite cytoplasm. This research provides the first evidence for use of chemically stabilized peptides with *P. falciparum*. Future work will aim to further develop this novel class of peptide-based antimalarial in order to enhance both specificity and parasite toxicity.
Magnetic Resonance Imaging, Histology, and Sensorimotor Analysis of a Novel Ischemic Stroke Pig Model

Stroke is the leading cause of long-term disability among adults in the United States. Despite hundreds of drugs going to clinical trials, only one has been approved by the Food and Drug Administration. One explanation for the high rate of translational failure is the lack of preclinical testing in a gyrencephalic animal model. The vast majority of stroke therapies are developed and verified in lissencephalic rodent models. Pigs possess a gyrencephalic brain and are more similar to humans than rodents with respect to gray-white matter composition and size. We hypothesized that cauterization of the middle cerebral artery (MCA) in pigs would lead to ischemic infarction and functional neurological deficits. Our objective was to develop a pig middle cerebral artery occlusion (MCAO) ischemic stroke model to address the need for a robust and repeatable gyrencephalic animal stroke model.

A right fronto-temporal craniectomy was performed on 8 adult male Yucatan miniature pigs. The MCA was permanently occluded with bipolar electrocautery. Magnetic resonance imaging (MRI) was performed 1 and 90 days post-MCAO surgery. A computational video capture system was used to assess changes in motor function pre- and post-stroke. Histological analysis was performed 90 days following MCAO surgery.

Diffusion weighted and apparent diffusion coefficient MRI images confirmed stroke damage 1 day post MCAO. T1-FLAIR MRI analysis showed a loss of 59.17 ± 10.06 cc of tissue from day 1 to day 90. Histological examination of the brain demonstrated severe atrophy of the affected right hemisphere. The white matter in the affected cortex could not be defined due to loss of normal elements, glial proliferation, and infiltration of gitter cells. Motor function analysis showed loss of gait symmetry and changes in stride length and maximum hoof height of the contralateral limbs.

MCA occlusion in pigs led to structural and sensorimotor changes that are compatible with an ischemic stroke injury. MRI and histological analysis demonstrated the evolution of a significant structural lesion that was repeatable and consistent between MCA occluded animals. Corresponding motor function analysis consistently confirmed a loss of gait symmetry in all MCA occluded animals.

The development of a pig MCAO model will allow stringent assessment of efficacy and safety of novel stroke therapies in an animal model that shares important neuro-anatomical and physiological features with humans.
Differentiation of Recent US *Mycoplasma gallisepticum* Isolates from Vaccine Using PCR-RFLP

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The widespread use of live vaccines to control *Mycoplasma gallisepticum* (MG) infection in poultry has increased the importance of differentiating wild-type MG strains from vaccine strains. Techniques currently used to diagnose MG (including serology, polymerase chain reaction (PCR), real-time PCR and isolation by culture) do not facilitate the differentiation of wild-type MG from vaccine strains. Targeted sequencing of certain MG genetic regions allows very good strain differentiation; however, the expense and availability of sequencing technology may limit its use. In addition, targeted genetic sequencing cannot differentiate mixed-strain infections, such as co-infection with wild-type MG and ts-11 vaccine strains. We have developed a simple PCR-restriction fragment length polymorphism (RFLP) procedure, which employs the restriction enzymes, *Hinf*I and *Ase*I, to digest the 16S-23S rRNA intergenic spacer region (IGSR) PCR amplicon of MG. *Hinf*I and *Ase*I target the sequences G^A_nTC and AT^TAAT respectively, and do not degrade IGSR PCR primers. Restriction enzyme digestion of IGSR PCR amplicons allowed differentiation of ts-11 from eight recent US MG samples, one reference and two vaccine strains analyzed in this study, and theoretically differentiates ts-11 vaccine strain from all 21 IGSR sequence types in the Poultry Diagnostic and Research Center (PDRC) database, originating from 173 farms in 18 US states between 2000 and 2013. This PCR-RFLP technique facilitates the differentiation of wild-type MG from ts-11 vaccine in both single-strain and mixed-strain infections.
MRI-Based Contrast Agents for Tracking Mesenchymal Stem Cells in a Large Animal Model of Tendon Injury

Alexandra Scharf, Shannon Holmes, Merrilee Thoreson, Jennifer Mumaw, Alaina Stumpf and John Peroni

The goal of this study was to compare the biocompatibility and signal enhancement properties of equine mesenchymal stem cells (MSCs) labeled with two common, MRI-based, contrast agents. Equine MSCs were treated with superparamagnetic iron oxide nanoparticles (SPIO) or a gadolinium (Gd) chelate and evaluated for viability as well as their ability to proliferate, undergo tri-lineage differentiation, and express a select number of cytokines. All labeled cells demonstrated characteristics comparable to controls (p<0.05), with the exception of chondrogenic differentiation in SPIO-labeled cells (p<0.0001) and osteogenic differentiation in Gd-labeled cells (p<0.01). In both cases all labeled cells successfully underwent differentiation and differences are likely due to variation in cell proliferation. Following injection of 10-25 million cells labeled with Gd, highly localized, hyperintense signal was visible on MR images. This hyperintense signal became notably more diffuse as cells distributed themselves along the length of the tendon over a 3 day-period. Cells labeled with SPIOs demonstrated hypointense signal with a noticeable blooming artifact at all time points. These cells demonstrated a similar pattern of migration over a 3-day period. Both systems appear adequate for tracking cells in equine tendon, although previous studies suggest that persistence of SPIOs after cell death may pose an issue in longitudinal cell tracking studies. For this reason, we suggest the alternative use of Gd-based cell labeling, as they appear to have similar biocompatibility with enhanced signal detection.
Interns and Residents

FEBRILE NEUTROPENIA IN CATS TREATED WITH CHEMOTHERAPY
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Introduction: Febrile neutropenia (FN) is rare in chemotherapy-treated cats and has not been described in the literature. The purpose of this study was to describe the clinical presentation, causative agents, treatment, and outcome of FN in cats treated with chemotherapy.

Materials & Methods: Medical records from 4 institutions were retrospectively reviewed.

Results: Twelve FN events in 10 cats were evaluated. Lymphoma was the most common cancer diagnosis. Associated clinical signs of FN included lethargy, inappetence, vomiting, and diarrhea. Median body temperature and absolute neutrophil count at presentation were 103.9°F (range: 102.4-105.1°F) and 200/uL (range: 5-1600/uL), respectively. A variety of agents caused the FN; lomustine and vincristine were most frequently implicated. Median number of days between chemotherapy administration and FN onset was 7 (range: 4-23 days). Nine of 10 cats were hospitalized and treated with intravenous fluids, broad spectrum antibiotics, and gastroprotectants. Fever resolved in all cases, and absolute neutrophil count returned to normal in all but one cat, a median of 10 days following the FN onset (range: 1-44 days). One cat with irreversible neutropenia was euthanized following the FN event; lymphoma was confirmed in the bone marrow at necropsy.

Conclusion: Clinical presentation of cats with FN is similar to other species, and recovery is relatively rapid with supportive care. Additional studies to determine risk factors of FN in cats receiving chemotherapy are needed.
Pharmacodynamic Evaluation of Oral Rivaroxaban in Healthy Adult Cats
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Cats with primary cardiomyopathy (CM) have a high incidence of arterial thromboembolism (ATE). A safe and effective oral anticoagulant for cats with CM has yet to be identified. Rivaroxaban (RVX) is an orally administered direct inhibitor of activated factor X (FXa). In people, RVX is used for prevention of deep vein thrombosis and ATE. Prothrombin time (PT), dilute prothrombin time (dPT) and activated partial thromboplastin time (aPTT) become linearly prolonged in people with increasing plasma RVX levels. We hypothesize that oral administration of RVX to cats will result in predictable plasma drug concentrations within the target range reported for efficacy in people and will result in dose-dependent prolongation of dPT, aPTT and anti-factor Xa activity (aXa). We also hypothesize short and long-term oral administration of RVX will be well-tolerated and safe for use in cats.

RVX tablets (10mg Xarelto®, Bayer Healthcare) were divided into halves and quarters. Tablets were weighed to estimate actual milligrams (mg) of drug, with the assumption that the active ingredient was evenly distributed. The 1.25mg tablets were prepared as tablet triturates from the commercially available tablets. A single oral dose of RVX was administered to 6 cats at 1.25mg (G1) and 2.5mg (G2.5) and to 3 cats at 5mg (G5). Blood samples were obtained at baseline and then at 3, 8, and 24 hours following the dose, except for G2 which was evaluated at 1, 3, and 12 hours. Citrated plasma was frozen at -80°C until batch analysis at the Cornell University Comparative Coagulation Laboratory. At each time point, aXa and dPT were evaluated, and aPTT was evaluated at each time point for G2.5 and G5. After a one-month washout period, once daily 1.25mg RVX was given to 6 cats for 30 days (G30). Citrated blood samples were analyzed for aXa, dPT, and aPTT at baseline, and on days 7, 14, 21, 28 and 42.

RVX administration, at all dosages studied, was well tolerated in healthy cats and resulted in dose-dependent prolongation of coagulation times. Peak anticoagulant effects were seen at the 3 hour time point for all assays. Oral RVX demonstrated dose-dependent aFXa activity in cats. A standard dose of 1.25mg resulted in a maximum mean aXa of 2.25 +/- 1.3 U/mL. In the higher dose groups (G2.5, G5), most coagulation effects returned to baseline by 24 hours. In the 1.25mg dose group (G1), coagulation effects were normal at the 12 hour time point. The duration of action of the 1.25mg dose are similar to those seen in studies that have shown positive outcomes in a number of different thrombogenic conditions in people. The higher doses, especially in smaller cats, may result in high aXa values, which may predispose to hemorrhage. Further studies to investigate the pharmacokinetics and pharmacodynamics of RVX in cats with heart disease are necessary.
Identification Of A Disseminated *Pseudallescheria boydii* Infection In A Golden Retriever Using DNA Sequencing

Fungal culture and DNA ITS sequencing were performed to further characterize a disseminated fungal infection in a 1-year-old, female spayed, Labrador Retriever. This dog presented to the University of Georgia, Emergency Service with a history of persistent pyrexia and neurologic signs including ataxia, nystagmus, and seizures. Magnetic resonance imaging findings and cerebrospinal fluid analysis were initially suggestive of an inflammatory disease confined to the central nervous system. Due to severe, progressive neurologic deterioration, euthanasia was elected. At necropsy, the brain, spinal cord, heart, kidneys, spleen, lungs, thyroid gland, and tracheobronchial lymph nodes had variably sized, widely scattered, well-demarcated, pale tan to yellow foci, with reddened centers. Histopathology revealed widespread pyogranulomatous inflammation with filamentous, parallel-walled, septate, and acute angle dichotomous branching fungal hyphae, morphologically similar to *Aspergillus* or *Fusarium* sp. Although fungal culture was initially suggestive of *Blastomyces dermatitidis*, DNA sequencing classified this fungal organism as *Pseudallescheria boydii*. *P. boydii* is primarily an emerging human pathogen, which has been rarely documented in veterinary species, typically dogs, in which it is often misdiagnosed as *Aspergillus* or *Fusarium* sp. due to their morphologic similarities. Accurate characterization of the fungal agent as *P. boydii* is critical since treatment modalities differ markedly from those for *Aspergillus* or *Fusarium* sp. Future work will include a retrospective study using DNA sequencing techniques to re-evaluate histologically diagnosed *Aspergillus* sp. cases received through the Athens Veterinary Diagnostic Laboratory between 2002-2013, in an effort to estimate the proportion of possible *P. boydii* cases.
Imaging the canine spine with SPACE, a 3D T2-weighted spin echo sequence with variable flip angle refocusing
Shaikh LS, Holmes SP, and Selberg KT

3D volume imaging in MRI has an advantage of allowing multiplanar reconstructions after image acquisition, providing for shortened total imaging time. 3D pulse sequences, until recently, have been limited to gradient-recalled echo (GRE) sequences. Fast-spin echo (FSE) sequences are the most commonly used pulse sequences, because they provide superior image contrast and are less susceptible to magnetic field inhomogeneities compared to GRE sequences. Newly developed FSE sequences with very long echo train lengths achieved by application of variable flip angle refocusing (SPACE) allow 3D volumetric scanning. The goal of this study was to optimize the use of the SPACE sequence in the canine spine and compare its image quality to conventional T2-weighted (T2w) FSE sequences.

Seven young adult, healthy and neurologically normal dogs were examined post-mortem using a 1.5T Siemens Symphony with TIM® MRI unit. Centered at the thoracolumbar junction, sagittal plane and transverse plane conventional 2D T2w FSE sequences and a 3D sagittal plane T2w SPACE sequence were acquired. Transverse reconstructed images of the thoracolumbar spine were made from the sagittal SPACE images to match the location of the conventional T2-weighted transverse images. Reconstructions were made using both identical slice thickness and slice overlap as the conventional 2D T2w FSE sequence as well as variably thin-slice and overlapped images. SPACE transverse images were evaluated for overall image quality and visibility of structures compared to the conventional pulse sequences.

The SPACE imaging time was less than the sum of the times for the sagittal and axial plane conventional FSE sequences. The sagittal SPACE was acquired in 6 minutes and 31 seconds, whereas the FSE sequences took 10 minutes and 46 seconds to acquire. Voxel resolution was better for the SPACE images (0.7 x 0.6 x 1.0mm) than for the sagittal conventional T2-weighted FSE sequences (sagittal 0.6 x 0.6 x 2.5mm, transverse 0.6 x 0.6 x 3.0mm). Multiplanar reconstructions of the 3D SPACE dataset were easily and quickly performed. The transverse reconstructed SPACE images were assessed by a radiology resident and two board-certified radiologists to have acceptable diagnostic image quality. There was good visibility of the spinal cord, epidural fat/fluid, intervertebral disc, articular processes and facet joints, vertebral venous sinuses, and spinal nerves. The thinner slice image reconstructions with higher overlap were preferred for evaluating small structures.

The 3D SPACE T2w pulse sequence with multiplanar and thin-slice reconstructions is a viable tool for MR examination of the canine spine. The fast acquisition times make this sequence an attractive option to be competitive with computed tomography (CT) exams, particularly in acute neurologic presentation.
A Retrospective Study of Thymic Hemorrhage in 16 Dogs

S. Coutermarsh-Ott

Idiopathic thymic hemorrhage is an uncommon lesion in young dogs that should be differentiated from that seen in dogs exposed to anticoagulant rodenticides. The purpose of this retrospective study was to review canine necropsy submissions at University of Georgia diagnosed with moderate to severe thymic hemorrhage as the primary lesion. Additionally, only those cases with results from rodenticide toxin testing were included. The case series included a total of 15 canine submissions from 2002-2013. Animals ranged in age from 10 weeks to 6.5 years, though the majority (11/15) were below 1 year of age. Ten breeds including mixed breeds were represented. In 9/15 cases, anticoagulant rodenticides were detected and, of these, just under half (4/9) described the thymus as the only site of significant hemorrhage. Histologic findings were similar in all cases and characterized by expansion of thymic lobules and interlobular septa as well as variable effacement of the thymic cortex and medulla with moderate to large amounts of hemorrhage. Two cases reported variable amounts of necrotic debris as well. Thymic hemorrhage, with or without additional lesions, is an uncommon cause of sudden death or collapse in young dogs. Its etiology is often multi-factorial and includes exposure to anticoagulant rodenticides, trauma, and idiopathic. Results of this retrospective study suggest that anticoagulant rodenticide exposure can manifest as only thymic hemorrhage and thus testing should always be performed to rule this out.
Hookworm Disease in South American fur seal (*Arctophoca australis*) pups at Guafo Island, Chile


Hookworms are highly pathogenic nematodes that parasite a wide range of mammals including several species of otariid seals.

During 2012 and 2013 austral summers necropsies and captures on South American fur seal pups at Guafo Island, Southern Chile were performed. On captured pups complete blood cell count, fecal eggs count and clinical examination were performed on the field.

On necropsies in carcasses that presented complete gastrointestinal tract, hookworm (*Uncinaria sp*) infection was found in all pups examined (28/28). In at least 15 of those cases *Uncinaria sp* infection can be considered as cause or contributory factor to the death of the pups. Common findings in these pups included severe hemorrhagic enteritis with hookworms deeply embedded in the mucosa and occasionally free in the abdominal cavity, enteric and respiratory bacteria (*E. coli, Streptococcus sp*) in the blood, lung and liver and gram positive or gram negative bacteria in hookworm feeding tracks and/or inside macrophages at the mesenteric lymph nodes and lungs. From 70 live pups with complete CBC, 16 presented anemia (RBC < 3,000,000 µl) and hookworm infection. Commonly these pups presented pale mucosa, diverse degrees of weakness, bloody diarrhea and mucopurulent conjunctivitis.

Hookworm disease associated with secondary bacterial infections remains as an important cause of morbidity and mortality in this population. This contrasts with South American fur seal rookeries in the Atlantic Ocean, where morbidity and mortality because of hookworms are very low.
Postdoctoral Fellows and Faculty

Development of Novel Diagnostic Tools for Early Detection of Mycoplasma synoviae in Chickens

Vijay Durairaj and Naola Ferguson-Noel

Mycoplasma synoviae (M. synoviae) is an infectious pathogen affecting chickens targeting the synovial membrane of joints and bursa. M. synoviae adversely affects the respiratory system and causes subclinical/clinical signs. M. synoviae causes devastating economic losses in the poultry industry. M. synoviae is transmitted by both vertical and horizontal routes. Aerosol exposure is one of the common routes for transmission of M. synoviae. It causes mucosal insult in the trachea and stimulates the production of IgA antibodies. It further leads to a systemic infection resulting in production of IgG antibodies. Serological assays have been used as first line diagnostic assays in monitoring and surveillance programs. The objectives of this research were to develop diagnostic assays for M. synoviae by the detection of (a) IgA antibodies by a fluorescence antibody culture test (FACT) and (b) IgG antibodies by the determination of serum neutralization titer. For the FACT test M. synoviae colonies were grown on Frey’s modified agar plate. Tracheal supernatant of the chickens was used as a primary antibody followed by a secondary anti-chicken IgA antibody conjugated with FITC. Based on presence and absence of immunofluorescence signals, the positive and negative reactivity of the samples were identified respectively. Three-week-old experimentally infected SPF chickens were swabbed from the trachea and the supernatant was used for FACT. Sample collection (blood, choanal cleft swabs, tracheal swabs) was performed from 3-days after infection to 11-days after infection. The samples were analyzed by serum plate agglutination test, hemagglutination inhibition test, ELISA, conventional culture test, real-time PCR and FACT. Positive reactions were observed as early as 3 days by FACT, conventional-culture test and real-time PCR. For the second objective of this research, serum neutralization antibody titers against M. synoviae were determined. Serum neutralization assay was performed by adding a standard concentration of M. synoviae culture to diluted levels of serum. The results of this assay were read ten days later based on color changing units. No color change indicated presence of neutralizing antibodies and color change to yellow indicated absence of neutralizing antibodies. This assay provides both quantitative and qualitative results.
Reproductive Capacity is Associated with Lifespan and Cause of Death in Companion Dogs
Jessica M. Hoffman, Kate E. Creevy and Daniel E.L. Promislow

Reproduction is a risky affair; a lifespan cost of maintaining reproductive capacity, and of reproduction itself, has been demonstrated in a wide range of animal species. However, little is understood about the mechanisms underlying this relationship. Most cost of reproduction studies simply ask how reproductive capacity influences age at death, but are blind to the subjects' actual causes of death. Lifespan is a composite variable of myriad causes of death and it has not been clear whether reproductive capacity influences all causes of death equally.

We compared causes of death among 41,045 sterilized and reproductively intact domestic dogs, using a previously-described pathophysiologic classification systema and stratified by age using a Cochran-Mantel-Haenzel (CMH) test. We found that sterilization increased life expectancy by 13.8% in males ($\chi^2 = 446, P < 10^{-6}$) and 26.3% in females ($\chi^2 = 1372, P < 10^{-6}$). Sterilized dogs were dramatically less likely to die of infectious disease ($\chi^2 = 184.4, P < 10^{-6}$), trauma ($\chi^2 = 268.7, P < 10^{-6}$), infectious disease ($\chi^2 = 184.4, P < 10^{-6}$), vascular disease ($\chi^2 = 8.25, P = 0.004$), and degenerative disease ($\chi^2 = 7.7, P = 0.006$). In contrast, sterilized dogs died more commonly from neoplasia ($\chi^2 = 300.4, P < 10^{-6}$) and immune-mediated disease ($\chi^2 = 167.2, P < 10^{-6}$).

These findings suggest that to better understand how reproduction affects lifespan, a shift in research focus is needed. Beyond the impact of reproductive capacity on when individuals die, we must investigate its impact on why individuals die.

Comparison of Gastrosplenic Entrapment of the Small Intestine to Other Small Intestinal Lesions in Horses
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Strangulating lesions of the small intestine are common reasons for surgical exploration of the equine abdomen. The objective of this study was to compare the clinical data of horses with gastrosplenic entrapment of the small intestine (ESIGL) to clinical data of horses with other strangulating small intestinal lesions.

Medical records of horses undergoing surgical exploratory celiotomy for acute abdominal pain associated with strangulating small intestinal lesions between January 2001 and December 2011 were reviewed. Signalment, presenting physical examination findings, clinicopathologic variables, surgical findings and surgical procedures performed, postoperative data and short term survival were recorded.

Clinical findings included excessive nasogastric reflux and abnormal abdominal fluid. Horses with ESIGL were significantly more likely to require intestinal resection and anastomosis and produced significantly less reflux post-operatively than horses with other strangulating small intestinal obstructions. Geldings were significantly more likely to develop ESIGL than mares or stallions. Quarter Horse or Quarter Horse type breeds were predisposed to ESIGL. Survival to hospital discharge in horses with ESIGL (16/22, 72.7 %) was significantly higher than that of horses with other strangulating small intestinal obstructions (92/183, 50%).

ESIGL was more prevalent in this population of horses evaluated for acute abdominal pain than in previous studies, accounting for 10.68% of all horses with strangulating small intestinal lesions. Geldings and Quarter Horse or Quarter Horse related breeds are predisposed to this condition. The prognosis to survival to hospital discharge was fair to good.
Evaluation Of NIRS, Serum Biomarker And Muscle Damage In A Porcine Model Of Extremity Compartment Syndrome

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Extremity compartment syndrome (ECS) can result in devastating consequences. Near Infrared Spectroscopy (NIRS) has been shown to provide continual, real time, non-invasive measurement of regional perfusion in an infusion model of ECS. The objective of the present study was to assess and correlate NIRS, tibial intra-compartmental pressure (TICP), tibial intra-compartmental perfusion pressure (TIPP), serum markers of inflammation and muscle injury in a balloon compression model of ECS. Six landrace swine were anesthetized and NIRS sensors were placed on each leg. Direct pressure transducer measurement of compartmental pressure was performed. A balloon catheter was placed between the tibia and the cranial tibialis (CT) muscle in the test limb and inflated to 30 mm Hg over mean arterial pressure. This pressure was maintained for six hours. Continual time synchronized measurements of systemic blood pressure were collected. At conclusion of the study, pigs were euthanized and the CT muscle was collected from both limbs. Serum was collected for measurement of inflammatory biomarkers creatine kinase (CK), myoglobin, TNF-α, IL-1β, and IL-6. All pigs were euthanized at the end of the experiment. Repeated-measures ANOVA evaluated TICP, NIRS and TIPP measurements between and within test and control limbs, and to compare CK, myoglobin, TNF-α, IL-1β, and IL-6 between time points. Multiple comparisons were adjusted using Tukey’s test. A Wilcoxon signed-rank test was used to compare muscle scores between test and control legs. Pearson’s correlations were calculated between muscle degeneration and edema and NIRS at the final measurement time. The model successfully created increased TICP and decreased TIPP consistent with ECS. NIRS also detected significant changes in tissue oxygenation at all the same points. Specifically, the test limb TIPP significantly decreased from baseline during balloon inflation and at 15min post deflation throughout the remainder of the study period. Test limb TICP significantly increased compared to baseline during balloon inflation and at 1-6 hours following deflation. NIRS measurements were significantly lower than baseline at balloon inflation and throughout the remainder of the study period. Myoglobin concentrations significantly increased over baseline at balloon deflation and remained high throughout the study period. CK significantly increased over baseline 2hrs post balloon deflation and remained high. No changes were seen in TNF-α, IL-1β, and IL-6. Significant muscle degeneration and edema were found in the CT muscle. There was a significant correlation of muscle degeneration and edema with NIRS at the final measurement time. Significant correlation coefficients existed between degeneration and NIRS ($r = -0.67$), edema and NIRS ($r = -0.60$), and hemorrhage and NIRS ($r = -0.67$). NIRS of the compartment provided a reliable, sensitive measure correlating to both an increase and decrease in TICP and TIPP in this porcine balloon model. CK and myoglobin significantly increased following balloon removal. Significant correlations between muscle degeneration, edema, hemorrhage and NIRS were found.