

2021 UTCVM RESEARCH DAY ABSTRACTS

SESSION A

Room A203: Sequoyah Graduate Classroom

Zoom Link: <https://tennessee.zoom.us/j/97260975618>

“Rat dental pulp stem cells as a multi-potent bio-additive for tissue engineering technologies”

Austin Bow, Thomas Masi, Madhu Dhar

Dental pulp-derived stem cell (DPSC) cultures presents a promising source for a multi-potent bio-additive, with these cells being neural crest origin. Dental pulp from rats was dissected and cultured in both α -MEM and DMEM/F12-based mediums. Characterization was conducted using flow cytometry to determine expression profiles of the key stem cell markers. Despite similar marker profiles, morphological differences were observed, with rounded cells present in α -MEM cultures resembling endothelial-like cells as opposed to the maintained spindled shape of cells in DMEM/F12. These data, as well as that on proliferation, indicated that DMEM/F12 cells may be superior as a multi-potent bioactive additive. Biomaterials fabricated using a pneumatic additive manufacturing system were examined in combination with cell populations. Composites consisting of a modified thermoplastic basal structure and a protein-rich gel coating showed promise as potential DPSC delivery vehicles in future in vivo studies for promoting repair of a robust selection of target tissues.

“Stem cell activity during osteoporosis”

Amber MacDonald, Lisa Amelse, Austin Bow, Steven Newby, Thomas Masi, Madhu Dhar

Osteoporosis is the most common bone disease whereby old bones are susceptible to severe fractures. The cells responsible for developing new bone are mesenchymal stem cells (MSCs), and are a targeted intervention for osteoporosis. The causes of osteoporosis involve cell signaling mechanisms whereby MSCs resist osteogenesis. Currently, information regarding genetic activity of MSCs from an osteoporotic model is limited. In this study, we obtained rats that were either sham-operated or ovariectomized (OVX). After eight weeks, bone marrow-derived MSCs were harvested and cultured with osteogenic stimulants. Afterwards, cells were stained for mineralization and underwent RNA sequencing at two time points. We found that MSCs derived from OVX animals lacked mineralization in comparison to control animals. Subsequently, specific gene clusters important to bone development were reduced in MSCs derived from OVX animals. This information is necessary for future drug development that could “boost” the production of new bone material in osteoporotic women.

“Poly lactic-co-glycolic acid and graphene oxide nerve conduits implanted with human adipose-derived mesenchymal stem cells to promote axonal growth in peripheral nerve defect”

Meaghan Harley, Alisha Pedersen, Steven Newby, Richard Steiner, Dustin Crouch, David Anderson, Madhu Dhar

The overall goal of this research is to develop a new medical device to improve the treatment for peripheral nerve injuries when a segment of the nerve has been lost. The current clinical standard for nerve gap injuries is an autograft, but harvesting of peripheral nerves can cause untoward effects. The goal of the current studies is to fabricate a nerve conduit using Poly Lactic-co-Glycolic Acid combined with 0.25% Graphene Oxide. We expect that the conduit will promote axonal growth and that this process can be accentuated with human adipose-derived mesenchymal stem cells. Towards this goal, we first validated markers including, Vimentin, Microtubule Associated Protein 2, β -Tubulin III, and Neuron-Specific Enolase which will be expressed by the cells as they undergo neural differentiation. Next, conduits were fabricated using 3D printing, and were demonstrated to be cytocompatible. Ultimately, the conduits will be used *in vivo* in a rat sciatic nerve defect model to evaluate the regenerative potential.

"Effects of acepromazine and butorphanol on propofol induction dose in healthy dogs"

Stephanie Dantino, Stephanie Kleine, Christopher Smith, Sayge M Smith, Xiaojuan Zhu, Reza Seddighi

Objective: Determine the effects of intravenous (IV) premedication with acepromazine, butorphanol, or their combination on the propofol induction dose. **Methods:** Six adult Beagles received six IV treatments given once weekly in a Latin square design: 0.9% saline, acepromazine (0.02 mg kg⁻¹ and 0.04 mg kg⁻¹), butorphanol (0.2 mg kg⁻¹ and 0.4mg kg⁻¹) and acepromazine 0.02 mg kg⁻¹ with butorphanol 0.2mg kg⁻¹ (ABC). Fifteen minutes later, propofol was administered (1 mg kg⁻¹) IV, then aliquots of 0.5 mg kg⁻¹ every 15 seconds, until intubation. Physiological variables, propofol dose, and adverse effects were recorded at baseline, post-premedication, post-induction and extubation. Data were analyzed using mixed-effects ANOVA ($p < 0.05$). **Results:** Mean propofol dose was lower in all treatments compared to saline with the largest decrease with ABC. Mean arterial pressure in acepromazine treatments was lower post-induction compared to baseline. ABC had the highest incidence of transient hypotension. **Conclusion:** ABC resulted in the largest decrease in propofol dose but was associated with the highest incidence of transient hypotension.

"Biometric assessment of gait following locking plate fixation bridging a segmental bone gap in the tibia of goats"

Kristin Bowers, Pierre-Yves Mulon, Steve Adair, Lori Terrones, Elizabeth Croy, Caroline Billings, Xiaocun Sun, David Anderson

The purpose of this study was to analyze the effect of locking plate stabilization on a tibia segmental ostectomy in goats. We hypothesized that stable fixation of a segmental bone defect, using a locking plate, would result in minimal changes in gait biometric variables. Boer-cross goats (n=17) were trained to walk through a pressure-sensing walkway, and a right hind tibia segmental ostectomy and bridging stabilization with a locking plate were performed on each goat. Biometric data was collected preoperatively and on days one and seven postoperatively. Statistical analysis revealed significant changes in the operated limb (decreased maximum peak pressure, maximum force, and stance time), but alterations of gait biometrics were not found in the other limbs.

"Comparison of protocols for in vitro drug elution characteristics"

Caroline Billings, Cassandra Downing, David Anderson

In vitro drug elution experiments are commonly performed when evaluating potential fitness of local drug delivery devices for in vivo use. Evaluation of drug elution characteristics spans many arenas including antimicrobial and chemotherapeutic drug delivery systems. Despite widespread utility, there is little agreement in methodology to perform such studies, and there are many limitations recognized in published works. We chose to evaluate two of the most commonly reported in vitro drug elution methods. We used a commercially available collagen matrix (Fibro-Gide, Geistlich) and a commonly used local antibiotic (gentamicin). The protocols used either 1) washing and replacement of media, or 2) replacement of media without washing. Results indicate no statistically significant difference in elution profiles between the two methods utilizing this delivery vehicle and drug. These results may provide the framework for moving towards more consistent methodology for in vitro elution experiments and address some acknowledged limitations in the literature.

"Pharmacokinetics of orally administered single-dose ponazuril in cats"

Becky DeBolt, Catherine Burlison, Sherry Cox, Joseph Smith, Jennifer Stokes, Jacqueline Whittemore

Ponazuril is commonly used to treat coccidiosis in shelter cats (using one to three daily doses), but its pharmacokinetics are still undetermined. Six healthy cats received a single dose of 50mg/kg ponazuril PO. Plasma was collected at predetermined intervals from time 0 to 336 hours after dosing. The samples were analyzed using high pressure liquid chromatography, and concentration data were analyzed using non-compartmental analysis. Mean \pm SD area under the plasma concentration time curve was $1,302 \pm 490$ h- μ g/mL with a peak concentration of 7.49 ± 2.06 μ g/mL at 14.67 ± 7.45 h post-administration. Elimination half-life was 136 ± 48 . Ponazuril was well-absorbed in cats following oral administration: it remained detectable through the final sampling timepoint, and no adverse effects were observed. The single dose produced plasma concentrations that inhibit growth in similar organisms in vitro and is effective against apicomplexans in other species. This study will enable further pharmacodynamic field studies to determine dosing optimization in cats.

SESSION B

Room A118: First Year Classroom

Zoom Link: <https://tennessee.zoom.us/j/95127116818>

“Investigation of the prevalence of *Toxoplasma gondii* in North American waterfowl”

Nicole Szafranski, Kyle Van Why, Janetta Kelly, John Veon, Charlie Bahnson, Bradley Cohen, Abigail Blake-Bradshaw, Richard Gerhold

The role of waterfowl has been largely overlooked in the epidemiological picture of the zoonotic parasite, *Toxoplasma gondii*. Infected waterfowl pose a threat to hunters who may consume undercooked meat and to wild predatory species. Samples of heart and sera from a variety of waterfowl species were collected from hunter-killed birds and wildlife management activities. Antibody titers were determined via the modified agglutination test (MAT). Parasitic DNA within the tissue was analyzed via polymerase chain reaction (PCR) and genetic sequencing. In total, samples from 737 birds were collected through the 2020-2021 hunting season from five different states (AR, MN, ND, PA, TN). Antibody titers of at least 1:5 were found in 25.8-43.2% of the birds, and PCR positivity ranged from 0-8.6%. As an important potential transmission source to other wildlife and to humans, investigating toxoplasmosis in waterfowl is warranted and can help inform future management decisions and education efforts.

“Metabolic profile of *Histomonas meleagridis* and undefined bacterial population in Dwyer’s media with and without rice starch”

Sawsan Ammar, Nicole Szafranski, Courtney Christopher, Rebekah Jones, Sree Rajeev, Hector Castro, Robert Campagna, Richard Gerhold

Since 1970, *Histomonas meleagridis*, an important pathogen of Galliformes, has been propagated in Dwyer’s media containing rice starch. To investigate the role of rice starch in Dwyer’s media, we conducted a metabolic analysis of intracellular metabolites of *H. meleagridis*, and undefined bacteria grown in Dwyer’s media with (SD) and without rice starch (NR). Metabolites were measured using ultra performance liquid chromatography-high resolution mass spectrometry and total of 170 known metabolites were detected. Dwyer’s media with rice starch significantly supported the growth of *H. meleagridis* in comparison to NR media while bacterial growth was not affected by the type of the media used. There was a significant difference in metabolites at 0 hours between SD and NR media while there was a minimal difference in the blank media. That was further investigated, and we found that riboflavin recorded the highest variable importance in projection score that was significant at all five components at blank and 0 hours. In the future, we will be investigating the role of riboflavin and if media supplemented with riboflavin can support *H. meleagridis* growth in absence of rice starch. Furthermore, these findings have potential for discovering chemotherapeutic agents to control and prevent blackhead in poultry.

“New technique to grow *Toxoplasma gondii* directly from animal tissue on cell culture”

Tania Dawant, Chunlei Su, Richard Gerhold

Toxoplasma gondii is a coccidian parasite that can infect most warm-blooded vertebrates and causes disease in a wide variety of species (including humans). Currently, the standard for growing the parasite for research purposes is through mouse bioassay. First, serological testing is done on a host animal of interest with the Modified Agglutination Test (MAT). Heart tissue is collected from animals with a high titer, prepared and injected into the peritoneum of mice. The parasite proliferates within the mouse and is collected via peritoneal lavage. The goal of this study was to develop a technique that eliminates the need for mice in this process. Cell culture flasks with Vero or HFF cells are directly inoculated with prepared heart tissue from an infected animal. This has been demonstrated successfully and has the potential to be broadly used to isolate *T. gondii* for research purposes.

“Detection of select vector borne agents of ticks collected from East Tennessee veterinary clinics”

Katy Wilkinson, Nicole Szafranski, Richard Gerhold, Sawsan Ammar, Eliza Baker, Nate Crilly, and Ashley Hartley

Cytauxzoon felis transmission occurs via infected ticks, and clinical disease in domestic cats from asymptomatic to life-threatening illness. Competent tick vectors are present in Tennessee, yet the prevalence of *C. felis* within these tick vectors is unknown. Our study objective was to determine the prevalence of *C. felis* DNA in Tennessee ticks and compare to clinical cytauxzoonosis in a feline referral population. Ticks (n=144) collected via a 2016 Tennessee passive surveillance program were extracted and tested against *C. felis*-specific DNA primers. Positive PCR results were present in four tick samples (4/144, 2.8%), all from *Amblyomma americanum* ticks in eastern Tennessee. Review of coded feline medical records (2010-2021) from a large referral hospital revealed 2 clinical cases (2017, 2019); two additional cases were identified (2020-2021) via a clinician case log search. *C. felis* is an emerging disease present in eastern Tennessee and infection should be considered in cats with tick exposure.

“Establishing a wildlife biobank at The University of Tennessee College of Veterinary Medicine with a pilot study of wildlife pathology”

Julie Bedwani, Michelle Dennis, Richard Gerhold

Wildlife populations have important roles in the ecology of emerging infectious diseases. Surveillance is essential for early detection and management of these diseases. Biobanking of tissue samples augments disease surveillance by facilitating retrospective comparisons which strengthen our understanding of disease ecology. The objective of this project was to initiate and develop a sustainable protocol for long term disease surveillance and biobanking of wildlife presenting through UTCVM. A selection of disposal wildlife carcasses were necropsied and tissues, serum, parasites, urine, and feces were sampled in replicates and stored at -80C, and 90% ethanol, and 10% neutral-buffered formalin. A total of 131 animals were examined from May-July, 2021, including 84 (64%) mammals, 34 (26%) birds, and 13 (10%) reptiles and amphibians. Etiologic categories included trauma (59%), infectious disease (5%), combined trauma and infectious disease (4%), and other/uncertain (32%). Ancillary diagnostics applied to biobanked tissues by researchers at UT allowed the diagnosis of leptospirosis in two species. The protocol was successfully trialed through student participation. Its continuation will likely require greater species focus, dictated by researcher interest and storage capacity.

“A retrospective review of spinal disease in non-domestic felids”

Abbie Metcalfe, Andrew Cushing, Michelle Dennis

This retrospective study characterized and determined the prevalence of spinal disease in non-domestic felids within a sanctuary population. A review of 294 necropsy reports in *Panthera* species from 2003 to 2021 revealed that 28% (81/294) of the population was diagnosed with spinal disease. Spinal lesions were categorized by the following pathologic processes: degenerative (91%), developmental (11%), inflammatory (8.6%), and neoplastic (8.6%). Intervertebral disc disease was found in 74% (60/81) of the study population, primarily affecting the cervical spine. A chi-square test of independence was used to identify signalment factors associated with the occurrence of spinal disease (significance level $p < .05$). Specifically, lions [$\chi^2 (N=26)=13.2, p < .001$], males [$\chi^2 (N=53)=7.1, p = .0079$], and aged ($\geq 14y$) animals [$\chi^2 (N=59)=5.3, p = .021$] were significantly more likely to have spinal disease. The spinal pathology documented in this study provides insight into high-risk signalment categories and predominant disease types.

SESSION C

Room A335: Third Year Classroom

Zoom Link: <https://tennessee.zoom.us/j/93149909456>

“Geographic disparities and temporal changes of diabetes mortality risk among adults in South Africa, 2002-2016”

Tamara Chavez-Lindell, Agricola Odoi

Background: Information on geographic disparities and temporal changes in diabetes mortality is important for health planning but is not currently available in South Africa. Therefore, this study investigated geographic disparities and temporal changes in diabetes mortality risks in South Africa between 2002 and 2016. **Methods:** Diabetes death records among adults were used and temporal trends investigated using one-tailed tests of proportions. Choropleth maps were used to investigate district-level spatial patterns. Flexible spatial scan statistics were used to identify high-risk spatial clusters. **Results:** Increasing temporal trends and winter peaks in diabetes mortality risks were identified. Overall, 59.6% of the districts had significant risk increases between 2002 and 2016. Two significant ($p=.001$) spatial clusters of high mortality risks (Relative Risks=1.30 and 1.36) were identified. **Conclusions:** There was evidence of geographic disparities, increasing temporal trends, and winter peaks in diabetes mortality risks. This information is useful for targeting interventions to reduce mortality.

“Geographic disparities and socio-demographic predictors of pertussis risk in Florida”

Corinne Tandy, Agricola Odoi

Objective: This study investigated the geographic disparities, temporal changes, and socioeconomic and demographic predictors of pertussis risk in Florida from 2010 to 2018. **Methods:** Spatial patterns and temporal changes of pertussis risk were assessed using county-level choropleth maps. Tango’s flexible spatial scan statistics were used to identify high-risk pertussis spatial clusters. Ordinary least squares regression was used to identify significant predictors of county-level pertussis risk. **Results:** A total of 11 clusters were identified. Geographic distribution remained relatively consistent over time with areas of high risk persisting in the western panhandle, northeastern coast, and along the western coast. Predictors of high county-level pertussis risk were rurality, higher median income, and higher percentage of females. **Conclusion:** This study highlights the importance and application of GIS technology and spatial statistical/epidemiological tools in identifying areas of highest disease risk to guide resource allocation to reduce health disparities and improve health for all.

“Antimicrobial, multidrug and methicillin resistance among *Staphylococcus* spp. isolated from canine clinical specimens between 2006 and 2017: A descriptive study”

Jennifer Lord, Nick Millis, Rebekah Duckett Jones, Brian Johnson, Stephen Kania, Agricola Odoi

Objective: This study investigated antimicrobial resistance among staphylococci from canine specimens submitted to the University of Tennessee College of Veterinary Medicine Bacteriology Laboratory. **Methods:** Antimicrobial susceptibility and *mecA* polymerase chain reaction results from 2006 to 2017 were obtained for canine *Staphylococcus* isolates. Temporal trends were assessed with Cochran-Armitage tests. Agreement between PCR and disk diffusion was assessed using kappa tests. **Results:** Commonly isolated species were *S. pseudintermedius*, *S. aureus*, and *S. schleiferi*. Almost half (45.5%) of *S. pseudintermedius* isolates were multidrug-resistant (MDR), and 30.8% were methicillin-resistant. There were significant ($p < 0.05$) increases in levels of methicillin and chloramphenicol resistance during the study period. For *S. aureus*, 40.9% were MDR and 37.4% were methicillin-resistant. Oxacillin was superior to cefoxitin for detecting *mecA*-mediated resistance in all species except *S. aureus*. **Conclusions:** These findings have implications for treatment decisions and public health. Ongoing surveillance and antibiograms should guide clinical practice.

“Prevalence and predictors of stroke among individuals with prediabetes and diabetes in Florida”

Md Marufuzzaman Khan, Agrícola Odoi

Objectives: The objectives of this study were to estimate the prevalence and identify predictors of stroke among persons who reported having either prediabetes or diabetes in Florida. **Methods:** Behavioral Risk Factor Surveillance System survey data, summary statistics, and a conceptual model of stroke predictors were used in the investigation. Two multivariable logistic models were built to investigate predictors of stroke among adults with prediabetes and diabetes. **Results:** The percentage of stroke among respondents that had prediabetes and diabetes was 7.8% and 11.2%, respectively. The odds of stroke were significantly higher among respondents with prediabetes that were ≥ 45 years old, had hypertension and hypercholesterolemia. On the other hand, the odds of stroke among respondents with diabetes were significantly higher if respondents were non-Hispanic Black, hypertensive, and had depression. **Conclusions:** Study findings are useful for guiding health programs aimed at reducing stroke burden among adults with prediabetes and diabetes in Florida.

“Mommy, why is grandma getting fat? A research plan to compare lean to overweight post-menopausal women”

Beth Wilson, Cristina Barroso

Long-ranging and comprehensive research into overweight and obesity has been conducted in the overall population with subgroups of children and pregnant women given special attention. However, few studies exist on the post-menopausal female population. The prevalence of obesity increases significantly in American women after they reach age 40, reaching 65% of women between 40 and 59 years and 73.8% in women over age 60 (Lizcano, 2014). Accompanying this prevalence are increases in obesity-associated co-morbidities such as metabolic syndrome, cardiovascular disease, and diabetes. With menopausal women being three times more likely to develop obesity and metabolic syndrome than premenopausal women (Eshtiaghi, Esteghamati and Nakhjavani, 2010), understanding causes of weight gain in older women is a direct method to improving worldwide overall health. How do always-lean women differ from those who become overweight with age? A research plan will be presented that uses existing literature, prior studies, and population-based longitudinal data.

“Comparing methodologies for the detection of Leptospira in environmental samples”

Myranda Gorman, Dhani Prakoso, Sreekumari Rajeev

Leptospirosis, a life-threatening disease of humans and animals, is a widespread global zoonosis. Contact with urine from infected animals and contaminated environment is the major source of transmission to humans and animals. We explored the usefulness of next generation sequencing with MinION when compared to culture-based methods and PCR to detect Leptospira in water and soil samples obtained from a local creek. Direct PCR on DNA extracted from water and soil samples were positive for Leptospira genus specific 23S and 16S ribosomal DNA targets but was negative for LipL32, a pathogenic Leptospira gene marker. However, PCR of the cultured samples were positive for LipL32. We have completed the next generation sequencing of the samples using Nanopore MinION device and are analyzing the data. Our study confirms that pathogenic and saprophytic Leptospira is in our environmental samples. Further characterization of Leptospira isolates by PCR and sequencing is underway.

“Controlling the HSV-1 infection by metabolic manipulation”

Engin Berber, Logan Miller, Barry T. Rouse

HSV is one of the causative viral agents of eye lesions and herpetic encephalitis. The role of immune-mediated latency and reactivation has been described but how metabolic changes can affect the outcomes of acute infection is poorly understood. Thus, we suspect that some changes in host metabolism could influence the function of the immune cells and outcomes can be either beneficial or detrimental. By using an encephalitis and eye model mice, we showed inhibition of glucose metabolism results in less ocular damage but reduced survival due to the development of encephalitis. Inhibition of immune cell functions in the trigeminal ganglia where virus establishes the latency suspected to cause the encephalitis. Furthermore, we showed that mice that received the glucose-controlling agent, metformin, had an increased survival rate. Together, we conclude that targeting metabolism during the acute HSV infection may be the key to controlling the HSV and outcomes of infection.

SESSION D

Room A203: Sequoyah Graduate Classroom

Zoom Link: <https://tennessee.zoom.us/j/97260975618>

"Effects of chronic endotoxemia on rectal and subcutaneous temperature in wethers"

Allison Renwick, Joseph Daniel, Brian Whitlock

Lipopolysaccharide (LPS) causes inflammation and fever in bacterial infections. Twenty-two wethers underwent chronic LPS challenge [control (CON;saline IV;n=4),single acute dose (SAD;400 ng/kg on D1 saline D2-7 IV;n=4),daily acute dose (DAD;400 ng/kg IV;n=5),daily increasing dose (DID;400 ng/kg with 20% increase each day IV;n=5),subcutaneous steady dose (SSD;20 ug/kg/day SQ;n=4)]. Subcutaneous temperature (SCT;continuous) sensors implanted and rectal temperatures (RT) determined multiple times a day. The RT in all IV treatment groups 3h post treatment and 5h after for SSD on D1 greater than CON. Compared to CON, RT was increased 3 h after treatment administration on D2,D3, D5, and D6 for DAD and D2 and D5 for DID. The SSD group had the greatest SCT followed by DID, DAD, SAD, and CON. Overall, chronic endotoxin did induce an increase in RT and SCT, but differently with RT changing over time.

"Efficacy of a Hypochlorous acid solution on bacterial re-colonization of surgical sites"

Channing Cantrell, Joe Smith, Sreemari Rajeev, Haley Cremerius, Pierre-Yves Mulon

A pillar of perioperative care is the usage of antiseptic solutions to prevent post-surgical infection by effectively reducing the commensal bacterial load present on the skin. Various surgical scrub products and protocols exist. Hypochlorous acid (HOCl) has been utilized in human and veterinary medicine for wound management. The residual effect of the surgical preparation protocol by the addition of HOCl has not yet been studied. Using goats as models in a cross over study, skin bacterial populations were quantified over time after standard and HOCl enhanced surgical preparation using two distinct methods: colony forming unit (CFU) counts and point-of-care swabs for ATP detection. Addition of HOCl showed a prolonged (4 hours) suppression of bacterial growth compared to the standard method (2 hours) using CFU counts. ATP was significantly lowered by usage of HOCl, compared to the standard group. No clear correlation between CFU counts and measured ATP was observed.

"Pharmacokinetics and pharmacodynamics of pantoprazole in neonatal calves"

Haley Cremerius, Joe Smith, Pierre-Yves Mulon, Jeff Olivarez, Channing Cantrell, Rebecca Rahn, Windy Soto-Gonzalez, Lainey Harvill, Joan Bergman, Lisa Abner, Sherry Cox

Abomasal ulcers can occur commonly in veal and hospitalized beef calves. Thus, the lack of efficacy data for gastroprotectants, such as pantoprazole, for ulcer prevention and treatment in cattle is a health and welfare issue. Six, approximately one month old, beef calves were cannulated with human gastrostomy tubes and randomly assigned to intravenous and or subcutaneous administration. Pantoprazole was administered on three consecutive days. Blood was sampled on days one and three, abomasal fluid was sampled on all days. After a ten-day washout period, treatments were crossed-over. High-pressure liquid chromatography was used to determine pantoprazole concentration in plasma. A pH meter was used to determine abomasal pH. Results showed a significantly higher pH for all calves at four, six, and, eight hours post-administration of pantoprazole as well as rapid elimination ($t_{1/2}$ of 1.19 and 1.73 hrs). Increased abomasal pH by pantoprazole could allow for improved ulcer treatment in calves.

“Dog with MRSP pyoderma converted to MSSP after *Staphylococcus pseudintermedius* vaccine administration”

Emma Faddoul, Stephen Kania, Linda Frank

Staphylococcus pseudintermedius is a gram-positive opportunistic pathogen that is the leading cause of canine pyoderma; it is also a commensal organism that can be found on the skin and mucous membranes. Methicillin-resistant *S. pseudintermedius* (MRSP) is an emerging and challenging problem in the veterinary community, with some MRSP patients being left with no antibiotics to treat their infection. Our study documents that a dog with pyoderma infected with MRSP and vaccinated with a four-protein *S. pseudintermedius* vaccine over a 4-week period converted to having methicillin-susceptible *S. pseudintermedius* (MSSP). The dog had a strong antibody response to each component of the vaccine with the strongest response from the last sample taken following three injections. Western blot analysis confirmed the presence of the vaccine components in the susceptible bacteria and multilocus typing suggested that the resistant bacteria were replaced with susceptible bacteria rather than a change in resistance within the MRSP.

“Development of a serum-based enzyme-linked absorbent assay detecting alpaca pregnancy associated glycoproteins (PAGs)”

Piper Gauthier, Stephen Kania, Andrea Lear

Current methods of pregnancy diagnosis in alpacas are limited and unreliable based on stage of gestation. Measurement of pregnancy-associated glycoproteins (PAGs) are a reliable method of pregnancy diagnosis in ruminants and have yet to be evaluated in alpacas. The objective of this study was to design a blood-based sandwich ELISA pregnancy test to detect PAGs and determine its accuracy in pregnant alpacas. To design the ELISA, the alpaca PAG gene was identified, synthesized using a His-tag, inserted into a plasmid within *E. coli*. The protein was expressed, isolated, purified, and concentrated. One thousand ug of PAG protein was collected and used to create anti-PAG polyclonal antibodies. To evaluate the accuracy of the test, blood was collected from pregnant (n=6) and non-pregnant (n=6) alpacas monthly and sera saved for analysis. Data collection is still ongoing. Following successful development of the ELISA, further validation is needed prior to clinical application.

“Gastrointestinal dysbiosis in dogs with congenital portosystemic shunts”

Nathan Squire, Cassie Lux, Jan Suchodolski

The microbiota of the mammalian gastrointestinal (GI) tract consists of trillions of organisms performing a variety of physiologic roles. Studies have characterized bacterial populations and profiles for GI flora in healthy humans and animals. Subsequently, extensive research has been performed evaluating the GI microbiome characteristics in human and animal populations with specific pathologies. The role of the GI microbiome in hepatopathies and hepatic encephalopathy has been frequently investigated in humans, but has not been characterized in dogs. The aim of the current study is to characterize the nature and degree of dysbiosis in dogs with congenital portosystemic shunts (CPSS), and to evaluate for associations between dysbiosis and clinical variables. Our hypothesis is that the various facets of medical management for CPSS will have a significant impact on the dysbiosis index, but that clinical outcome of the patient will not be affected regardless of the level of dysbiosis.

SESSION E

Room A118: First Year Classroom

Zoom Link: <https://tennessee.zoom.us/j/95127116818>

“Serological surveillance of various pathogens in Middle Tennessee wild turkey population”

Laura Horton, Lindsey Phillips, David Buehler, Tania Dawant, Richard Gerhold

This collaborative study between the NWTf, TWRA, and two research labs at the University of Tennessee involves disease screening for multiple viral, bacterial, fungal, and parasitic pathogens which may be associated with population declines in Middle Tennessee Wild Turkeys. Serum was collected from tagged birds from two county groups—one experimental group experiencing perceived declines and one control group not experiencing perceived declines. Commercial ELISA tests were performed to detect Newcastle Disease Virus (NCDV), Avian Influenza Virus (AIV), and *Mycoplasma* spp. in serum. Modified agglutination tests (MAT) were performed to detect *Toxoplasma gondii* in serum. Results showed 8.5% seropositivity for NCDV in control counties and 10.8% seropositivity in experimental counties; 10.3% seropositivity for *Mycoplasma* spp. in control counties and 17.9% seropositivity in experimental counties; AIV was not detected. *Toxoplasma gondii* was detected in 82.6% of all samples, with 34.4% of positives from control counties and 65.5% of positives from experimental counties.

“Health survey of coyotes from South Carolina and Tennessee”

Eliza Baker, Richard Gerhold, Alex Jensen, Debra Miller

Coyotes can be sentinels for diseases of veterinary and public health importance, and frequent monitoring is needed to understand pathogen prevalence. Blood, stool, and ectoparasite samples were taken from 10 and 41 coyotes from Tennessee and South Carolina, respectively. Additionally, bodies were opportunistically collected for necropsy. Plasma from 26 coyotes was tested for *Trypanosoma cruzi* (20% positive) and *Toxoplasma gondii* (84% positive). *Dirofilaria immitis* adults were found in 12 of 20 necropsied coyotes, and SNAP 4Dx tests performed on 30 whole blood samples disclosed 37% and 27% prevalences for *D. immitis* and *Ehrlichia* sp, respectively. *Hepatozoon* sp. was sequenced in 24% of samples, and cysts were seen in muscle in 6 out of 20 necropsied coyotes. *Hepatozoon* has not been reported in South Carolina and represents a rare but important risk for dogs. This survey found that coyotes are important carriers for both zoonotic and domestic diseases of concern.

“In vitro examination of novel therapeutic compounds to control *Histomonas meleagridis*”

Megan White, Richard Gerhold, Sawsan Ammar

Histomonas meleagridis is a parasite that causes blackhead in poultry, a disease with high morbidity and mortality rates. Currently there are no approved treatments or preventatives for this disease. The lack of treatment has led to the appearance of significant outbreaks of the disease across the country (Jones et al, 2020). There is a need for a new drug or feed supplement. In this study, five novel therapeutic compounds were tested *in vitro* in order to determine if any have effects on the growth of *H. meleagridis*. Two of the compounds showed significantly lower mean cell counts of *H. meleagridis* when compared to a control and may have suppressive effects on growth. These compounds are worthy of further *in vivo* trials and have the potential to serve as novel therapeutic agents for the parasite.

“Serological diagnosis of *Parelaphostrongylus tenuis* infection in aberrant hosts”

Jessie Richards, Manasi Balachandran, Abigail Wilson, Richard Gerhold, Stephen Kania

Parelaphostrongylus tenuis is an extrapulmonary lungworm common among deer, elk, moose, camelids, goats, and horses. The parasites migrate through the central nervous system (CNS), resulting in variable morbidity and mortality in aberrant hosts. Techniques currently available for definitive diagnosis involve a necropsy to detect adult parasites in the CNS with or without polymerase chain reaction (PCR) analysis. Due to inconsistent serological results using previously published novel proteins and suspected cross reactivity with other similar organisms, we have pursued full genomic analysis of *P. tenuis* to identify new novel proteins. Isolation of IgG antibodies from known positive sera, formation of antigen-antibody complexes using digested *P. tenuis* organisms, and cross-reference to our previously acquired transcriptome has since allowed us to identify a new, more specific, antigen that shows significant promise. Testing and development of an enzyme-linked immunosorbent assay (ELISA) using synthetic peptides of this identified antigen is currently underway.

“Role of tick exosomes in acquisition or transmission of tick-borne pathogens”

Waqas Ahmed, Wenshou Zhou, Girish Neelakanta, Hameeda Sultana

Development of an animal model to study acquisition/transmission dynamics of vector-borne pathogens into and from ticks is essential to establish successful blood-feeding on a suitable vertebrate host. We provide evidence that murine model with higher infectious-dose can be successfully used to study acquisition dynamics of tick-borne Langat virus (LGTV), a tick-borne flavivirus member. Our study showed that both larval and nymphal ticks that fed on murine host successfully acquired LGTV. Also, we found that after molting LGTV was transstadially-transmitted from larval to nymphal stage. Our previous studies have shown that tick exosomes play crucial roles in facilitating pathogen transmission. However, the molecular mechanism(s) that enables feeding and transmission of tick-borne flaviviruses via exosomes are poorly understood. We have identified that cement protein facilitates exosomes-mediated acquisition/transmission during tick blood-feeding. Collectively, we have established a murine model for tick-borne flavivirus infection, and have identified a tick exosomal-enriched protein that facilitates pathogen transmission.

“Rickettsial pathogen uses tryptophan metabolite xanthurenic acid to facilitate tick cell survival”

Prachi Namjoshi, Mustapha Dahmani, Hameeda Sultana, Girish Neelakanta

Anaplasma phagocytophilum, an obligate intracellular rickettsial pathogen, is a causative agent of human anaplasmosis. The blacklegged tick, *Ixodes scapularis*, serves as a primary vector for this pathogen. Previous studies from our laboratory have shown that exogenously added xanthurenic acid (XA), a tryptophan metabolite, increased *A. phagocytophilum* loads in both ticks and tick cells (ISE6). In this study, we found that XA-treated uninfected, or *A. phagocytophilum*-infected tick cells exhibited significantly less cell death when compared to the respective mock-treated controls. QRT-PCR analysis revealed that transcript levels of cell death markers were significantly reduced in *A. phagocytophilum*-infected tick cells and ticks upon XA-treatment. In addition, we noted that transcript levels of several cell death markers were significantly reduced in *A. phagocytophilum*-infected ticks when compared to uninfected controls. This study will be further explored to understand how *A. phagocytophilum* modulates arthropod tryptophan pathway to facilitate tick cell survival.

SESSION F

Room A335: Third Year Classroom

Zoom Link: <https://tennessee.zoom.us/j/93149909456>

“Viral, vector-borne, and blood-borne pathogen prevalence of free-ranging ocelots (*Leopardus pardalis*) and bobcats (*Lynx rufus*) in southern Texas”

Ashley Reeves, Clayton Hilton, Michael Tewes, Kelsey Carrier, Trey Kennedy, Melissa Kennedy, Nicole Szafranski, Richard Gerhold, Jessica Kern, Tyler Campbell, Debra Miller

Ocelot populations in southern Texas have shown loss of genetic variability over the last few decades. Declining immune fitness due to loss of genetic heterozygosity can allow for an increased susceptibility to pathogens and diseases that, in a normal individual, may not cause significant disease or mortality. Blood smear analysis, serum and whole blood testing for evidence of viral, vector-borne, and blood-borne pathogens were conducted on 35 ocelots (n=15) and bobcats (n=20) spanning 35 years. Additionally, general health monitoring profiles and physical examinations were completed to assess clinical effects of disease. Positive results were found for Feline Immunodeficiency Virus (FIV) (14%), *Ehrlichia* sp. (6%), *Hepatozoon* sp. (29%), *Toxoplasma gondii* (68%), and *Cytauxzoon felis* (31%). These findings will provide a better understanding of the population’s general health and assist wildlife managers in planning strategies that aid in reducing or preventing transmission of infectious disease agents to these endangered populations.

“Finding normal in a warming world: Baseline PCV and TS data and its relation to incubation temperature in sea turtle hatchlings and post hatchlings from Boca Raton, Florida”

Samantha Kuschke, Debra Miller, Jeanette Wyneken

There are seven extant species of sea turtles, all of which are imperiled. As such, large research efforts are aimed at understanding the effects of climate change on the health, physiology, and disease processes of these sentinel animals to aid conservation efforts across the world. A small portion of these efforts are aimed at establishing normal values for blood parameters in adults but, a large knowledge gap exists for these same values in hatchlings and post hatchlings. We present preliminary data gathered to establish reference ranges for packed cell volume (PCV) and total solids (TS) in leatherback and loggerhead sea turtle hatchlings and post hatchlings. In leatherback hatchlings, we found a mean PCV of 24.78 (n = 50) and mean TS of 3.4 (n=47), while leatherback post hatchlings three to four weeks of age had a mean PCV of 20 (n=30) and a mean TS of 1.9 (n=26). In loggerhead hatchlings the mean PCV was 26.9 (n=45) and the mean TS was 3.6 (n = 42). Additionally, we analyzed the effects of incubation temperature and found positive correlations with PCV and TS. These data provide baseline values to assess hatchling health and possibly an explanation for decreased survival in hatchlings incubated at higher temperatures.

“Use of implants for Terbinafine administration to treat and prevent chytridiomycosis in amphibians”

Anastasia Towe, Rebecca Hardman, Wesley Siniard, Joseph DeMarchi, Edward Davis Carter, Matthew Gray, Debra Miller

Batrachochytrium dendrobatidis (Bd) is a chytrid fungus that infects amphibians and is present in habitats worldwide. Bd chytridiomycosis has been documented to cause mortalities in sirens (*Siren lacertina*). This study evaluated the efficacy and safety of an anti-fungal drug (Terbinafine) along with a novel delivery method, a Terbinafine-impregnated intracoelomic implant, in prevention and clearance of Bd infection in sirens. Four sirens received Terbinafine implants and four received blank implants. They were exposed to Bd zoospores at one and two months post-implant placement. Blood was collected monthly for plasma terbinafine levels, and skin swabs performed weekly for Bd PCR. Animals with terbinafine implants showed increased levels of terbinafine in plasma; however, treatment did not prevent infection and the clearance rate of Bd infection was not different from control animals. These findings indicate that intracoelomic drug implants are a safe method for antifungal drug delivery in amphibians; however, Terbinafine efficacy remains unclear.

“Classification and implications of bacteria within the GI tract of leatherback (*Dermochelys Coriacea*) hatchlings from the East Coast of Florida: An exploratory study”

Samuel Good, Debra Miller

As conservation efforts continue to rise for sea turtle species, the leatherback (*Dermochelys Coriacea*) population, more specifically the pacific population, are still critically endangered and are estimated to go extinct within 60 years. Florida Atlantic University's Marine Laboratory is designed and permitted to house and grow leatherback hatchlings for further research purposes. Understanding the uniqueness of this facility and the need to meet nutritional demands of the hatchlings, dead-in-nest hatchlings were used to take samples from the stomach, large intestine, and yolk sac to better understand the gut microbiome. Swabs taken from these organs were gram stained and then described and quantified based on shape and gram stain appearance. Out of a sample size of 33 hatchlings, gram + rods were most abundant (~90-95%) and gram - rods were also prevalent (~5-10%). Trends saw a higher overall bacterial abundance in nests with a higher average incubation temperature.

“Fungus among us: The impact of *Batrachochytrium salamandrivorans* (Bsal) on reproductive fitness”

Mariah Dee, Anastasia Towe, Wesley Siniard, Matthew Gray, Debra Miller

Batrachochytrium salamandrivorans (Bsal) is a fungal pathogen which has devastated populations of salamanders in Europe. Several studies have outlined the physiological consequences of chytridiomycosis, but it is still unclear what long-term sublethal effects, such as impacts to reproductive fitness, result in animals that survive infection. We utilized a preserved population of North American newts, which included the Eastern newt (*Notophthalmus viridescens*, n=18), the Black Spotted newt (*Notophthalmus meridionalis*, n=10), and the Rough-skinned newt (*Taricha granulosa*, n=17), to determine whether there is a difference between reproductive quality and/or output of those individuals who were infected with Bsal versus uninfected individuals. Preliminary results indicate a negative correlation between reproductive fitness markers and Bsal infection. This finding will be useful in the event of a Bsal epidemic, especially when conservationists convene to discuss repopulation efforts.

“Environmental determinants of bovine anaplasmosis in Tennessee cattle”

Shamim Sarkar, Brian Whitlock, Lew Strickland, Justin Rhinehart, John Beever, Katie Reif, Chika Okafor

The study objective was to identify environmental determinants of bovine anaplasmosis (BA) in Tennessee cattle. Between April and November 2020, 1,117 TN cattle were tested for BA. Environmental indices (temperature, rain days, humidity) were obtained for counties from which these animals originated. Multivariable logistic regression was used to estimate the effect of these environmental indices on BA. The overall apparent and true seroprevalence of BA was 20.41% and 19.8%, respectively. The odds of BA diagnosis were 12 times as high in areas with ≤ 10 rain days/month and 3.3 times in mixed breed cattle compared to areas with > 10 rain days/month and pure breed cattle, respectively. Future surveillance and control measures for BA should be targeted to the identified areas.

“Occurrence of antibiotic residues in beef, eggs, and honey sold in East Tennessee farmers’ markets, 2020”

Shamim Sarkar, Marcy Souza, Tomas Martin-Jimenez, Mohamed Abouelkhair, Stephen Kania, Chika Okafor

The study objective was to assess the occurrence and concentration of tetracycline, erythromycin, and sulfonamide residues in beef, eggs, and honey sold at East Tennessee farmers' markets. Between July 2020 and September 2020, beef (n=9), eggs (n=18), and honey (n=9) samples were purchased from the selected East Tennessee farmers' markets. The residues were detected using an enzyme-linked immunosorbent assay. In beef, the median concentration of tetracycline, erythromycin, and sulfonamides was 0.41 ppb, 2.5 ppb, and 81.2 ppb, respectively. In egg, the median concentration of tetracycline and sulfonamides were 2.15 and 87.64 ppb respectively. In honey, the median concentration of tetracycline and erythromycin were 3.55 ppb and 6.0 ppb respectively. Study findings indicate the concentrations of tetracycline, erythromycin, and sulfonamides residues were within the allowable maximum residue limit. However, the presence of the residues in animal products could have a negative health effect (antimicrobial drug resistance bacteria) among consumers.