2024 Research Day Program and Schedule

Abstracts are listed by session in order of presenter appearances, with presentations in room A118 being listed first in the document and presentations in the Teaching and Learning Center Ann and John Tickle Lecture Hall listed in the second half of the document.

Session 1: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Julia Albright, Richard Gerhold, and Ashley Stokes
Moderator: Laura Horton
Presenters (in order of presentation): Heather Smith, Annalisa Wager, Gabriela Bastos, and Tatiana Delecave Dias

Cracking the shell: How climate change impacts neonatal loggerhead (*caretta caretta*) health through blood analysis

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

All loggerhead sea turtles (*Caretta caretta*) are considered endangered. Climate change is one of multiple factors causing this status partly due to elevated temperatures negatively impacting sea turtles both in the ocean and on the beaches. While it is known that increased incubation temperatures adversely affect hatchling health, their long-term impacts remain unclear. This study aimed to fill these knowledge gaps by assessing the relationship between incubation temperatures and neonatal health in loggerhead turtles at 4-5 weeks of age. Blood samples were collected from 114 neonates across 12 nests from Boca Raton and Juno Beach, Florida. Incubation temperatures were used to categorize each nest as "cool" or "hot" based on time spent at or above 32°C. Key blood analytes, including packed cell volume (PCV), total protein (TP), agarose gel electrophoresis (AGE) values and capillary zone electrophoresis (CZE), were measured. Results revealed significant physiological differences between neonates from hot and cool nests, including elevated PCV, and decreased TP, albumin, and gamma globulins in neonates from hot nests. These alterations suggest possible dehydration, malnutrition, or immunodeficiency in neonates that emerged from hot nests. These physiological changes could reduce neonatal fitness and survival in the wild and may be a contributing factor leading to the decreased success rate observed in hatchlings incubated at higher temperatures.

Incidence and radiographic appearance of the os clitoridis (baubellum) in female domesticated ferrets (*Mustela putorius furo*)

Annalisa Wager, Dr. Silke Hecht, Dr. Linden Craig, Dr. Emi Knafo, Dr. Danielle Tarbert, and Dr. Robert Reed

There is limited published information on the os clitoridis in veterinary species. This retrospective study aims to provide information on its incidence and radiographic appearance in client owned female domesticated ferrets (Mustela putorius furo) presented to an academic veterinary medical center, and investigate any association with age, intact versus spayed status, and adrenal disease. Sixty-six ferrets (37 intact and 29 spayed) with a median age of 39.5 months (range, 2 – 118 months) met inclusion criteria. An os clitoridis was identified in 49/66 animals (74.24%). The mean length was 3.06 ± 1.20 mm and the mean width at the widest point of the bone was 0.68 ± 0.18 mm. An os clitoritis was significantly more common in intact animals ($p=0.0229$) and in older animals ($p=0.01454$). There was no association between an os clitoridis and adrenal disease ($p=0.2188$). The os clitoridis is a normal anatomic structure on radiographs of female ferrets and should not be misinterpreted as an abnormality.

Assessment of dual-platform technology for bone regeneration and local antibiotic delivery in a goat osteomyelitis model

Dr. Gabriela Bastos, Dr. Tatiana Delecave Dias, Lori Terrones, Dr. Silke Hecht. Dr. Pierre Yves-Mulon, and Dr. David Anderson

Osteomyelitis (OM) poses significant treatment challenges, making local drug delivery devices a promising technology. This study introduces a dual-platform device designed to promote bone healing and deliver antibiotics to prevent OM. Our hypothesis was that an established bone tissue regeneration scaffold could deliver antibiotics sufficient to eliminate bacteria from the site of bone injury. Adult, female, boar-cross goats were allocated into four treatment groups: 1) SA: Vancomycin loaded scaffolds (n=5); 2) SB: Staph. aureus contaminated scaffolds (n=6); 3) SBA: SA+B (n=6); 4) NS: native scaffolds (n=6). Scaffolds, +/- vancomycin and +/- bacteria, were implanted in the right tibia of each goat. Over a period of 3 months, radiographs, CT imaging, and bacteriology on bone, scaffold and soft tissues were performed. Radiographs and CT were scored by a blinded radiologist for OM. All SB group goats were removed within the first 6 weeks because of OM. Radiograph OM scores differed significantly across groups, with SA and NS having similar outcomes, SB having OM, and SBA having less sever and delayed on-set of OM. CT comparison findings aligned with radiograph OM scores and both radiographs and CT provided consistent healing assessments with no significant difference between methods. These results indicate that vancomycin-loaded scaffolds may offer a distinct advantage in contaminated surgeries, underscoring the importance of antibiotic integration in treating osteomyelitis.

Assessment of dual-platform technology for bone regeneration and local antibiotic delivery in a goat osteomyelitis model
Dr. Tatiana Delecave Dias, Dr. Gabriela Bastos, Lori Terrones, Elizabeth Croy, Dr. Pierre-Yves Mulon, Dr. Sherry Cox, and Dr. David Anderson.
Orthopedic infection is a common complication of orthopedic injury, and effective treatment remains a challenge. Synthetic bone scaffolds that support bone regeneration while also effectively delivering antibiotics to contaminated tissues to prevent infection hold promise for improving patient outcomes. This study aimed to evaluate the elution of vancomycin from a dual-platform bone scaffold. The *in vitro* drug elution was performed using an established protocol, with complete turnover of media, collected for analysis at days 0, 1, 2, 3, 5, 7, and 10. For the *in vivo* vancomycin essay, the vancomycin-loaded scaffolds were implanted into segmental tibial defects in goats as a bone healing model. Plasma samples were collected at the same time points as in the *in vitro* experiment. Vancomycin was measured during the 10-day *in vitro* study period, with the vast majority of drug eluted within 3 days. *In vivo,* no vancomycin was detected after day 3 in the samples. These findings suggest that the scaffold did not achieve sustained antibiotic release *in vivo* beyond 72 hours. However, it is possible that vancomycin may have been sequestered in the dead space within the bone defect and resulting disruption of blood supply. This may have limited absorption and systemic distribution of the antibiotic. Further studies are needed to elucidate the scaffold's potential for effective *in vivo* antibiotic delivery, including collection of fluids immediately surrounding the implant.

Session 2: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Julia Albright, Timothy Chamberlain, and Ashley Stokes
Moderator: Laura Horton
Presenters (in order of presentation): Laura Antizzo, Michael Rivera Orsini, Sarah Fiedler, and MaCayla Clements

A retrospective review of pancreatic islet cell neoplasia in non-domestic felids
Laura Antizzo, Dr. Andrew Cushing, Dr. Michelle Dennis, and Dr. Dalen Agnew
Several cases of pancreatic islet neoplasia in non-domestic felids have been diagnosed at UTCVM and other institutions; however, published reports are sparse within the literature. The aim of this study was to describe the pathology of islet neoplasia in non-domestic felids and any associations with clinical signs and laboratory diagnostics. Seven cases of pancreatic islet neoplasia in non-domestic felids (6 tigers [*Panthera tigris*] and 1 cougar [*Puma concolor*]) were identified in the UTCVM pathology database, and pathological reports and histological sections pertaining to each case were systematically reviewed. Mean (range) age at necropsy was 19 (13-23) years. No vomiting, hypochloremia, hypoglycemia, or glycosuria were observed in performed diagnostics in any cases. In all cases, islet cell neoplasia was an incidental finding diagnosed postmortem. AT UTCVM, incidence of islet cell neoplasia at necropsy by species was 0.023 in tigers (95% CI [0.0083, 0.049]) and 0.031 in cougars (95% CI [0.00079, 0.17]) over the course of 20 years (2004-2024). Microscopically, neoplasms were composed of polygonal cells with finely granular cytoplasm arranged in packets separated by fibrous stroma. Metastasis was identified in 4 cases. Churukian Schenk staining revealed the presence of argyrophilic granules in all 7 cases. Minimal amyloid was observed in 1 case following Congo Red staining. Immunohistochemistry for insulin, glucagon, gastrin, and somatostatin production by tumor cells is in progress.

CD90+ and MHCII- canine adipose tissue-derived MSCS as potential for off-the-shelf application

Michael Rivera Orsini, Emine Berfu Ozman, Alyssa Miles, Dr. Steven Newby, Dr. Nora Springer, Dr. Darryl Millis, and Dre. Madhu Dhar

Cell-based therapies are increasingly used in both human and veterinary medicine to treat patients with diseases and injuries. Mesenchymal stem cells (MSCs) are adult tissue-derived multipotent cells with self-renewal capability and the ability to differentiate into specific tissue lineages. The availability of methods for isolation, characterization, and *ex vivo* expansion of MSCs presents them as the most commonly used cells in regenerative medicine. The use of autologous MSCs has been the golden standard due to the donor and the recipient being the same. However, the efficacy of MSCs depends on various factors, including lack of uniform isolation, donor source, age, and health. Hence, a fully characterized repository of allogenic sourced MSCs with consistent performance, such as an off-the-shelf bank for immediate application, is needed. Based on our data, we hypothesize that MSCs that express CD90 and lack the expression of MHC II, with viability of ≥90%, can be used safely as an allogenic source of cells in the treatment of a multitude of injuries or diseases. We have isolated and characterized adipose-derived MSCs from a 13-month-old female Pitbull mix and demonstrated the expression of CD29, CD44, and CD90 with >80% viability in saline post-cryopreservation and under extreme temperatures up to 75 hours. Further investigation is underway to ensure the *in vivo* safety and efficacy of these MSCs. In conclusion, we have a cryobank of CD90+ and MHCII- canine adipose tissue-derived MSCs, with the potential to be used as an off-the-shelf therapy either in the clinic or shipped to other practices.

Comparison of serological and histological findings in the diagnosis of Toxoplasma gondii in various wildlife species

Sarah Fiedler, Katie Riese, Dr. Jennifer Riley, Dr. Richard Gerhold, Dr. Michelle Dennis, and Dr. Chunlei Su

Toxoplasma gondii is a zoonotic protozoan parasite with a broad host range known to infect most endothermic species. Although *T. gondii* is one of the leading causes of foodborne illnesses in humans across the globe, it is considered a neglected parasite. Conducting more research on *T. gondii* could provide information to the general public on the risk factors and how to avoid the spread of disease. Our research compared two testing methods for detecting *T. gondii* in various wildlife species. The first diagnostic method we utilized was a Modified Agglutination Test (MAT) which detects *T. gondii* antibodies in serum samples. The other method to detect *T. gondii* was screening for tachyzoites and bradyzoites in formalin-fixed tissue samples histologically. The MAT results showed that 19/110 (17%) of samples were seropositive. We took the 19 seropositive samples and looked at the respective formalin-fixed tissue samples which include heart, brain, spleen, and liver samples. We found that 1/19 (5%) of formalin-fixed tissue samples showed *T. gondii* histologically. Although not conclusive, based on these results, MAT may be a more sensitive diagnostic method than histology alone. Further research will be conducted by continuing serology and histology testing on additional sample sets, including PCR testing on fresh frozen tissues, and looking closely for patterns of transmission such as diet (carnivore vs. herbivore).

Using blow flies as biological drones to remotely detect outbreaks of *Histomonas meleagridis* in wild turkey populations in Tennessee
MaCayla Clements, Dr. Charity Owings, and Dr. Richard Gerhold
Histomonas meleagridis is a protozoal parasite that is a causative agent of blackhead disease in turkeys. This disease is devastating to our wild turkey populations because once infected the death rate is over 80%. The aim of this study was to use real-time polymerase chain reaction (PCR) to determine if black blow flies (*Phormia regina*), a ubiquitous carrion-feeding fly, could feed on *H. meleagridis* infected turkey carcass and act as biological drones to alert researchers to local outbreaks. Naturally infected turkey liver and cecum were used in a feeding experiment with a lab grown colony of blow flies. Flies were grouped into four treatments (unfed, exposure to uninfected turkey breast, exposure to infected liver tissue, exposure to infected cecum; *N*= 10/treatment, 5 male, 5 female) and allowed to feed for four hours before they were frozen and their crops were collected for DNA extraction. PCR was run on each sample using primers to detect *Histomonas*. 40% of all the flies (both male and female) exposed to the infected liver came back PCR positive and 80% of all the flies (both male and female) exposed to infected cecum came back PCR positive. DNA sequencing was performed on these positive samples it was determined that they were all indeed positive for *H. meleagridis.* We can conclude that blow flies can be used as biological drones to detect *Histomonas* outbreaks in wild turkey populations and this may prove useful in further wildlife disease research.

Session 3: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Madhu Dhar, Timothy Chamberlain, and Ashley Stokes
Moderator: Dr. Kim Newkirk
Presenters (in order of presentation): Samuel Calabrese, Lindsey Rice, Amy Webb, Logan White, Matthew Knight

Tick-borne pathogen prevalence and the standardization of necropsy protocols In southeastern North American black bears (Ursus americanus)
Lindsey Rice, Katie Riese, Dr. Julie Sheldon, Dr. Sarah Linn-Peirano, and Dr. Richard Gerhold
Black bears (*Ursus americanus*) can serve as a host for multiple tick species that may harbor various pathogens potentially impacting wildlife, livestock, and public health. In more urbanized areas, bears may pose both as a sentinel and source of tick-borne pathogens for humans. Currently, there is a lack of knowledge pertaining to tick-borne pathogen prevalence in our population of black bears in the Southeast, and how these pathogens may inflict disease or impact the bears clinically. The morphologically identified ticks were collected off both live and dead bears that either presented to the zoological medicine service or necropsy, as well as whole blood samples. DNA extraction of the ticks and bear blood were followed by DNA amplification via PCR, and subsequent sequencing, to determine if molecular evidence of pathogen DNA was present. This study intends to provide data on tick species commonly found on the bears, but also tick-borne pathogen prevalence which can be used to assess potential health implications to the bears in this region. Alongside tick collection and analysis, a standardization of necropsy protocols with a standardized aging scheme is being created to aid in proper disease identification as it correlates with age in this species. Since the black bear may act as a sentinel in this region, a better understanding of pathogen transmission and overall health will have lasting implications for both humans and the health of our wildlife populations.

Histopathology of corals showing tissue loss during an unusual coral mortality event in Jamaican north coast reefs

Dr. Amy Webb, Dr. Anna Becker, Dr. Judith Lang, Dr. Esther Peters, and Dr. Michelle Dennis

An unusual mortality event affecting coral reefs along the northern coast of Jamaica was discovered in 2018. Based on gross lesions and species affected, this mortality event appeared similar to what had been described for stony coral tissue loss disease (SCTLD) in Florida, which is characterized by rapidly progressing tissue loss with high mortality rate affecting >30 species of scleractinian corals. The objective of this study was to describe microscopic lesions of diseased corals and determine the extent to which the lesions documented were similar to SCTLD. Biopsies were taken from diseased and apparently healthy colonies from 2 sites along the northern coast of Jamaica in spring and summer 2019. Histologic lesions were nonspecific, and the characteristic form of lytic necrosis previously associated with SCTLD was not observed, nor were etiologic agents histologically observed. It is unclear if this mortality event is the result of a different disease or if it was SCTLD but presenting differential histological changes due to stage of disease when biopsied, host species response to disease, or changes in disease ecology. This study highlights the importance of histology in defining coral diseases and the need for selective sampling of active lesions early during a coral disease epidemic.

Investigation of brown pigmented lesions in Mountainous Star Coral (*Orbicella faveolata*)

Logan White, Dr. Amy Webb, and Dr. Michelle Dennis

Focal brown pigmented lesions are one of the many observed conditions in coral leading to their increase in mortality. They are characterized by an increase in brown to yellow pigmentation and are sometimes coupled with chronic tissue loss. Due to the significant role of symbionts in the production of coral pigment, our study's goal was to compare the density of symbionts in healthy and lesioned tissue. Paired samples (n=8) were collected and used to form a tissue slurry, which was used in a haemocytometer to count the number of symbiont cells present in each sample. The density was then calculated using the total number counted. Our results showed that 87.5% of lesioned tissues have a decrease in symbiont density when compared to healthy tissues from the same colony. This unfortunately does not explain the cause of the increased pigmentation but does provide a foundation for a few different theories that could help us understand the condition. Future research should be done to further investigate these potential causes.

The Effect of Animal-Assisted Intervention on Preoperative Anxiety and Dose of Sedation in Children

Mtthew Knight, Dr. Zenithson Ng, Dr. Julia Albright, Dr. Marcy Souza, Dr. Elizabeth Strand, and . Jennifer Lord

Background: Preoperative anxiety in pediatric patients is highly prevalent, with nearly 75% of the five million children undergoing elective surgeries each year in North America experiencing significant stress. Anesthesia is among the most anxiety-inducing medical procedures for children, often leading to fear, agitation, and difficulties in cooperation, such as crying, spontaneous urination, and the need for physical restraint during anesthetic induction. A growing body of literature suggests that dogs may help reduce stress in these settings. This study explores the impact of animal-assisted intervention (AAI) on preoperative anxiety and sedation doses in children. **Methods:** A total of 49 patients between 2 and 15 years of age were enrolled in a randomized cross-over design, where participants received a 10-minute interaction with a therapy dog or iPad tablet before undergoing a sedated procedure. Anxiety was measured using the Modified Yale Preoperative Anxiety Scale (mYPAS). The sedation dose was recorded as the volume of ketofol administered by the anesthesiologist divided by body weight. **Results:** The therapy dog intervention demonstrated significantly lower preoperative anxiety than the iPad tablet intervention, with reduced mYPAS scores ($p = 0.0096$), particularly in the domains of vocalization and emotional expressivity. Ketofol dosage did not differ significantly between groups ($p = 0.1995$). **Conclusion:** These preliminary findings suggest AAI potentially reduces preoperative anxiety in pediatric patients, particularly in vocalization and emotional expressivity. While sedation dose was not different between interventions, AAI may still enhance the hospital experience, warranting further investigation.

Session 4: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Debra Miller, Pierre Yves-Mulon, and Sarah Schmid
Moderator: Dr. Marcy Souza
Presenters (in order of presentation): Mayzie Miller, Leo Spadafino, and Taryn Harris

The ADVIA 2120 Myeloperoxidase Cytogram Does Not Distinguish Acute Myeloid From Acute Lymphoblastic Leukemia In Dogs

Dr. Mayzie Miller, Dr. Nora Springer, Dr. Dorothee Bienzle, Dr. Kristina Meichner, and Dr. Tracy Stokol

Background: Acute myeloid leukemia (AML) cannot be reliably distinguished from acute lymphoblastic leukemia (ALL) by morphology. Different chemotherapeutic protocols may be recommended for AML versus ALL and immediate therapy is often needed due to rapid disease progression. Immunophenotyping may result in treatment delays; therefore, a rapid method for distinguishing AML from ALL at time of diagnosis is desirable. As myeloid cells are expected to have more myeloperoxidase activity than lymphoid cells, the ADVIA 2120 myeloperoxidase (MPX) cytogram may be able to differentiate AML from ALL. **Hypothesis:** Large unstained cells (LUC) on the ADVIA 2120 MPX cytogram will create a more acute angle with the x-axis in AML versus ALL. **Methods:** Dogs with acute leukemia were prospectively enrolled and phenotyped via flow cytometry and cytochemistry, including MPX, as part of a multi-institutional study. Fifty-eight dogs had available ADVIA MPX cytograms. A blinded observer estimated a line-of-best fit through LUC with FIJI software in triplicate and recorded the mean angle. Patients were then grouped as AML (N=43) or ALL (N=15) for comparison with a Mann-Whitney U test. Correlation between LUC angle and MPX cytochemical staining was assessed by Spearman Rank. **Results:** There was no significant difference in the MPX LUC angle between AML and ALL (p=0.9). MPX cytochemical staining results did not correlate with LUC angle (R^{sp}=0.02 p=0.9). All cases with LUC angle <78°were diagnosed as AML (10/43, 23%). **Conclusions:** The ADVIA MPX cytogram does not reliably distinguish between AML and ALL in dogs, although an angle <78° was suggestive of AML.

Canine Blastomycosis: A Retrospective Study of 126 cases

Leo Spadafino and Ashley Hartley

Blastomyces dermatitidis, a fungus endemic to Tennessee, infects both dogs and humans. Canine blastomycosis was last characterized in East Tennessee with 78 cases presenting to the University of Tennessee Veterinary Teaching Hospital (UTVTH) during 1977-1999. In 2008, a Blastomyces antigen quantitative (BdAg) test emerged as a less invasive yet sensitive diagnostic for blastomycosis. Study goals were to identify cases of canine blastomycosis at UTVTH and characterize the presentation, clinicopathologic and imaging abnormalities, and treatments selected during a 10-year period (2014-2023). One hundred thirty-one (131) cases of canine blastomycosis were identified using laboratory database searches for BdAg and pathology (i.e. cytology, histopathology, and necropsy) submissions. Electronic medical record and imaging report screening confirmed clinical diagnosis in 126 cases. Clinical cases were all ages, gender, and size, yet most often <5 years of age (n=65/126, 52%) and weighing >10 kg (n=57/126, 45%). Emergency (n=57/126, 45%), Ophthalmology (n=28/126, 22%), and Internal Medicine (n=25/126, 19.8%) services were most visited at initial presentation. Respiratory, ocular, and dermatological manifestations were most commonly involved, both as primary and total

organ systems. Notable clinicopathologic abnormalities included leukocytosis, neutrophilia, hyperproteinemia, hypoalbuminemia, hyperglobulinemia, elevated C-reactive protein, and low iron. Thoracic imaging commonly revealed structured interstitial patterns (50/86, 58.1%), with miliary (20/86, 23.3%) and nodular (38/86, 44.2%) features. Itraconazole was the most frequently prescribed antifungal (89/106, 84%). Veterinarians should consider *Blastomyces sp.* infection for a variety of clinical presentations.

Evaluation of the effects of gabapentin on stress and pain in shelter cats undergoing ovariohysterectomy

Taryn Harris, Emily Reger, and Dr. Stephanie Kleine

The majority of cats entering shelters require ovariohysterectomy (OVH) prior to adoption. Transportation, handling, surgery, and anesthesia can be sources of stress in this population of cats, which can result in illness and numerous physiologic and behavioral changes. Therefore, stress reduction prior to and during OVH is of paramount importance. Gabapentin has been shown to reduce stress during veterinary visits in client-owned cats and shelter cats undergoing behavioral modification. The purpose of this randomized, masked, placebo-controlled trial is to evaluate the efficacy of preoperative gabapentin on stress, tolerance to handling, and pain in shelter cats undergoing OVH. Thirty-four healthy female cats were randomized to receive gabapentin (20 mg/kg) or a placebo orally, 2-4 hours prior to anesthesia. Cats were scored by two masked individuals for stress, sedation, tolerance to handling, and pain using the cat stress score, feline multiparametric sedation score, a previously published handling score, and the feline grimace scale, respectively. Scores were assigned prior to the first dose of medication, at anesthetic induction, and 30, 60-, 90-, 120-, and 180-minutes following recovery. Data were analyzed with a mixed-effects ANOVA to evaluate the effect of treatment, time and the treatment by time interaction. Preliminary results show no significant difference between treatment groups or treatment by time, with some significant time points compared to baseline within each treatment group. Further data analysis will determine if differences between surgeons, institutions, time from treatment administration to premedication, and cat's demeanor resulted in the lack of statistical differences between treatment groups.

Session 5: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Michelle Dennis, Debra Miller, and Pierre Yves-Mulon
Moderator: Dr. Carla Sommardahl
Presenters (in order of presentation): Grace Malla, Olivia Escher-Price, Jessica Lynch, and Sydney Craig

The pharmacokinetics, relative bioavailability, and pilot pharmacodynamics of subcutaneous levamisole in goats

Grace Malla, Dr. Joe Smith, Dr. Meggan Graves, Dr. Lisa Ebner, Ryan Branham, Jessica Lynch, Laura Gilliard, Julia Cutchin, Madeline Duncan, Rebecca Rahn, Dr. Michelle Buckley, Dr. Cassie Klostermann, Jessy Shanks, and Dr. Sherry Cox

Anthelmintic resistance is a major welfare issue in goats, and the longevity of anthelmintic drugs lies in their responsible use. Recently, an injectable combination (levamisole/doramectin) product labeled for cattle became available. The goal of this study was to compare the pharmacokinetic parameters of this new combination drug to a commercial oral levamisole formulation in goats. Six adult goats were injected with a 9mg/kg dose of the combination product. Blood samples were collected at 14 time points over 48 hours. After a washout period, the same goats were given a 12mg/kg dose of the oral formulation and sampled following the same timepoints. Levamisole concentrations were measured via liquid chromatography. A non-compartmental analysis was used to generate PK parameters for both formulations. After one subcutaneous injection, the maximum plasma concentration (Cmax), time to Cmax (Tmax), area under the curve (AUC), and elimination half-life (T1/2) was 468.57 ± 151.12 ng/mL, 2.24 ± 1.58 hours, 3206.42 ± 1189.73 h*ng/mL, and 2.36 ± 2.07 hours. After a single oral administration, Cmax, Tmax, AUC, and T1/2 were 573.21 ± 149.01 ng/mL, 0.5 ± 0.41 hours, 2995.47 ± 2203.93 h*ng/mL, and 3.74 ± 2.19 hours. Fecal samples taken before and after subcutaneous administration had an average egg count reduction of 61.97% (P = 0.0625). The relative bioavailability was 185%. Considering its bioavailability and egg count reduction, the combination product may be a good choice for goat management, however, a larger field trial is needed to determine its true reductive ability and other pharmacodynamic parameters.

The Bioavailability and Pharmacokinetics of Intravenous and Subcutaneous Famotidine in Domestic Goats (Capra aegagrus hircus)

Olivia Escher-Price, Dr. Joseph Smith, Kamryn Christopher, Bryan Hogan, Lainey Harvill, Makenna Hopson, Madeline Duncan, Kya Logan, Hannah Luu, Keren Hernandez, Dr. Jessica Gebert, Dr. Mona Bandyopadhyay, Dr. Sherry Cox

Abomasal ulceration can occur in goats due to stress, inappetence, and adverse reactions to medications. There is currently limited information regarding treatment and prevention for ulceration. Famotidine is a histamine type-2 (H-2) antagonist drug that works by decreasing gastric acid production. Famotidine is currently labeled for use in companion animals for treatment of stomach ulceration, acid reflux and gastritis. Reports in companion animal and human studies have shown famotidine to be an effective gastroprotectant. The purpose of this research was to report the pharmacokinetic parameters after a single intravenous (IV) and single subcutaneous (SC) administration of famotidine in goats. Six healthy goats were used in each study and famotidine was dosed at 0.6mg/kg IV and 1.2mg/kg SC. Blood samples were collected over 24 hours (IV) and 12 hours (SC). The plasma was analyzed using reverse phase high performance liquid chromatography. Pharmacokinetics were modelled with a noncompartmental approach. The elimination half-life for IV was 0.31 hours and for SC was 1.31 hours. The mean residence time for IV was 0.33 hours and for SC was 1.62 hours. The area under the curve for IV was 903.83 h*ng/mL and for SC was 1101.90 h*ng/mL. The elimination half-life of the IV administration was significantly faster than what has been reported in cattle and horses. The bioavailability for SC administration was determined to be 62.97%. With the results determined from this study, future studies including pharmacodynamic analysis can be performed to help determine the efficacy of famotidine in goats.

Therapies to get behind: the effect of rectal fluid therapy on biochemical analytes in the hospitalized porcine

Jessica Lynch and Dr. Joseph Smith

Intravenous (IV) fluid administration can be challenging in swine. Rectal fluid administration (proctoclysis) has been used successfully in other species, and is commonly utilized in hospitalized pigs with limited IV access. Recently, a 2022 study in healthy swine reported that proctoclysis did not significantly affect serum biochemical analytes compared to IV fluids, casting doubt to the efficacy of proctoclysis. Given that proctoclysis is typically employed in unhealthy patients, we evaluated pigs receiving proctoclysis at UTCVM from 2016-2024. Based on the recent study, we hypothesized there would be no changes before and after proctoclysis. Patient demographics and signalment were identified, and medical records were scoured for electrolyte (Na, K, Cl), hydration (HCT, TS, Lactate) and renal (BUN, Creatinine) parameters recorded before and after proctoclysis in hospitalized pigs. Data was statistically compared for differences ($p < 0.05$). Twenty-five cases fit the admission criteria, with mean age of 3.3±3.2 years and mean mass of 54.5±49.7 kg. Proctoclysis duration averaged 35.5 ±19.6 hours, with an average rate of 161±209 ml/h. The most commonly administered fluid types were tap water, LRS, Hartmann's, and saline. Statistically significant changes were identified after proctoclysis for hematocrit (p=0.0005); and creatinine (p=0.0019). Mean reductions and P values were NA (1.39mmol/L; p=0.4262), K (0.33mmol/L; p=0.4464), Cl (2.26mmol/L; p=0.0680), HCT (8.79%; 0.0009), TS (0.415g/dL; 0.0576), Lactate (1.77mmol/L, p=0.4609), Creatinine (1.19mg/dL; p=0.0002), and BUN (6.46mg/dL; p=0.1297). Given these results, proctoclysis may be beneficial in unhealthy patients, but additional research utilizing uniform treatment strategies is indicated to further evaluate its efficacy.

Biological variation of basal cortisol levels in healthy canines

Sydney Craig, Dr. Luca Giori, Dr. Julia Albright, Dr. Alex Estellar-Vico

Biological variation (BV) represents the central tendency of homeostatic regulation of any blood measurand, unique to individual (CV_I) and group (CV_G). The relationship between CV_I, CV_G, and analytical variability (CV_A) are used to calculate the Index of Individuality (IoI) which determines whether a reference change value (RCV) could be a more sensitive tool to assess clinically relevant deviation from "the individual normality" compared to population-based reference intervals (pRIs). Canine basal cortisol BV has yet to be established. This prospective study aims to characterize CV_I, CV_G, (CV_A), IoI and RCV of basal cortisol concentration in healthy dogs and assess usefulness of pRIs for interpretation of test results.18 clinically healthy, privately owned, dogs were sampled for hormonal analysis once weekly for 6 weeks. Samples were collected from the jugular vein, centrifuged within 45 minutes of collection. Sera were stored at -80°C. All samples will be processed in triplicate at the end of the collection period using a chemiluminescence analyzer (Siemens Immulite 2000XPI). A nested ANOVA is used to identify significant variations due to intra-individual, group, and analytical causes, assuming the three components are independent and normally distributed with constant variances. Knowledge of BV will add to understanding of canine cortisol physiology and will help to assess the number of samples needed to estimate the basal cortisol homeostatic set point, to set quality specifications (i.e. Total Allowable Error, TEa), and to determine whether patient cortisol data are better assessed with pRIs or through significant changes in serial results from each individual patient.

Session 6: Teaching and Learning Center (TLC) Ann and John Tickle Lecture Hall

Judges: Drs. Michelle Dennis and Angela Rollins
Moderator: Dr. Carla Sommardahl
Presenters (in order of presentation): Evie Yazbec, Conner Hayes, Caroline Moses, and Connor West

Hematological and Biochemical Parameters in Mexican Axolotls (Ambystoma mexicanum)

Evie Yazbec, Dr. Emi Knafo, Dr. Bente Flatland

Mexican axolotls (Ambystoma mexicanum) have a longstanding place in scientific research as they been used for over 150 years in studies of evolution, embryology, endocrinology and the like. Their popularity in more recent years has grown as a subject for research in regeneration and stem cells, in conservation efforts as they are critically endangered, and as a household pet. Due to this increase in attention, it has become evident that there is a lack of clinical research which enables the veterinary study and care of these animals. In a previous study of axolotl biochemical parameters, samples were collected using heparinized syringes, which was shown in a separate study of crocodilian hematological parameters to dilute samples. As such, this study will evaluate hematological and biochemical parameters in healthy axolotls without using heparinized syringes to help determine baseline values for these tests. To establish these reference intervals, 40 axolotls will have samples collected from their tail vein under manual restraint. Hematologic and biochemistry reference ranges (mean, standard deviation) will be established using guidelines set forth by the American Society of Veterinary Clinical Pathology (ASVCP).

Hypoalbuminemia Associated with Decreased Survival in Cats Presenting to a Tertiary Hospital: Retrospective Case-Control Study

Dr. Conner Hayes, Xiaojuan Zhu, and Dr. Sarah Schmid

Background- Hypoalbuminemia (HA) is associated with morbidity and mortality in people. Little is known about hypoalbuminemia in cats. **Objective-** To describe the characteristics of cats with mild (2.6-2.9 g/dL), moderate (2-2.5 g/dL) or severe (< 2 g/dL) HA and compare their survival rates to cats without HA (> 2.9 g/dL). **Animals-** 832 cats with HA and 832 age, breed, and sex-matched cats without HA. **Methods-** Retrospective case-control study. Clinical data and outcome were reviewed, and cats were classified into disease categories based on clinical diagnosis. Duration of hospitalization, presenting disease, and survival rates were compared among groups. **Results-** Overall prevalence of HA was 31% (917/2927). Of the 832 cats included for further analysis, severity of HA was graded as mild, moderate, and severe in 540 (65%), 199 (24%), and 93 (11%) cats, respectively. Cats with HA had a longer duration of hospitalization compared to cats without HA ($p < .001$). The primary disease in cats with HA most often affected the gastrointestinal system (162, 19%) with infectious (215, 26%) and neoplastic (196, 24%) processes being most common. Cats with severe HA had a shorter in-hospital survival rate compared to cats with mild ($p = .021$) or moderate HA ($p = .048$), and cats without HA ($p < .001$). For cats that survived to discharge, survival rates were lower for cats with any severity of HA compared to cats without HA ($p < .001$ for all). **Conclusions and Clinical Importance-** Hypoalbuminemia is a clinically relevant negative prognostic indicator in cats.

Enhanced Membrane Composition for Lipid Nanoparticle Vascular Drug Delivery

Caroline Moses, Connor West, and Dr. Diedra Mountain

Vascular disease is the most prevalent pathology in the United States and the most common cause of surgical intervention. Up to 60% of vascular interventions fail within the first 12 months due to dysfunctional vascular remodeling. Our research team is developing targeted lipid nanoparticles (LNPs) to deliver therapeutics to the vessel wall at the time of surgery, aiming to prevent remodeling-induced restenosis. In previous research, our team demonstrated the efficacy of these targeted LNPs in the delivery of gene therapeutics, using PEGylated distearoyl-glycerol-phosphoethanolamine (DSPE-PEG) as a membrane component to improve particle stability and drug retention. However, current literature suggests that dimyristoyl-glycerol derived PEG (DMG-PEG) may provide greater LNP functionality. Our objective here is to determine if substituting DMG-PEG for DSPE-PEG in our LNP formulation will improve in vitro cellular delivery, without sacrificing critical quality attributes (CQA). Cellular uptake in human aortic smooth muscle cells is investigated using fluorescently tagged LNPs and quantified by fluorometry. CQAs are determined using standard characterization assays to measure siRNA drug loading and retention (EE%), LNP size, homogeneity, and particle stability. LNPs with DMG-PEG at 1-10mol% membrane incorporation significantly improved LNP cellular association compared to DSPE-PEG. Furthermore, LNPs with either DMG-PEG or DSPE-PEG maintain stable particle size and homogeneity (<100nm; PDI<0.2) and retain their full siRNA drug load (EE%>90%) for up to 28 days at 4°C storage. In conclusion, substituting DMG-PEG for DSPE-PEG may enhance in vitro efficacy of our LNP formulation without significantly affecting nanoparticle characterization or stability.

Formulation of Non-Cationic Lipid Nanoparticles for Enhanced siRNA Drug Encapsulation and Delivery

Connor West, Caroline Moses, and Dr. Diedra Mountain

Vascular disease is the most prevalent pathology in the United States and the most common cause of surgical intervention. Up to 60% of vascular interventions fail within the first 12 months due to dysfunctional vascular remodeling. Our research team is developing targeted lipid nanoparticles (LNPs) to deliver therapeutics to the vessel wall at the time of surgery, aiming to prevent remodeling-induced restenosis. In previous research, our team demonstrated the efficacy of these targeted LNPs in the delivery of gene therapeutics, using PEGylated distearoyl-glycerol-phosphoethanolamine (DSPE-PEG) as a membrane component to improve particle stability and drug retention. However, current literature suggests that dimyristoyl-glycerol derived PEG (DMG-PEG) may provide greater LNP functionality. Our objective here is to determine if substituting DMG-PEG for DSPE-PEG in our LNP formulation will improve in vitro cellular delivery, without sacrificing critical quality attributes (CQA). Cellular uptake in human aortic smooth muscle cells is investigated using fluorescently tagged LNPs and quantified by fluorometry. CQAs are determined using standard characterization assays to measure siRNA drug loading and retention (EE%), LNP size, homogeneity, and particle stability. LNPs with DMG-PEG at 1-10mol% membrane incorporation significantly improved LNP cellular association compared to DSPE-PEG. Furthermore, LNPs with either DMG-PEG or DSPE-PEG maintain stable particle size and homogeneity (<100nm; PDI<0.2) and retain their full siRNA drug load (EE%>90%) for up to 28 days at 4°C storage. In conclusion, substituting DMG-PEG for DSPE-PEG may enhance in vitro efficacy of our LNP formulation without significantly affecting nanoparticle characterization or stability.

Session 1: Room A118

Judges: Drs. Agricola Odoi, Paolo Pazzi, and Troy Rowan
Presenters (in order of presentation): Alyssa Wilson, Heather Thomasovich, Morgan Adkins, Caroline Griffin, and Haley Richardson

Respiratory disease in calves post weaning exposed to an in-utero BVDV infection

Alyssa Wilson, Dr. Andrea Lear, Dr. Marc Caldwell, Dr. Korakrit Poonsuk, Dr. Nora Springer

The in-utero environment, including maternal viral infection, can have profound impacts on fetal immune development and neonatal susceptibility of disease(s) in humans and livestock. Calves infected in-utero with bovine viral diarrhea virus (BVDV) suffer from decreased growth rates and dysfunctional immune responses increasing their susceptibility to early life infections such as bovine respiratory disease. The objective of this study is to elucidate the mechanisms behind the long-term immunological impact of an in-utero BVDV transient infection. To meet this objective, weaned calves exposed to BVDV in-utero were inoculated with *Mannheimia haemolytica* (MH) or media via broncho-alveolar lavage (BAL). A two-by-two study design was used: control calves, not exposed to BVDV in-utero, inoculated with media (CRTL/CRTL, n=5), control calves inoculated with MH (CRTL/MH, n=5), transiently infected calves, exposed to BVDV in-utero with media inoculation (TI/CRTL, n=2), and transiently infected calves with MH inoculation (TI/MH, n=3). All groups were assigned clinical illness scores daily with thoracic ultrasound scoring throughout the study. Cytokine and MH antibody concentrations were assessed from BAL, serum, and saliva samples collected periodically throughout the study. Results found that the severity of clinical symptoms, including rectal temperature, correlates with the presence of lesions in the lungs and their severity. However, the difference between transient infected calves and control calves has so far yielded unconclusive when comparing clinical illness and thoracic ultrasound. Further statistical analysis results are still pending.

Biomarker discovery in pregnant cattle infected with bovine viral diarrhea virus

Heather Thomasovich, Dr. Jon Beever, Dr. Ky Pohler, Dr. Andi Lear, Michael Rivera Orsini

Exosomes are a subset of extracellular vesicles (EVs) that carry proteins, nucleic acids, and lipids and act as intercellular communication. Placenta-derived exosomes are released from the trophoblast along with pregnancy-associated glycoproteins (PAGs); both can be fetal health indicators. 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. This study aims 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, 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, 120, and 250 days of gestation and serum and plasma isolated. Exosome population was further isolated from plasma and 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 pregnancy-associated glycoprotein (PAG) concentration using a commercially available ELISA. No trend was observed in PAG concentrations between PI and CTRL groups. CD63+ placenta-derived exosomes increased over time in both PI and CTRL groups, but no significant differences in CD63+ or PLAP+ exosome

concentrations were found between treatment groups. 188 proteins, primarily involved in inflammatory and immune response, were differentially expressed between PI and CTRL groups. We expect differentially expressed RNA between PI and CTRL groups.

Targeted transcriptome analysis of beef cattle persistently infected with Bovine Viral Diarrhea Virus

Dr. Morgan Adkins, Dr. Jon Beever, Dr. Sonia Moisa, Dr. Andrea Lear

Bovine Viral Diarrhea Virus (BVDV) is an endemic virus of North American cattle populations with significant economic and animal health impacts. While BVDV infection has a myriad of clinical manifestations, a unique and problematic outcome is the establishment of a persistently infected (PI) animal following in-utero viral infection. While it is well established that PI animals serve as a constant reservoir of BVDV, the mechanism for the maintained infection remains unknown despite multiple theories. The purpose of this study was to use transcriptome analysis to further define long term immune status of adult PI cattle and offer insight into the potential mechanistic establishment of persistent BVDV infection. Peripheral blood mononuclear cells were collected from PI beef cattle (N=6) and uninfected controls (N=6) for targeted RNAseq analysis using 54 genes associated with immune function and followed by pathway enrichment analysis. Analysis revealed 29 differentially expressed genes (FDR < 0.05, fold change $\geq$ 2) representing 14 significant KEGG pathways between PI and control animals (FDR < 0.05). Transcriptome changes indicate chronic upregulation of interferon gamma (IFNG) with unexpected expression of related genes, suggesting a maintained stimulation of the PI immune system resulting from virus-mediated dysregulation of immune function.

Interferon stimulated genes as predictive morbidity markers in calves following in utero viral infection

Dr. Caroline Griffin, Dr. Andrea Lear, Dr. Jon Beever

During pregnancy, viral infections cause a potent inflammatory response which is linked to adverse pregnancy outcomes with increased risk of fetal morbidity and mortality. Interferon stimulated genes (ISGs) and their proinflammatory products are increased in placental and fetal tissues following in-utero viral infection. An over-active pro-inflammatory response by the fetal placenta may induce lasting changes to the fetal immune system, resulting in lifelong consequences. In this study we compared changes in placental and circulating ISG products in calves exposed to in utero viral infection and in healthy control calves. We hypothesized that gestational viral infection would result in an increased type I interferon (IFN) response with a measurable increase in ISG expression in the placenta of infected calves versus healthy controls. We also hypothesized that calves exposed in utero would have increased circulating ISG products during the neonatal (birth) and calfhood (3 months) phases of life. Circulating ISG products were measured via cytokine/chemokine magnetic bead panel, and differential expression and coregulation of ISGs in the fetal placenta was determined via RNA sequencing. Preliminary results indicate no treatment by time effect for any of the measured circulating ISG products. RNA sequencing results are pending. The information gained from this study is intended to provide scaffolding for further work investigating ISGs as biomarkers for determining placental and fetal health during compromised pregnancy and predicting neonatal outcomes following birth.

An investigation into alternative forages for grazing sheep

Haley Richardson, Dr. Andrea Lear, Dr. Patrick Keyser

Grazing native warm-season forages present opportunities for improved animal health and pasture management in small ruminants. Benefits include improved rates of gain from reduced parasite exposure and enhanced forage production with heat tolerant species. This study consists of two experiments evaluating grazing alternative forages in sheep. Experiment 1 evaluates nutritional benefits and parasite loads in sheep grazing native warm season grasses (NWG) versus a tall fescue (TF) control. The hypothesis is sheep grazing NWG will have lower parasite burdens due to taller grazing height. Experiment 2 examines the health impact of grazing switchgrass, a saponin-containing forage. For experiment 1, 32 yearling Katahdin ewes were blocked based on fecal egg counts (FEC) and randomized into two treatment groups, NWG (n=16) or TF (n=16). Animals grazed on assigned forages from early July through mid-August. During the trial, FEC, FAMACHA, body condition score, and packed cell volume were performed every 2 weeks. For experiment 2, 24 yearling Katahdin ewes were blocked based on body weight and randomized into three treatment groups: grazing switchgrass for 3 (SG3, n=6), 6 (SG6, n=6), or 9 weeks (SG9, n=6). Liver function was assessed via biochemical analysis and histopathology monthly. There is no significant difference in FEC and PCV values between sheep grazing NWG versus TF; however, there is a significant difference between time in GLDH after grazing SG, likely from ongoing acute hepatocellular damage. Completion of this experiment provided insight into parasitic and nutritional benefits of NWG, and the extent of toxic effects of switchgrass in sheep.

Session 2: Room A118

Judges: Drs. Agricola Odoi, Paolo Pazzi, and Troy Rowan
Moderators: Sree Rajeev
Presenters (in order of presentation): Micah Roberts, Prachi Namjoshi, Lichao Liu, and Mahesh Puttiyottu Poyil

Biomechanical evaluation of VetWelding resorbable pin and plates on cadaveric simulated metatarsal fractures

Micah Roberts, Dr. Darryl Millis, Dr. Pierre-Yves Mulon, Dr. Greg Woo

Fracture fixation with standard metal implants in small animals may have complications which necessitate removal after surgery. Refracture after implant removal is a complication that occurs due to open screw holes left behind. Biodegradable implants eliminate these complications by using fully resorbable implants that are broken down via hydrolysis. These implants are slowly resorbed over time which progressively loads the bone and results in the disappearance of any foreign material that the body might react to. The purpose of this study was to evaluate the biomechanical properties of rabbit metatarsal bones post fracture fixation with the VetWelding resorbable pin and plate system. 30 rabbit metatarsals were harvested and used in this study. An osteotomy was made in the center of the diaphysis and then repaired using the VetWelding resorbable pin and plate system. Groups were Control (C), 6 (6P, 3 pins on either side of the osteotomy) or 7 pins (7P with the 7th pin placed in the osteotomy). Metatarsals were subjected to 4-point bending biomechanical testing until failure. The strength and elastic modulus were determined. There were no treatment differences among groups for load at failure. There was no significant difference in modulus of elasticity between 6P and 7P groups however both were significantly lower than the C group. This initial study suggests that the VetWelding system has strength that is comparable to intact rabbit metatarsals. However, this system has lower elastic modulus resulting in easier deformation of the system than that of an intact metatarsal.

Tick vesicular-associated membrane proteins assist in the entry and survival of rickettsial pathogen in the arthropod vector

Dr. Prachi Namjoshi, Jaydeep Kolape, Avni Patel, Dr. Hameeda Sultana, Dr. Girish Neelakanta

Anaplasma phagocytophilum, an obligate intracellular rickettsial pathogen, is a causative agent of human anaplasmosis and a blacklegged tick, *Ixodes scapularis*, serves as a primary vector for this pathogen. Upon entering the host cell, *A. phagocytophilum* creates a host-derived vacuole called morulae. Within these morulae, *A. phagocytophilum* replicates to establish the infection. Very little information is known on how *A. phagocytophilum* enters and forms intracellular *Anaplasma phagocytophilum*-occupied vacuoles in tick cells, or the arthropod molecules associated with the morulae formation. In this study, we provide evidence that arthropod SNARE proteins such as VAMP3 and VAMP4 are critical for *A. phagocytophilum* entry, formation of morulae, and establishment of infection in tick cells. Quantitative real-time polymerase chain reaction (QRT-PCR) analysis showed that both *vamp3* and *vamp4* transcripts were significantly upregulated at early time points of *A. phagocytophilum* infection of tick cells. RNAi-mediated knockdown of *vamp3* and/or *vamp4* expression followed by confocal microscopy and QRT-PCR analysis showed a significant reduction in *A. phagocytophilum* burden in tick cells. Our study indicates that VAMP3 and VAMP4 are not only important for bacterial entry into tick cells/acquisition of bacteria from infected host into ticks but also in the persistent survival of this

bacterium in ticks and tick cells. This study provides further insights on how obligate intracellular bacteria exploit host SNARE proteins to establish its infection, making SNAREs as important tool to curb bacterial foothold.

Rickettsia parkeri infection in human endothelial cells is affected by NAD+ and a NAD-dependent enzyme

Lichao Liu, Dr. Shahid Karim, Dr. Chris Paddock, Dr. Hameeda Sultana, Dr. Girish Neelakanta

Rickettsia parkeri is a gram-negative, obligate intracellular bacterium responsible for causing rickettsiosis in humans. It is mainly transmitted by the Gulf Coast tick, *Amblyomma maculatum*. The symptoms of *R. parkeri* rickettsiosis are generally milder compared to Rocky Mountain spotted fever, which is caused by the more virulent *R. rickettsii*. Research on *R. parkeri* could enhance our understanding of other pathogens in the spotted fever group of rickettsiae. This study is focused on the involvement of NAD+ (nicotinamide adenine-dinucleotide) in the interaction between *R. parkeri* and its major target cell type, human endothelial cells. NAD+ plays a role in many cellular processes including an essential role in cellular oxidation-reduction reactions, the main source for NADP+, and a cofactor for various non-redox NAD-dependent enzymes. We found that *R. parkeri* infection regulates the transcription level of NAD+ related genes involved in the salvage pathway which maintains the intracellular NAD+ level, and the ROS pathway. Also, NAD+ treatment of the host cells and/or the bacterium results in significant changes in the bacterial burden. In addition, CD157, a NAD-dependent enzyme also known as BST1, was significantly upregulated upon *R. parkeri* infection of endothelial cells. The siRNA-mediated silencing of CD157 resulted in increased bacterial burden in endothelial cells. Taken together, these data indicated an important role for NAD+ and its signaling in *R. parkeri* infection of human endothelial cells.

Effect of active immunization with an arthropod protein on the transmission of Anaplasma phagocytophilum from ticks to the vertebrate host
Dr. Mahesh Puthiyottu Poyil, Dr. Prachi Namjoshi, Dr. Hameeda Sultana and Dr. Girish Neelakanta
Anaplasma phagocytophilum is a rickettsial pathogen that causes human Anaplasmosis. This bacterium is transmitted by a black-legged tick *Ixodes scapularis*. We recently reported that passive immunization targeting a *I. scapularis* organic anion transporting polypeptide (OATP) impairs transmission of *A. phagocytophilum* from ticks to the murine host. In the current study, we generated recombinant GST-tagged C-terminal fragment of the OATP protein (rGST-OATP) and performed active immunization studies in mice. Control mice were immunized with recombinant GST protein alone (rGST). Using ELISA and immunoblotting analysis, we detected high levels of the OATP antibodies in the rGST-OATP-immunized mice serum and in *A. phagocytophilum*-infected ticks that were fed on these mice. In addition, we detected decreased bacterial loads in blood and spleen of rGST-OATP-immunized mice compared to the levels noted in rGST-immunized mice. We also detected reduced bacterial loads in *A. phagocytophilum*-infected ticks that were fed on rGST-OATP-immunized mice. Similar observation in the bacterial loads was noted in molted ticks. Furthermore, we observed reduced molting of nymphal ticks that were fed on rGST-OATP-immunized mice. Increased cell death was noted in tick cells upon treatment with rGST-OATP antiserum. Our current results indicate that increased cell death in tick cells could be due to apoptosis. Taken together, these data indicate that the tick protein under study is an important candidate for the development of an anti-tick vaccine.

Session 3: Room A118

Judges: Drs. Braidee Foote and Andrea Lear
Moderator: Meaghan Harley-Troxell
Presenters (in order of presentation): Jeremy Turck, Kehinde Fasae, Durga Neupane, Swarnendu Basak, and Biswajit Bhowmick

Anaplasma phagocytophilum modulates arthropod autophagy molecules for its survival in Ixodes scapularis
Jeremy Turck, Dr. Hameeda Sultana, and Dr. Girish Neelakanta
Anaplasma phagocytophilum is the agent of human anaplasmosis. *Ixodes scapularis* is one of the tick species that is responsible for transmitting this bacterium to humans. *Anaplasma phagocytophilum* employs several strategies for its continued survival in host cells. This bacterium develops a host-derived vacuolar structure called morula and modulates the signal transduction of certain processes such as autophagy for its survival in host cells. Autophagy is a process of cell self-degradation, and it is an essential process to remove damaged or dysfunctional cells. In *Ixodes scapularis* unfed nymphal ticks and ISE6 tick cells, there are several autophagy molecules that are significantly upregulated upon *A. phagocytophilum* infection. Some of these autophagy molecules are essential for the initiation and formation of the autophagosome in order to begin the process of autophagy. We observed that when the expression of autophagy molecules such as ULK1 and Atg14 were silenced, the *A. phagocytophilum* loads were significantly decreased in tick cells. This study not only delineates tick cell signaling important for *A. phagocytophilum* survival but also provides evidence that this bacterium requires certain autophagy molecules for its survival in the vector host.

hTERT immortalized mesenchymal stem cell-derived EVs treatment reduces ZIKV-induced cortical neuronal infection, exosome-mediated transmission, and death

Kehinde Fasae, Ana Melentijevic Eckert, Dr. Girish Neelakanta, Dr. Hameeda Sultana

Mesenchymal stem cells (MSC) derived-extracellular vesicles (MSC-EVs) are paracrine effectors of MSCs, which play strategic roles in mediating intercellular communication between MSCs and target cells. Since MSC-EVs take on the function and properties of their mother cells and have lower immunogenicity, they have demonstrated beneficial effects in several preclinical and clinical disease models including viral infections and neurological disorders. In this study, we examined the neuroprotective effects of MSC-EVs on primary cortical neurons infected with Zika Virus (ZIKV). Neuronal cell death has been observed following ZIKV infection. The American Culture Type Collection (ATCC) 2022 challenge award to Dr. Sultana (call for proposals to use the product "ATCC® SCRC-4000-EXM ™", hTERT immortalized MSC-EVs in new, interesting, or daring applications) allowed us to test these hTERT MSC-EVs on ZIKV-infected neurons.
Our results show increased cell viability of hTERT MSC-EVs treated ZIKV-infected neurons compared to the untreated infected neurons with a decrease in markers of apoptosis including Caspase 3/9, Bax, and Bcl-2). Further, ZIKV transcripts were reduced in neurons treated with hTERT MSC-EVs, along with increased Interferon-beta (IFN-B) and decreased TNF-alpha expression. Inhibition of EVs production and/or release by GW4869 treatment followed by hTERT-MSC-EVs incubation affected exosome-mediated ZIKV transmission in primary cortical neurons. Our findings suggest a potential neuroprotective, pro-survival, and antiviral effect of hTERT MSC-EVs treatment on neurological dysfunction following ZIKV infection. This study sheds light on the promising therapeutic potential of hTERT MSC-EVs in ZIKV-mediated complications such as microcephaly in newborns and Guilian barre syndrome in adult humans.

Mosquito Exosomal tetraspanin CD151 regulates ZIKA and DENV2 infection

Durga Neupane, Dr. Hameeda Sultana, Dr.Girish Neelakanta

Mosquito-borne flaviviruses such as Dengue virus (DENV), Zika virus (ZIKV) and West Nile virus (WNV) infection has a significant impact worldwide, causing asymptomatic or mild fever to hemorrhagic disease, multiple organ failure, microcephaly, encephalitis, and death. Apart from the geographical spread of vectors and their contact with human population, a major factor contributing to the global threat of flaviviruses is the lack of effective vaccine and treatment strategies. Recent studies have highlighted the importance of tetraspanins for the development of novel therapeutic. Despite the importance of tetraspanins in numerous diseases, it is still unknown how tetraspanin proteins play roles in arthropod-borne flaviviral infections. In this study, we used two important human pathogens DENV2 (serotype 2) and ZIKV to study the role of mosquito tetraspanins combed from the *Aedes aegypti* genome. Among the seven tetraspanin selected, CD151 showed significant upregulation upon both ZIKV and DENV2 infection in mosquito cells and in EVs-derived from these cells. To further understand the role of CD151, we used RNAi-mediated silencing that significantly reduced the viral burden. Co-immunoprecipitation and immunofluorescence analyses also showed direct interaction and co-association of CD151 with ZIKV and DENV2 viral proteins . Furthermore, the inhibition of exosome release by GW4869 (inhibitor of exosome biogenesis) significantly reduced the viral burden and transmission via EVs. Overall, our study suggested that the tetraspanin CD151 acts as a therapeutic to block DENV2 and ZIKV infection.

Bovine Endometrial Epithelial Cells protects against ZIKA virus infection by modulating the antiviral interferon tau response

Swarnendu Basak, Katie Peterson, Emma Hessock, Dr. Girish Neelakanta, Dr. Daniel J. Matthew and Dr. Hameeda Sultana

Efficient and eco-friendly ruminant farming is crucial for maintaining the global food production especially as the world population is growing in developing countries. However, one of the major challenges in ruminant reproduction is early embryonic mortality, which occurs most often in the first few months of pregnancy and is a key factor in reproductive failure. The causes of early embryonic mortality are complex and multifaceted. One significant factor is the inadequate communication between the developing embryo (conceptus) and the lining of the uterus (endometrium). This communication is vital for the successful attachment and development of the embryo. There is also growing concern about the potential impact of ZIKA virus (ZIKV) on ruminant reproduction. The Centers for Disease Control and Prevention (CDC) notes that there hasn't been enough research to determine whether ZIKV can affect bovine animals. This raises questions about whether ZIKV could pose a serious risk to ruminants. In bovine species, the endometrial epithelial cells play a critical role in the attachment of the conceptus to endometrial wall through the induction of interferon-tau (IFN-tau), which is secreted by the trophectoderm of the conceptus. Our study demonstrates that ZIKV can successfully replicate within bovine endometrial epithelial cells. Additionally, transwell migration assays indicated that ZIKV can replicate and subsequently infect underlying cell layers through transcellular transport. Importantly, ZIKV loads increased from day 1 to 3 but gradually decreased from day 5 to 7. Moreover, we observed that ZIKV infection significantly upregulated the expression of interferon-stimulated gene 15 (ISG-15), interferon tau, and several other antiviral and retroviral genes, which may correlate with the observed decrease in ZIKV loads from day 3 to 7. Interestingly, we found an increase in ZIKV loads in extracellular vesicles (EVs) derived from infected bovine endometrial epithelial cells. Treatment of these infected cells with EVs biogenesis inhibitor, GW4869, resulted in a gradual decrease in ZIKV loads from day 1 to 7. This suggests that ZIKV may heavily rely on cell-secreted infectious EVs for its propagation. Our findings indicate that GW4869 could be a potential therapeutic agent for treating ZIKV infections in bovine species.

Mosquito Exosomal tetraspanin CD151 regulates ZIKA and DENV2 infection

Durga Neupane, Dr. Hameeda Sultana, Dr.Girish Neelakanta

Mosquito-borne flaviviruses such as Dengue virus (DENV), Zika virus (ZIKV) and West Nile virus (WNV) infection has a significant impact worldwide, causing asymptomatic or mild fever to hemorrhagic disease, multiple organ failure, microcephaly, encephalitis, and death. Apart from the geographical spread of vectors and their contact with human population, a major factor contributing to the global threat of flaviviruses is the lack of effective vaccine and treatment strategies. Recent studies have highlighted the importance of tetraspanins for the development of novel therapeutic. Despite the importance of tetraspanins in numerous diseases, it is still unknown how tetraspanin proteins play roles in arthropod-borne flaviviral infections. In this study, we used two important human pathogens DENV2 (serotype 2) and ZIKV to study the role of mosquito tetraspanins combed from the *Aedes aegypti* genome. Among the seven tetraspanin selected, CD151 showed significant upregulation upon both ZIKV and DENV2 infection in mosquito cells and in EVs-derived from these cells. To further understand the role of CD151, we used RNAi-mediated silencing that significantly reduced the viral burden. Co-immunoprecipitation and immunofluorescence analyses also showed direct interaction and co-association of CD151 with ZIKV and DENV2 viral proteins . Furthermore, the inhibition of exosome release by GW4869

(inhibitor of exosome biogenesis) significantly reduced the viral burden and transmission via EVs. Overall, our study suggested that the tetraspanin CD151 acts as a therapeutic to block DENV2 and ZIKV infection.

Dopamine Pathway in Ticks: Its Role in Exosome Production, Feeding Behavior, and Viral Transmission

Biswajit Bhowmick, Katarina Jones, Alison Fujii, Hector F. Castro, Shawn Robert Campagna, Girish Neelakanta, Hameeda Sultana

Dopamine signaling plays a crucial role in the survival of an organism. The dopamine receptor 1 (DR1) and aromatic L-amino acid decarboxylase (AADC) are the key components in this signaling process. Our research investigated the significance of dopamine signaling pathway in *Ixodes scapularis* ticks and its interaction(s) with Langat virus (LGTV), a model pathogen for the tick-borne encephalitis flavivirus. We observed that treating tick cells or ticks with L-dihydroxyphenylalanine (L-DOPA) or dopamine hydrochloride (DHCL) led to an increased expression of DR1 and AADC transcripts. Similarly, blood feeding in uninfected ticks resulted in elevated levels of DR1 and AADC transcript levels. Interestingly, LGTV infection caused a significant downregulation of both DR1 and AADC in tick cells, extracellular vesicles (EVs), and in unfed nymphal ticks. Silencing of DR1 and AADC expression through RNA interference (RNAi) had multiple effectson tick blood feeding, reduced LGTV transmission from infected ticks to naive hosts, and decreased viral loads in tick cells, EVs, and ticks. Super-resolution imaging revealed AADC distribution in EVs. Exogenous L-DOPA or DHCL treatment enhanced EVs secretion from both uninfected and LGTV-infected tick cells, while silencing of DR1 and AADC had the opposite effect. LGTV infection led to significantly reduced endogenous dopamine levels in tick cells, EVs, unfed nymphs, and in adult ticks,salivary glands (SG), and SG-derived EVs. Additionally, AADC enzyme activity was diminished in LGTV-infected nymphal and adult ticks. In conclusion, our findings suggest that dopamine is highly essential in ticks.

Session 4: Room A118

Judges: Drs. Braidee Foote, Andrea Lear, and Girish Neelakanta
Moderator: Meaghan Harley-Troxell
Presenters (in order of presentation): Avni Patel, Bryanna Fayne, and Swetha Madesh

Preliminary in vitro assessment of Leptospira-MDBK cell interaction.

Avni Patel, Dr. Liana Barbosa, Bryanna Fayne, Dr. Sreekumari Rajeev

Leptospirosis caused by the spirochete bacteria *Leptospira* is a worldwide neglected zoonotic disease with a global human and animal health impact. A comprehensive understanding of host-pathogen interactions is crucial to characterizing the pathogenicity of this organism. The basic characterization of pathogenicity aspects can be evaluated through *in vitro* cell culture-based systems. In this study, we evaluated *Leptospira's* effect on MDBK (Madin-Darby Bovine Kidney) cells. The cell culture plates were seeded with 10^5 MDBK cells/well and after 24 hours, the adherent cells were infected with *L.interrogans* serovar Copenhageni at a dose range from 10^8 to 10^2/ ml. We also cocultured *Leptospira* with MDBK cells immediately after combining both. We observed the culture plates for 7 days. In both conditions, MDBK cells viability and growth were not affected by *Leptospira.* The presence and growth of *Leptospira* was confirmed in the cell cultures using immunofluorescence assay. It is intriguing that the presence of pathogenic bacteria is not affecting the growth of a cell culture, and further studies will be needed to evaluate the conditions that allow the growth of cells and the bacteria. Further investigations may unravel the factors leading to renal *Leptospira* reservoir status commonly observed in animals.

Outcome of Leptospira interrogans serovar Copenhageni infection in resistant and susceptible C3H mice strains

Bryanna Fayne, Dr. Liana Barbosa, Avni Patel, Dr. Sreekumari Rajeev

Leptospirosis is a significant, underrecognized global zoonoses caused by *Leptospira*. The C3H/HeJ mouse is a well-recognized lab animal model for *Leptospira* studies due to the strain's spontaneous mutation in the toll-like receptor 4 (*tlr4)* gene and ability to develop leptospirosis. However, inconsistencies exist between studies on the effect of *Leptospira* on C3H/HeJ mice. The objective of this study is to compare the outcomes of *Leptospira interrogans* infection in C3H/HeJ mice and its wild-type mouse strain C3H/HeN. Eight-week-old male and female mice (n = 8) from the strains C3H/HeJ and C3H/HeN were intraperitoneally infected with $6x10^6$ *Leptospira interrogans* serovar Copenhageni strain Fiocruz L1-130. An uninfected group (n=4) from each strain was kept as negative controls. Mice were observed daily for up to 28 days post-infection to evaluate clinical scores and were humanely euthanized at IACUC-approved endpoints. Heart, lung, liver, spleen, kidneys, and blood were collected for evaluating *Leptospira* colonization (PCR and culture), host response (ELISA and Microscopic Agglutination Test (MAT), and cytokine evaluation). The C3H/HeN strain had a higher IgM and IgG response after infection compared to the C3H/HeJ strain, but both strains had a minimal agglutination response to *Leptospira* by MAT. Kidney cultures were positive for all infected C3H/HeN mice and seven infected C3H/HeJ mice. All uninfected mice were negative for *Leptospira* DNA and had no significant antibody responses against *Leptospira*. The C3H/HeN mouse had been previously described as resistant to leptospirosis but interestingly showed clinical signs of leptospirosis and a higher antibody response after *Leptospira* infection.

A pilot study to Characterize Immunogenic proteins from Ehrlichia canis and Anaplasma platys

Swetha Madesh, Dr. Liana N Barbosa, Dr. Alejandro Llanes, Dr. Sreekumari Rajeev

Ehrlichia canis and *Anaplasma platys* are significant tick-borne pathogens in dogs, transmitted by Rhipicephalus sanguineus, leading to severe conditions such as Canine Monocytic Ehrlichiosis and Canine Cyclic Thrombocytopenia. The challenge of culturing these obligate intracellular bacteria complicates vaccine and diagnostic development. This study aimed to identify and characterize shared immunogenic proteins of E. canis and A. platys to aid in vaccine and diagnostic test advancement. We utilized a high-throughput microarray containing 5000 B cell epitopes and screened the immunoreactivity using serum from dogs infected with *E. canis* and *A. platys.* Among 406 identified shared epitopes, the top ten reactive peptide epitopes were traced back to their respective proteins for further analysis. Using Uniprot, Interproscan, I-TASSER, and the Protein Data Bank (PDB), we predicted the functional and structural properties of three key proteins: surface antigen msp4 (P1), a conserved hypothetical protein (P2), and protein translocase subunit yidC (P3).P1, identified as part of the Msp4/OMP-like beta barrel family, is associated with bacterial surface antigens. P2, predicted to belong to the TrbC/VirB2 family, may play a role similar to VirB2, an immunogenic component in vaccines against Anaplasma marginale. P3 is linked to the YidC/ALB3/OXA1/COX18 family, which includes proteins involved in membrane insertion and assembly. In summary, peptide microarray analysis enabled the identification of protein candidates with value in vaccine and diagnostic development against *E. canis* and *A. platys*. Initial characterization through protein predictions software provided useful insights but require further refinement to select optimal targets for vaccine and diagnostic applications.

Session 5: Room A118

Judges: Drs. David Anderson, Stephanie Kleine, and Hameeda Sultana
Moderator: Dr. Girish Neelakanta
Presenters (in order of presentation): Demi Striligas, Rachel Hofer, Natalie Azzolini, and Chessa Brown

Use of a Distal Penile Urethrostomy with Partial Phallectomy in Standing Sedated Horses

Demi Striligas, Dr. Jim Schumacher, Dr. Henry Adair, Dr. Phillip Jones, Dr. Elizabeth Collar

Equine partial phallectomy is utilized to treat various penile conditions. Data is scarce comparing standing phallectomy and distal penile urethrostomy to phallectomy under general anesthesia. We hypothesized that standing sedated distal penile urethrostomy would have no increase in complication rates in the short- or long-term relative to other phallectomy methods and would still lead to high levels of owner satisfaction. Data was collected from 34 horses (37 procedures) over 15 years, including demographics, procedure types, cost, complications, and owner satisfaction. Owner follow-up via telephone gathered information on long-term complications, their management, tumor recurrence, patient mortality, and satisfaction. Data was analyzed using one-way ANOVA and Chi-square tests with significance set at $p<0.05$. Results showed no difference in cases between procedures performed standing or under general anesthesia in case demographics, procedure type, complications in hospital, or satisfaction as perceived by owners. However, standing procedures were cheaper than procedures under general anesthesia ($p=0.0069$). 32 of 37 cases had none to minimal short-term complications that resolved within 24

hours post-operatively. Long-term follow-up reported no observed complications in 61% of the 23 cases where follow-up was available; when complications were noted, they were described as manageable issues at home. Owners were highly satisfied with both standing (100%) and general anesthesia (96%) procedures. The findings suggest that phallectomy with distal penile urethrostomy performed under standing sedation is a viable option for equids, particularly where financial or physiological constraints exist, with no significant increase in risks of short-term or long-term complications or owner dissatisfaction.

Canine Plasmalipomas: a Retrospective Study

Rachel Hofer and DeNae Lobato

Plasmalipomas are skin tumors in canines consisting of two cell lineages: adipocytes and plasma cells. Skin tumors consisting of adipocytes or plasma cells alone–lipomas and extramedullary plasmacytomas, respectively–are common, well-documented, and typically benign. There has been a single case report on plasmalipomas but relatively little literature covers these specific entities. A subjective increase in the diagnosis of plasmalipomas in canine skin biopsies submitted to the UTCVM biopsy service was noted in recent years. This retrospective study sought to identify all plasmalipomas submitted to the UTCVM biopsy service from 2013-2023, determine if there has been a true increase in occurrence, and identify trends in tumor location and signalment. We hypothesized that this is not a true increase in the prevalence of plasmalipomas but rather an emerging recognition of a specific tumor that has been historically misclassified.

Pharmacodynamics of Intravenous and Oral Esomeprazole in Horses Administered Phenylbutazone and Dexamethasone

Natalie Azzolini, Melissa Hines, Rebekah Johnson, Rebecca Bergee, and Elizabeth Collar

Performance horses are at high risk for equine gastric ulcer syndrome (EGUS). The use of common horse show medications may exacerbate ulcer development. Esomeprazole is effective in horses, is shown to treat ulcers more effectively than omeprazole in people and has intravenous (IV) and oral formulations. Over 2 phases, 6 adult horses received phenylbutazone (bute; 2 grams SID) administered days 1 and 2, bute and dexamethasone (dex; 10 mg SID) administered days 3 and 4, and bute, dex and esomeprazole (IV or oral, randomized crossover design; 0.5 mg/kg) administered days 5 and 6. Gastric juice pH was measured daily and on day 7, and 1 hour after each new drug was introduced. Squamous and glandular ulcers were scored every other day (days 1-7). Data was analyzed using mixed model analysis for repeated measures. Significance was set at $p \leq 0.05$. Squamous ulcer scores increased from day 1 (2.0±0.63) to days 5 (2.67±0.52), and 7 (3.33±0.82) ($p \leq 0.044$), and from day 3 (2.5±0.55) and day 5 to day 7 ($p \leq 0.044$). Glandular ulcer scores increased from day 1 (0.33±0.52) to day 7 (1.50±1.22) ($p = 0.05$). Esomeprazole increased pH 1 hour after its administration ($p = 0.0089$). When separated by treatment, pH increased in horses receiving IV, but not oral, esomeprazole. However, there were no significant differences between IV and oral treatments in both pH and ulcer scores. Overall, squamous ulcer scores severely increased with administration of bute and dex. Esomeprazole was effective at increasing gastric pH with concurrent bute and dex administration.

Subchondral bone disease and treatment outcomes in non-racing horses

Chessa Brown, Stacie Aarsvold, Rebecca Bergee, and Elizabeth Collar

Subchondral bone disease is often diagnosed on MRI, but no standardized treatment protocol exists. Our objectives were to review lesions and treatments for subchondral bone disease in a population of horses referred for MRI and to assess outcomes (soundness and return to work). MRI (1.5T) reports from 2014-2023 and follow-up information from clients and referring veterinarians were reviewed. Lesions were categorized as either Grade 0 (cartilage), 1 (sclerosis/minimal bone loss), or 2 (bone loss/STIR hyperintensity). Treatments were grouped into rest/shoeing changes, conservative management >rest/shoeing (e.g., Injections), and surgical treatment. Data was analyzed using chi-square analysis with significance at p£0.05. Eighty-seven subchondral bone lesions in 67 limbs in 41 horses were evaluated with only the primary lame limb included for analysis. Follow-up was obtained in 36/41 cases, with 22 cases having bone lesions as the primary cause of lameness. Following rest or shoeing changes, 71% (5/7) became sound, but 86% (6/7) improved and returned to work. Following conservative management, 75% (6/8) of cases became sound and returned to work. After surgical treatments, 86% (6/7) became sound and returned to work, with 100% (7/7) improvement in all cases. No treatment allowed more than 50% (conservative management) of cases to return to the same level of performance. No significant differences were found between treatments. Treatment choice appears to depend on the severity or type of lesion. As long as some targeted treatment is provided for the diagnosed subchondral lesions, most horses appear to improve.

Session 5: Room A118

Judges: Drs. David Anderson, Stephanie Kleine, and Tena Ursini
Moderator: Dr. Hameeda Sultana
Presenters (in order of presentation): Emily Travis, Lily Davis, Logan Kirby, and Victoria Stancy

Compound 6, a synthetic kisspeptin analog, increases plasma luteinizing hormone levels in cows with low plasma progesterone concentrations.

Emily Travis, Dr. Brian Whitlock, Dr. M Beltramo, Dr. Casey Nestor, Dr. Alex Esteller-Vico, Dr. Kara Brady, Xiaocun Sun

Kisspeptin (KP) regulates reproduction by controlling the release of gonadotropin-releasing hormone, which stimulates luteinizing hormone (LH) secretion, essential for ovarian follicular development and ovulation. Compound 6 (C6), a synthetic analog of KP, has structural modifications and an albumin-binding addition, enhancing its potency and reducing degradation in the body. This study aimed to evaluate the effect of C6 on plasma LH concentrations in cows with a low plasma progesterone concentration. Cows were administered one of two treatments [control (2 mL saline) or C6 (100 nmoles in 2 mL of saline); n=10 per treatment)] in crossover experiments (one replication with about one month between treatments). Treatments were administered intramuscularly on Day 6 of follicular wave emergence and blood samples were collected every hour for 12 hours (1 hour pre- to 11 hours post-treatment) for analysis of plasma LH concentration. Intravaginal progesterone-releasing devices were used in all cows so that differences in endogenous hormone profiles would not confound results. There was an effect of treatment ($p < 0.0001$) and an interaction of treatment and time ($p < 0.001$) on plasma concentrations of LH. Compared to the control group, C6 increased plasma LH concentrations

overall ($p < 0.05$) and specifically at Hours 5 and 7 ($p < 0.05$). Additionally, C6 increased plasma LH above baseline values (Hour 0; C6 treatment group) from Hours 4 through 7 ($p < 0.05$). The findings suggest that C6 is an alternative to KP with potential applications to improve reproduction cattle.

Comparison of the effects of hydromorphone alone and combined with medetomidine-vatinoxan or dexmedetomidine on the induction dose of alfaxalone and selected cardiopulmonary variables in healthy dogs anesthetized with sevoflurane.

Drs. Lily Davis, Chiara Hampton, Stephanie Kleine, Christopher Smith, Genevieve Bussières, Xiaojuan Zhu, and Reza Seddighi

The primary goal of this study was to determine the effect of pre-anesthetic sedation with combinations of medetomidine-vatinoxan, dexmedetomidine and/or hydromorphone on the induction dosage of alfaxalone in dogs. A secondary aim was to compare the effects of these combinations on sedation and recovery scores, and on cardiopulmonary and biochemical variables during 60-min of sevoflurane anesthesia. Eight healthy, adult Beagles were enrolled in a randomized, masked, crossover study. Dogs received intramuscular hydromorphone (H; 0.1 mg kg^{-1}), dexmedetomidine + hydromorphone (DH; 0.005 mg kg^{-1}+ 0.1 mg kg^{-1}, respectively), or medetomidine + vatinoxan + hydromorphone (MVH; 0.01 mg kg^{-1} + 0.2 mg kg^{-1} + 0.1 mg kg^{-1}, respectively). Anesthesia was induced with alfaxalone and maintained for 60 minutes with sevoflurane. Sedation and recovery scores were rated, and selected cardiopulmonary and biochemical variables were measured by a masked observer. Mixed effects ANOVA and ANOVA on ranks were used to evaluate differences between treatments, time, and their interaction, and Tukey-Kramer method was used for post-hoc analysis ($p<0.05$). Dogs receiving MVH had greater sedation scores compared to DH and H ($p<0.01$ and $p<0.001$, respectively), required a lower mean dosage of alfaxalone (0.77 ± 0.4 mg kg^{-1}) compared to DH and H (1.16 ± 0.34 mg kg^{-1};1.13 ± 0.18 mg kg^{-1}, both $p=0.02$), but had lower systolic and mean arterial pressures compared to DH. Treatment did not affect biochemical variables clinically, nor recovery scores ($p=0.26$).

Evaluating the Cerebrospinal Fluid Pharmacokinetics of Sapropterin Dihydrochloride in Sheep: A Model for Human Depression Therapy

Logan Kirby, Dr. Joseph Smith, Dr. Bhavya Sharma, Dr. Keith Hyland, Dr. Keith LeVert, Kevin Ledford, Dr. Brian Whitlock

Fifteen percent of the 21 million Americans diagnosed with clinical depression are non-responsive to current treatment options, classifying them as having treatment refractory depression. Tetrahydrobiopterin (BH4) is a precursor to dopamine, serotonin, and norepinephrine. In patients with depression, these neurotransmitters are often at a deficient level. Kuvan®, sapropterin dihydrochloride, is a synthetic form of BH4 used to treat phenylketonuria. Recently, administration of Kuvan® was found to improve symptoms of treatment refractory depression. However, there is no pharmacokinetic data on cerebrospinal fluid (CSF) concentrations of BH4 following oral administration of Kuvan®. We hypothesize that oral administration of Kuvan® will increase CSF concentrations of BH4. In this study, Kuvan® was administered to sheep, 1500 mg daily via bard button for 5 days and CSF samples were collected, allowing for both acute and steady-state pharmacokinetic

analysis. Following acute (single dose) Kuvan® administration, there was a 2.54 ng/mL increase of BH4 from baseline and the time to maximum concentration was 2.63 hours. The elimination half-life for acute Kuvan® administration was 3.32 hours. Steady-state (multiple dose) Kuvan® administration elevated CSF BH4 by 6.09 ng/mL and the time to maximum concentration was 2.35 hours. The elimination half-life of Kuvan® at this state was 2.47 hours. The accumulation ratio of Kuvan® throughout the study was 2.16. In future studies, it would be beneficial to administer Kuvan® to subjects with centrally low BH4, to restore these levels as a model of treatment resistant depression.

Mild Traumatic Brain Injury and Neuroinflammation: Biomarker Discovery in a Yucatan Pig Model

Victoria Stancy, Dr. Brian Whitlock, Dr. Chiara Hampton, Dr. Bhavya Sharma, Dr. Pierre-Yves Mulon DVM, Dr. Jon Beever, and Dr. Allison Renwick PhD, Laura Crispell

Traumatic brain injury (TBI) is an epidemic likely causing both physical and mental diseases. Currently, there are no clinically available diagnostics for mild TBI (mTBI), although the most common form of TBI is mTBI. We recently used 10 male castrated Yucatan pigs in a mTBI model so that new diagnostics may be tested. Prior to treatment administration animals were surgically instrumented with jugular and intrathecal catheters for collection of blood and cerebrospinal fluid (CSF). A few days after instrumentation, while the pigs were anesthetized, a shock wave tube, charged with nitrogen gas to a pressure of ~413 kPa, was discharged approximately 5 cm from the right temporal bone to induce a mTBI. Pigs were recovered from anesthesia and blood, saliva and CSF was collected over the next 10 days. Subsequently animals were euthanized, their brains were collected, sectioned, observed for gross lesions and prepared for immunohistochemistry to evaluate expression of three biomarkers of neuroinflammation (ß-APP, GFAP, and IBA-1). Additionally, brain samples will be assessed using RNA-sequencing to determine changes in the transcriptome. Lastly, plasma concentrations of circulating biomarkers associated with neuroinflammation will be determined using Single Molecule Array immunoassays. We hypothesize that the pigs that received the shock tube-induced mild TBI, will have increased concentrations of neuroinflammatory biomarkers in their brains and plasma, as well as changes consistent with neuroinflammation in their brain transcriptome. After completion of this study, we believe that the results that are obtained from analyzing these neuroinflammatory biomarkers will result in new diagnostic treatments for mTBI.