

## 2023 Research Day Abstracts

Abstracts are listed in alphabetical order by the presenters' last names.

### Efficacy of diet, antibiotics, or probiotics for the treatment of canine acute uncomplicated gastroenteritis

Francis Aduku, Maryanne Murphy, and Angela Rollins

Background: Even though most cases of canine acute uncomplicated gastroenteritis are mild and self-limiting, owners and veterinarians often seek treatments such as antimicrobial agents, probiotics, and dietary modifications to obtain a quicker resolution. To date, however, there has been no comparison of each of these treatments versus a placebo to determine if they do in fact lead to resolution sooner than awaiting the self-limiting nature of the condition. Objective: Evaluate if diet modification, antibiotics or probiotics are more effective than each other and/or placebo in treating canine acute uncomplicated gastroenteritis. Animals: 40 client-owned dogs with acute uncomplicated gastroenteritis. Procedures: Dogs presenting to their regular veterinarian with a fecal score of 2.5 or lower on the Royal Canine fecal scoring system and confirmed to have no parasites detected on a routine fecal panel (sugar and zinc) will be randomly assigned to one of four treatment groups: diet, metronidazole, probiotic, or placebo. Owners will submit daily fecal score assessments over the subsequent 5-day period via an electronic questionnaire. Data will be analyzed to compare the initial fecal score and timing for a fecal score to improve by 1.5 points. Clinical relevance: The practice of nonspecific antibiotic use could be reduced if diet or probiotic is proven to be as effective as metronidazole. Additionally, if there is no difference in time to fecal score improvement in the placebo versus other treatment groups, the data would represent a strong argument to question the utility of these treatments in canine acute uncomplicated gastroenteritis.

### Evaluation of the effect of bilirubinuria on urine dipstick results

Amanda Alli, Sarah Schmid, and Xiaojuan Zhu

The urinalysis is essential in the assessment of canine health. Despite urine dipstick manufacturers warning that pigmenturia can influence results, the effect of bilirubinuria is unknown. This study sought out to determine the effect of bilirubinuria on urine dipstick results. Urine samples were spiked with a water-based bilirubin standard (Sigma-Aldrich). Urine samples from five healthy dogs and eight clinical pooled canine urine samples were spiked to bilirubin concentrations of 20, 8, 4, 2, 1, 0.5, and 0 mg/dl. For each urine-bilirubin mixture, urine specific gravity (USG) was measured, and visual and automated urine dipstick evaluations were performed. A mixed effect ANOVA was used to analyze the differences in automated urine dipstick analyte readings among bilirubin concentrations. The agreement between visual and automated urine dipsticks was compared with the interclass correlation coefficient (ICC). Urine glucose, ketones, and bilirubin were positively associated with urine bilirubin concentration ( $p < 0.0001$ ,  $p < 0.0001$ , and  $p = 0.014$ , respectively). USG was negatively associated with bilirubin concentration ( $p < 0.0001$ ). Urine protein was positively correlated with USG ( $p = 0.038$ ). Agreement between visual and automated urine dipstick results were excellent for bilirubin (ICC 0.946) and blood (ICC 0.914), good for pH (ICC 0.854) and protein (ICC 0.886), moderate for glucose (ICC 0.550), and poor for ketones (ICC  $< 0.001$ ). Our findings indicate that although bilirubin may cause a mild increase in urine dipstick glucose and ketone readings, the change in the color pad was so small that it could not be detected by visual inspection.

## Echocardiographic estimates of shunt volumes in dogs with patent ductus arteriosus and their association with measures of cardiac volume overload and outcome.

Allie Asbury, Liza Koster, and Cary Springer

Patent ductus arteriosus (PDA), a common congenital cardiovascular anomaly in dogs, can cause left-sided congestive heart failure (L-CHF) and pulmonary hypertension (PH) in puppies. We evaluated 53 client-owned dogs who presented to the UTCVM for echocardiography and diagnosed with a PDA. Our aims were to describe murmur grade, and shunt patterns; to compare measures of shunt volume between Standard Left-to-Right shunts (LR) with those dogs that have PH, L-CHF, and Hemodynamically insignificant shunts (HDI); and examine the association of the echocardiographic markers of shunt volume with indices of left chamber size. Most of the dogs had standard left-to-right shunts (76%), with a grade 4/4 heart murmur (69%), closing Doppler pattern (71%) and color flow Doppler (CFD) pattern of the shunt reaching the pulmonary valve (51%). Murmur grade was correlated with measures of left ventricle in systole ( $\rho=0.423$ ,  $p=0.004$ ) and diastole ( $\rho=0.47$ ,  $< 0.01$ ) and left atrial dimensions ( $\rho=0.477$ ,  $<0.01$ ). Traditional shunt volume estimate (Qp:Qs) was higher in dogs with LR shunt as compared to dogs with PH ( $p=0.007$ ) and minimum ductal diameter normalized to body weight (BW) also larger in dogs with PH ( $p=0.005$ ). Dogs with L-CHF had larger CFD width compared to LR dog group ( $p=0.009$ ), which was larger than the PH group ( $p=0.004$ ). The BW normalized CFD width was larger in dogs with L-CHF as compared to standard LR shunting dogs ( $p=0.002$ ). Novel Doppler techniques, specifically CFD width, are helpful in assessing severity of shunt volume in dogs with patent ductus arteriosus.

## Characterizing *Mycoplasma* species isolated from dogs and cats with non-respiratory clinical presentations

Jaclyn Azelby, Porsha Reed, Rebekah Jones, Brian Johnson, Rajeev Nair, and Sreekumari Rajeev

*Mycoplasma* spp. are cell wall-deficient bacteria that have been classified as commensal organisms of mucosal surfaces and opportunistic pathogens that can cause a broad spectrum of disease in both humans and animals. Although most commonly associated with respiratory illness, non hemotropic Mycoplasmas have been reported in association with cases of urogenital tract disease, keratoconjunctivitis, polyarthritis, and soft tissue infections in dogs and cats. *Mycoplasma* species' specific growth requirements for culture often allows them to go undetected and are commonly isolated from patients who fail to respond to standard beta-lactam antibiotic therapy. Given their commensal nature, their characteristics and significance as a causative pathogen in many non-respiratory infections is not fully understood. In this study, we characterized *Mycoplasma* isolates obtained from dogs and cats representing both in house and referral cases submitted between 2021 and 2023 at the University of Tennessee Veterinary Bacteriology and Mycology Laboratory. The isolates included were from vaginal samples, urine, ears, wounds, cornea, conjunctiva, semen, joints, abscesses, and lymph nodes. We performed 16s rRNA amplification and sequencing for the identification of these isolates. The sequencing identifications made were *Mycoplasma canis* (n=14), *Mycoplasma maculosum* (n=2), *Mycoplasma spumans* (n=1), *Mycoplasma gaetae* (n=1), *Mycoplasma felis* (n=9), *Mycoplasma arginini* (n=1), and *Mycoplasma edwardii* (n=4).

## Long-term infectivity of dengue virus in mosquito cells revealed implications for persistent viral replication in the vector host

Swarnendu Basak, Girish Neelakanta, and Hameeda Sultana

Dengue viruses (DENV) cause severe diseases such as dengue fever (DF), dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), multi-organ failure and sudden death in human. According to World Health Organization (WHO), mosquito-borne dengue transmission impose a significant public health concern and outcome challenges suggest this virus to be an emerging pathogen. Case reports have documented that human who were infected with DENV and showed no symptoms or signs of illness for many years suddenly experienced severe neurological complications in life. Arguably, the concept of DENV entering a latent state within the human host for prolonged durations remained negligible. Moreover, the absence of an appropriate model system for investigating the ability of long-term infection and maintaining DENV propagation is essential. Consequently, understanding the intrinsic potential of viral replication in host cell is critically important. In this study, we interrogated at what extent DENV can proliferate within, *Aedes albopictus* C6/36 cells (an in vitro cell line used for propagation of DENV in laboratories). We quantified DENV2 (serotype 2) loads in cell culture supernatants collected on days 19, 33, 60, 90, and 120 (referred as viral isolates). The tissue culture infectious dose 50 (TCID<sub>50</sub>) assay consistently revealed a significant reduction in viral infectivity and titers from days 19 to 120. Furthermore, our infection kinetics with above viral isolates demonstrated declining viral loads in both C6/36 cells and human endothelial cells. These results confer the viral replication competence and persistency of DENV2 infection from days 19 to 120 in mosquito cells. Additionally, extracellular vehicles (EVs) isolated from these C6/36 cells infected with DENV isolates showed declining viral loads but the EVs remained infectious. In pursuit of elucidating the underlying cause for persistent viral infectivity, we further investigated the presence of genetic mutations in viral genes (Envelope (E) fusion loop and NS5). No mutations were noticed at the amino acid level. In conclusion, DENV exhibits the capacity for persistent long-term infection in mosquito cells, thereby potentially serving as a crucial model for investigating the mechanisms that govern long-term sustained viral infections within the vertebrate host cells.

## Isolation, expansion and characterization of rabbit tendon-derived mesenchymal stem cells

Emine Berfu Ozmen, Michael Rivera Orsini, Steven Newby, David Anderson, and Madhu Dhar

Tendon injuries are common musculoskeletal injuries that are difficult to manage and cause permanent disability. Autografts, allografts, and xenografts are currently used as treatments. Biologically derived grafts however, have several disadvantages such as graft failure, rejection, and disease transmission. Therefore, there is a need to develop synthetic alternatives to overcome the challenges of the current strategies. Recent focus on regenerative medicine has driven the exploration of bioactive technologies that help restore tissue features (biomimicry), together with stem cell therapy that promotes tissue healing through tissue regeneration. Our hypothesis in this study is that the combination of biomaterials and stem cell therapy using mesenchymal stem cells (MSCs) will provide a novel treatment for tendon injuries. The current study describes the first step to prove our hypothesis and achieve our goals. We describe here the isolation, expansion, and characterization of rabbit tendon-derived MSCs. Tendons were collected from eight rabbits during surgery. Tissue was digested using collagenase I and dispase II. The digested tissue was seeded in culture with DMEM F12 medium containing 10% fetal bovine serum, and adherent cells were observed within 7 days (passage 0). Spindle shaped cells were ex vivo expanded until passage 6. At each passage, cells were collected and cryopreserved to generate a cryobank. Cells were characterized using immunofluorescence to evaluate specific cell surface markers such as CD29, CD44, and CD90. While cells were CD29 and CD44 positive, CD90 showed robust expression, which suggests that these cells are mesenchymal stem cells and will be referred to as tMSCs.

## Tick secrets: Decoding the role of aromatic L-amino acid decarboxylase, dopamine receptor, and dopamine in exosome biogenesis and during tick-borne flavivirus infection

Biswajit Bhowmick, Girish Neelakanta, and Hameeda Sultana

Dopamine signaling via the dopamine receptor 1 (DR1, a G protein-coupled receptor), triggers essential cAMP cascades crucial for insect antiviral defense. Aromatic L-amino acid decarboxylase (ADDC), an enzyme in insects, facilitates dopamine synthesis. In this study using the medically important vector tick *Ixodes scapularis*, we investigated whether introducing exogenous L-DOPA (precursor of dopamine) and dopamine hydrochloride (DHCL), as well as tick blood-feeding and Langat Virus (LGTV) infection, could enhance DR1 and ADDC activity, consequently boosting dopamine production and the release of extracellular vesicles (EVs). Our data revealed the presence of both DR1 and AADC in tick EV samples. Furthermore, we observed that both DR1 and ADDC genes were down-regulated during LGTV infection, and their silencing led to down-regulation, indicating the host's defense response against LGTV infection. In vivo analysis indicated that the knockdown of both genes reduced blood intake by ticks. Quantitative measurement suggested decreased dopamine levels during LGTV infection, implying hindered dopamine metabolism due to tick-borne viral infection. These findings could potentially pave the way for innovative strategies aimed at disrupting tick feeding behavior, interfering with viral replication, and ultimately mitigating the transmission of tick-borne viral diseases.

## Cancer progression is impacted by modulating a feature of glycolytic metabolism

Nicholas Britt, Matthew Vander Heiden, and Anna Barbeau

Pyruvate kinase (PK) is the key enzyme involved in the conversion of phosphoenolpyruvate to pyruvate during the final step of glycolysis. The PK isoforms PKM1 and PKM2 have both been shown to support tumor growth in a context and tumor-specific manner. PKM1, the high activity isoform, supports the energetic needs of the cell, while PKM2, the low activity isoform, contributes to cell growth through diversion of glycolytic pathway intermediates towards biosynthesis of nucleotides and amino acids. Pancreatic ductal adenocarcinoma (PDAC) is one tumor type out of numerous that highly expresses PKM2. However, previous work has shown that pancreas-specific knockout of Pkm2 in the KP-/-C mouse model of PDAC (KrasG12D;Tp53fl/fl;Pdx1-Cre;Pkm2fl/fl) does not slow tumor growth and leads to an upregulation of Pkm1. Here, we aim to assess how loss of both Pkm1 and Pkm2 influences PDAC progression and survival in the KP-/-C mouse model. We demonstrate that Pkm1 and Pkm2 knockout in PDAC cells significantly extends survival of KP-/-C mice. Although there are no differences in tumor size at their humane endpoint, there is a significant reduction of tumor size in Pkmfl/fl mice assessed at an earlier timepoint. These data lend insight into the metabolic dependencies of PDAC tumors and may indicate a potential avenue in targeting metabolic pathways to potentiate tumor therapy success.

## Clinical effect of multidose oral administration of firocoxib and *t*-TUCB alone and in combination for the treatment of osteoarthritis in horses

Chessa Brown, Alexandra Carlson, Elizabeth Collar, and Melissa Hines

Osteoarthritis is a degenerative and debilitating joint disease. Development of effective new drugs without adverse side effects is important for improving animal welfare. Soluble epoxide hydrolase (sEH) inhibitors represent an alternative method of pharmaceutical pain control that could be used alone or synergistically with nonsteroidal anti-inflammatory drugs (NSAIDs). The objective of the study was to evaluate the clinical effect of the sEH inhibitor, *trans*-4-{4-[3-(4-trifluoro-methoxy-phenyl)-ureido]-cyclohexyloxy}-benzoic acid (*t*-TUCB), compared to the NSAID, firocoxib, in horses with osteoarthritis. A 3-way crossover study (n=6) was performed with 10 days each of oral administration of 1) *t*-TUCB (500mg), 2) firocoxib (57mg), and 3) *t*-TUCB/firocoxib (500 mg/28.5 mg). Lameness evaluations utilizing a body-mounted inertial sensor system (Equinosis Q™) and gastroscope exams utilizing the Equine Gastric Ulcer Council grading system were conducted before and following treatment periods. Daily activity was monitored continuously using tri-axial accelerometers. Daily activity and gastric grades did not differ between or within treatment. Lameness grades significantly improved from baseline across all treatments (p<0.001). Treatment with *t*-TUCB and firocoxib alone improved lameness grades (p=0.0022, p=0.0128). Firocoxib combined with *t*-TUCB did not significantly improve lameness grades from baseline (p=0.0627). Lameness grades remained improved from baseline two weeks following discontinuation of *t*-TUCB alone (p=0.01). Lameness grades worsened two weeks following treatment discontinuation with firocoxib alone (p=0.0084). Horses did not consistently return to pre-study lameness grades for all treatment periods. No adverse events occurred. The sEH inhibitor, *t*-TUCB, is a promising novel analgesic for horses with osteoarthritis when used alone or synergistically with NSAIDs.

## Cholecystitis Secondary to *Hammondia* spp. Tachyzoite Infection

Skyler Caldwell, Eliza Baker, Richard Gerhold, Chunlei Su, Lisa Neufang, Nora Springer, Jake Salzman, Jillian Smith, and Ashley Hartley

Background: Biliary protozoal infections are rare in dogs. Sarcocystidae protozoal infections (i.e. *Toxoplasma*, *Neospora*, *Hammondia* spp., etc.) are uncommon and host-specific. PCR amplification and genetic sequencing provide techniques to expedite diagnosis and speciation in clinical protozoal infections. Objectives: To characterize a series of canine protozoal cholecystitis cases. Animals: Two dogs with biliary protozoal infections consistent with *Hammondia* spp. Methods: Limited case series of two dogs presenting with mixed hepatopathies and hyperbilirubinemia. Biliary tachyzoites were identified via cholecystocentesis cytology during diagnostic investigations. Bile aerobic and anaerobic culture, Sarcocystidae serology, and protozoal gene PCR amplification were completed. Results: Crescent-shaped protozoal organisms (4-5µm x 0.5µm) consistent with tachyzoites were identified on bile cytology in both dogs. Bile aerobic and anaerobic cultures would have no growth. Sera were positive for anti-*Toxoplasma* sp. (n=1/2) and anti-*Neospora* sp. (n=2/2) antibodies. Genomic isolation from bile followed by pan-Sarcocystidae (18S rRNA) PCR amplification were consistent with *Hammondia* spp. Gene-specific amplification and sequencing to discriminate *Hammondia triffittae* (Ht) versus *Hammondia heydorni* (Hh) were similar but not all identical for reported cytochrome b (99.7% Ht vs. 98.9% Hh; 99.4% Ht vs. 99.1% Hh), alpha tubulin (98.7% Ht vs. 97% Hh; 97.9% Ht vs. 100% Hh), and LSU rRNA (99.3% Ht vs. 100% Hh; 99.3% Ht vs. 100% Hh) genes in the two samples. Conclusions and clinical importance: Molecular techniques are key adjunctive techniques for protozoal identification in rarer infections. Sequence data suggests the infective *Hammondia* spp. strains are closely related but different from reported *H. triffittae* and *H. heydorni* strains.

## Retrospective evaluation of squamous cell carcinoma in non-domestic felids

Madison Callicott and Denae LoBato

Squamous cell carcinoma (SCC), and its association with feline papillomavirus and/or UV light exposure, is well-documented in domestic cats, but little has been reported in non-domestic felids. Here we describe 14 cases of SCC diagnosed in non-domestic felids at the University of Tennessee College of Veterinary Medicine. Animals most commonly initially presented for evaluation of a primary tumor (12/14), with 2/14 cases identified only at necropsy. Affected species included tigers (9/14), lions (4/14), and a snow leopard (1/14). The average age affected was 16.6 years. No gender predisposition was identified. Cutaneous tumors arose from the ventral abdomen (4/15), eyelid (2/15), tail (1/15), digit (1/15), hindlimb (1/15), and pinna (1/15); additional primary tumors arose from the oral mucosa (3/15), tongue (1/15), and lung (1/15). Cutaneous tumors were typically ulcerated (13/15). Common histologic features were keratin formation (16/16), intratumoral inflammation (15/16), and a scirrhous response (13/16). Metastasis was identified in 6/14 cases, affecting the lymph nodes (5/6), lungs (3/6), liver (3/6), small intestines (2/6), spleen (1/6), adrenal gland (1/6), kidney (1/6), stomach (1/6), and peritoneum (1/6). Average survival time after diagnosis was 7.4 months; the most common cause of death was euthanasia due to local and/or metastatic SCC (8/14). Papillomavirus was not detected by PCR on formalin-fixed tissue in any sample (0/19 samples; 0/14 cases). SCC is important to consider in non-domestic felids due to its negative impact on lifespan. Unlike in domestic cats, papillomavirus infection and UV light exposure do not appear to be predisposing factors in non-domestic felids.

## Evaluation of soluble epoxide hydrolase inhibitor in an in vitro osteoarthritis model with equine synovial fluid derived mesenchymal stem cells

Alexandra Carlson, Elizabeth Collar, and Madhu Dhar

Synovial fluid-derived mesenchymal stem cells (sMSCs) and soluble epoxide hydrolase inhibitors (sEHi) are novel therapies with evidence they may individually provide disease-modifying effects for the treatment of osteoarthritis. An in-vitro model of osteoarthritis was utilized to evaluate the effect of an sEHi (*t*-TUCB). Equine sMSCs were cultured (n=3) and treated with one of five primers: phosphate buffered saline (PBS), dimethyl sulfoxide (DMSO), *t*-TUCB at 200 ng/ml, 1 ug/ml, or 4 ug/ml. Primed cells were induced into inflammation with interleukin 1 $\beta$  (10 ng/ml) or non-inflammation with PBS (0.01%), and then treated with one of six treatments: PBS, DMSO, phenylbutazone (2 ug/ml), *t*-TUCB at 200 ng/ml, 1 ug/ml, or 4 ug/ml. Cells were evaluated for viability using a Cell Proliferation Assay (MTS). Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) was measured in culture supernatant. Inflammation reduced sMSC viability and increased PGE<sub>2</sub> levels (p<0.0001). Under inflammatory conditions, viability of sMSCs was greater when cells were primed with *t*-TUCB at 200 ng/ml, 1 ug/ml, or 4 ug/ml and/or treated with phenylbutazone, *t*-TUCB at 1 ug/ml, or 4 ug/ml compared to PBS and DMSO (p<0.01). Viability of sMSCs did not differ between primers or treatments under non-inflammatory conditions. Prostaglandin E<sub>2</sub> levels were lower in both inflammatory and non-inflammatory conditions following treatment with phenylbutazone compared to all other treatments except *t*-TUCB at 4 ug/ml under inflammatory conditions (p<0.02). Treatment with *t*-TUCB at 4 ug/ml reduced PGE<sub>2</sub> levels compared to the DMSO vehicle-control (p<0.02). Data suggests that the combination of *t*-TUCB with sMSCs may be beneficial as a potential disease-modifying approach to osteoarthritis treatment.



## Lignin derived carbon quantum dots as a novel material for promoting mesenchymal stem cell proliferation

Eli Christoph, David Harper, Madhu Dhar, David Keffer, Steven Newby, Lu Yu, Michael Orsini, and Jakob Scroggins

Carbon quantum dots (CQDs) have been investigated for biomedical applications recently in medical imaging due to their fluorescent properties, overall long-term stability, and excellent biocompatibility. Further uses of CQDs have begun to be investigated by adhering citric acid-derived CQDs onto a substrate and examining cellular responses to CQDs. What has not yet been explored is the impact of CQDs, derived from lignin, on stem cell migration, proliferation, and differentiation. The ability to control the ultimate fate of stem cells through nanoscale patterning of the scaffolding surface remains an outstanding challenge in the field of regenerative medicine. A novel approach to that challenge was carried out in this study, in which lignin derived CQDs were synthesized, characterized, and assessed on their ability to stimulate human mesenchymal stem cell survivability, attachment, and proliferation. It was observed that lignin derived CQDs had good cytocompatibility and promoted cell attachment and proliferation after 24 hours and 7 days.

## Geographic disparities and temporal changes of diabetes-related mortality risks in Florida: An ecological study

Nirmalendu Deb Nath and Agricola Odoi

Background: Diabetes is a growing public health problem in the United States. Although risk of deaths related to diabetes has been increasing in recent years in Florida, little is known about the geographical disparities of diabetes-related mortality risk (DRMR) and yet this information is important for guiding control efforts to reduce disease burden. Therefore, the aim of this study was to investigate the geographic disparities and temporal changes of DRMR in Florida. Method: Mortality data were obtained from the Florida Department of Health. Tenth International Classification of Disease codes E10-E14 were used to identify diabetes-related deaths. County-level mortality risks were computed and presented as number of deaths per 100,000 persons. Spatial Empirical Bayesian smoothing was performed to adjust for spatial autocorrelation and the small number problem. High mortality risk clusters were identified using Tango's flexible spatial scan statistics. County-level DRMR and clusters were displayed using ArcGIS while seasonal patterns were visualized in Excel. Results: A total of 54,684 deaths were reported during the study period. County-level DRMR varied geographically across the state, ranging from 12.6 to 81.1 deaths per 100,000 persons. High mortality risk spatial clusters were identified in the central and northern parts of the state. A seasonal pattern of DRMR was detected, with the highest risks occurring during winter. Conclusion: There is evidence of geographic disparities and seasonal patterns of DRMR in Florida. The findings are helpful in guiding allocation of resources to control the disease, reduce disparities, and improve population health.

### ***In vitro* feasibility of commercially-prepared canine whole blood and packed red blood cells as a source of xenotransfusion in swine**

**Victoria Diaz, Deanna Schaefer, Pierre-Yves Mulon, Luca Giori, Joe Smith, and Chiara Hampton**

Sourcing porcine blood for transfusion is difficult for multiple reasons, but canine blood may be a feasible source of xenotransfusion in miniature pigs. The primary objective of this study was to determine the frequency of incompatible crossmatch (CM), major (CMMa) and minor (CMMi), using canine whole blood or packed red blood cells (cPRBCs), and whole blood from miniature pigs via standard saline agglutination (SSA) method. A secondary objective was to determine the agreement between SSA and a quick slide (QS) method, which is easier to perform stall-side. Blood was sourced from 3 canine donors, 3 bags of DEA1.1 negative cPRBCs, three bags of DEA1.1 positive cPRBCs, and 7 miniature pigs. Sixty-three pairs of CM were performed. Incompatibility was defined as any macroscopic or microscopic agglutination or hemolysis. Mann Whitney U-test verified the effect of porcine blood group on compatibility with canine products. Kappa statistics tested the agreement between SSA and QS. Significance was set at  $< 0.05$ . Incompatibility was documented in 100% of both CMMa and CMMi via SSA and QS with all canine products tested. Agreement between methods and accuracy could not be calculated due to lack of compatible CM pairs, which also affected our ability to test the effects of porcine blood type on compatibility. Based on *in vitro* results, canine blood products are contraindicated for xenotransfusion in swine. Quick slide results should be interpreted with caution. Due to the clinical significance of incompatibility on major crossmatching in other species, *in vivo* studies pursuing canine blood sources are discouraged.

### ***In vitro* feasibility of commercially-prepared canine whole blood and packed red blood cells as a source of xenotransfusion in swine**

**Victoria Diaz, Deanna Schaefer, Pierre-Yves Mulon, Luca Giori, Joe Smith, and Chiara Hampton**

Sourcing porcine blood for transfusion is difficult for multiple reasons, but bovine blood may be a feasible source of xenotransfusion in swine. The primary objective of this study was to determine the frequency of incompatible crossmatch (CM), major (CMMa) and minor (CMMi), using bovine whole blood and whole blood from production pigs via standard saline agglutination (SSA) method. A secondary objective was to determine the agreement between SSA and a quick slide (QS) method. Blood was collected from 12 heifers (pooled by three into eight bags) and eight commercial-cross pigs. Sixty-four pairs of CM were performed. Incompatibility was defined as any macroscopic or microscopic agglutination or hemolysis. Mann Whitney U-test verified the effect of porcine blood group on compatibility with bovine blood. Kappa statistics tested the agreement between SSA and QS. Significance was set at  $P < 0.05$ . Agglutination occurred in 9.4% of CMMa and 100% of CMMi via SSA, and in 9.4% and 98.4%, respectively, via QS. Accuracy of QS for agglutination was 87.5% on CMMa and 98.4% on CMMi. Agreement between methods was fair ( $\kappa=0.36$ ) on CMMa but could not be calculated for CMMi due to lack of compatible pairs. Porcine blood type O ( $P=0.0107$ ) was more likely to be incompatible on CMMa compared to type A, with no effect on CMMi. Based on these results, bovine blood is a promising source of xenotransfusion in commercial swine. Quick slide cannot replace SSA in pre-transfusion testing. *In vivo* studies are warranted to elucidate the clinical significance of incompatibility on minor crossmatching.



### Point of care (POC) lactate meter field trial in farm animal species

Ryan Flynn, Pierre-Yves Mulon, Kati Houser, Jessica Garcia, and Joe Smith

Introduction: Plasma lactate concentration can provide useful information in our farm animal species. Increased levels of plasma lactate stem from several etiologies including poor perfusion in the body, dehydration, and hypovolemia. Practitioners benefit from devices that give quick and accurate plasma lactate concentrations for farm animal patients. Objective: Determine the relationship between lactate measurements from a human POC blood analyzer validated in the use of large animal species vs a human POC lactate meter in farm animal species. Methods: Blood samples were collected from farm animal patients which were presented to the University of Tennessee College of Veterinary Medicine (UTCVM) and needed a blood gas analysis to be run during any point while in hospital. Following sample collection, each sample was run on the device already validated in several large animal species. Using the residual blood, each sample was run on the human POC device that has not been studied in farm animal species. Data was then compiled and analyzed using a commercially available statistical software to statically compare the two devices and their respective readings. Results: Currently there have been a total of 70 samples. After statistical analysis, regression identified a relationship of  $Y = 1.178X + 0.2338$ . Bland-Altman analysis demonstrated a bias of  $0.4513 \pm 1.712$  mmol/L (95% limits of agreement: -2.904 to 3.806) when comparing the human POC device not studied in farm animal species compared to the other POC device. Conclusion: Practitioners should be aware of the bias and variation the human POC device has when reporting plasma lactate concentrations for farm animal species, which are elevated, compared to that of the already validated device.

### Pharmacokinetics of nalbuphine administered intravenously and subcutaneously in goats (*Capra aegagrus hircus*)

Jessica Garcia, Joe Smith, Makenna Hopson, David Minich, Rebeca Rahn, Meggan Graves, Geneviève Bussières, Pierre-Yves Mulon, Joan Bergman, Lisa Ebner, and Sherry Cox

Pain caused by routine surgical procedures and unforeseen disease is common in goats, yet there is a scarcity of pharmacokinetic data available on the use of opioids, like nalbuphine, in this species. The purpose of this study was to evaluate the pharmacokinetics (PK) of intravenously (IV) and subcutaneously (SC) administered nalbuphine in domestic goats. To do this, nalbuphine hydrochloride was administered at 0.8 mg/kg for both IV and SC administrations in six goats with a ten day wash out period in between sample collection periods. Eighteen plasma samples were collected over a 36-hour period then analyzed using reverse phase high-performance liquid chromatography (HPLC). PK parameters were analyzed using a compartmental model. After IV nalbuphine administration elimination rate constant, area under the plasma concentration time curve from time 0 to infinity ( $AUC_{0-\infty}$ ), maximum plasma drug concentration, and total body clearance were  $0.0024 \pm 0.0017$  ( $\text{min}^{-1}$ ),  $17311.01 \pm 7227.32$  ( $\text{min} \cdot \text{ng} \cdot \text{mL}^{-1}$ ),  $675.6 \pm 337.13$  ( $\text{ng} \cdot \text{mL}^{-1}$ ),  $1844.48 \pm 572.15$  ( $\text{mL} \cdot \text{min}^{-1}$ ), respectively. After SC nalbuphine administration elimination rate constant, area under the plasma concentration time curve from time 0 to infinity ( $AUC_{0-\infty}$ ), maximum plasma drug concentration, and total body clearance were  $0.0054 \pm 0.002$  ( $\text{min}^{-1}$ ),  $20826.5 \pm 14376.2$  ( $\text{min} \cdot \text{ng} \cdot \text{mL}^{-1}$ ),  $368.03 \pm 503.78$  ( $\text{ng} \cdot \text{mL}^{-1}$ ),  $1853.57 \pm 864.43$  ( $\text{mL} \cdot \text{min}^{-1}$ ), respectively. Nalbuphine may be a safe analgesic opioid option for goats in the future following pharmacodynamic investigation.

### Comparison of fecal storage methods on hormone metabolite concentrations in domestic cats

Bethany Garland, Amy Miller, Julie Barnes, and Lindsey Vansandt

Fecal steroid analysis is a widely used tool for non-invasive monitoring of health, physiology, and reproductive status of animals in both captive and field studies. As evaluation of these parameters must be performed in a laboratory, fecal samples must be stored and transported in a manner that prevents the degradation or alteration of hormonal metabolites. In this study, we evaluated how different storage temperatures for various durations prior to freezing affected fecal metabolite concentrations. 84 fecal samples from seven domestic cats were collected and divided into four treatment groups. Freezing immediately after collection at  $-20^{\circ}\text{C}$  is the gold standard method for fecal sample storage and served as our control treatment. Treatment B samples were kept at ambient temperature ( $20\text{-}22^{\circ}\text{C}$ ) for 48 hours and then frozen. Treatment C samples were refrigerated at  $4^{\circ}\text{C}$  for 48 hours and then frozen. Treatment D samples were refrigerated at  $4^{\circ}\text{C}$  for two weeks before freezing. All samples remained in  $-20^{\circ}\text{C}$  for at least two weeks before being dried in a lyophilizer and extracted. Cortisol, estrogen, progesterone, and testosterone concentrations of fecal extracts were quantified using enzyme immunoassays (EIAs). EIA testing is still in progress for testosterone and cortisol concentrations. Comparison of steroid values among each treatment is ongoing.

### Characterization of scleral rupture of canine globes following compression via mechanical testing unit

Jennifer Goldreich, Diane Hendrix, Pierre-Yves Mulon, and Daniel Ward

**Purpose.** To determine the load (Newtons) at failure of canine intact cadaveric eyes and to describe the anatomic location and length (mm) of the rupture following external compression. **Methods.** Enucleated cadaveric globes ( $n = 33$  dogs,  $n = 66$  globes) were subjected to an axial force impacting the cornea or equator using a commercially available Instron 5900 Series mechanical testing unit. Following rupture, eyes were inspected to document the anatomical site and length of rupture. **Results.** The average force necessary to induce scleral rupture was  $299 \pm 115$  N (63-538) or approximately  $1457 \pm 496$  mmHg (530-2653). Increasing body weight ( $P = 0.000088$ ) as well as cranial-to-caudal ( $P = 0.00024$  OD,  $P = 0.0053$  OS) and medial-to-lateral ( $P = 0.0002$  OD,  $P = 0.0062$  OS) globe diameter were associated with a higher compressive force necessary to induce rupture. Average length of scleral rupture was  $9.5 \pm 4.6$  mm (0.7-22) located  $7.5 \pm 5.3$  mm (0-25) posterior to the limbus at the most cranial extent. Rupture orientation in relation to the limbus was perpendicular ( $n = 36$ ), parallel ( $n = 14$ ), or other ( $n = 16$ ). Globe lateralization ( $P = 0.368$ ), sex ( $P = 0.083$ ), and age ( $P = 0.089$ ) did not have a significant influence on the force necessary to induce rupture. **Conclusions.** Following external compression, the canine globe frequently ruptures in a region approximating the equator extending one centimeter posteriorly. Traumatic ruptures may not be apparent on clinical examination, and occult scleral rupture must be considered in cases of blunt ocular trauma.

## Evaluating long-term systemic toxicity of graphene nanoparticles and xenogeneic human mesenchymal stem cells using a sciatic nerve defect rat model

Meaghan Harley-Troxell, Madhu Dhar, and Mohamed Abouelkhair

The overall goal of this research is to evaluate the biocompatibility of graphene oxide (GO) nanoparticles, and to prove it is a safe component to treat neural injuries. Towards this goal, this study aims to evaluate the long-term systemic toxicity of 0.25% graphene oxide (GO), and poly (lactic-co-glycolic acid) (PLGA) nerve guidance conduits (NGC) with xenogenic human mesenchymal stem cells (MSCs) when implanted in a sciatic nerve defect rat model for 6-months. We hypothesize that a PLGA/GO NGC +/- MSCs implanted in vivo, will express normal patterns and levels of T-cells, B-cells, natural killer (NK) cells, and macrophages throughout the 6-month study. The NGC was 3D printed using an extrusion-based technique. To prove our hypothesis, a 10-mm long sciatic nerve defect model was created in 8-10-week-old Lewis rats. Rats were randomly divided into 4 treatment groups. Group one was the autograft, which served as a positive control. Groups two through four consisted of 3D NGC conduits containing the polymer PLGA alone, PLGA/GO alone, and the PLGA/GO NGCs seeded with  $1 \times 10^6$  human MSCs. Tail blood specimens were collected pre-surgery, and at 24-hours, 11-days, and 2, 3, 5, and 6-months post-surgery. Hematological analyses for 8 different combinations of immune cell markers were carried out and evaluated. Results show that all NGCs exhibit cell levels equivalent to the autograft. Results, for the first time, demonstrate the lack of nano-toxicity of the graphene nanoparticles and any adverse effect due to the human MSCs.

## Prevalence of bacterial pathogens in ticks collected from deer in Tennessee

Matthew Katzmarek, Jeremy Turck, Jessie Richards, Heidi Wyrosdick, John Schaefer, Richard Gerhold, Hameeda Sultana, and Girish Neelakanta

Tick borne diseases pose both a health and economical risk to human and animal populations across the globe. These diseases account for more than 75% of vector-borne disease cases reported annually in the United States to the Centers for Disease Control and Prevention. The goal of this study is to determine the prevalence of *Rickettsia* sp. *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, and *Borrelia mayonii* in *Ixodes scapularis* ticks collected from deer in Tennessee. These pathogens are the agents for rocky mountain spotted fever, anaplasmosis, babesiosis, and Lyme disease, respectively. It is important to know the distribution of these pathogens to be able to better protect humans and animals alike. Twenty-one ticks were collected mid feeding from deer at the Oak Ridge deer hunt check station, Tennessee. From each deer, three ticks were collected. DNA extraction, qPCR, and sequencing was performed on these tick samples. Based on qPCR analysis, it is likely that most of the ticks are positive for *Rickettsia* sp. Ticks from deer 2 may be positive for *A. phagocytophilum*, and ticks collected from deer one through five may be positive for *B. burgdorferi*. Based on DNA Sequencing results, the ticks collected from deer one, four, five, and six, tested positive for *Rickettsia japonica*-like organism with a 95% match at the nucleotide sequence level. Further sequencing needs to be performed to confidently identify the pathogens in the other tick samples.

### Investigation of alopecia lesions in gray bats

Megan Kinsella, Ashleigh Cable, Emma Wilcox, and Richard Gerhold

Alopecia lesions in the endangered gray bat, *Myotis grisescens*, have been noted and documented but minimal literature exists on this process. This research aimed to establish prevalence, examine infection trends, and investigate potential pathogens. It was hypothesized that the alopecia lesions affecting gray bats are most prevalent among lactating or post-lactating females in summer months with potential connections to opportunistic infections. Samples were collected during three separate rounds from affected and control bats to perform cultures and look for ectoparasites. Prevalence and severity data were also analyzed. Alopecia lesions appear to be a significant disease process. Findings indicated overall higher prevalence in female bats than males. Alopecia also appears to be more severe in female gray bats than in male bats. Several potential pathogens were isolated from cultures that could be related to alopecia lesions. Further research is needed to better understand population impacts, pathogen causation, and the role of any additional factors in this disease process.

### Using natural remedies to reduce fungal (*Batrachochytrium salamandrivorans*) infection in salamanders

Kaitlyn Linney, Davis Carter, Carmen Merolle, Merrie Urban, Matthew Gray, and Debra Miller

*Batrachochytrium salamandrivorans* (*Bsal*), a fungal pathogen identified in 2013, is a causative agent of lethal chytridiomycosis in amphibians. The introduction of *Bsal* to Europe has devastated salamander populations, and it has the potential to spread worldwide. Previous experiments in our laboratory indicate that curcumin, a compound found in turmeric, demonstrates inhibitory and fungicidal effects on *Bsal* growth. Other experiments have shown that survival of salamanders exposed to *Bsal* is temperature dependent, with greater mortality and zoospore load seen when temperatures are closest to the optimum for *Bsal* growth (15°C). In order to determine the safety and efficacy of curcumin as a treatment for *Bsal* chytridiomycosis, *Notophthalmus viridescens* adults were collected and individually housed at two different temperatures (13°C and 20°C). Selected animals were exposed to a *Bsal* dose of  $5 \times 10^3$  zoospores/mL for 24 hours. Exposed animals at each temperature were assigned to one of three treatment groups: simultaneous treatment with 7 µg/mL curcumin, post-exposure (PE) treatment (3 days PE) with 7 µg/mL curcumin, or no fungicide treatment. Each animal was swabbed every 6 days to determine the presence of *Bsal* DNA via qPCR. Prevalence of *Bsal* detection in animals treated simultaneously with curcumin was lower than in both post-exposure treated and non-treated animals. Additionally, simultaneous treatment with curcumin reduced average infection load for animals at both temperatures (13°C and 20°C). Our results suggest that curcumin may be effective at preventing infection and reducing loads of *Bsal* in *N. viridescens* but may be less effective at treating animals with established infections.

## Role of *Rickettsia parkeri* membrane assembly protein in the interactions of this bacterium with mammalian and tick cells

Lichao Liu, Shahid Karim, Christopher Paddock, Hameeda Sultana, and Girish Neelakanta

*Rickettsia parkeri* is a gram-negative obligate intracellular bacterium that causes rickettsiosis in humans. This bacterium is primarily transmitted by Gulf coast tick *Amblyomma maculatum*. *Rickettsia parkeri* rickettsiosis has milder symptoms compared to Rocky Mountain spotted fever caused by *R. rickettsii*. Study on *R. parkeri* helps advancing the understanding of other spotted fever group rickettsial pathogens. This study is focused on a membrane assembly protein of *R. parkeri*. The infection dynamics was confirmed in multiple mammalian cell lines and a tick cell line. Immunoblotting and QRT-PCR analysis confirmed expression of *R. parkeri* membrane assembly protein and transcripts, respectively, at various conditions. A polyclonal antibody was generated against the epitope in the extracellular loop of the *R. parkeri* membrane assembly protein. ELISA assay showed specificity of this antibody binding to the peptide from *R. parkeri* membrane assembly protein. Furthermore, antibody-blocking experiments showed that bacterial growth was significantly affected in tick cells, HL60 cells, and mouse raw macrophages upon treatment with an antibody generated against *R. parkeri* membrane assembly protein. These results provide evidence that the membrane assembly protein is important for *R. parkeri* infection of mammalian and tick cells. Current research efforts are centered to delineate the mechanistic role of *R. parkeri* membrane assembly protein in interactions with mammalian and tick cells.

## Mapping the male and female rhesus monkey by computed tomography: Sexual dimorphism and aging-related pathology

Jessica Lynch, John Olson, George Schaaf, Brendan Johnson, and Mark Cline

The NIH expects that researchers will include sex as a biological variable when designing and reporting vertebrate animal studies. Implications of sexual dimorphic characteristics in research are not fully known, but are important in developing translational studies and increasing the rigor and reproducibility of experimental design. To date, there are few studies describing the secondary sex characteristics of male and female rhesus monkeys (*Macaca mulatta*). A few useful anatomical references for this species exist, but no computed tomography (CT) atlas is currently available. The creation of an atlas will allow for visualization of anatomic structures with important clinical and research implications. Documenting common aging pathologies will provide examples of age-associated lesions that might appear frequently on clinical CT scans. This study analyzed CT scans from 51 rhesus macaques, including 4 females and 47 males. Age at most recent evaluated scan ranged from 8.2 to 24.6 years. Axial, coronal and sagittal planes were assessed for each monkey. Any common pathologies associated with aging were documented. Relevant clinical history and pathology reports were compared to CT findings for each animal. The most common aging pathologies found in these animals were spondylosis deformans, osteoarthritis, and colonic diverticulosis, documented in 61%, 51%, and 49% of the cohort respectively. Characterized sexual dimorphisms include trachea length, skull thickness, craniofacial structure, and laryngeal air sac size. The full body axial CT scan of an adult male rhesus was annotated and is to be posted on the lab's website at a later date.

### Development of a liposomal amphotericin B induced lameness model in goats (*Capra aegagrus hircus*)

Grace Malla, Joe Smith, Jessica Garcia, Denae LoBato, and Pierre-Yves Mulon

The goat industry has experienced a steady rise in popularity in recent years, and as such, knowledge of caprine lameness behaviors is essential for animal welfare. Induced lameness models exist using the antifungal Amphotericin B (AB), however, these are typically shorter duration. The goal of this study was to assess lameness behaviors in goats free of lameness, and then again after inducing lameness through an intraarticular injection of liposomal Amphotericin B. Ten healthy 6-month-old Kiko-cross goats were used. The goats' pre-induction gait and baseline physiological characteristics were evaluated using tri-axial accelerometry, pedometers, and visual lameness scoring, as well as cortisol and fibrinogen blood analysis. A brief synovitis was induced through an intraarticular injection of liposomal AB to the right rear lateral distal interphalangeal joint. The lameness responses were assessed with the same implements at intervals of 6, 12, 24, 48, 72, and 96-hours post-injection. A statistically significant difference ( $P = 0.0129$ ) was found for pre-induction and post induction pedometer results ( $8628 \pm 2779$  steps pre vs  $2315 \pm 1809$  steps day 1 post induction). No changes in lameness scores were observed between pre-induction and post-induction. No statistically significant changes were noted in 24hr mean or sum results of the tri-axial accelerometer findings. No significant changes were noted for cortisol or fibrinogen concentrations compared to baseline. While step counts were significantly reduced, more analysis will be necessary for tri-axial accelerometry data. Due to the sustained release nature of liposomal AB, it may not be effective for lameness induction in this model.

### Blocking of arthropod transporter reduces *Anaplasma phagocytophilum* replication in *Haemaphysalis longicornis* tick

Prachi Namjoshi, Donald Lubembe, Hameeda Sultana, and Girish Neelakanta

*Haemaphysalis longicornis* (also known as the Asian longhorned tick) are parthenogenetic and are reported to be infected with several human pathogens, including spotted fever group *Rickettsia*, *Anaplasma*, *Borrelia*, and *Babesia* species. The infestations with these ticks are of medical and veterinary concern because of their ability to transmit multiple pathogens to a wide range of hosts including humans, pets, and livestock. *Ixodes scapularis* (also known as a deer tick) is known to transmit *A. phagocytophilum* (a causative agent of Human anaplasmosis) to human hosts. Our previous studies reported that *A. phagocytophilum* modulates *I. scapularis* Organic anion transporting polypeptides (IsOATP4056) and tryptophan pathway for its survival and transmission to vertebrate host. Antibody against the extra-cellular loop 6 (EL-6) of *I. scapularis* IsOATP4056 protein was previously reported by our laboratory to block the transmission of *A. phagocytophilum* from infected ticks to murine hosts and clear bacterial loads in *I. scapularis* ticks. In this study, we report that *H. longicornis* ticks encode and express OATPs. We also report a new method to generate *A. phagocytophilum*-infected *H. longicornis* ticks in vitro. QRT-PCR analysis revealed that *A. phagocytophilum* modulates expression of certain *H. longicornis* OATPs. Treatment of *A. phagocytophilum*-infected *H. longicornis* ticks with tryptophan metabolite, xanthurenic acid, modulates expression of certain OATPs in these ticks. We noted that *I. scapularis* EL-6 antibody efficiently cross-reacted with *H. longicornis* OATP. In addition, treatment with anti-EL6 antibody significantly affected *A. phagocytophilum* loads in *H. longicornis* ticks. Collectively, these results provide evidence that EL6 region of OATP could be considered as a promising candidate for the development of universal transmission blocking vaccine to target ticks and tick-borne pathogens.



### Modulation of macrophage cytokine response from tick proteins

Krittika Nandy, Daniel Sonenshine, Hameeda Sultana, and Girish Neelakanta

Ticks secrete a salivary cocktail of immunomodulatory molecules into the host and around the feeding site that facilitates prolonged periods of blood feeding. Some of these secreted molecules modulate immune responses at the bite site facilitating tick feeding. In this study, we measured cytokines secreted by murine RAW macrophages upon treatment with salivary gland lysates generated from unfed or fed *O. turicata americanus* ticks. The cytokine array results showed that IGFBP-3 protein and transcripts were significantly upregulated upon treatment of RAW macrophages with salivary gland lysates generated from fed ticks compared to the levels noted in cells treated with salivary gland lysates generated from unfed ticks. IGFBP-3 is a multifunctional protein that is found to play a variety of roles in circulation, in the extracellular environment and inside the cell. One of the ways in which IGFBP-3 can exert its actions on target cells is by inducing apoptosis or programmed cell death. We hypothesized that ticks secrete molecules in their saliva to induce IGFBP-3 leading to apoptosis of the immune cells at the tick bite site. Microscopic studies, MTT, Live/Dead and QRT-PCR assays indicates that IGFBP-3 is implicated in the apoptosis response observed in the raw macrophage cultures treated with the tick lysates generated from fed ticks. Current experiments are planned to identify tick molecule(s) that induces IGFBP-3-mediated apoptotic response in these cells. Collectively, these results indicate that during blood feeding ticks secrete molecules to modulate cytokine responses at the bite site.

### Proof-of-concept of a newly developed rapid, stall-side portable assay for the measurement of equine adrenocorticotropin hormone

Lisa Neufang, Joseph Ramos, Luca Giori, and Shigetoshi Eda

Background: Pituitary pars intermedia dysfunction (PPID) is a neurodegenerative disease of senior horses. Loss of dopaminergic inhibition on the melanotropes of the pars intermedia leads to increased concentrations of proopiomelanocortin (POMC)-derived peptides. Diagnosis is challenging due to pre-analytical variables. Recent studies in human medicine, indicate that the Immulite 2000 detects and measures not only ACTH, but also other POMC-derived peptides. Objectives: Develop an assay that could be performed under field conditions with similar sensitivity and specificity to the current gold standard Immulite 2000. Methods: Using capture ELISA, two ACTH-specific monoclonal antibodies, CBL57 and EPR20361-248, were selected based on the recognition of separate epitopes, strong and rapid color change, minimal background interference, and no cross-reactivity. CBL57 serves as the solid surface for capturing and enriching ACTH from plasma. Biotin-conjugated EPR20361-248 served as the detection antibody. The concentrations of probes, dilutions and volumes of plasma, and incubation durations were optimized. Results: The immunoassay detected unglycosylated human recombinant ACTH. However, the assay did not detect ACTH using plasma from positive PPID equine samples, as determined by the Immulite 2000. Conclusions: The assay detects unglycosylated human recombinant ACTH, but not equine ACTH. Further studies are ongoing to identify 1) possible plasma matrix interferents, 2) determine whether the Immulite 2000 detects POMC-derived peptides in equine plasma, similar to people, and 3) whether the assay antibodies are unable to detect glycosylated forms of ACTH. This information would aid in optimization of the assay to be used in the for rapid and accurate identification of PPID-affected animals.

## Tetraspanins mediate flaviviral infection in mosquitoes

Durga Neupane, Waqas Ahmed, Girish Neelakanta, and Hameeda Sultana

Tetraspanins are a vast superfamily of membrane proteins with four transmembrane domains (TMs), a large extracellular domain (EC2) and a small extracellular domain (EC1). Tetraspanins are evolutionarily conserved proteins with over thirty-seven members in *Drosophila* and thirty members found in humans with homologues that are conserved through distantly related species including fungi, insects, and sponges. These proteins are crucial for coordinating a variety of cell-cell, matrix-cell interactions as well they play essential roles in regulation of different cellular processes such as signal transduction, vesicular trafficking, exosome biogenesis, cell adhesion, differentiation, and motility. Additionally, studies have shown that tetraspanins are found to be involved in various pathological conditions. We identified several tetraspanin members in mosquitoes, the medically important vectors. The redundant presence of tetraspanins promoted us to hypothesize that these molecules are perhaps involved in the transmission and pathology of flaviviral infections in mosquitoes. We studied both ZIKA virus (ZIKV) and dengue virus (serotype 2, DENV2), which have extensive global burden causing severe and potentially fatal disease ranging from various congenital anomalies to hemorrhagic fevers and ultimate death. Among several identified tetraspanins, we selected seven for this study. Our QRT-PCR and immunoblotting analysis showed that mosquito tetraspanin CD151 is highly upregulated in both ZIKV, and DENV2-infected cells and exosomes derived from these infected cells. In addition, our RNAi mediated silencing of CD151 resulted in reduced ZIKV and DENV2 loads in both cells and exosomes. Moreover, treatment with GW4869 known as exosome release inhibitor affected both ZIKV and DENV2 loads, thus suggesting a crucial role for CD151 in exosome-mediated viral transmission and pathogenesis. Overall, our study suggests CD151 as a potential therapeutic candidate for blocking mosquito-borne transmission of flaviviral infections to vertebrate host including human.

## Effect of immunization with a recombinant *Leptospira* chemotaxis protein in C3H/HeJ mice

Liana Nunes Barbosa, Cheri Bonnell, and Sreekumari Rajeev

**Introduction.** Leptospirosis is an important zoonosis with estimates of more than 1 million of new confirmed human cases a year. Commercially available vaccines cannot protect animals from all pathogenic *Leptospira*, do not induce long-term protection and are not available for humans, reinforcing the need of a new effective and safe vaccine. The goal of this study was to test the effects of immunization with a recombinant *Leptospira* chemotaxis protein in C3H/HeJ mice. **Methods.** C3H/HeJ mice were immunized three times with *Leptospira* recombinant chemotaxis protein. Non-immunized and mice immunized with Phosphate Buffered Solution were kept as controls. Two weeks following the last immunization, the animals were challenged with a lethal dose of *Leptospira*, and were monitored daily for clinical signs of leptospirosis. Humoral immune response elicited by protein was measured by indirect ELISA. **Results and discussion.** Mice immunized with a single dose of the vaccine containing *Leptospira* recombinant protein developed significant IgG antibody levels when compared to control groups ( $p \leq 0.001$ ). Sera from control groups did not present significant levels of antibodies, suggesting that the vaccine candidate induced a robust and specific humoral immune response in C3H/HeJ mice. The humoral immune response elicited by the vaccine may suggest a potential protective effect against lethal leptospirosis. **Partial conclusions and future perspectives.** The vaccine candidate elicited a significant humoral immune response in the C3H/HeJ mice. The seroreactivity against native protein in *Leptospira* extracts, protection against lethal acute infection and carrier status will be evaluated in the following steps of the present study.

## Evaluation of the analgesic efficacy of grapiprant compared to robenacoxib in cats undergoing elective ovariohysterectomy

Elizabeth Pisack, Stephanie Kleine, Chiara Hampton, Christopher Smith, Genevieve Bussieres, Reza Seddighi, Jennifer Weisent, Rebecca DeBolt, and Cambrie Schumacher

Objectives: The objective was to compare post-operative analgesic effects of grapiprant with robenacoxib in cats undergoing ovariohysterectomy. Methods: Thirty-seven female cats (4 months-10 years, weighing  $\geq 2.5$  kg) were enrolled in a prospective, randomized, masked, non-inferiority clinical trial. Cats received oral robenacoxib (1 mg /kg) or grapiprant (2 mg/kg), two hours prior to ovariohysterectomy. Analgesia was assessed via Feline Grimace Scale (FGS), Glasgow Composite Pain Scale-feline (GCPS-f), and von Frey monofilaments (vFF) at 2 hours prior to treatment administration, extubation, and T2, T4, T6, T8, T18, and T24 hours post-extubation. Hydromorphone ( $\pm$  12 hours post-operatively) or buprenorphine ( $\pm$  12 hours post-operatively) were administered to cats with scores of 5 on GCPS-f and/or 4 on FGS. Non-inferiority margins (NI) for GCPS-f and vFF were set at 3 and -0.2, respectively. A mixed-effect ANOVA was used for FGS ( $p < 0.05$ ). Data are reported as mean  $\pm$  SD. Results The data from 33 cats were analyzed. The upper limit of the 95% confidence interval (CI; 0.35) was less than NI of 3 for GCPS-f and the lower limit of the 95% CI (0.055) was greater than the NI of -0.2 for vFF, indicating non-inferiority of grapiprant. The FGS scores were higher than baseline at extubation for both treatments (1.65  $\pm$  0.63;  $p=0.001$ ), however there was no difference between treatments. There was no difference between treatments nor treatment by time for GCPS-f or vFF ( $p < 0.001$ ). Conclusions and relevance: These results support that grapiprant provided non-inferior analgesia compared to robenacoxib for treating post-operative pain associated with ovariohysterectomy in healthy cats.

## The role of organic anion transporting polypeptide in tick immunity and in the interactions with *Anaplasma phagocytophilum*

Mahesh Puthiyottu Poyil, Hameeda Sultana, and Girish Neelakanta

Our laboratory recently generated an antibody targeting the extracellular loop-6 (EL-6) of *Ixodes scapularis* Organic Anion Transporting Polypeptide-4056 (IsOATP4056). We reported that targeting IsOATP4056 with EL-6 antibody impairs transmission of *Anaplasma phagocytophilum* from ticks to the vertebrate host and significantly reduces the bacterial load both in ticks and tick cells. We noted that arthropod Toll pathway was activated both in ticks and tick cells upon treatment with EL-6 antibody. We performed an immunoprecipitation assay with tick lysate and EL-6 antibody and identified a 22kDa hypothetical protein (HP) as a possible interacting partner of IsOATP4056. The expression of HP was downregulated in *A. phagocytophilum* infected tick cells and upregulated when these cells were treated with EL-6 antibody or silenced for isoatp4056 expression. In addition, Pelle, a protein component of Toll pathway was downregulated upon HP silencing in tick cells. In addition, *A. phagocytophilum* load was increased when tick cells were silenced for HP expression. The GST co-precipitation assay revealed that GST tagged HP protein interacts with IsOATP4056. Furthermore, we noted that exogenous addition of tryptophan pathway metabolite, xanthurenic acid (XA), downregulated the expression of HP and upregulated isoatp4056 expression in tick cells. The electrophoretic mobility shift assay showed that the transcription factor Aryl Hydrocarbon Receptor (AhR, known to bind XA) binds isoatp4056 promoter and activates this gene. The presence of XA increased binding of AhR on isoatp4056 promoter. Collectively, these results elucidate that *A. phagocytophilum* modulates IsOATP4056 to inactivate HP-mediated activation of innate immune signaling for its survival in ticks.

## Diagnosing wildlife: The creation of immunoassays and necropsy protocols

Lindsey Rice, Jessie Richards, and Richard Gerhold

Diagnosing wildlife can be challenging due to lack of research, literature, sampling opportunity and funding, which can lead to unrecognized disease and parasite transmission amongst economically important species. Currently, there is a lack of antemortem testing for devastating parasites such as *Paralaphostrongylus tenuis* and *Elaeophora schneiderii* that can prove deadly for wild cervids and domestic ungulate species. These parasites have been linked to significant morbidity and mortality in susceptible populations of wild cervids and even post-mortem diagnosis is challenging in affected hosts. The same techniques used for the creation of an indirect ELISA for the detection of *P. tenuis* antibodies, was applied to the preliminary investigation into the creation of an indirect ELISA for the detection of *E. schneiderii* antibodies. Alongside ELISA testing, the importance of necropsy techniques with regard to postmortem diagnoses was analyzed through opportunistic wildlife submissions to the UTCVM Biobank. Through multifactorial testing and clinical presentation, we can better understand wildlife species from a herd health standpoint and how parasite transmission via these species can have an impact on economically important fauna.

## Black bear (*Ursus americanus*) population health monitoring in the southeast

Kathleen Riese, Richard Gerhold, Eliza Baker, and Monica Lee

Recent growth of the American black bear (*Ursus americanus*) population in the southeast raises concerns about the potential spread of density-dependent diseases among bears. However, research on the health of bears in this area is limited. We analyzed samples from 169 bears in the region. We performed Knotts tests, skin scrapes, and postmortem exams. We found that 69% (63 of 91) had microfilaria; genetic analysis revealed these microfilariae to be 85% similar to *Mansonella ozzardi*, a human filarial nematode. We identified *Ursicoptes* spp. mites on six skin scrapes (6.5%). Parasites found on histology or grossly during postmortem exams were morphologically identified; 16.2% (6/37) had *Baylisascaris transfuga*, 13.6% (3/22) had *Gongylonema* spp., 5.4% (2/37) had *Macracanthorhynchus ingens*, 2.7% (1/37) had *Physaloptera* spp., 2.7% (1/37) had *Dirofilaria immitis*, 5% (1/20) had *Trichinella* spp., 3.9% (1/26) had *Pelodera* spp., 3.6% (1/28) had *Sarcocystis* spp., and 3% (1/33) had *Angiostrongylus vasorum*. This research will lay the foundation for future research on bear diseases in the southeast and aid wildlife managers with management decisions on free-ranging black bear populations.

## The effect of butorphanol and dexmedetomidine on serum cortisol concentration

Jake Salzman, Shelly Olin, Alejandro Esteller-Vico, and Luca Giori

The adrenocorticotrophic hormone (ACTH) stimulation test is commonly used to assess the hypothalamic-pituitary-adrenal axis (HPA) in dogs with suspected hyper/hypoadrenocorticism. Dogs routinely receive sedation, including butorphanol, with or without dexmedetomidine, to decrease patient anxiety, minimize radiation exposure to personnel, and improve diagnostic yield. In healthy dogs, butorphanol (0.3 mg/kg IV) increases basal and post-ACTH cortisol concentrations by 90-minutes, but dexmedetomidine (4 µcg/kg IV) does not alter cortisol concentrations. The effect of using both drugs on the HPA, duration of drug effects on the HPA, and optimal amount of time in-between sedation and testing is unknown. It is clinically relevant to know if dogs that undergo sedation have an accurate same-day ACTH stimulation test. Therefore, the objective of this study is to assess the duration of impact on the HPA axis in healthy dogs caused by the administration of butorphanol, with or without dexmedetomidine, in comparison to a control group. In a pilot study of 3 healthy dogs, cortisol concentration returned to within 30% of baseline by 6 hours (Tp) following administration of butorphanol (0.3 mg/kg IV). Subsequently, 11 healthy beagles were included in a prospective, randomized, blinded, 3-period cross-over design study with a 1-week washout. Cortisol concentrations at baseline, Tp, and post stimulation test, were determined after butorphanol (0.3 mg/kg IV), butorphanol (0.3 mg/kg IV) and dexmedetomidine (5 µcg/kg IV), and 0.9% saline (0.5 mL IV). Data analysis will include descriptive statistics, mixed model analyses of variances, and Shapiro-Wilk test of normality and QQ plots with statistical analysis pending.

### ***Fusarium* spp.: The unseen threat to Florida's leatherback (*Dermochelys coriacea*) population**

Heather Smith, Samantha Kuschke, Jeanette Wyneken, and Debra Miller

All leatherback populations are considered endangered, but some isolated subpopulations of leatherbacks are at higher risk of extinction. One major challenge facing leatherbacks is poor hatchling success. The cause of low hatchling success in leatherbacks is likely multifactorial but a link between decreased hatching success and the presence of *Fusarium* spp. in the nest has been made in all 7 species of sea turtles. The *Fusarium solani* species complex is known to cause sea turtle egg fusariosis (STEF), which causes death in sea turtle eggs. The causative agents of STEF are also known to cause mycotic dermatitis in leatherback sea turtle neonates. Using fungal cultures of sand and skin samples from leatherback nests and neonates we aim to relate the detection of *Fusarium* spp. across these samples to the development of mycotic dermatitis in post hatchlings, emergence success, and incubation temperature. We found *Fusarium* spp. in 40% of nest sand samples at excavation and on the skin of 18% of apparently healthy post hatchlings. We identified a relationship between the development of mycotic dermatitis in post hatchlings and low nest emergence success. Additionally, we found that nests with *Fusarium* spp. detected in one or more samples spent more time above 32°C. We concluded that *Fusarium* spp. are present in leatherback sea turtle nests in Florida and are affecting neonatal leatherbacks even after emergence. These data provide initial information to begin assessing hatchling health and possibly elucidate a cause for decreased emergence success and survival in hatchlings incubated at higher temperatures.

### **A cranial approach to the elbow for osteophyte removal**

Stephanie Steuri, Darryl Millis, and Tim Chamberlain

Introduction: Currently described approaches to the cranial elbow joint are invasive and require tenotomies and osteotomies. While effective in gaining access, a limited cranial approach may result in less morbidity and faster return to function postoperatively. The purpose of this study was to develop a surgical approach to the cranial elbow that would provide appropriate exposure for osteophyte removal from arthritic elbows with restricted range of motion (ROM), while decreasing surgical trauma. Materials and Methods: 10 cadaveric canine forelimbs were used for anatomical dissection to develop a surgical approach to the cranial elbow joint. Results: An approach to the cranial elbow joint allowing access to the radial head and cranial ulna was achieved. A craniomedial skin incision was made extending from the distal humerus to the proximal radius and ulna. Dissection and retraction of surrounding muscles allowed visualization of the joint capsule. Larger vessels and nerves were isolated and preserved. A stab incision was made through the joint capsule, and manipulation of the limb allowed small rongeurs or a burr to enter the space and remove osteophytes. Discussion/Conclusions: This initial study suggested that it may be possible to access the cranial elbow joint through a cranial approach, enabling the removal of osteophytes to improve ROM. Additional studies, to assess ROM pre and post operatively will determine the value of this approach and its clinical applications in the treatment of elbow arthritis. To date, we have had some success in two clinical cases with severe arthritis and decreased ROM.

## Comparison of 2 jig types in the precision of torsional correction during distal femoral osteotomies for the correction of medial patellar luxation

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**Introduction:** The distal femoral osteotomy is a surgical technique used for the correction of patellar luxations, occurring secondary to significant structural femoral deformities. Previously, the use of the Slocum tibial plateau leveling osteotomy (TPLO)-jig during femoral osteotomy has been described, as it serves as a temporary stabilizer of the osteotomy site. More recently, the use of a novel deformity reduction device (DRD)- jig has been described by Panichi et. al, for use in surgical management of patellar luxation. The objective of the current study is to further investigate the use of the DRD jig in comparison to the Slocum-TPLO jig for correction of varying degrees of torsional deformities of the distal femur when a set valgus deformity around 15 degrees is present. We hypothesize that there will be no significant difference in the precision of correction of the torsional deformities between the two jigs.

**Materials and Methods:** 3-Dimensional printed femur models (n=60) were developed and a standard varus deformity of 15° was applied to the 3D reconstructed model. Next, 10, 20, or 30 degrees of external rotation were applied to the model, resulting in a representative 3D reconstruction for femurs included in each of the three groups. CORA methodology was used to obtain measurements and plan the procedure on a single representative model for each of the three deformity groups. The Slocum-TPLO jig or DRD-jig was applied to the assigned bone models, and a closing wedge osteotomy was performed. **Results:** Study in progress. Preliminary results will be presented.

## In vivo efficacy of vancomycin-loaded bone filler scaffold for prevention of osteomyelitis in a caprine model

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Osteomyelitis remains a frequent complication following orthopaedic surgery and traumatic bone injury. Biomaterials that act as local antibiotic delivery devices (LADDs) could be an advantageous alternative to traditional osteomyelitis treatment. In the present study, we evaluated the ability of a novel bone filler scaffold as a LADD for vancomycin (VANC) in a Staph. aureus contaminated caprine tibial defect model. 15 goats were divided into three treatment groups: vancomycin-treated scaffold only (n=4), scaffold and inoculated with Staphylococcus aureus (SC-SA; n=5), and vancomycin-loaded scaffold with S. aureus (SC-VANC-SA; n=6). A unicortical tibial defect was created surgically wherein a scaffold with or without vancomycin was implanted with or without a S. aureus inoculum, depending on treatment group. Over a 60-day period, all goats were scored for lameness; assessed via thermographic and fluorescent imaging; digital radiographic imaging; bacterial culture; and end-of-study CT imaging. No significant difference was noted between treatment groups in lameness severity or thermographic and fluorescent imaging. Radiographic analysis determined goats with vancomycin-treated scaffolds had significantly less evidence of osteomyelitis as compared with those without vancomycin. Mid- and end-of-study bacterial cultures indicated that S. aureus was reduced in the tissues of goats with vancomycin-treated scaffolds compared to the non-vancomycin treated, SA challenged group. End-of-study CT assessment found there was significantly less evidence of osteomyelitis in the vancomycin-scaffold only goats compared to the other two groups. Bone histology results are pending. Further research is needed to assess the potential of this scaffold for use as a LADD.



### Biomarker discovery in pregnant cattle infected with bovine viral diarrhea virus

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Placenta-derived exosomes are released from the trophoblast along with pregnancy associated glycoproteins (PAGs), and both can be indicators of fetal health. Exosomes are a subset of extracellular vesicles (EVs) that carry proteins, nucleic acids, and lipids and act as intercellular communication. Higher numbers of exosomes can be measured in pregnant females than in non-pregnant females, but it is unknown how pathology affects exosome content in pregnant cattle. Bovine viral diarrhea virus (BVDV) can invade the fetal compartment, causing disease in the fetus without the dam displaying symptoms. The objective of this study is to identify biomarkers in the blood of the dam that are associated with fetal BVDV infection. Nulliparous pregnant beef heifers were divided into 2 treatment groups, a PI group (heifers carrying a PI fetus, n=4), and a CTRL group (heifers carrying a non-infected fetus, n=4). Cattle were intranasally inoculated with BVDV-1b strain (BJ6) or sham media at 75 days of gestation. Whole blood was collected at 45, 95, and 120 days of gestation and plasma isolated. Using density gradient centrifugation, the exosome population was isolated from plasma and placental exosomes were further isolated by immunoprecipitation, using placental alkaline phosphatase (PLAP) conjugated dynabeads. Flow cytometry was used to confirm the presence of CD63+ and PLAP+ placenta-derived exosomes, which were then evaluated by proteomics and RNA extraction. Serum was used to determine PAG concentration using a commercially available ELISA. We expect that PAG concentrations and EV content will differ between heifers with healthy and BVDV infected fetuses.

### Anaplasma phagocytophilum modulates SHP-2/mTOR signaling for its survival in ticks

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*Anaplasma phagocytophilum* is the agent of human anaplasmosis. *Ixodes scapularis* and *Ixodes pacificus* ticks transmit this bacterium to humans. *Anaplasma phagocytophilum* is an obligate intracellular bacterium that employs several strategies for its survival in the host cells. Inside the host cells, this bacterium persists in a host-derived vacuolar structure called a morula. This vacuolar structure is crucial for *A. phagocytophilum* survival in host cells. Our study shows the impact of *A. phagocytophilum* infection on the modulation of cell signaling in the vector host. *A. phagocytophilum* causes a significant increase in SHP-2 tyrosine phosphatase and mTOR serine/threonine kinase transcript levels in unfed nymphal ticks and ISE6 tick cells. SHP-2 phosphatase helps regulate the PI3K pathway which leads to the activation of mTOR kinases. mTOR activation inhibits autophagosome formation and plays a role in autophagy regulation within the cell. The inhibition (with functional inhibitors) and genetic silencing (via RNAi) of SHP-2 and mTOR expression significantly increased *A. phagocytophilum* loads in ISE6 tick cells. We also noted differential expression of several autophagy markers that are linked to the initiation and maturation of the autophagosome in the vector host upon *A. phagocytophilum* infection. Taken together, our study provides evidence that *A. phagocytophilum* modulates SHP-2/mTOR signaling for its survival in the vector host.

### Cytokeratin expression by sarcomas does not indicate synovial origin

Annalisa Wager, Linden Craig, Cary Springer, and Rebecca Bergee

A previously accepted diagnostic tool for suspected synovial sarcomas was evaluation of cytokeratin expression; however, cytokeratin expression occurs in tumors not of synovial origin. This retrospective study consisted of analyzing 25 canine subcutaneous soft tissue sarcomas and 7 canine articular soft tissue sarcomas by immunohistochemistry for expression of cytokeratin. Additional case history including survival time, breed, weight, and sex was obtained for 19 subcutaneous sarcoma cases and 7 articular sarcoma cases. Eight of the 25 subcutaneous sarcomas (32%) expressed cytokeratin in 1-50% of the neoplastic cells, while 1 of the 7 articular sarcomas (14%) expressed cytokeratin. Through the use of the Kaplan-Meier test, the mean survival time of the dogs with subcutaneous sarcomas (28.1 months [CI:17.8,38.4]) did not significantly differ from survival of dogs with articular sarcomas (24.8 months [CI: 0.5, 29.0]). Additionally, the survival of dogs with cytokeratin expression in their sarcomas (22.0 months [CI: 8.4, 35.6]) did not differ significantly from those without cytokeratin (31.2 months [CI: 17.8,44.6]). Therefore, cytokeratin expression does not depend on synovial origin ( $p=0.64$ ) and neither the sarcoma location ( $p=0.76$ ) nor cytokeratin expression ( $p=0.53$ ) affects patient survival. The use of cytokeratin immunohistochemistry is not helpful to determine synovial origin or to predict the behavior of sarcomas in dogs.

### Exploring the role of childhood obesity in cancer: structural changes in adipose tissue stroma

Jessica Wakeman, Alyssa Miles, and Nora Springer

In the United States, childhood obesity has reached epidemic levels. With the increase in incidence of colorectal cancer correlating to childhood obesity, an understanding of the underlying mechanisms between obesity and adipose tissue is needed to develop preventive and interventional treatment modalities for cancer. It is known that in obesity, structural and biochemical changes occur in adipose stroma cells (ASCs) and extracellular matrix (ECM) that mimic the tumor microenvironment. Given this evidence, it is predicted that juvenile obese and aged adipose tissues will share features such as ASC cellular aging and ECM fibrosis; essentially, obesity will result in premature aging of ASCs, and this effect is irreversible with weight loss. ASCs in this study were isolated via a diet-induced obesity (DIO) mouse model consisting of the following cohorts: lean/low fat diet (LFD) juvenile mice, obese/high fat diet (HFD) mice, formerly obese mice, and lean aged mice. For each ASC line, immunofluorescence microscopy was used to assess myofibroblast differentiation, cellular proliferation by BrdU incorporation, and ECM composition by fibronectin, collagen I and VI immunostaining. Myofibroblast differentiation is increased in the HFD cell lines relative to lean, formerly obese, and aged cell lines. HFD cells were less proliferative than other cell lines. Data for ECM composition is pending analysis. The effects of obesity studied in this project appear to be reversible with weight loss. Aged lean ASCs were similar to lean and formerly obese ASCs isolated from younger mice.

## Early development and pre-clinical evaluation of a fluorine-18 labeled peptide p5+14 for PET/CT imaging of cardiac amyloidosis

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Background: Systemic amyloidosis is a rare protein misfolding disorder, where patients can present with varied symptoms due to the diverse organ involvement. Currently there are no FDA-approved methods that allow visualization of amyloidosis in patients and early diagnosis. Methods: Isotopic exchange chemistry using a silicon-fluoride-acceptor (SiFA) method was used to label peptide p5+14 with F-18. After standard purification of the product, radiochemical stability studies demonstrated that it was stable, with no significant loss of F-18 for at least 5 hours. Radiofluorinated p5+14 was assessed in bioactivity “pulldown” assays to ensure binding to synthetic AL amyloid-like fibrils and human AL amyloid extracts. The radiolabeled peptide was injected in the lateral tail vein of mice with severe systemic AA amyloidosis. After 1 h and 4 h mice were euthanized small animal PET/CT imaging performed and tissue biodistribution measurements were conducted using an automated gamma counter. Results: Using optimized synthesis conditions, the non-decay corrected radiochemical yield was  $55 \pm 4.5$ . The binding of  $^{18}\text{F}$ -p5+14 to synthetic AL amyloid like fibrils composed of a  $\lambda 6$  variable domain was 96.2%, similar to data using the radioiodinated peptide, and 87.2% to human AL amyloid extracts. In mice, accumulation of  $^{18}\text{F}$ -peptide in the liver and spleen, the organs with most AA amyloid deposits in this mouse model, was readily evident in PET images taken 1 h post injection. There was also evidence of hepatic clearance of the peptide, manifest as a prominent gall bladder in the images.

## Evaluation of a new platelet-rich plasma collection system

Greg Woo and Darryl Millis

Platelet-rich plasma (PRP) is increasingly used in small animal practice for the treatment of a variety of musculoskeletal conditions. Most sources state that there should be at least a 2 to 3-fold increase in platelet concentration in PRP as compared to those in peripheral whole blood (WB) with minimal presence of white blood cells (WBCs). The proposed Dechra PRP kit aims to yield PRP from canine whole blood using a total of 90 second centrifugation system, which is quicker than other commercially available systems. Eight healthy adult laboratory dogs were used in this study. Forty-five mL of whole blood was collected per dog to yield two samples of PRP. Cytology of WB and PRP samples were performed along with quantification of growth factors and cytokines (bFGF, TGF- $\beta$ 1, VEGF, PDGF-BB, IGF-2, IL-2, and TNF- $\alpha$ ). The PRP samples were hemolyzed with a 50.4% reduction of red blood cells compared to WB. WBC concentrated by mean of 5.7-fold between WB and PRP while platelet concentrated by average of 6.6-fold. PDGF-BB and IGF-2 were statistically different ( $p < 0.01$ ) between EDTA and ACD-A samples while VEGF and IL2 showed no difference (VEGF:  $p = 0.99$ ; IL2:  $p = 0.53$ ). The Dechra kit yielded higher platelet concentrations while WBC count showed similar results when compared to other kits. This study showed improved concentrations of platelets and VEGF compared to other commercially available systems. However, some caution is prudent because of the relatively high number of red blood cells and white blood cells in the PRP product.