

2018 UTCVM Research Day Abstracts

Presenter: Mohamed Abouelkhair

Authors: Mohamed A. Abouelkhair, David A. Bemis, Richard J. Giannone, Linda A. Frank, Stephen A. Kania

Title: Characterization of SpEX, a new candidate *Staphylococcus pseudintermedius* exotoxin

Abstract: Staphylococci have evolved numerous strategies to evade their hosts' immune systems. We analyzed *Staphylococcus pseudintermedius* secreted proteins using liquid chromatography mass spectrometry (LC-MS) guided by genomic data and identified a potential exotoxin, SpEX, that is conserved among *S. pseudintermedius* isolates. SpEX kills polymorphonuclear leukocytes (PMN) and peripheral blood mononuclear cells, binds to complement C5, inhibits complement activation and inhibits chemotaxis at sublethal concentrations. Thus, it may play an important role in suppression of innate and adaptive immunity. Molecular modeling was used to identify functional domains and an attenuated recombinant version of the protein was produced through amino acids substitutions at selected points. Attenuated SpEX had diminished cytotoxicity and antibody produced against it in dogs reduced the inhibitory effect of native SpEX on canine PMN chemotaxis and protected canine leukocytes from the toxic effects of the native recombinant protein. SpEX is being evaluated as a component in a vaccine designed to treat dogs with pyoderma.

Presenter: Sawsan Ammar

Authors: Sawsan Ammar, Janetta Kelly, Graham Hickling, Chunlei Su, and Richard Gerhold

Title: *Toxoplasma gondii* prevalence in various waterfowl species

Abstract: Toxoplasmosis is a zoonotic disease caused by the protozoan parasite *Toxoplasma gondii* which infects mammals and birds. We are investigating *T. gondii* prevalence in numerous wild game birds to better understand the parasite's host, geographical distribution and zoonotic potential. Waterfowl are hunted for human consumption and thus represent a potential risk factor for human infection with *T. gondii*. We collected 25 serum samples from various waterfowl species from eastern Tennessee and found a seroprevalence of 72% (n=18) using modified agglutination test (MAT). PCR was performed on 35 heart tissue samples (including the 25 samples tested serologically) for *T. gondii* and 25.7% (n=9) were PCR positive. Eight birds were positive by both MAT and PCR. Our results suggest that waterfowl may be important in the transmission of *T. gondii* to humans. Further research is needed to elucidate the transmission dynamics of *T. gondii* in waterfowl and other aquatic mammals and birds.

Presenter: Caroline Billings

Authors: Caroline Billings, Alexandru Biris, David Bemis, Sherry Cox, David Anderson

Title: The Effect of Porosity on Drug Elution from Novel Polymeric Devices

Abstract: Osteomyelitis is a major challenge in the successful recovery of patients suffering bone infections. However, current methods of antibiotic therapy are often insufficient. Utilizing local drug delivery devices for targeted antibiotic therapy offers advantages compared with systemic administration in osteomyelitis cases.

Unfortunately, currently available local delivery devices leave much to be desired. The aim of this study was to determine the effect of porosity on antimicrobial drug elution from a degradable bone regeneration scaffold composed of polyurethane, nano-hydroxyapatite and demineralized bone particles, in vitro. Four variations of the polymeric device were utilized, varying in porosity from 55 to 70%. Results indicated efficient elution of antimicrobial from the device, a positive correlation between drug elution and bioactivity of the drug but no significant difference between porosities within the range of 55 to 70%. The results of this study suggest that these bone regenerative devices may serve a dual role to deliver antibiotics within the porosity range of 55 to 70%. Effects of greater or lesser porosity need to be investigated independently.

Presenter: Austin Bow

Authors: Austin J Bow, Bailey K Jackson, Sara M. Howard, Hector F. Castro, Shawn E Bourdo, David E Anderson, Alexandru S Biris, Madhu S Dhar

Title: Pathway Development for Effects of an Osteogenic Construct on Adipose-derived Human Mesenchymal Stem Cells through Metabolomic Analysis

Abstract: The use of biomaterials for regenerative medicine has been a widely pursued area of research; however, the precise mechanisms by which many of these materials operate is still largely unexplored. The field of metabolomics is rapidly emerging as a powerful analytical tool for studying pathway mechanics in biological systems through the characterization of small molecule concentrations. The interaction between human MSCs and a scaffold, designed for enhancing bone repair, was assessed to identify potential metabolic pathways effected. Samples were processed using liquid chromatography-mass spectrometry (LC-MS), and output analyzed with MAVEN software to obtain concentrations of identified metabolites. An online statistical analysis tool, Metaboanalyst, was then utilized to evaluate sample groups and generate graphical representations for data. Metabolites of interest (MoIs) determined through this assessment were used to construct metabolic building blocks (MBBs), directly related components within a metabolic pathway, using the KEGG database and relevant literature.

Presenter: Samantha Collins

Authors: Samantha Collins, Marc Caldwell

Title: Microenvironmental sampling techniques of the nasal cavity of cattle and experimental nasal colonization of *Mannheimia haemolytica*

Abstract: Cattle have a number of unique microenvironments within nasopharyngeal cavity that are known to harbor bacterial pathogens associated with the bovine

respiratory disease complex. However, to date there have been no culture independent studies to define the microbiomes within these sites. The purpose of this study was to characterize the microbiomes of the upper respiratory tract of post-weaned calves before and after experimental colonization with *Mannheimia haemolytica*. Qualitatively, calves in this study displayed consistent populations within the tonsillar crypts, cranial nasal cavity and nasopharyngeal area. Moreover, these populations were unique to each microenvironment. Introduction of *Mannheimia haemolytica* created a predominance of that species within the nasal cavity and nasopharyngeal cavity and was isolated from those sites in all of nine infected animals for an average of 4-10 days post inoculation. Animal origin, treatment group, and day of study showed no significant differences.

Presenter: Nicole Corder

Authors: Nicole Leanne Bidaud Corder, Jane Woodrow, Elizabeth M Lennon

Title: Mast Cells Exhibit Bone Morphogenetic Protein Signaling in Response to Inflammation

Abstract: Mast cells are innate immune cells most infamous for their roles in anaphylaxis and allergy. Recently, they have been recognized as orchestrators of anti-inflammatory responses. We have demonstrated that mast cells produce bone morphogenetic proteins (BMPs), cytokines in the TGF- β ² superfamily with anti-inflammatory and anti-fibrotic effects, making them attractive therapeutics for inflammatory bowel disease (IBD). The objective of this study was to determine stimuli that trigger upregulation of BMPs and elucidate downstream signaling pathways within mast cells. We hypothesized that inflammatory stimuli upregulate BMPs and that signaling occurs via the Smad1/5/8 pathway. Murine bone marrow derived mast cells (BMMCs) were used to characterize expression of BMPs and their signaling molecules in response to pro-inflammatory stimuli. BMP7 expression was 2.45-fold increased in BMMCs stimulated with lipopolysaccharide (LPS) and IgE versus control cells ($p=0.008$), and expression of phosphorylated Smad1/5/8 was elevated in the nucleus. These findings indicate that acute inflammatory stimulation of mast cells causes BMP upregulation and signal transduction through the Smad pathway.

Presenter: BreeAnna Dell

Authors: BreeAnna Dell, Adam Willcox, Richard Gerhold, Charles Masembe, Chika Okafor, Marcy Souza

Title: Species Deception in Bushmeat at Point of Sale and Perceptions of Zoonoses Potential in Nwoya District, Uganda

Abstract: In northern Uganda, consumption of primates and bats is perceived to be infrequent; however, hunters reportedly frequently butcher bushmeat into unidentifiable pieces to sell as more culturally accepted species. We conducted surveys of 301 female cooks and 181 hunters in Nwoya district between 2016 and 2017 and purchased 229 bushmeat samples and performed polymerase chain reaction (PCR) and sequencing. Cooks and hunters indicated primates and bats as highest risk, with

livestock and other wildlife as lower risk. 58%, 69.1%, and 63.5% of hunters reported hunting baboons, monkeys, and bats, respectively. Most hunters reported baboon (48.6%) and monkey (55.8%) meat being sold every week and 38.7% report bat meat being sold every week. In contrast, 79.7%, 83% and 87.7% of cooks believed that baboons, monkeys, and bats, respectively, were never available. 50.2% and 23.3% of cooks responded that primate meat is "never and sometimes disguised as another kind, while 95% of hunters responded that it is usually disguised. Preliminary molecular results disclosed a 28% mismatch between species reported and molecular identification of bushmeat samples.

Presenter: Vincent Doré

Authors: Vincent Doré, Derek M. Foster, Geof W. Smith

Title: Comparison of different resuscitation therapies for calves with neonatal diarrhea

Abstract: Producers and veterinarians are exposed to a large variety of on-farm resuscitation therapies. The objective was to compare four resuscitation therapies in calves. Osmotic diarrhea and dehydration were induced in 32 neonatal Holstein bull calves using a combination of diuretics and hyperosmolar milk replacer. Calves were randomly divided in four treatment groups: oral electrolyte solution (OES) (Diaque); OES + hypertonic saline (4 ml/kg, IV); intravenous LRS (2 liters), or subcutaneous LRS (2 liters). Treatment was administered at 0 and 12 hours. Changes in plasma volume, acid-base status, electrolyte concentrations and physical examination parameters were recorded for 24 hours. Increase in plasma volume was observed in all groups between time 0 and 2. The OES groups showed the greatest increase of blood pH and glucose. In conclusion, OES with or without hypertonic saline appeared to be an excellent option for resuscitation of calves with moderate diarrhea and acidosis.

Presenter: Naomi Falconnier

Authors: Naomi Falconnier, Sherry Cox, David Bemis, Kadum Alghazali, Alexandru Biris, David Anderson

Title: Effect of Hydrophilicity elution of Ceftiofur from various Polyurethanes Substrates

Abstract: Data regarding the effect of hydrophilicity on drug elution from polyurethanes are limited. We hypothesized that drug elution would be greater in magnitude and duration with increasing hydrophilicity. Hydrophilic PUs (hPUs: 1%, 60%, 90%) were selected and eluents collected at pre-determined time points. Eluents were analyzed for drug concentration (HPLC analysis) and bioactivity (disc diffusion using Staph. aureus). hPUs with 60% hydrophilicity eluted significantly greater concentrations for the longest period of time above MIC ($p < 0.01$). Interestingly, 90% hPUs eluted less drug and for a shorter period compared with 60% hPUs. Bioassay proved that eluted drug was active and bioactivity was consistent with the measured drug concentration. Hydrophilic PU polymers may be suitable for use in drug delivery devices to improve drug elution characteristics. Future studies include determination of

drug loading capacity, effect of a range of hydrophilicity, and validation using in vitro and in vivo models of bacterial infection.

Presenter: Trey Fisher

Authors: Richard K. Fisher, Samuel I. Mattern-Schain, Stacy S. Kirkpatrick, Michael D. Best, Raymond A. Dieter, Joshua D. Arnold, Michael R. Buckley, Michael M. McNally, Michael B. Freeman, Oscar H. Grandas, Deidra J.H. Mountain

Title: A scalable assembly method for siRNA-loaded Stealth liposomes

Abstract: PEGylated liposomes (PLPs) are promising drug delivery agents due to biocompatibility and pharmacokinetics. However, pitfalls in liposomal-siRNA assembly limit translational success. Cationic lipids incorporated to enhance siRNA delivery are associated with cytotoxicity and poor half-life. Surface-conjugated PEG improves pharmacokinetics, but hinders target cell delivery. PLPs equipped with cell-penetrating peptides (CPP-PLP) overcome PEG-induced hindrance without requiring cationic lipids. However, CPP-PLP assembly must be optimized for siRNA loading efficiency. Octaarginine was conjugated to stearic acid (STR-R8) and incorporated to PLPs for CPP-PLP formulations. Liposomes were characterized for size, surface charge, polydispersity index, and siRNA encapsulation efficiency (%EE). STR-R8 enhanced encapsulation in a concentration-dependent manner, with 10mol% achieving siRNA loading at ~75%EE. There was a synergistic increase in %EE when combining STR-R8 with Ca²⁺. Lipid:siRNA was positively correlated with encapsulation, with 20:1 achieving ~80%EE. EE% was inversely related to injection rate. The optimized parameters herein represent an efficient assembly method for siRNA-loaded CPP-PLPs.

Presenter: Remigiusz Grzeskowiak

Authors: Remigiusz Grzeskowiak, Rebecca Rifkin, Pierre-Yves Mulon, David Harper, Steve Adair, David Anderson

Title: In Vitro Biomechanical Testing of the Equine Metacarpophalangeal Collateral Ligaments and the Collateral Ligaments Repair using Suture Anchors

Abstract: Metacarpophalangeal collateral ligaments (CL) injuries in horses result in guarded to poor prognosis for their athletic performance due to the secondary osteoarthritis in the metacarpophalangeal joint. The current treatment of choice is a conservative therapy including a limb immobilization with the fiberglass cast. This study introduces a novel surgical technique using the suture anchors to provide additional stabilization of the joint after trauma. The 4-point bending test of the intact collateral ligament (control group) was performed and the following parameters were recorded: force at maximum load (8.54 kN), and maximal flexure displacement (8.99 mm). The test resulted in the ligaments' tear as well as proximal phalanx failure. The treatment group consisted of the completely severed CLs which were surgically repaired with four different types of suture anchors. The surgical repairs with the anchors underwent then the same test obtaining an average of 14% of the intact CLs force at maximum load and 95% of the CLs maximal flexure displacement. Furthermore, the method of the anchor

failure was also described. The suture anchors held only 14% of the intact ligaments strength, however under clinical circumstances the anchors will not be exposed to such a high amount of force like during the in vitro tests because of the surrounding soft tissue additional stabilization. The conclusion of the study is that horses may benefit from additional joint stabilization with the suture anchors and the procedure is relatively quick and easy to perform.

Presenter: Ashley Hand

Authors: Ashley Hand, Dan Su

Title: The impact of food-dispensing toys on the activity level of dogs

Abstract: Canine obesity is the most common nutritional disorder seen in dogs in the United States, affecting 59% of the population. This study's goal was to determine if using food-dispensing toys instead of food bowls impacted a dog's activity level and could therefore be implemented into a weight loss program. It was hypothesized that using food-dispensing toys would increase a dog's activity level, because they transform a sedentary part of the dog's day into an active period. This study used a 2-way, 2 period, and repeated measures mixed-effects crossover design. The dogs in the study were client owned and housed primarily indoors. For the duration of the study, dogs wore an activity monitor to track how walking, running, and total activity were impacted. It was found that using food-dispensing toys significantly increased the dogs' total activity. Additionally, it was found that age impacted their total activity level regardless of the treatment.

Presenter: Wendy Harmon

Authors: Wendy A. Harmon

Title: The Impossible Salamander: Aberrant Coloration as a Result of Metal Toxicity, Crypsis, or Light Exposure?

Abstract: The Buck Creek Serpentine Barrens in Clay County, North Carolina is an unusual habitat comprised of a pine savannah with endemic plant species underlain by serpentinite rock. The area is drained by a stream with a mostly open canopy. Most *Desmognathus monticola* (Seal Salamanders) living in the Barrens stream have bright yellow patches on their skin, although this population is genetically similar to normally-colored populations of *D. monticola*. My research explored whether this unusual coloration was due to: 1) trace metals from the serpentinite rock, 2) phenotypic plasticity for crypsis against the lightly colored serpentine rock on the stream bottom, or 3) excessive light exposure and decreased shade. I found that metals were unlikely to cause the yellow coloration. Moreover, there were no differences in white blood cell counts or liver mass between Barrens and control populations, suggesting that there was no ongoing immunological response to or accumulation of toxic metals. The Barrens salamanders did have less epithelial pigment than control salamanders. Salamanders from both Barrens and control populations became more yellow in every crypsis lab experiment, whether exposed to light, dark, yellow, or red backgrounds. I

also found that the yellow coloration of salamanders was affected by salamander population source, light level they were exposed to, and calcium concentration, but luminosity (brightness) of the salamanders was not affected by any factors in that experiment. Understanding the environmental stimuli that induce this morphological change in these salamanders' integument, and if these morphological changes are unique to this population, will help to shed insight upon a unique part of North Carolina's landscape diversity.

Presenter: Conner Hayes

Authors: Conner Hayes, Geneviève Bussières

Title: Retrospective study on morbidity and mortality associated with general anesthesia in Potbellied-Pigs: Preliminary Results

Abstract: The purpose of this retrospective study was to describe mortality rate, complications and predisposing factors associated with general anesthesia in potbellied-pigs. Medical records of potbellied pigs undergoing anesthesia at UTCVM over three years (2015-2018) were reviewed. Data collection from records included per-anesthetic management, anesthetic agents, complications and outcome. All data were evaluated as predisposing factors to pre-identified complications. 87 records (44 males, 42 females) were analyzed with age ranging from 4 months to 15 years and weight, from 4.8 to 136 kg. The study identified specific complications to potbellied pigs and their incidence. Results demonstrate that hypothermia, hypotension and partial intravenous anesthesia significantly increased the number of complications and abdominal surgery significantly increased hypoventilation. Patient's age was positively correlated to duration of recovery. Obesity was not a predictor of mortality/complications. All patients survived anesthesia. Data collection is incomplete, but complications associated with general anesthesia in potbellied-pigs were identified.

Presenter: Katherine Hedges

Authors: Katherine Hedges, Adesola Odunayo, Josh Price, Silke Hecht, M. Katherine Tolbert

Title: Evaluation of the Efficacy of a Famotidine Continuous Rate Infusion in Healthy Dogs

Abstract: Famotidine administered intravenously as a continuous rate infusion (CRI) is used to treat upper gastrointestinal ulceration in dogs; however, the efficacy of this practice is unknown. To evaluate the efficacy of a famotidine CRI to increase intragastric pH in dogs, a randomized, crossover study was performed in which 9 dogs received 0.9% saline for 24 hours, followed by 1.0 mg/kg IV twice-daily famotidine or CRI famotidine at 8.0 mg/kg/day for 3 consecutive days. Intragastric pH was continuously monitored during all treatments. The clinically important parameters, mean percentage time (MPT) that intragastric pH were 3 and 4, were compared between groups using ANOVA. There was a dramatic difference ($p < 0.05$) in MPT 3 and 4 between the CRI and twice-daily groups for all treatment days. Only the famotidine CRI achieved pH goals for

treatment of acid-related disorders. Famotidine CRI may be an alternative to treatment with intravenous proton pump inhibitors in dogs.

Presenter: Niloofar Kazerooni

Authors: Niloofar Kazerooni, Aaron Bauman, Dianne Mawby, Melissa Kennedy

Title: Virucidal activity of select disinfectants used in veterinary practices

Abstract: Nosocomial infections are hospital acquired and can have serious consequences. This is true for veterinary as well as human medicine. Proper disinfection is critical to prevent nosocomial infections but can be difficult in a setting where animals are housed. The gold standard remains sodium hypochlorite (Clorox) but this product is very toxic and corrosive, and may evolve toxic gases upon contact with other cleaning products or urine. Newer disinfectants have as their active ingredient accelerated hydrogen peroxide, which is much less toxic and corrosive. The aim of this study was to evaluate the effectiveness of four different disinfectants commonly used in veterinary practices (Wysiwash, PREempt, REScue, and ProVet Logic Professional) against common veterinary viral pathogens of varying disinfectant susceptibility (CPV, FHV, FCV) and compare these with a Clorox control. Our investigation showed efficacy of some of these disinfectants approaching that of Clorox. Implications will be discussed

Presenter: Calvin Kidd

Authors: Calvin Kidd, Karen Tobias, Pierre-Yves Mulon

Title: Comparison of Suture Tensile Strengths

Abstract: Purpose: To compare baseline maximal load of two sizes (2-0, 3-0) of three midterm absorbable sutures (Monocryl, Securocryl, Biosyn) and three long-term absorbable sutures (Maxon, PDS*II, Securodox) and to compare loss of strength over time.

Materials and Methods: Maximal load of suture loops of each material and size were measured with an Instron unit at day 0 and after 7, 14, 21, or 28 days of incubation in sterile saline.

Results: At baseline, PDS*II (2-0 and 3-0), Securodox (2-0 and 3-0), and Securocryl (3-0) were significantly weaker than the other sutures. Most long-term absorbable sutures maintained strength over 28 days of incubation. Midterm absorbable sutures lost strength over time, although Biosyn maintained strength longer than Monocryl and Securocryl.

Conclusion: Tensile strength of suture materials varies significantly. Knowledge of maximal load at baseline and over time will facilitate selection of appropriate materials for surgery.

Presenter: Kristen Maxwell

Authors: Kristen Maxwell, Elizabeth Lennon

Title: Evaluation of fat-soluble vitamin status in dogs and cats with chronic enteropathy

Abstract: Chronic enteropathies such as inflammatory bowel disease (IBD) are becoming increasingly common. IBD is characterized by intestinal inflammation, loss of intestinal barrier function, and nutrient malabsorption. Fat-soluble vitamins dampen immune responses and promote immune tolerance. Deficiencies of these vitamins, which occur in humans with IBD, can exacerbate inflammation. Little research has been performed to assess fat-soluble vitamins in dogs and cats with IBD. In clinical patients with intestinal failure, fat-soluble vitamin supplementation is sometimes considered, but no evidence exists to support this practice. We hypothesize that, like humans, some pets with chronic enteropathy develop vitamin deficiencies. The purpose of this study was to measure blood concentrations of fat-soluble vitamins in pets with chronic enteropathy compared to healthy pets, using radioimmunoassay and ultraperformance liquid chromatography. Consistent with previous reports, cats with chronic enteropathy had decreased serum concentrations of 25-hydroxyvitamin D (67.7 ± 26.9 nmol/L) compared to healthy cats (172.4 ± 17.4 nmol/L; $p < 0.02$). Dogs with chronic enteropathy had decreased serum concentrations of retinyl palmitate (440.0 ± 146.8 ng/ml) compared to healthy dogs (1020.0 ± 180.8 ng/ml; $p < 0.05$). Some individual patients had markedly low concentrations of 25-hydroxyvitamin D and retinyl esters that were not apparent in analysis of the entire population. No differences in serum concentrations of α -tocopherol were identified. In conclusion, some patients with chronic enteropathy develop fat-soluble vitamin deficiencies that could exacerbate inflammation. Further studies are necessary to investigate the significance of these results and the impact of supplementation on inflammation.

Presenter: Jeremy McKeever

Authors: Jeremy McKeever, Jeanette Wyneken, Alexandra Lolavar, Ashley Reeves, Debra Miller

Title: Effects of Incubation Temperature on Intestinal Length in Loggerhead Sea Turtle (*Caretta caretta*) from Boca Raton, Florida.

Abstract: Incubation temperatures affect the sex, size, and survivability of hatchling marine turtles and rising ambient temperatures are being recorded at marine turtle nesting beaches. We evaluated the effect that incubation temperatures have on the intestinal length and length fraction available to absorb yolk sac nutrition in the immediate post-hatch period. We necropsied 41 loggerhead sea turtle (*Caretta caretta*) hatchlings incubated in situ, in relocated nests, or in artificial incubators from Boca Raton, Florida. The carapace length, total alimentary length, and intestine length were measured, and intestine length fraction was calculated. The mean temperature for the middle third of incubation duration was negatively correlated with intestine length. The mean temperature for the middle third of incubation and the maximum temperature experienced in the initial third of incubation were negatively correlated with intestine length fraction. This data provides a possible explanation for decreased survival in hatchlings incubated at higher temperatures.

Presenter: Megan Miller

Authors: Megan D. Miller, Stephen Kania, Richard W. Gerhold

Title: Serological Diagnosis of *Elaeophora schneideri* infection in moose (*Alces alces*)

Abstract: *Elaeophora schneideri* is a nematode which inhabits the arteries of its ruminant hosts. It is the etiological agent of elaeophorosis, a known cause of morbidity and mortality in moose. Moose numbers are declining in many states, and it is possible that *Elaeophora* is a factor. Available methods for diagnosing elaeophorosis require finding nematodes or arterial lesions in deceased moose. The lack of a diagnostic test has made the significance and geographical distribution of this parasite difficult to assess. This research is an investigation of antigenic potential of *Elaeophora* proteins to develop an enzyme-linked immunosorbent assay (ELISA) to detect anti-*Elaeophora* antibodies in moose serum. Genome sequencing and transcriptome analysis were effective in identifying two genes encoding peptides that are predicted to be immunogenic. Synthetic genes encoding these peptides were prepared and are being used to produce recombinant proteins. These proteins will be used in an ELISA to detect antibodies in moose.

Presenter: Steven Newby

Authors: Steven D. Newby, David Anderson, Madhu Dhar

Title: Evaluation of cell material interaction using decellularized bovine and porcine based scaffolds for bone tissue repair

Abstract: Autogenic and allogenic bone grafts are the gold standards in orthopedic reconstruction, but suffer from certain challenges. As an alternative xenogenic bone grafts are being developed. They have a potential to satisfy the current demand in orthopedic medicine. Fresh bovine and porcine bone samples were harvested from the epiphysis of the femur immediately after slaughter from a local slaughterhouse. The bone samples were decellularized, delipidated via multiple cycles of thermal shock ranging from 121°C to -196°C, followed by washing with Triton X-100. Histomorphometric analyses confirmed that the samples were devoid of any donor cellular material. SEM showed that the microarchitecture of the trabecular bone that is important for osteoconductive potential was preserved in the decellularization process. Ongoing studies include coating the decellularized sample with functionalized graphene nanoparticles, and seeding them with fat-derived human MSCs to evaluate the nanocomposite scaffold as a potential bone graft substitute.

Presenter: Lindsey Parker

Authors: Lindsey Parker, Andrew Loveridge, Jessica Dawson, Roger Parry, Patricia Pritchard, Stephen Kania, Melissa Kennedy

Title: Prevalence of Feline Immunodeficiency Virus in Lions of Northwest Zimbabwe

Abstract: Feline Immunodeficiency virus (FIV), a lentivirus of felids similar to HIV of humans, is endemic in many Felidae species, some being endangered or threatened with extinction. The lentivirus of lions (*Panthera leo*; FIV_{ple}) entered various lion

populations in which it is now endemic thousands of years ago and is found in lion populations of eastern and southern Africa. Some populations of lions remain FIV-free while others have unknown infection status. It is imperative for management of wild lion populations that FIV infection status of different populations be known. The purpose of this study was to quantify the seroprevalence of FIV in Northwest Zimbabwe measuring FIV-specific antibody using two different Elisa tests. Over 200 banked lion serum samples and 20 domestic cat samples were tested. More than 75% of samples tested had detectable antibody to FIV. We concluded that FIV is endemic in this population and potential implications will be discussed.

Presenter: Pawat Pattarawat

Authors: Pawat Pattarawat, Lenora A. Pluchino, Hwa-Chain Robert Wang

Title: Synergistic induction of cell death and reduction of drug resistance in human urinary bladder cancer cells by a triple combination of gemcitabine, cisplatin, and romidepsin

Abstract: Urinary bladder cancer (UBC) is one of the most common malignancy in the United States. Combination of cisplatin and gemcitabine is commonly used to treat UBC. However, recurrence of UBC is still high with the current treatment regimens, which necessitate the development of new therapies. In this study, we investigate on the efficacy of a novel combination of cisplatin, gemcitabine and romidepsin in treating UBC cells. We observed that the combination synergistically induce cell death and reduce drug resistance in vitro. Cell death was mediated by the activation of the Ras-ERK-Nox pathway through production of reactive oxygen species, and decreased drug resistance was attributed to reduced glutathione levels. In vivo studies verified the efficacy of the combination treatment in xenograft tumors. Overall, our results demonstrate that combination of cisplatin, gemcitabine and romidepsin is a novel and effective treatment regimen for the UBC.

Presenter: Jessie Richards

Authors: Jessie Richards, Manasi Balachandran, Richard Gerhold, Stephen Kania

Title: Serological Diagnosis of *Parelaphostrongylus tenuis* Infection

Abstract: *Parelaphostrongylus tenuis* is a parasitic nematode common among deer, elk, moose, and horses. The parasites attack the central nervous system, laying their eggs in meningeal tissue resulting in high morbidity and mortality. Techniques currently available for a definitive diagnosis involve a necropsy to detect adult worms in the brain and spinal cord. In order to detect *P. tenuis* antemortem, a gene encoding Pt-API-1 was inserted into an expression plasmid and propagated in *E. coli*. Western blots were utilized to identify anti-*P. tenuis* antibody present in blood, serum and spinal fluid using sera from known positive and negative animals. However, due to inconsistent results using this protein and cross reactivity with other similar organisms, we have moved our attention to a full genomic analysis of *P. tenuis* and have since identified a new, more definitive antigen that shows significant promise.

Presenter: Rebecca Rifkin

Authors: Rebecca Rifkin, Remigiusz Grzeskowiak, Pierre- Yves Mulon, H. Steve Adair, Alexandru S. Biris, Madhu Dhar, David Anderson

Title: Quantitative assessment of lameness in goats

Abstract: OBJECTIVE: To quantitatively assess gait characteristics and weight-bearing forces during ambulation in healthy goats using a pressure sensing mat as a biometric tool for gait analysis.

METHODS: Gait analysis was performed by training goats to walk over a pressure-sensitive mat, which recorded quantitative measurements. Measurements evaluated for gait analysis were as follows; maximum force per body weight (%bw), maximum pressure (kg), maximum peak force (Kpa), stance time (sec), stride length (cm), stride time (sec), stride velocity (cm/sec), impulse (%BW*sec), and impulse (kg*sec).

RESULTS: ANOVA revealed significant differences ($p < 0.05$) between the limbs in the variables maximum force per body weight (%BW) and maximum pressure (Kg). The Tukey's HSD test determined that the hindlimb and forelimb means were similar for each paired limbs ($p < 0.05$) for maximum force by body weight (%BW) and maximum pressure (kg). The hindlimb means were significantly different from that of the forelimbs ($p < 0.05$). When evaluating non-normally distributed data, there was no significant difference between the left front and right front in terms of impulse (%BW*sec), impulse (kg*sec), maximum peak force (Kpa), stride velocity (cm/sec), stance time (sec), and stride time (sec) ($p < 0.05$). The same variables showed no significant differences between the left and right hindlimbs.

CONCLUSIONS: Pressure mat sensing provides detailed analysis of forces in goats based on statistical analysis. This tool will be valuable for clinical monitoring of response to treatment, research regarding animal welfare, and quantitative assessment of goat models for orthopedic disease. Use of objective measurements will improve lameness assessment and minimize errors associated with numeric scoring systems.

Presenter: Owen Schumacher

Authors: Owen Schumacher, Carla Sommardahl, Bente Flatland, Melissa Hines

Title: Validation of point of care blood ammonia meter, PocketChem BA meter, in horses

Abstract: This study investigated analytical performance of the PocketChem BA meter, a point-of-care ammonium meter validated for use in dogs. Meter precision was investigated at low, normal, and high ammonium concentrations using equine whole blood and spiked plasma. Precision increased as ammonium concentration increased, and precision at normal and high ammonium concentrations was within ASVCP allowable total error recommendations. Ammonium concentrations were higher in plasma than in paired whole blood specimens, an expected artifact given the meter's analytical methods. Recovery was investigated using spiked plasma; recovery decreased as ammonium concentration increased but was acceptable overall. The PocketChem BA

meter is a useful screening tool to determine ammonium concentration in equine whole blood.

Presenter: Jillian Smith

Authors: Jillian Myers Smith, Elizabeth Lennon

Title: Myeloperoxidase and Xanthine Oxidase as Possible Biomarkers for Urinary Tract Infections in Canines.

Abstract: Bacterial urinary tract infections (UTIs) can be challenging to differentiate from other lower urinary tract diseases, with a waiting period of up to 3 days for culture results. Therefore, a rapid bedside test that could diagnose a UTI with high accuracy would be useful and could avoid inappropriate use of antibiotics. We hypothesized that two biomarkers of UTI, xanthine oxidase (XO) and myeloperoxidase (MPO) may be able to distinguish between UTI and non-infected urine. The objective of this study was to determine if XO or MPO could be used to differentiate dogs that had UTI from dogs who were suspected to have a UTI but were culture negative. MPO and XO were measured in urine samples from dogs with culture-confirmed UTI or negative controls. Urine XO concentration was not statistically different between UTI samples (0.37 ± 0.06 mU/ml) and negative controls (0.36 ± 0.04 mU/ml; $p=0.86$). MPO was significantly increased in dogs with UTI (7.7 ± 1.6 ng/ml) compared to negative controls (2.7 ± 1.1 ng/ml; $p<0.001$). Despite this fact, there was substantial overlap in MPO concentration between groups. Based on a receiver operator characteristic, the cutoff to achieve maximum sensitivity and specificity was < 1.5 ng/ml, with a sensitivity of 79.5%, and specificity of 47.1% in diagnosing UTI. Therefore, cannot recommend the use of this test in patients with complex medical conditions at a tertiary care referral center. Future studies will evaluate MPO as a biomarker of UTI in otherwise healthy dogs.

Presenter: Richard Steiner

Authors: Richard Steiner, Tom Masi, David E Anderson, Madhu Dhar, David Harper, Alex Biris, Shawn Bourdo

Title: In-vitro immunofluorescence analysis of MSC culture on PCL +GO films for nerve regeneration

Abstract: Nerve regeneration continues to be a challenging problem. Utilization of biomaterial scaffolds to serve as devices for the delivery of stem cell therapies offer a potential to improve healing of damaged nerves. We developed an electro-spun polycaprolactone (PCL) substrate coated with graphene oxide (GO) to support rat mesenchymal stem cells (rMSC). We hypothesize that PCL+GO films with either smooth or fiber surface topographies will support human mesenchymal stem cells (hMSC) delivery. Static and trans-differentiated hMSC cell lines were assessed for cytotoxicity with DiI staining and immunofluorescence assays for vimentin and S100 expression. hMSC cultured on both PCL+GO fibers, and smooth films showed positive cell attachment and supported hMSCs under neural trans-differentiation stimulation. The

study has provided preliminary data in support of an in-vivo rat study to assess use of the biomaterial as a neural wrap for repair of severe peripheral nerve injury.

Presenter: Riley Thompson

Authors: Riley Thompson, Aime Johnson, Tulio Prado, Christopher Premanandan, Megan E Brown, Brian Whitlock, Budhan Pukazhenth

Title: Influence of Dimethyl Sulfoxide Concentrations and In Vitro Culture on Equine Endometrial Tissue Explants

Abstract: This study was designed to better understand cryopreservation of equine endometrial tissue. Previous studies in the human and horse have focused on cryopreservation of dissociated endometrial cells. To our knowledge, there are no studies on cryopreservation of endometrial explants. Our objective was to determine the influence of differing concentrations of the permeating cryoprotectant dimethyl sulfoxide (DMSO) on viability and structural integrity (histology) of cryopreserved endometrial tissues over a 5-day culture interval. Endometrial tissue was cryopreserved using a slow cooling method in Minimum Essential Medium (MEM) containing 20% fetal bovine serum and 0%, 10%, or 20% (v/v) of DMSO. Cell viability, histology, and gene expression were all assessed prior to and following cryopreservation. Fresh and cryopreserved endometrial tissues also were subjected to a short-term culture (375% CO₂) on agarose blocks in two different media (Dulbecco's Modified Eagle Medium [DMEM] and MEM) to determine tissue viability and function. Results demonstrate the sensitivity of equine endometrial tissue to low temperature storage and the capacity to protect the structural integrity but not cell viability with DMSO. In conclusion, although equine endometrial tissue can be cryopreserved in DMSO, further studies are warranted to minimize loss of cell viability.

Presenter: Anastasia Towe

Authors: Anastasia E. Towe, Robert Ossiboff, Matthew J. Gray, E. Davis Carter, Markese Bohanon, Debra L. Miller

Title: Investigating the pathology of *Batrachochytrium Salamandrivorans* in salamanders

Abstract: *Batrachochytrium salamandrivorans* (Bsal) is a chytrid fungus that infects amphibians and has not yet been reported in North America. It attacks keratinized epithelium, obliterating the epidermis, resulting in death in less than 2 weeks. The other species within this genus is *B. dendrobatidis* (Bd), which is found throughout the globe. Lesions can be coinfecting but organisms cannot be definitively distinguished by routine histological staining. Because Bsal is a foreign pathogen and both pathogens are OIE reportable, it is imperative that we can positively identify them in cases of infection. In situ hybridization (ISH) is a molecular test that detects pathogens in tissues. We performed ISH on cut tissue sections from 18 animals, representing 5 different salamander species. Three of these animals were co-infected with Bsal and Bd, while the

other 15 were singly infected with Bsal. In all cases, the assay was able to distinctly stain Bsal and Bd.

Presenter: Tena Ursini

Authors: Tena Ursini, L. Amelse, H. Elkhenany, A. Odoi, J. Carter-Arnold, S. Adair, M. Dhar

Title: Retrospective Analysis of Adverse Reactions Associated With 230 Allogenic Administrations of Bone Marrow-Derived Mesenchymal Stem Cells in 164 Horses

Abstract: Bone marrow derived mesenchymal stem cells (BM-MSCs) are used to treat musculoskeletal injuries. Cells that are available for immediate use is ideal. Allogenic BM-MSCs can satisfy the need for rapid treatment. Our objectives were to characterize BM-MSCs from a donor horse, *in vitro*, and to identify and describe adverse reactions that occurred following their injection. We hypothesized that BM-MSCs could be implanted into another without significant adverse reactions and the frequency of adverse reactions would be similar to that of autologous BM-MSCs. BM-MSCs were characterized and cryopreserved. Post injection reaction was defined as increased pain, swelling, or heat at or near injection site, or increased lameness. Ten of 230 (4.35%) injections were associated with an adverse reaction. Adverse reactions occurred in synovial structures (n=3) and in soft tissues (n=7). In conclusion, fully characterized BM-MSCs may be safely administered to other horses with various musculoskeletal injuries.

Presenter: Alex Viere

Authors: Alex Viere, Linden Craig, Andrew Cushing

Title: A Retrospective Study of Brain Lesions in Captive Non-Domestic Felids

Abstract: This retrospective study identified and characterized brain lesions in captive non-domestic felids from a large cat sanctuary, submitted to the University of Tennessee College of Veterinary Medicine for postmortem analysis. Necropsy reports from January 2006 through June 2018 were examined, and gross images and microscopic slides were reviewed from cats where available. In total, 238 cats met the inclusion criteria, of which 43 cats (18%) were determined to have brain lesions. These lesions were grouped into six etiologic categories: congenital (13%), idiopathic (8%), inflammatory (21%), metabolic (9%), neoplastic (30%), and vascular (9%). Not included in the brain lesions categorized above were previously undescribed amphophilic globules in the cerebral cortex of many cats with and without other brain lesions. The gross and histopathologic brain changes documented in this study provide insight into specific diseases and pathologic processes that affect the brains of captive populations of non-domestic felids.

Presenter: Rachael Wolters

Authors: Rachael Wolters, Malgorzata Rychlowska, Luis Schang

Title: The effect of polynucleotide kinase phosphatase (PNKP) on ZIKV replication

Abstract: This study investigated the effect of a DNA damage repair enzyme, polynucleotide kinase phosphatase (PNKP) on Zika virus (ZIKV) replication. As indicated by preliminary studies from the Schang lab, ZIKV induces cytoplasmic relocalization of PNKP, where it co-localizes with viral replication compartments and small molecule PNKP inhibitors inhibit ZIKV replication. The hypothesis of this study is that PNKP is a necessary host factor for ZIKV replication. The approach was to test ZIKV replication in a PNKP knockout (KO) cell line created with CRISPR/Cas9. The PNKP knockout efficiency was tested with western blotting. ZIKV infection assays (strain R103451) were performed and used to analyze viral infectivity by immunofluorescent imaging of ZIKV NS1 expressing cells, and ZIKV replication was studied by titrating viral progeny released from PNKP-KO and control cells using a plaquing assay.

Presenter: Jane Woodrow

Authors: Jane Woodrow, Elizabeth Lennon

Title: Counter-regulatory roles of mast cell derived bone morphogenetic protein (BMP)-7 during inflammation

Abstract: Mast cells are strategically located at mucosal surfaces where antigen exposure is concentrated, such as the gut. Our group and others have shown that mast cells have anti-inflammatory roles in chronic spontaneous colitis. Our preliminary data demonstrates that their protective effect may be mediated by bone morphogenetic protein (BMP)-7, a cytokine in the TGF β 2 superfamily with anti-inflammatory and anti-fibrotic roles in the intestine. We hypothesized that, in response to constant antigenic stimulation in the gut, mast cells synthesize BMP7 as a counter-regulatory mechanism to limit inflammation. We further hypothesized that this BMP7 acts locally in autocrine and paracrine manners to mediate its anti-inflammatory effects. The aims of this study were to describe: 1) BMP7 production by mast cells upon stimulation with mediators that mimic antigenic stimulation in the gut (lipopolysaccharide (LPS) and immunoglobulin (Ig)-E, 2) BMP receptor expression by mast cells, 3) anti-inflammatory actions of BMP7 on mast cells and 4) the influence of BMP7 on T regulatory cell differentiation. Mast cells significantly up-regulated BMP7 in response to LPS and IgE versus no stimulus ($P=0.0016$), and express BMP receptors type 1 and type 2. BMP7 pretreatment of mast cells resulted in down regulation of TNF production in response to LPS and IgE ($P<0.0001$). BMP7 treatment of naive T cells resulted in a trend of increased T regulatory cell differentiation. These results support a role for anti-inflammatory actions of mast cells in colitis via BMP7 production, both on themselves and on T cells. This work demonstrates a role of mast cell-derived BMP7 as a counter-regulatory mechanism for inflammation.

Presenter: Ellis Wright

Authors: Ellis Wright, Pierre-Yves Mulon

Title: Effect of Plate to Bone Length on Mechanical Properties Using Locking Plate Constructs

Abstract:

Purpose: To compare stability and failure modes of orthopedic constructs stabilized by partial and complete bone-spanning locking plates using an osteotomy gap model on goat tibiae.

Materials and Methods: A 1-cm mid-diaphysis osteotomy was stabilized by either an 18-cm (N=9) or 14-cm (N=8) plate. Tibiae measurements were collected and constructs were mounted on an Instron unit for a single compression test to failure. Maximum load and failure mode were recorded.

Results: The two tibiae populations were not statistically different. No significant difference was present for the maximal load to failure or mode of failure between the two groups.

Discussion: As shorter plates appeared to provide similar stability to the constructs, these plates may be beneficial to the patients as they may lower the surgical time, and reduce invasiveness of the surgery. However, further study would be necessary to evaluate non-buttress plates on more immature bone.