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ASSOCIATIONS BETWEEN IRREGULAR TRIPLOID OYSTER GAMETOGENESIS AND DIGESTIVE DIVERTICULA PATHOLOGY

Sarah Amblard (Veterinary Student)
Tal Ben-Horin, PhD

College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina.

Triploids are widely used in commercial fruit, fish, and mollusk production around the world. Having three rather than the normal two sets of haploid chromosomes leads to many beneficial commercial traits, including improved growth and infertility. Triploid oyster production has fueled oyster aquaculture’s global expansion, as triploids provide a consistent, year-round product compared to diploids, which are typically commercially unavailable during and after spawning. Nevertheless, triploid oysters still undergo partial gametogenesis, with little known physiological consequences. The past decade has seen widespread triploid mortality events timed with peaks in gametogenesis but unclear pathology. Occasional erosion of gill epithelium is seen in affected oysters, which suggests an environmental cue, as is widespread sloughing and necrosis of epithelial cells in oyster digestive diverticula. Here we conducted an environmental study investigating triploid gametogenesis and associated pathology during an oyster mortality event, hypothesizing that triploid mortality is a secondary response to environmental stress occurring during gonad reabsorption. The results showed little association between gonad stage and reabsorption with the observed pathology. We found cell sloughing and necrosis in the digestive diverticula to be tightly coupled with observed oyster mortalities. Etiology remains unknown and may be more generally related to triploid oyster physiology. Our histopathologic findings are serving as the basis of our future research in collaboration with cooperative programs to develop oyster stocks for sustainable aquaculture production in North Carolina and across the Atlantic coast.

Research Grant: NC Department of Environmental Quality (Contract # CW19141)
Student Support: North Carolina State College of Veterinary Medicine Veterinary Scholars Program

Topic: Gastroenterology
Contact Information: seamblar@ncsu.edu (Sarah Amblard), talbenhor@ncsu.edu (Tal Ben-Horin)
ENTERIC GLIAL CELL-SECRETED FSTL3 PROMOTES ATM-DEPENDENT CHEMoresISTANCE IN COLON CANCER STEM CELLS

Gregory Bacola1 (Graduate student)
Simon Valès2, Alice Prigent2, Kelsie A. Dougherty1, Deanna M Peperno1, Shaian Lashani1, Bradley A. Wieland1, Melissa Touvron1, Lisa Oliver3, François M Vallette3, Michel Neunlist2, Laurianne Van Landeghem1

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(1) NCSU CVM, Raleigh, NC, USA
(2) UMR Inserm 1235, IMAD, Nantes University, Nantes, France
(3) UMR Inserm 1232, CRCINA, Nantes University, Nantes, France

Enteric glial cells (EGC) have recently been shown to impact tumor development. Here, we investigate the impact of EGC on colon cancer stem cell (CSC) chemoresistance. EGC promoted growth of CSC-derived tumors in the presence of 5-FU in vivo and in vitro. EGC-conditioned medium (CM) reduced 5-FU-induced apoptosis in CSC. Ataxia telangiectasia mutated (ATM) mRNA was significantly enriched in 5-FU-treated CSC grown with EGC vs. alone. EGC pro-chemoresistance effects were reduced in CSC derived from cell lines knocked-down for ATM. Inhibition of ATM activity using KU-55933, or upstream MRN activity using mirin abolished EGC-induced CSC resistance to 5-FU. Mass spectrometric analyses identified Follistatin Like 3 (FSTL3) was highly enriched in the CM of 5-FU-treated EGC. Treatment with recombinant FSTL3 protein increased tumor formation and reduced apoptosis in ATM-expressing CSC in the presence of 5-FU. FSTL3 treatment did not promote tumor formation in CSC knocked-down for ATM, or in CSC treated with KU-55933. EGC knocked-down for FSTL3 expression did not promote CSC tumor formation in the presence of 5-FU.

Altogether our data show that EGC stimulate CSC chemoresistance by promoting activity of the MRN/ATM pathway, potentially through the release of FSTL3. Future studies will determine if EGC-induced ATM signaling promotes increased DNA damage repair in CSC.

Category: Cell Biology
DOMESTIC PIGS REPRESENT A NOVEL TRANSLATIONAL ANIMAL MODEL FOR EOSINOPHILIC ESOPHAGITIS

David Brodsky\textsuperscript{a,g} – Graduate Student – Oral Presentation Preference

Lizette M. Cortes\textsuperscript{b,g}, Jessica Proctor\textsuperscript{b,g}, Cecilia Schaaf\textsuperscript{c,g}, Caroline McKinney\textsuperscript{c,g}, Jack Odle\textsuperscript{d,g}, Anthony Blikslager\textsuperscript{c,g}, Liara Gonzalez\textsuperscript{c,g}, Harry Dawson\textsuperscript{e}, Evan S. Dellon\textsuperscript{f}, Scott M. Laster\textsuperscript{f,g} and Tobias Käser\textsuperscript{b,g}.

Email – dmbrodsk@ncsu.edu, lmlorenz@ncsu.edu.

\textsuperscript{a}Department of Biological Sciences, NCSU, Raleigh, NC, USA
\textsuperscript{b}Department of Population Health and Pathobiology, NCSU, Raleigh, NC, USA
\textsuperscript{c}Department of Clinical Sciences, NCSU, Raleigh, NC, USA
\textsuperscript{d}Laboratory of Developmental Nutrition, Department of Animal Science, NCSU, NC, USA
\textsuperscript{e}USDA, ARS, Diet, Genomics and Immunology Laboratory, Beltsville, MD, USA
\textsuperscript{f}Center for Esophageal Diseases and Swallowing, Department of Medicine, Division of Gastroenterology and Hepatology, UNC School of Medicine, Chapel Hill, NC, USA
\textsuperscript{g}Comparative Medicine Institute, NCSU, Raleigh, NC, USA

Funding – NIH (NIAID) R21, CGIBD, CMI

Subject Category – Immunology

Food allergy affects ~8% of the world’s population and is caused by excessive immune responses against food allergens. These responses result in pathological consequences such as eosinophilic esophagitis (EoE) which has yearly associated costs in the US of $1B. EoE is a T-helper 2 cell driven disease leading to accumulation of eosinophils in the esophagus. The resulting inflammation and fibrosis cause failure to thrive, dysphagia and food impaction. There are no FDA-approved treatments for EoE partly explained by the limitations of the standard mouse model for translational research. Therefore, our goal was to develop the pig as a biologically relevant translational model for EoE.

Food allergy was induced by intraperitoneal sensitization with hen egg white protein (HEWP) with cholera toxin as adjuvant followed by seven daily oral HEWP challenges. Systemic IgG, IgE and CD4 T cell responses were monitored weekly. At necropsy, esophageal tissue was assessed for pathology and eosinophil infiltration and RNA was isolation for qPCR and RNAseq analysis.

Sensitized and challenged pigs showed clinical signs of food allergy, increased serum IgG and IgE levels, and a strong systemic CD4 T-cell response; their esophagi also revealed RNA and pathological changes seen in human EoE patients; moreover, these pigs developed the hallmark of EoE – esophageal eosinophilia. This study establishes the pig as a relevant animal model for EoE: this model has the potential to highly improve the development of new diagnosis and treatment strategies for EoE.
INTERROGATING MARKS INHIBITION AS A STRATEGY TO ALTER BOVINE NEUTROPHIL RESPONSES TO S. TYPHIMURIUM.

**Chalise Brown**: Veterinary Student
Haleigh Conley, Katie Sheats

Department of Clinical Sciences, North Carolina State University College of Veterinary Medicine, Raleigh, North Carolina

Salmonellosis is a disease of major importance in both livestock and humans. *Salmonella* infection in cattle causes economic loss due to animal death and human food supply contamination. Neutrophils play a key role in *Salmonella* enteritis, causing inflammation that ultimately enables *Salmonella* to colonize the gut. With growing concerns regarding antibiotic resistance, particularly in animal agriculture, non-antimicrobial interventions/treatments for salmonellosis are needed. One proposed method for treatment is to target the host response by reducing the inflammation that supports *Salmonella* colonization with the gut. Previous research in our lab has shown that MARCKS protein plays an essential role in neutrophil inflammatory functions, including respiratory burst and adhesion. Therefore, we investigated whether MARCKS protein inhibition would be a strategy to alter bovine neutrophil responses to *S. typhimurium*. We hypothesized that treatment of isolated bovine neutrophils with a MARCKS-specific inhibitor peptide, known as MANS, would attenuate *Salmonella*-induced respiratory burst and adhesion. Whole blood was collected from healthy, lactating Holstein cows for neutrophil isolation (IACUC #20-115). Neutrophils were stimulated with late exponential phase *S. Typhimurium* strain HA420 (MOI 50:1). Plate-based fluorescence assays using dihydrorhodamine (DHR) were used to measure the production of reactive oxygen species (ROS). Static plate-based assays with calcein-labeled neutrophils were used to measure adhesion. Preliminary results with MANS peptide treatment show a trend for a concentration dependent attenuation of respiratory burst in *Salmonella*-stimulated bovine neutrophils.

Research Grant: USDA

Student Support: VSP Scholarship Program, FFAR Veterinary Fellowship

Subject Category: Immunology, Infectious Disease
Multidrug resistant *Vibrio* and *Aeromonas* in seafood from international sources sold in North Carolina

Jaime Calcagno, Erin Harrell, Lyndy Harden, Megan Jacob, Paula Cray and Siddhartha Thakur

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina.

The National Antimicrobial Resistance Monitoring System (NARMS) monitors the multidrug resistance trends of bacterial pathogens in retail meats, humans and animals. The objective of this study is to determine the phenotypic and genotypic characterization of *Vibrio* and *Aeromonas* species in seafood products sold in North Carolina retail markets. We hypothesize that seafood from larger countries will experience an increased concentration of antimicrobials during their growth phase due to unrestricted use, leading to a higher incidence of multidrug resistance (MDR) in bacterial pathogens that cause human infections. 45 *Vibrio* and 39 *Aeromonas* isolates were obtained from tilapia, shrimp, and salmon sourced from 15 countries. Sensititre™ autoinoculator dispensed diluted isolates into gram negative minimum inhibitory (MIC) plates. OptiRead™ analyzed the MIC, or the lowest concentration at which a drug inhibits the growth of bacteria. *Vibrio* DNA was extracted and speciation occurred through PCR. MDR, or resistance to 3 or more classes of antibiotics, trends were as follows: China (nMDR=9), USA (nMDR=8), Argentina (nMDR=2), Indonesia (nMDR=2), Columbia (nMDR=1), Chile (nMDR=2), Ecuador (nMDR=2), New Zealand (nMDR=1), Bangladesh (nMDR=2), Honduras (nMDR=1), and Panama (nMDR=3). A higher incidence of MDR seafood pathogens was found in larger countries, however, Panama displayed a high level of MDR. A tilapia sample from China showed extreme resistance to 12 out of 14 antibiotics. 33 of the 48 samples displayed MDR. *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus* were identified. Antimicrobial agents used to treat seafood infections are losing effectiveness due to unrestricted administration facilitating mutations/gene transfers that promote resistance.

Research Grant: None

Student Support: Veterinary Summer Scholars Program
Title: EARLY EVALUATION OF THE FOOD AND DRUG ADMINISTRATION (FDA) REGULATIONS ON ANTIBIOTIC USE IN FOOD ANIMALS ON ANTIMICROBIAL RESISTANCE MONITORING SYSTEM (2006-2018)

Author: Liton Chandra Deb1 (Graduate Student)

Co-Authors: Manuel Jara1, and Cristina Lanzas1*

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Abstract: Antimicrobial resistance (AMR) is one of the biggest challenges to global health. To address this issue in the US, governmental agencies have implemented system-wide guidelines to reduce antimicrobial use. In 2012, the Food and Drug Administration (FDA) prohibited the extra-label use of cephalosporins in food animals. In addition, it issued guidelines about establishing a framework to phase out the use of all medically important drugs for growth promotion. To assess the potential early effects of these FDA guidelines, we compared the temporal patterns of the phenotypic minimum inhibitory concentration (MIC), Resistance prevalence, and genotypic resistances for selected antimicrobials (i.e., ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, and tetracycline) across different enteric pathogen species *Escherichia coli* (poultry), *Salmonella enterica* serotype Dublin (cattle), *S. ent.* serotype Typhimurium (poultry, cattle, and humans), *S. ent.* serotype Typhi (humans), as reported by the National Antimicrobial Resistance Monitoring System (NARMS). Additionally, using a Bayesian phylogenetic approach, we reconstructed the evolutionary patterns (through effective population size) in *S. ent.* Typhimurium using whole-genome sequences (WGS). Our phylogenetic results indicated that variations in FDA regulations may have impacted *S. Typhimurium* diversification, particularly at the population level. In addition, we observed strong variations in short and specific periods that coincide with the implementation of FDA regulations. While at the phenotypic level, most of the antimicrobials analyzed showed statistically significant differences in their MIC values across most species/hosts between pre and post-FDA implementation. This study represents the first attempt to evaluate the impact of FDA regulations on reducing antibiotic use in food animals.

Funding Source: R35GM134934

Primary Subject Area: Other (Population and Global Health)
Numerous diseases in rabbits may result in severe anemia and necessitate a blood transfusion. If a rabbit blood donor is not immediately available, a transfusion from another species or xenotransfusion may be a lifesaving alternative. Canine and feline blood products are often available at veterinary specialty centers, making them a convenient option for emergent xenotransfusion. This study evaluated the major crossmatch compatibility between rabbit recipients, a conspecific donor, and the major canine and feline blood types. Blood samples were collected from 11 healthy New Zealand white rabbits (Oryctolagus cuniculus) with no prior transfusion history. Each rabbit recipient underwent a major crossmatch using standard tube crossmatch methodology with itself and the following donor blood types: rabbit, dog erythrocyte antigen (DEA) 1.1-positive, DEA 1.1-negative, feline Type A, and feline Type B. Self-crossmatches and crossmatches between rabbit recipients and conspecific donors were negative for hemolysis and agglutination. Crossmatches between rabbit recipients and canine and feline donors were negative for hemolysis but produced varying degrees of agglutination. Canine blood donors had 1.4 (95% CI: 1.1-1.8) times the risk of macroscopic agglutination than feline blood donors. No significant difference in agglutination was found between DEA 1.1-positive and DEA 1.1-negative or feline Type A and Type B donors. These findings support allogenic blood transfusions between rabbits being highly compatible and suggest rabbits have naturally occurring alloantibodies against both canine and feline red blood cells. However, feline red blood cells may have better in vitro serologic compatibility if an emergent xenotransfusion is needed.

Category: Clinical Medicine
USING HIGH-THROUGHPUT APPROACHES TO IDENTIFY FUNCTIONAL FC GAMMA RECEPTOR SNPS.
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Abstract:
Genetic research into clinical diseases relies on Genome-Wide Association Studies (GWAS) to identify significantly associated Single Nucleotide Polymorphisms (SNPs). Previous HIV-1 vaccine research has associated multiple genetic variations in the Fcγ Receptor (FcγR) region with disease susceptibility and vaccine protection. However, complex genetic variations in this region present a challenge for functional analyses and many FcγR SNPs exist within non-coding regions. Therefore, the identity and regulatory role of functional SNPs (fSNPs) in the FcγR region have been limited.

To systematically identify fSNPs from the FcγR region, we have utilized the recently developed high-throughput Reel-Seq screen with a custom-designed synthetically constructed oligo library containing a large number of polymorphisms (>4,300 SNPs) of high interest to HIV-1 vaccine efficacy. Allele-specific binding alterations at an individual fSNP are indicative of changes in cell-specific protein binding that may modulate transcriptional regulation of genes in proximity. To evaluate the molecular mechanisms for candidate fSNPs, we have identified unique protein interactions by proteomic analysis of alleles that show altered protein binding patterns. Using the interactive evidence between the fSNP and nuclear proteins, complementary assays will be performed to establish the functional roles of individual fSNPs in the regulation of FcγR gene expression. The regulatory mechanisms of fSNPs may be utilized to guide the development of novel treatments and vaccines for HIV-1 and other complex diseases.

Funding Sources: CVM Intramural Grants 2021-2022.
Primary Subject Category: Genetics (Immunology, secondary)
Presentation Preference: Oral Presentation
ASSESSMENT OF THE HYPOTHALAMIC-PITUITARY-ADRENOCORTICAL AXIS UTILIZING A VASOPRESSIN STIMULATION TEST IN HEALTHY FOALS

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Background: Sepsis is a major cause of death in foals and associated with hypothalamic-pituitary-adrenal axis (HPAA) dysfunction. HPAA function can be evaluated by an arginine-vasopressin (AVP) stimulation test. Hypotheses/Objectives: Administration of AVP will stimulate a rise in systemic adrenocorticotropin-releasing hormone (ACTH) and cortisol in healthy foals; no clinically adverse effects will be detected.

Methods: HPAA function was assessed in 12 healthy foals utilizing three doses of AVP (2.5, 5, 7.5 IU), administered between 24-48h of age in this randomized cross-over study. Cortisol, ACTH, and corticotropin-releasing hormone (CRH) were measured at 0 (baseline), 15, 30, 60 and 90 minutes after AVP administration with immunoassays. A fold increase 15 and 30 minutes from baseline was calculated for cortisol and ACTH.

Results: All doses of AVP resulted in a significant increase of cortisol concentration over time, and a dose-dependent increase of ACTH concentration over time. ACTH and cortisol increased 15 and 30 minutes, respectively after all three doses of AVP compared to baseline (P<0.01). A 50-fold increase in cortisol was seen with 5 and 7.5, and a 20-fold increase with 2.5 IU. A 10-fold increase in ACTH was seen with 7.5 IU, 8-fold increase with 5 IU, and 4-fold increase with 2.5 IU (P<0.05). There was no effect of AVP on endogenous CRH.

Conclusion: Administration of AVP is safe and results in a significant rise in ACTH and cortisol in healthy foals. A stimulation test with AVP (5 IU) can be considered for HPAA assessment in septic foals.

Funding Sources: Intramural Seed Grant

Subject Category: Clinical Medicine

Oral presentation
VISUAL PERFORMANCE OF DOGS IN LOW LIGHT LEVELS
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Abstract
Dogs exhibit superior vision in low light due to several factors, including the presence of a tapetum lucidum. The purpose of this study was to assess visual performance of dogs in low light levels. We hypothesize that dogs can see low light levels up to 0.005 cd/m². Normal beagle and hound dogs (n=14) completed trials in a four-choice vision testing device. This device consisted of a junction box with four tunnels. Dogs were placed in the box and given one vision-based choice for exit. The time to exit and first-choice tunnel were recorded and analyzed. Seven trials were completed for each light intensity: 0.005, 0.01, 0.1, 1, and 10 cd/m². The control trial consisted of the dogs wearing bilateral opaque contact lenses, when tolerated, or completed runs at 0 cd/m² to mimic blindness. Data was analyzed via one- and two-way ANOVA, non-parametric Friedman test, and Mann-Whitney U test with statistical significance set at p<0.05. Results revealed that there was no significant difference in time to exit and first choice tunnel between light levels. There was a significant difference between time to exit and first choice tunnel when comparing the control trial to all other light levels (p<0.0001). Factors such as age, age-related lens changes, and fearful behavior did not affect measured parameters. In conclusion, beagle and hound dogs can see low light levels up to 0.005 cd/m². For future studies, visual performance at lower light intensities such as 0.003, 0.001 cd/m², and ultraviolet light will be assessed.

Funding resources: Army Research Office (ARO) 2021-2420

Primary subject category: Ophthalmology
RADIATION LOWERS HUMERAL CORTICAL BONE DENSITY BUT ALSO OSTEOARTHRITIS INCIDENCE IN NON-HUMAN PRIMATES

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Total body irradiation (TBI) has long-term health effects; TBI-treated childhood cancer survivors are more likely to develop diabetes mellitus (DM). DM induces a pro-inflammatory environment conducive to osteoarthritis (OA). Radiation is associated with arthropathy and lowered bone mineral density (BMD). This study utilized a non-human primate (NHP) model to test the hypothesis that osteoporotic changes and OA measured by computerized tomography (CT) occur with increased incidence in DM TBI NHPs when compared to TBI-only, DM-only, and control NHPs. Rhesus macaques (n=134) were previously exposed to 1.1-8.5 Gy; other NHPs (n=32) were unexposed. At the time of imaging, 9 irradiated NHPs had DM, while zero non-irradiated NHPs were diabetic; however, two developed DM fifteen months later. CT analysis of the proximal humerus revealed lower cortical volume (p<0.001), lower cortical BMD (p<0.001), and shorter humeral length (p=0.003) in TBI-only primates vs other groups. Trabecular BMD was higher (p=0.006) in TBI-only NHPs vs control. Greater cortical volume, marginally higher cortical BMD, and similar trabecular BMD for DM TBI vs control indicated a differential response related to DM status. After sub-cohort analyses (n=86), NHPs with shoulder OA had higher cortical and lower trabecular BMD (p<0.05). Shoulder OA severity was higher in DM-only and control, while reduced in TBI-only NHPs (p=0.009). TBI in non-DM NHPs yielded expected bone deficits. However, irradiated NHPs with DM did not exhibit similar bone changes but rather opposite responses. As OA was not associated with TBI, a correlative link between TBI and DM status with skeletal deficits was not supported.

NIH U01AI150578, T35 OD010946
Other
There is growing interest in using machine learning to predict phenotypic outcomes from microbiome data. Highly accurate machine learning models could allow for more efficient ways to detect diseases in humans and animals. For example, there are numerous studies that link the human gut microbiome to colorectal cancer, and the ability to predict colorectal cancer from microbiome profiles would allow for screening using easy to obtain and non-invasive stool samples. However, traditional machine learning algorithms often have poor predictive performance on microbiome data due to several properties of the data. Community sequencing methods typically only provide information about relative abundances in microbial communities. Microbiome data is usually sparse with small sample sizes. It is difficult to combine data from different studies because the measurements have bias that depend on DNA extraction and sequencing protocols. All these properties provide challenges to typical machine learning algorithms. We have created novel random forest algorithms that are specifically designed to account for the properties of microbiome data. We evaluate our methods on many data sets across several diseases. We particularly focus on cross-study prediction between data sets from studies with different population cohorts and sequencing and extraction protocols to ensure that our methods are robust. These methods have the potential to increase the effectiveness of using microbiome data for screening and prediction of disease.

Subject Category: Other
HEMOLYMPH BIOCHEMICAL VALUES IN A MANAGED POPULATION OF FEMALE THORNY DEVIL STICK INSECTS (*EURYCANTHA CALCARATA*)

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Abstract (250 words maximum, current = 250):

Invertebrates, including insects, are becoming increasingly important as research, collection, and food animals. Establishment of hematologic and biochemical parameters in healthy individuals is crucial to the interpretation of results in diseased animals. The objective of this study was to develop a hemolymph sampling protocol in the thorny devil stick insect (*Eurycantha calcarata*) and report hemolymph biochemical values. Eighteen clinically healthy stick insects (female n=14, male n=4) were enrolled. In pilot testing, various hemolymph collection strategies were attempted; none were successful in male insects. In females, hemolymph was most reliably collected from the lateral abdomen immediately caudal to the proximal hindlimb. A small puncture wound was created using an 18g needle and hemolymph was passively collected via gravity. Hemolymph was allowed to clot, centrifuged, and the serum immediately analyzed (Avian-Reptile rotor, Abaxis Vetscan VS2). All samples (n=14) processed successfully. Median (minimum-maximum) values for analyzed biochemical parameters included: aspartate aminotransferase 12 (0-45) U/L, creatinine kinase 25 (0-76) U/L, uric acid 7.5 (3.1-13.7) mg/dL, glucose 12 (8-22) mg/dL, calcium 18.6 (17.2-19.4) mg/dL, phosphorous 15.0 (n=1) or >30.0 (n=13) mg/dL, total protein 2.7 (1.6-2.9) g/dL, albumin 0.9 (0.2-1.2) g/dL, globulin 0.0 (0-1.8) g/dL, potassium 10.6 (9.0-11.8) mmol/L, and sodium <100 (n=14) mmol/L. Bile acids were not detected in any sample. This study is the first report of biochemical parameters in healthy female thorny devil stick insects. Further investigation could optimize hemolymph collection in male stick insects, establish reference intervals for this species, and determine the clinical relevance of these values to stick insect health.

Funding Sources: None
Subject: Clinical Medicine
Presentation Preference: Oral
HERBICIDE INDUCED OXIDATIVE STRESS AND METABOLIC DYSFUNCTION LEADS TO DEFECTS IN INTESTINAL MORPHOGENESIS

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The herbicide atrazine (ATR) has been epidemiologically associated with structural birth defects in multiple organ systems; however, the mechanism behind this association is unknown. We have found that frog embryos exposed to ATR exhibit intestinal malrotation, a common human birth defect. In addition, ATR-exposed intestines are shorter, suggesting that ATR perturbs the process of tissue elongation. Consistent with this idea, immunostaining of ATR-exposed guts revealed abnormalities in cell shape and polarization at early stages, and abnormalities in cell division at later stages, indicative of a failure to execute the cell rearrangement and proliferation events that together drive intestine elongation. As an herbicide, ATR inhibits the photosynthetic electron transport chain (ETC) in chloroplasts, leading to the overproduction of deleterious reactive oxygen species (ROS) in the target plant; however, ATR can also elicit oxidative stress and dysregulate metabolism in animal cells. As both cell polarization and division are energy-dependent processes, ATR-induced intestinal defects may therefore be a result of inhibiting the mitochondrial ETC. Indeed, ATR exposure increased ROS levels in the embryo. Importantly, the intestinal shortening and malrotation phenotypes exhibited by ATR can be rescued by pretreatment with an antioxidant. RNA-sequencing analysis also revealed an enrichment for genes involved in metabolism, the oxidative stress response, and cell cycle regulation. Finally, metabolomic profiles confirmed predicted changes in mitochondrial metabolites after ATR exposure. Our results reveal a potentially novel mechanism of action of ATR on intestinal development and suggest that oxidative stress and metabolic dysfunction may underlie the etiology of a common birth defect.

This work was funded by the National Institute of Environmental Health Sciences (T32ES007046) and the National Institutes of Health/NCSU Biotechnology Program (T32GM133366).

Primary Subject Category: Cell Biology
BLOCKING ARTEMIN SIGNALING REVERSES OSTEOARTHRITIS ASSOCIATED PAIN AT EARLY AND LATE TIME POINTS

Presentation Preference: Oral Presentation

Ankita Gupta¹,²,³ (Graduate Student)

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Osteoarthritis (OA) is a leading cause of disability, with ~100 million US adults suffering from chronic joint pain, widespread sensitization, and decreased mobility. Clinically efficacious and safe therapeutics for OA-pain are limited. Thus, there is an urgent need to develop novel, clinically relevant analgesics for OA-pain. We have linked synovial fluid concentrations of a neurotrophic factor, artemin, to naturally occurring joint pain in dogs. Further, expression of GDNF family receptor alpha 3 (GFRα3, artemin’s receptor) was increased in dog sensory neurons serving OA joints compared to controls. Despite our compelling data, no studies have elucidated the role of artemin/GFRα3 signaling in the development and maintenance of OA-pain. This study explores the functional role of artemin/GFRα3 signaling in OA-pain. We used the monoiodoacetate (MIA)-induced model of stifle OA-pain to evaluate sensitivity to mechanical, hot, and cold stimuli and limb use at early inflammatory (day 7) and late OA (day 28) time points. At both time points, we assessed MIA-induced hypersensitivity and limb disuse at 2-, 5-, and 24-hrs. post-anti-artemin monoclonal antibody or isotype control administration. MIA-injected mice developed hypersensitivity to mechanical and thermal stimuli and had decreased limb use compared to the saline-injected controls. Artemin sequestration reversed MIA-induced hypersensitivity and limb disuse at early inflammatory and late OA-pain time points. This is the first evidence investigating the functional role of artemin/GFRα3 signaling in MIA-induced OA-pain at multiple disease time points. Our ongoing work elucidates the role of artemin/GFRα3 signaling in OA-pain and defines putative targets for developing safe and effective treatments.

Research Funding and Student Support: Donations to the Translational Research in Pain Program; salary release for Lascelles.

Category: Pain
POTASSIUM CHLORIDE ADMINISTERED VIA FOUR ROUTES FOR EUTHANASIA OF ANESTHETIZED Goldfish (CARASSIUS AURATUS)

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Abstract:

Immersion overdose in tricaine methanesulfonate (MS-222) is ineffective for goldfish euthanasia. This study investigated potassium chloride (KCl) via four routes for goldfish euthanasia. Thirty clinically healthy goldfish were anesthetized via immersion in buffered MS-222 (300 mg/L) and randomly administered one of five treatments: KCl (330 mg/mL) via intracardiac injection at 10 mEq/kg (IC), intracoelomic injection (ICe) or bilateral topical delivery over the gill filaments (T) at 100 mEq/kg, 90-minute immersion at 4500 mEq/L dissolved in induction solution (W), or no treatment (X). Following treatment, all fish were moved to anesthetic-free freshwater. Serial heart rates were collected via Doppler device until sound cessation or recovery. Median (range) time to perform treatments was 315 (73-480), 3 (3-3), and 10 (10-10) seconds in IC, ICe, and T, respectively. Doppler cessation occurred in 6/6, 6/6, 6/6, 6/6, and 0/6 fish in median (range) times of 3 (0-210), 18 (10-45), 118 (90-390), and 150 (60-240) minutes, in IC, ICe, T, W, and X, respectively. Following treatment, 1/6, 2/6, 6/6, and 4/6 fish in IC, ICe, T, and W, respectively, exhibited intermittent brief jerking movements. Median (range) time to recovery in X was 5.5 (3.5-6.5) minutes. All administration routes were effective, but time to Doppler cessation varied and transient movements were noted. Preliminary follow-up research revealed that return to MS-222 following treatment abolished movements. Intracoelomic KCl at 100 mEq/kg was technically simple and produced a rapid, consistent time to Doppler sound cessation in 6/6 anesthetized goldfish. Return to MS-222 following KCl administration may be warranted.

Funding: NCSU CVM Veterinary Scholars Program and the Balko Laboratory
Primary Subject Category: Clinical Medicine
Presentation Preference: Oral
LOCATION-SPECIFIC MUTATIONS IN CHD7 INDUCE SPECIFIC SENORIMOTOR PHENOTYPES IN A ZEBRAFISH CHARGE SYNDROME MODEL

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CHARGE syndrome is a rare congenital disorder characterized by a spectrum of physical manifestations including eye, heart, craniofacial, and ear defects. CHARGE patients frequently present with a range of behavioral difficulties such as autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (AD/HD), obsessive-compulsive disorder (OCD), anxiety, and sensory deficits. Most CHARGE cases arise from de novo, loss-of-function mutations in a master transcriptional regulator, chromodomain-helicase-DNA-binding-protein-7 (CHD7). CHD7 is an ATP-dependent chromatin remodeling protein that regulates key neurodevelopmental factors such as SOX10, ALDH1A3, TWIST, and RELN and promotes neuronal differentiation, neural crest cell development, and is required for cerebellar organization and other neural processes. While it is clear that CHD7 is required for normal neural development, how it affects neural circuit formation and function to regulate behavior is unknown. To investigate the pathophysiology of behavioral symptoms associated with CHARGE syndrome, we established a mutant chd7 zebrafish line using CRISPR/Cas9. With a panel of unbiased and high-throughput behavioral assays, we have defined multiple sensorimotor behavioral phenotypes. Our data show chd7 mutants have specific auditory and visually-driven behavioral deficits that are independent of defects in sensory structures, implicating chd7 in the regulation of underlying brain circuits. Additionally, morphological, and behavioral phenotypes depend on the location of the mutation in the gene, providing a novel insight to phenotype penetrance. To identify brain regions impacted by chd7 loss of function, we are analyzing brain-wide activity and morphometry in vivo. Together, these studies will define mechanisms of chd7-dependent neurobehavioral phenotypes and empower future work to identify potential therapeutic targets.

Funding: The CHARGE Syndrome Foundation; NIH R21-NS120079-01A1

Category: Neurosciences, Genetics
Judicious antimicrobial use (AMU) is important for preventing the evolution of antimicrobial resistance in bacterial pathogens, making subsequent use of these drugs less effective in both human and veterinary medicine. The COVID-19 pandemic required many practices to alter their operations and decrease the number of patients seen. Adding in potential shifts in pet care seeking behaviors by owners, such changes may have impacted AMU during the pandemic. The goal of this research is to quantify any changes in prescribing practices arising from the pandemic. To do so, a retrospective study was performed using prescribing data from the pharmacy at NC State College of Veterinary Medicine’s referral hospital and primary care centers for dogs and cats. This data contained records of all antimicrobial prescriptions from NC State from 2019-2020 (n= 32,034), and were used to categorize each as occurring before or during the pandemic. Each instance of AMU was counted to generate overall usage totals for antimicrobial classes in the dataset, which were then classified into tiers according to the FDA’s system for ranking drugs of human medical importance. Using ordinal logistic regression, we calculated an odds ratio (OR) quantifying the risk of each tier being prescribed during the pandemic versus beforehand. Preliminary analysis found patients seen during the pandemic were significantly more likely to receive more important antimicrobials than before the pandemic (OR= 1.1, p= 0.02). Incorporation of mixed effects and confounding variables resulted in the same OR but proved insignificant (p= 0.13) at the 0.05 level.

FDA U01FD007057
NIH Interdisciplinary Biomedical Research Training Program T35-T35OD011070

Pharmacology
Atopic dermatitis (AD) is an inflammatory skin disorder with characteristics of severe pruritus (or itch). AD individuals with chronic itch experience intense detriment to their ability to function, unbalanced and distracted sleep, and many other symptoms that lead to a significant impact on their quality of life for which the treatment options remain limited due to lack of understanding of the mechanisms behind them. Until recently, pruritus is linked with a plethora of mediators at the skin released from immune and non-immune cells and activate receptors that are expressed on neurons innervating the skin. However, the role of neuropeptides released by the sensory neurons in the skin and modulating the function of skin cells types are beginning to understand. Here, we explore to understand cellular and molecular mechanisms involved in the sensory-immune interactions that lead to skin inflammation and itch. In my graduate work, I am focusing on how epidermal nerve endings from a subset of sensory peptidergic neurons expressing brain natriuretic peptide (BNP) regulate mast cell number and function. Our preliminary using three different staining methods demonstrate how BNP-peptidergic neurons regulate mast cells that is linked to chronic itch and perhaps in skin inflammation. Overall, our study will reveal an unexpected neuroimmune mechanism behind chronic itch in AD individuals.
DIFFERENTIAL INHIBITION OF C. DIFFICILE BY THE MICROBIAL DERIVED SECONDARY LITHOCHOLATE AND ITS DERIVATIVES RESULTS IN DIVERSE MECHANISMS OF ACTION

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Primary Subject Category: Infectious Disease

Disruption of the indigenous gut microbiota and the loss of microbial derived secondary bile acids are associated with increased susceptibility to C. difficile infection (CDI). Previous work has shown that secondary bile acid isolithocholate (iLCA), a lithocholate (LCA) derived isomer has potent inhibitory activity against C. difficile strain R20291. Our objective was to further characterize LCA and its derivatives to determine if and how they can inhibit toxin production in C. difficile while sparing commensal members of the gut microbiota. After initially testing the inhibitory activity of the LCA derivatives iLCA, 3-oxo-LCA, and iso-allo-LCA (iaLCA) against C. difficile strain R20291 and a commensal gut microbiota panel, we shifted to determining the mechanism of action by which LCA and its derivatives inhibit C. difficile. For each bile acid, we identified the minimum inhibitory concentration (MIC), bacterial killing, membrane integrity disruption, and effects on toxin expression. We also assessed effects on Caco-2 cell apoptosis, viability, and permeability to determine the cytotoxicity of these compounds against the host. Our findings suggest that both iLCA and iaLCA have potent inhibitory activity against C. difficile while sparing commensal members of the gut microbiota. Although iLCA and iaLCA are both epimers of LCA, they have distinct mechanisms for inhibiting C. difficile. The properties of iLCA and iaLCA suggest their potential use as novel compounds that can target C. difficile while sparing gut commensals that are important for colonization resistance. These findings warrant future efficacy studies in a mouse model.
Title – In all CAPITAL letters (line #1)
• Author Name (Bold) with Category: undergraduate, veterinary student, house officer, graduate student, postdoc, staff (line #2)
• Co-Author(s), Faculty Mentor (underline) (line #3)
• Email address(es)
• Affiliation(s): Use NCSU CVM when appropriate
• Abstract: Limited to 250 words.
• Funding Source(s) if applicable
• Please provide primary subject category for presentation: Biomedical Engineering, Cell Biology, Clinical Medicine, Gastroenterology, Genetics, Immunology, Infectious Disease, Neurosciences, Pain, Pharmacology, Regenerative Medicine or Other
REVERSING OPIOID INDUCED RESPIRATORY DEPRESSION WITH A CANINE MODEL BY HOLLOW MICRONEEDLE DELIVERY OF NALOXONE AND NALMEFENE

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Abstract (abstract limit 250 words)
The aims of this study was to develop a canine model to stimulate opioid induced respiratory depression (OIRD) and to assess the feasibility of using a hollow microneedle array as an effective method of naloxone or nalmefene delivery for reversal. We hypothesized that canines could be used as a model for OIRD and the hollow microneedle array delivery of naloxone and nalmefenes would effectively reversal respiratory depression. Fentanyl was administered intravenously at a dose of 50 mcg/kg over 5 minutes to stimulate respiratory depression in 6 healthy Beagle dogs. Respiratory rate, oxygen saturation and heart rate were monitored continuously until oxygen saturation was less than 90%, fentanyl was discontinued. Naloxone (dose 0.04 mg/kg) or nalmefene (dose 0.2 mg/kg) was administered via hollow microneedle array in the left or right, shaved, inguinal area in a randomized crossover study. Blood samples were obtained at specific intervals over 6 hours from a aseptically placed central venous catheter. Plasma samples are being analyzed for naloxone, nalmefene and fentanyl concentrations via mass spectrometry. Plasma concentrations will then be modeled for pharmacokinetic analysis. All but one dog were reversed successfully with hollow microneedle array. Results thus far indicate that a canine model can be used to effectively stimulate OIRD and the hollow microneedle array shows potential as an alternative method of drug delivery for life-threatening opioid reversal.

Funding: National Institute of Health

Category: Pharmacology
IDENTIFYING CTENOCEPHALIDES FELIS MICROBIOME GENERA AND ASSOCIATED VECTOR PHYLOGENETIC DIVERSITY

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Subject Category: Infectious Disease

Abstract: As a cause of flea allergy dermatitis and vector for two genera of zoonotic pathogens (Bartonella and Rickettsia spp.), Ctenocephalides felis is of substantial medical importance to both human and veterinary medicine. The aim of this study was to assay the pathogenic and commensal microbial communities of individual C. felis from multiple geographic locations and analyze these findings by location, pathogen prevalence, and host diversity. 16S Next Generation Sequencing (NGS) was employed to describe the microbiome of fleas collected from free roaming cats. The cox1 gene was used for flea phylogenetic analysis. Samples included 168 individual fleas from 7 locations within the United States and United Kingdom. Given inconsistency in the genera reported to infect C. felis, we applied the decontam package and literature review to identify candidate microbiome members resulting in the selection of Peptoniphilus, Hathewaya, and Rhodococcus based on metric calculations for known C. felis microbiome members (Wolbachia, Rickettsia, and Bartonella). A single dominant amplicon sequence variant (ASV) was identified from each known C. felis genera reported and was detected in most geographical locations. Multiple minor ASVs were detected in a single or two geographically close locations. Inter- and intra-genus coinfection was common. We described location-based variation in flea phylogeny, identifying seven novel haplotypes. NGS sensitivity and specificity for Bartonella and Rickettsia spp. DNA detection was compared to targeted qPCR. Collectively, our findings confirm the presence of multiple variant ASVs whose presence vary by location and flea haplotype, with important implications for flea transmission and flea-borne pathogen control.

Funding Sources: NCSU’s Comparative Medicine Institute Summer Interdisciplinary Research Initiative, Comparative Medicine and Translational Research Program of the NIH, UW SVM Companion Animal Fund Grant Award. Funding sources had no role in study conceptualization, data collection, or data analysis.
CHEMOGENETIC ACTIVATION OF ENTERIC GLIAL CELLS PROMOTES GROWTH OF THE SMALL INTESTINAL EPITHELIUM IN HEALTHY ADULT MICE AND REPAIR AFTER CHEMOTHERAPY-INDUCED INJURY

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The intestinal epithelium undergoes continuous renewal driven by intestinal stem cells (ISC). ISC functions are regulated by extrinsic signals emanating from niche cells, which comprises enteric glial cells (EGC) in the enteric nervous system. While there is a growing interest in the influence of EGC on ISC activity, EGC impact on ISC-driven epithelial regeneration after genotoxic stress is unknown. ISC-reporter and chemogenetic Sox9-EGFP/GFAP-hM3Dq mice were used. Discrete expression of the eGFP transgene allowed for Sox9-EGFPLow ISC and Sox9-EGFPHSublow progenitors quantification. Expression of the DREADD receptor hM3Dq in GFAP-expressing cells, including EGC, allows for EGC activation via increased calcium signaling using the synthetic drug clozapine-N-oxide (CNO). To study the impact of EGC activation on ISC-driven epithelium renewal in homeostasis, Sox9-EGFP/GFAP-hM3Dq mice were given CNO for 7 days. Epithelium growth and ISC/progenitor density was assessed by H&E and flow cytometry in healthy guts. To study the impact of EGC activation on ISC-driven epithelium repair after genotoxic stress, mice received CNO 24h prior to and during chemotherapy treatment. Mice received 5 daily consecutive injections of the chemotherapeutic drug 5-fluorouracil (5-FU) at 40mg/kg or one single dose of 150mg/kg. The impact of EGC activation on epithelial regeneration was assessed on morphometric parameters and on crypt proliferation using the S-phase marker EdU.

Morphometric analyzes in uninjured intestines demonstrated that EGC chemoactivation led to increased villus/crypt density and height/depth in the small intestine. Flow cytometry data showed an increased progenitor proportion in CNO-treated mice versus controls. Finally, EdU staining showed an increase in the number of crypt cells in S-phase following both chronic and acute 5-FU treatment.

Our preliminary results suggest that EGC activation promotes intestinal epithelium growth during homeostasis and regeneration after genotoxic stress. Future studies will identify the EGC-derived factor(s) involved.

Fundings: UNC Linebeager Comprehensive Cancer Center (Developmental grant); UNC CGIBD (Pilot/Feasibility grant NIH NIDDK P30 DK034987); NCSU CVM (Seed Funding).

Subject category: Neuroscience-Gastroenterology
EFFICACY AND SAFETY OF LONG-TERM IMEPITOIN TREATMENT FOR CANINE STORM ANXIETY WITH INDIVIDUAL DOSE TITRATION

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Storm anxiety is a common disorder and impacts the welfare of affected dogs. Many dogs with storm anxiety show signs of fear well before storms begin, making this condition more complex compared to other noise aversions. Due to this early display of signs and unpredictability of storms, current drug treatment protocols are often insufficient. Previously, Pexion (imepitoin) was found to significantly reduce storm anxiety in dogs, although mild adverse effects (AE) occurred at the approved dosage (30mg/kg BID). The objective of this pilot study was to use a dose-titration approach to determine the optimal efficacious and safe dose range. We hypothesized that the range between 10-30mg/kg BID would be sufficient in effect with few AE’s. Thirty-three dogs with storm anxiety enrolled in this open-label trial. Over 12 weeks, participants completed a 2-week baseline period, 6-week dose-titration period, and 4-week stable dosing period. Owners completed weekly surveys and storm logs using the Canine Anxiety Scale (CAS). Scores from the CAS were analyzed for change from baseline at each dosage. All participants started at 10mg/kg BID; every two weeks, owner global scores were used to determine whether a dose increase was warranted. AE’s were recorded throughout. Significant decreases in CAS scores and storm logs were seen across all dosage groups. The most common reported AE was ataxia (14/33), followed by increased hunger (13/33); no serious AEs occurred. This study suggests an individually titrated dose was safe and effective, with a recommended starting dosage of 10mg/kg BID.

Research Grant: Ingelheim am Rhein, Boehringer-Ingelheim Vetmedica GmbH Ludwigshafen am Rhein, Germany

Student Support: VSP of North Carolina State College of Veterinary Medicine
ELIMINATION KINETICS OF SUBCUTANEOUSLY ADMINISTERED EPRINOMECTIN IN PLASMA AND MILK IN DRY-OFF DAIRY CATTLE

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Eprinomectin is a derivative compound of abamectin belonging to the macrocyclic lactone family. Historically, eprinomectin has been administered topically for treatment against endo- and ectoparasites and has been shown to be effective for approximately 30 days. The injectable formulation of eprinomectin has been increasingly utilized due to its significantly longer efficacy of around 150 days. While there is zero milk withdrawal time for topical eprinomectin, more research is necessary to establish the residues present in milk following subcutaneous administration. The hypothesis of this project is that dairy cattle given a label dose of 1 mg/kg of injectable eprinomectin (LongRange) at the start of their dry off period, 60 days prior to calving, will have milk residues of eprinomectin below the U.S. milk tolerance level of 12 ppb. 13 mature, pregnant dairy cattle were subjects of which plasma was collected daily for 7 days following eprinomectin administration, then at regular intervals for a period of 90 days. Once each cow calved, samples of colostrum and milk were collected daily for compartmental and noncompartmental pharmacokinetic analysis. This analysis is in progress and results will be published when acquired.

Research Grant: Center for Chemical Toxicology and Research and Pharmacokinetics, USDA (FARAD)
Student Support: Veterinary Scholars Program
Subject Category: Pharmacology
ACELLULAR PLACENTAL EXTRACT IMPROVES NEONATAL PORCINE INTESTINAL EPITHELIAL RECOVERY FOLLOWING TRAUMATIC INJURY

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Introduction: Necrotizing enterocolitis (NEC) is a cureless gastrointestinal condition with 20-30% mortality rate in pre-term, low birth-weight infants. Premature infants lose exposure to amniotic fluid known to reduce inflammation and accelerate intestinal maturation. Therefore, an acellular placental extract resembling amniotic fluid may be a novel NEC therapeutic. We hypothesized that extract application would enhance neonatal porcine intestinal epithelial cells’ capacity to repair after injury.

Methods: Ileum was collected from neonatal pigs (n=5). Epithelial monolayers were derived from intestinal crypt culture, scratched, and treated with media (control) or concentrations of acellular placental extract: 0.075, 0.15, or 0.5 mg/mL. Scratches were monitored for cell migration for 24H. Percentage-closure over time was compared between treatment groups using two-way ANOVA and Tukey multiple comparison (p<0.05). Immunofluorescence was used to evaluate stem cell proliferation. Separately, monolayers were exposed to 1% hypoxia followed by application of the same extract concentrations or control media. Transepithelial Electrical Resistance (TEER) was measured every 6-12H for 24H.

Results: Twelve hours post-injury, untreated monolayers achieved a mean epithelial wound closure of 43% compared to 48% (0.15 mg/mL), 58% (0.5 mg/mL), and 75% (0.075 mg/mL) in treated wounds (n=3-5; p<0.05). After 24H, all extract-treated monolayers were 100% closed; untreated wounds only achieved 76.8% closure (n=5; p<0.001). Preliminary evaluation demonstrated increased stem cell proliferation in treated scratch-wounds. Post-hypoxia TEER results pending.

Conclusions: Acellular placental extract application increased the rate of scratch-wound closure in neonatal porcine intestinal epithelial monolayers. As NEC induces epithelial damage, this product may enhance epithelial-mediated repair and combat this disease.

Funding: NIH R44HD100243, K01 OD019911-01A1
Ethical animal research: NCSU IACUC Approved
Subject Category: Gastroenterology
Consideration: To be considered for oral or poster presentation
Bacteroides theaiotaomicron (B. theta) is a Gram-negative gut microbe that encodes enzymes that alter the bile acid pool in the gut. Primary bile acids are synthesized by the host liver and can be conjugated with an amino acid. Bacterial derived bile acids include deconjugated bile acids in which the amino acid is removed, and secondary bile acids in which the sterol core has been modified. These bacterial derived bile acids can influence both the host and the microbiota. B. theta encodes two different bile salt hydrolases (BSHs) which deconjugate bile acids, as well as a hydroxysteroid dehydrogenase (HSDH) which modifies the sterol core. Few bacteria can perform both of these bile acid modifications. We hypothesize that B. theta modifies the bile acid pool so it is advantageous for its own growth. While Gram-negative bacteria are more resistant to the toxic effect of bile acids, B. theta was found to be relatively more sensitive to them, specifically CDCA and DCA when grown in media containing these bile acids. Knockouts of the bile acid altering enzymes were generated through allelic exchange. These knockouts were then grown in the presence of bile acids. Death of wildtype cultures not seen in knockout cultures were observed in stationary phase. qRT-PCR analysis showed bile acid altering genes are highly expressed in stationary phase, indicating their potential use in nutrient limited conditions. These findings could inform multiple areas of research including rational design of probiotics and shaping the gut microbiome through modification of the bile acid pool.
INVESTIGATING FELINE ENDOGENOUS RETROVIRUSES AS IMMUNOTHERAPY TARGETS FOR SQUAMOUS CELL CARCINOMA

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One key to developing immunotherapies for cancers resistant to traditional treatments is identifying tumor-exclusive proteins (antigens) that serve as targets for killer T cells. In a human study, intratumoral T cells were attracted to both patient-specific mutations and de-repressed endogenous retroviruses (ERVs), which would be shared by patients with the same tumor type and valuable for “off-the-shelf” therapy. ERVs constitute ~10% of genomic DNA as relics of germ cell infections. Their expression is normally repressed by epigenetic and intracellular immune mechanisms. Our hypothesis is that some ERVs that are prevalent in the feline genome are expressed in feline oral squamous cell carcinoma (FOSCC) but not in normal tissue, making them an antigen target for this incurable cancer. Since some feline ERVs are ancient and genetically fixed while others are still invading the genome at a variable prevalence, our first objective was to define the genomic prevalence of three ERVs, gamma4-X2, enFeLV, and DC10, in cats from the US. Our second objective was to compare expression of any prevalent ERVs in FOSCC samples and normal peripheral blood mononuclear cells (PBMCs). We found that the prevalence of the gamma4-X2, FeLV, and DC10 ERVs was 100%, 100%, and 18.75%, respectively, via PCR in cat gDNA. We probed for expression in normal PBMCs and FOSCC tumor samples (n=1-3) via RT-PCR and discovered that none were transcribed in either tissue types, despite being gDNA-positive for the ERVs. Additional research is needed to determine if ERVs could be a target for an anti-cancer vaccine in cats.

Research Grant: NC State Coat of Excellence Award
Student Support: VLN NC State Veterinary Scholars Program and the BIVSP
Primary Subject Category: Genetics
INVESTIGATION OF POTASSIUM CHLORIDE FOR THE EUTHANASIA OF ANESTHETIZED AFRICAN CLAWED FROGS (*XENOPUS LAEVIS*)

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Abstract:

Euthanasia is frequently performed in amphibians, but current techniques are limited and variable in efficiency. This study investigated the use of potassium chloride (KCl) for euthanasia of anesthetized African clawed frogs (*Xenopus laevis*). Twenty adult, female African clawed frogs were anesthetized via immersion in buffered tricaine methanesulfonate (MS-222) for 5 minutes beyond loss of righting reflex. Frogs were randomly assigned to receive one of four treatments: KCl via intracardiac injection at 10 mEq/kg (IC, n=5), intracoelomic injection at 100 mEq/kg (ICe, n=5), or immersion at 4500 mEq/L (IMS, n=5) or no treatment (C, n=5). Following treatment, serial Doppler readings were performed until loss of Doppler sounds, 60-minute endpoint (IC, ICe, IMS), or recovery (C). Times to loss of righting reflex, loss of Doppler sounds, and/or recovery were recorded. Plasma potassium concentrations were assessed immediately after Doppler sound cessation in frogs in IC (n=2), ICe (n=2), and IMS (n=5). Failure to inject occurred in one IC frog. One frog in ICe regained spontaneous movement 4 minutes post-injection. Both frogs were removed from data analysis. Doppler sound cessation occurred in 4/4, 4/4, 0/5, and 0/5 frogs in IC, ICe, IMS, and C, respectively. Median (range) time to Doppler sound cessation in IC and ICe was 0.5 (0-16) seconds and 17.5 (10-15) minutes, respectively. Plasma potassium concentration was >9.0 mmol/L in sampled frogs. Intracardiac KCl at 10 mEq/kg and intracoelomic KCl at 100 mEq/kg were effective for euthanasia of anesthetized African clawed frogs. Return to MS-222 following KCl administration may be warranted.

Funding Source(s) if applicable: Balko Laboratory

Primary subject category for presentation: Clinical Medicine (Euthanasia)
CORTICOSTERONE AS A PROGNOSTIC INDICATOR IN EASTERN BOX TURTLES (TERRAPENE CAROLINA CAROLINA) WITH TRAUMATIC INJURIES ADMITT ED TO A WILDLIFE REHABILITATION HOSPITAL

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Abstract: Eastern box turtles (EBT, Terrapene carolina carolina) commonly present to wildlife hospitals for traumatic injuries, as they have increased exposure to people and vehicles due to their terrestrial lifestyle. While it is known that wildlife in captivity experience stress, it is unknown how glucocorticoids could reflect survivorship in hospitalized box turtles.

For this study, small volumes of blood were collected from 37 EBTs admitted to TRT for traumatic injuries on days 0, 1, 7, 14, 21, and 28. Packed cell volume (PCV), total solids, leukocyte differentials, and corticosterone concentrations were measured, and triage scores were assigned at intake based on injury severity, reflexes, and attitude. Logistic regression was used to determine which parameters influenced survival on days 1, 7, and 28.

Turtles which did not survive for 24 hours had higher lactate on intake (p<0.05) and had higher triage scores (p<0.01) than turtles which survived past 24 hours. Turtles which did not survive the first week were more likely to have a decrease in corticosterone over the first 24 hours after intake, while corticosterone increased or stayed the same in surviving turtles, though this relationship was not statistically significant (p=0.09). Survival at one month was dictated by triage score (p<0.01). Lactate (p<0.05), PCV (p<0.01), and the heterophil:lymphocyte ratio (p<0.05) were highest at intake and decreased significantly throughout hospitalization.

These results implicate the need to reduce stress in the first 24 hours of hospitalization and demonstrates the importance of injury severity when assessing prognosis in EBTs with traumatic injuries.

Funding Sources: This study was funded by the Morris Animal Foundation Veterinary Student Scholar Program and the Triangle Community Foundation George H. Hitchings New Investigator Award in Health Research.

Primary Subject Category: Clinical Medicine
Fungal keratitis (FK) is an infectious corneal disease with worldwide increasing prevalence, necessitating models that reliably assess efficacy of topical applied drugs. Current models such as in vitro minimum inhibitory concentration (MIC) assays are difficult to interpret and may fail to correlate with in vivo application. This study compares a novel ex vivo corneal model to a recent MIC study, testing the hypothesis that this model provides an accurate and biologically representative evaluation of therapeutic drug efficacy.

Archived isolates of 4 fungal species sourced from equine FK patients at NCSU CVM were used. Porcine cadaver globes were inoculated with conidia via intrastromal injection. Corneas were excised and incubated in DMEM with one of four antifungal drugs (AMB, LUL, NAT, VOR) at increasing concentration equivalents of the previously reported MIC. Radial fungal growth was monitored for 72 hours total.

All antifungal drugs examined significantly inhibited growth of Aspergillus fumigatus and Fusarium keratoplasticum at or below the MIC at all time points. With few exceptions, all drugs inhibited growth of A. flavus below the MIC at all time points. AMB did not inhibit growth of F. falciforme, while all other drugs inhibited growth at or below the MIC for all time points except for LUL at 24 hours.

Inhibition of radial fungal growth in corneas injected with conidia and incubated in AMB, LUL, NAT, or VOR corresponded well with in vitro MICs in each isolate. With few exceptions, ex vivo fungal growth was significantly inhibited at 1/2 of the established MIC.

Subject category: Pharmacology
Abstract Title
VASOPRESSIN STIMULATION EFFECTS ON PHYSICAL EXAM AND CORTISOL RESPONSE IN HEALTHY FOALS

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Abstract Content:
The hypothalamic-pituitary-adrenal axis (HPAA) is responsible for homeostatic control of blood cortisol response to stress. HPAA derangement can cause poor stress responses known as relative adrenal insufficiency (RAI), defined by cortisol insufficiency contributing to severe pathologies such as shock. Sepsis, the life threatening dysregulation of the body organs in response to infection, is the leading cause of foal mortality. RAI is a sequelae of sepsis and an important pathology to diagnose in septic foals. This study’s goal was to create an effective protocol for evaluation of the HPAA in healthy foals utilizing an optimized vasopressin dose for stimulation. We hypothesized that endogenous cortisol will increase significantly with arginine vasopressin (AVP) administration without significant adverse effects. This crossover study utilized AVP stimulations at 3 doses (2.5, 5, and 7.5 IU), given 12 hours apart. These quantified AVP’s effects on cortisol response in 13 healthy foals. Baseline blood samples were collected at 24h of age, then at 15, 30, 60, and 90 minutes after stimulation. Vital parameters were taken at 0, 5, 10, 20, 30, 60, and 90 minutes. Cortisol was quantified with a radioimmunoassay. Cortisol concentrations were increased up to 45 times baseline at all doses, peaking 30 minutes after stimulation. This study showed that all 3 doses of AVP were safe and effective in foals to test HPAA function. This could be a more sensitive test for RAI in septic foals, better informing veterinarians on the best plan for foal sepsis and RAI treatment.

Funding Sources
Intramural Seed Grant
NC State CVM VSP and BIVSP

Subject Category
Clinical Medicine
EFFECTS OF PORCINE INTESTINAL NORMOTHERMIC MACHINE PERFUSION AND COLD STORAGE ON EPITHELIAL BARRIER INTEGRITY

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Abstract:

Intestinal failure (IF) is a loss of intestinal function and inability to absorb nutrients. Inflammatory bowel disease, vascular thrombosis, or short bowel syndrome can all result in IF. Intestinal transplantation is the final therapeutic option for IF patients. Cold storage (CS) is the current gold-standard method for preservation prior to transplant. However, CS damages the epithelium, causing impaired mucosal barrier function, bacterial translocation, sepsis, and graft rejection. Tight junction (TJ) proteins maintain epithelial integrity and their loss has been associated with CS in other organs. Normothermic Machine Perfusion (NMP) is an alternative preservation method that has been demonstrated to be less damaging than CS to kidney and liver grafts. Our objective was to compare the effects of intestinal CS and NMP on epithelial barrier integrity, focusing on structural proteins. We hypothesized that NMP better preserves epithelial barrier integrity than CS. Seven porcine intestines were preserved with CS or NMP for 6hrs. Jejunal biopsies were obtained prior to and after storage. Immunofluorescence for apoptotic cells (CC3) and TJ proteins claudin-3 and -4 was performed. Cells undergoing apoptosis were significantly greater in both CS (P=0.0026) and NMP (P=0.0061) compared to control, but no significant differences were found between NMP and CS. Claudin-4 expression in NMP and CS appeared decreased compared to control. Claudin-3 appeared more cytoplasmic in villous areas for both T6 NMP and CS. Using the current methods to evaluate barrier integrity, an improved preservation of tight junction proteins was not identified in NMP treated intestine.

Funding: U.S. Department of Defense PR181265, NIH K01OD010199 SERCA, NIHP30 DK034987

Student support: NIH-T-35 Interdisciplinary Biomedical Research Training Program (IBRTP)
Intestinal ischemia is the most common cause of gastrointestinal-related morbidity and mortality within the NICU. Ischemic-induced loss of the intestinal epithelial barrier predisposes patients to life-threatening sepsis unless that barrier is rapidly restored. Early barrier restoration relies on epithelial restitution to achieve wound closure, followed by rearrangement of tight junction proteins to reseal the barrier. To inform putative therapeutics that promote rapid barrier repair, we have developed a highly-translational model of intestinal ischemia in the neonatal pig. Previous studies have demonstrated a significant defect in post-ischemic repair of the neonatal pig (2-weeks-old) jejunum relative to the juvenile (6-weeks-old) pig. Therefore, I hypothesized that the neonatal colon will demonstrate a similar defect in epithelial barrier repair. To test this hypothesis, segments of spiral colon were subjected to surgically-induced ischemia following by ex vivo recovery on Ussing chambers. Unexpectantly, the neonatal pig colon undergoes robust, post-ischemic repair of its epithelial barrier. Ischemically-injured colonic mucosa demonstrated a significant increase in restitution (p<0.01) and barrier resistance (p<0.05). These findings suggests the presence of a mechanism of intestinal epithelial barrier repair unique to the colon. Given that colonic mucosa contains significantly more goblet cells than jejunal mucosa, goblet-cell secretory products are a feasible target for identifying mechanisms of this site-dependent discrepancy. Future endeavors will aim to elucidate inter- and intracellular mechanisms of barrier repair that may be mediated by TFF-3, one of the primary goblet cell secretory products, so as to inform putative treatment modalities that may mitigate the devastating consequences of neonatal intestinal ischemia.

**Funding sources:** NIH K01 OD 028207, NIH-NICHD R01 HD095876, U01 TR002953

**Category:** Gastroenterology
Oral Abstract

DEVELOPMENT OF A NOVEL POINT-OF-CARE PROGNOSTIC TEST OF NEURAL INJURY FOR DOGS

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Acute spinal cord injury (SCI) is a common neurological emergency in dogs, yet prognosis for recovery of ambulation is difficult to predict. Two nervous system cytoskeletal proteins, glial fibrillary acidic protein (GFAP) and phosphorylated neurofilament heavy chain (pNF-H) can be detected in the serum of dogs with severe SCI due to intervertebral disc disease (IVDD) and their levels predict outcome. We hypothesize that a new, inexpensive, rapid immunoassay point of care multiplex test can measure GFAP and pNfH (D4POC) accurately in canine serum and that serum concentrations of these biomarkers will predict outcome. The first study aim is to measure GFAP and pNfH in a subset of samples using both ELISA and D4POC to determine intraclass correlation between tests, and the second is to utilize the D4POC in remaining samples to determine predictive accuracy of serum biomarker concentrations for outcome. Thus far, 326 samples from dogs with severe SCI have been banked, of which 98 have recovered ambulation. Testing of the samples has started. We anticipate that the D4POC will be as specific and more sensitive than the ELISA tests and will predict outcome with high accuracy. Further, we anticipate the D4POC to be an efficient and cost-effective way to quantify biomarkers in dogs with injury to the central nervous system. If correct, the D4POC can be used in the clinic to determine the severity of SCI in paraplegic dogs, assisting owners and veterinarians in decision making and optimal patient triage.

Funding Sources: Morris Animal Foundation, NIH-T-35 Interdisciplinary Biomedical Research Training Program
Primary Subject Category: Neurosciences
THE PHARMACOKINETICS OF INTRANASAL ADMINISTRATION OF FLUNIXIN MEGLUMINE IN GROWER PIGS

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Abstract:
Flunixin meglumine is approved for the treatment of pyrexia associated with respiratory disease by intramuscular (IM) injection in swine, but is used extralabel for pain management since there are no drugs approved for analgesia in pigs. The objective of this study was to determine the pharmacokinetics of a novel intranasal (IN) route because a non-invasive administration method could have a major impact on the animal welfare and economics of the swine industry. In a randomized crossover study, six grower pigs received 2.2 mg/kg of flunixin by both IM and IN routes. Plasma samples were collected for 60 hours post administration and were analyzed by ultra performance liquid chromatography with tandem mass spectrometry to determine drug concentrations over time. Mean pharmacokinetic parameter estimates obtained using noncompartmental analysis for the IM (control) and IN (experimental) routes, respectively, were area-under-the-curve to infinity $6.75 \pm 1/33$ hr$\times$μg/mL and $5.28 \pm 2.13$ hr$\times$μg/mL; maximum concentration $4.18 \pm 0.88$ μg/mL and $3.35 \pm 1.36$ μg/mL, which occurred at time $0.22 \pm 0.08$ hours hrs for both routes. Terminal half-life was $7.49 \pm 1.40$ hours and $7.45 \pm 1.37$ hours. Relative bioavailability of IN administration compared to IM administration was estimated to be $78.8 \pm 26.7\%$. The IN administration method of flunixin administration to pigs appears to be relatively well absorbed and with plasma levels corresponding to analgesic efficacy in piglets.

Research Grant: Veterinary Scholars Program (Smith); Center for Chemical Toxicology Research and Pharmacokinetics, USDA (FARAD)

Primary subject: pharmacology
PLATELET-RICH PLASMA LYSATE AS A TREATMENT FOR BIOFILM ASSOCIATED PLACENTITIS

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Placentitis-induced pre-term fetal loss has caused hardships and financial losses to the equine breeding industry for decades. While traditional multi-modal treatments including a combination of gentamicin, penicillin, altrenogest, firocoxib, and pentoxifylline, have shown some success, treatment failures still occur. The understanding of potential biofilms in these reproductive infections has found a pitfall in traditional treatment options, in which the antimicrobials are ineffective at penetrating and killing biofilms. Platelet-rich plasma lysate has begun to be a widely accepted approach as a new treatment option for biofilm associated infections across species. It has been proven to be successful in treating equine synovial fluid biofilm infections with Staphylococcus aureus, especially when combined with an aminoglycoside. With these promising results, this study aims to look at different isolates of Escherichia coli from the equine uterus. Biofilm formation was tested amongst 10 clinical isolates followed by treatment with PRP-L, using a crystal violet assay. On average a 60% reduction of in vitro biofilm formation occurred as a result of the PRP-L treatment. For some isolates, nearly all biofilm formation was eliminated. Although E. coli was the bacteria used in this study, it is expected that similar results will be found from other gram negative as well as gram positive bacterial infections found in the equine reproductive tract. Moving forward, our laboratories aim to compare traditional multi-modal therapy for placentitis, with multi-modal therapy augmented with PRP-L.

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Subject Category: Clinical Medicine
PEPTIDE INHIBITORS OF MARCKS SUPPRESS ENDOTOXIN INDUCED UVEITIS IN RATS

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Abstract

Purpose: To determine if inhibition of Myristoylated Alanine Rich C Kinase Substrate (MARCKS) protein, using novel MARCKS inhibitor peptides, will reduce the severity of endotoxin-induced uveitis (EIU) in rats.

Methods: EIU was induced in Lewis rats via subcutaneous administration of lipopolysaccharide (LPS). In the first phase of the study, three different novel MARCKS inhibitor peptides that mimic the N-terminal region of MARCKS (BIO-11006, or lower molecular weight analogs BIO-91201 or BIO-91202; Biomarck Pharmaceuticals, Ltd., Newtown, PA), were administered intravitreally (IVT) at 50 μM and 100 μM. In the second phase, BIO-91201 was administered IVT at 10 μM, 50 μM and 100 μM and topically at the 100 μM concentration. The efficacy of MARCKS inhibitor peptides was assessed via clinical examination using slit lamp biomicroscopy, optical coherence tomography anterior chamber cell counts, histopathology, and aqueous humor cytokine analysis.

Results: Clinical scores were significantly reduced 24 hours following uveitis induction in the first phase of the study in the following treatment groups: BIO-11006 50 μM IVT and 100 μM IVT, BIO-91201 50 μM IVT, and BIO-91202 100 μM IVT (p<0.05). OCT anterior chamber cell counts were significantly reduced in the first phase of the study in all treatment groups (p<0.001). OCT anterior chamber cell counts and histopathology scores were significantly reduced in the second phase of the study in the BIO-91201 50 μM IVT group (p<0.05). No effect was seen with topical administration.

Conclusion: MARCKS inhibitor peptides were effective in reducing the severity of ocular inflammation and cellular influx in EIU.

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Subject category: Cell biology
MURIBACULACEAE COMPETE WITH CLOSTRIDIODES DIFFICILE FOR MUCIN-DERIVED SUGARS

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Primary Category - Infectious Disease

Clostridioides difficile is a Gram-positive pathogen that causes C. difficile infection (CDI). Infection is often initiated when antibiotic administration perturbs the intestinal microbiome, resulting in a loss of colonization resistance (CR) against C. difficile. Commensals compete with C. difficile for nutrients to support CR by restricting pathogen colonization and growth. Our prior work identified the Muribaculaceae as potential drivers of CR in a mouse model of CDI. Muribaculaceae are a recently cultured family of Gram-negative bacteria indigenous to mammalian guts and are thought to be particularly well equipped for glycan acquisition and they have been suggested to compete with C. difficile for mucin monosaccharides. Therefore, we hypothesize that several Muribaculaceae species are involved in the prevention and restriction of C. difficile colonization through depletion of mucus-derived sugars.

To explore the competition between Muribaculaceae and C. difficile, we developed a supplemented Fastidious Anaerobe broth to support the growth of Muribaculum intestinale and Duncinella muris, two Muribaculaceae species. We then co-cultured M. intestinale, D. muris, or both species together with C. difficile to test for competition for mucosal glycans. Media was supplemented with water, sialic acid, or N-acetylglucosamine and co-cultures were plated for C. difficile after 12 and 24 hours. C. difficile growth was most inhibited by the Muribaculaceae in the NAG-supplemented growth condition after 24 hours of growth, suggesting these bacteria can compete with C. difficile in a monosaccharide-dependent manner. We plan to test if Muribaculaceae can compete with C. difficile in vivo to provide CR and limit CDI severity.
CANINE UROPATHOGENIC AND AVIAN PATHOGENIC *ESCHERICHIA COLI* HARBORING CONJUGATIVE PLASMIDS EXHIBIT AUGMENTED GROWTH AND EXOPOLYSACCHARIDE PRODUCTION IN RESPONSE TO *ENTEROCOCCUS FAECALIS*

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Uropathogenic *Escherichia coli* (UPEC) and avian pathogenic *Escherichia coli* (APEC) are common extraintestinal pathogenic *Escherichia coli* (ExPEC) that infect dogs and poultry. ExPEC infections are often accompanied by co-infection with *Enterococcus faecalis* (EF); however, it remains unclear how EF co-infection modulates virulence of UPEC and APEC. To further characterize interspecies interactions among these bacteria, *in vitro* and *in vivo* models of co-infection were used to identify UPEC, APEC, and EF clinical isolates for whole-genome sequencing. Seventy-one UPEC strains were screened under iron-limited conditions for increased growth in mixed culture with EF. Sixty-nine UPEC exhibited a growth advantage with EF (*P* < 0.01) but were attenuated when grown alone. Phylogenetic analyses indicated the growth-response phenotype was highly conserved in ExPEC despite high strain diversity. Comparative genomic analysis of strains with growth-response and non-responding strains indicated that growth was associated with the presence of siderophore, exopolysaccharide (EPS), and plasmid conjugative transfer genes. Two matched pairs of responding and non-responding ExPEC (one matched pair each of UPEC and APEC) were selected for further characterization in macrocolony proximity assays. EF-responsive ExPEC produced 5 to 16 times more EPS compared to non-responsive strains (*P* < 0.01). Interestingly, a responsive APEC strain cured of its conjugative plasmid encoding the *tra* genes lost the enhanced growth and EPS production response to EF. These data demonstrate that growth augmentation by EF occurs in diverse UPEC and APEC strains and may be linked to conjugative virulence plasmids and EPS production, which are common virulence determinants of ExPEC.

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**Category:** Infectious Diseases
Objective: To investigate bacterial and fungal growth dynamics in extrinsically contaminated bupivacaine liposomal injectable suspension (BLIS) in comparison to bupivacaine 0.5% and propofol.

Methods: Vials of liposomal bupivacaine, bupivacaine 0.5%, and propofol were inoculated with known quanta of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Over 120 hours, aliquots were withdrawn, plated, and incubated to quantify fungal and bacterial growth via manual count of colony forming units. Data were analyzed by using a mixed effects procedure with multiple comparisons.

Results: BLIS did not support significant growth of *S. aureus* and *C. albicans* at any time. BLIS supported significant growth of *E. coli* and *P. aeruginosa* beyond the 48 hour time point. Bupivacaine 0.5% did not support significant growth of any organisms. Propofol supported significant growth of all organisms.

Conclusion: The growth of bacterial and fungal contaminants in extrinsically contaminated vials of BLIS was variable.

Clinical significance: Bacterial and fungal contaminant growth in BLIS is variably supported over time. Aseptic technique should be used when handling liposomal bupivacaine suspension.

Funding sources: CVM Intramural Grant 2021

Primary Subject Category: Clinical Medicine
Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is a canine pathogen of concern and is commonly associated with urinary tract infections, pyoderma, and other skin diseases. Antimicrobial resistance is one of the biggest challenges in both human and animal health. The mecA gene confers resistance to methicillin and other commonly used Beta-lactam antibiotics in *S. pseudintermedius*, and co-occurrence of other resistance genes may allow for further selection of multi-drug resistant MRSP. The objective of this study was to understand how previous antimicrobial prescriptions contribute to the development of MRSP infections. A retrospective cohort study of 153 records of patients with *S. pseudintermedius* cultured between 2014-2016 were reviewed for antimicrobial prescriptions, date of prescription, and presence of mecA. Analysis was accomplished by logistic regression, and AIC was used for model selection and to compare fit of models with prescription time windows. Covariates not included for fit were assessed for confounding. The final model included patient age, sex, and weight in addition to a variable for any antimicrobial prescribed in the 30, 60, or 90 days before culture. Time frame did not appear to meaningfully change the model (AIC = 155.98, 156.13, 156.28 respectively). When comparing antimicrobial exposure at days 30, 60, and 90, no significant change was seen (p = 0.53 or higher) which suggests that recent treatment with an antimicrobial did not increase the risk of being colonized by MRSP as compared to mecA negative *S. pseudintermedius*. Further analysis will look at the relationship between specific drug classes and mecA positive *Staphylococcus pseudintermedius*. 

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Subject category: 
Antimicrobial resistance
ALTERNATIVE ANALYTICAL FRAMEWORK FOR QUANTIFYING HOST EXPOSURES AND GENETIC RISK FACTORS FOR ANTIMICROBIAL RESISTANCE AMONG SWINE CAMPYLOBACTER COLI

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Antimicrobial resistance (AMR) is a major public health challenge today. Consequently, understanding risk factors driving AMR epidemiology is of critical importance. However, analytical methods traditionally used in epidemiology are poorly suited for evaluating AMR epidemiology for several reasons. First, these methods are well-suited for evaluating risk factors for a single outcome, but AMR is a system with multiple-outcomes. Second, these methods are poorly suited for complex systems like AMR where factors across diverse scales (i.e., environment, host, microbial) interact to influence AMR outcomes. Here we propose an alternative analytical approach using multi-layered chain graphs, a type of probabilistic graphical model, which is more suitable for AMR. We applied this approach to identify host exposures and microbial resistance genotypes associated with resistance phenotypes among Campylobacter coli isolated from longitudinally sampled swine cohorts from conventional and antibiotic-free (ABF) farms. Among all isolates, known important resistance mutations on the 23S rRNA and gyrA genes were associated with significantly higher minimum inhibitory concentrations (MICs) for macrolide and fluoroquinolone antibiotics, respectively. Unexpectedly, genes known to confer resistance to aminoglycosides were also significantly associated with macrolide resistance among ABF C. coli. Most swine antimicrobial exposures were not associated with higher MICs to tested antibiotics among conventional C. coli. Among all isolates, biosecurity practices particularly related to interspecific exposures were associated with significantly higher and lower MICs for macrolides and fluoroquinolones. We demonstrate an analytical framework that can be applied to other epidemiological research aiming to understand risk factors for AMR.

Funding Source: NIH F30OD030022

Primary Subject: Infectious Disease
Prostate cancer (PC) remains the second leading cause of human cancer deaths in the United States, and carries a similarly grave prognosis in canines. Most human mortalities are due to castration-resistant prostate cancer (CRPC), which is an aggressive form of PC with limited treatment options. Canines develop spontaneous PC with many characteristics similar to CRPC, including a geriatric onset, progression despite castration, and frequent metastases. The hypothesis for this study is that somatic mutations are conserved between a subset of canine PC and human CRPC, which may identify novel diagnostic and therapeutic opportunities for both species.

Whole exome sequencing was performed on 29 canine carcinomas involving the prostate. Approximately 47 million variants were detected and filtered for frequency and gene function. The BRAF V595E mutation, which has been identified previously in 85% of canine urothelial and prostatic carcinomas, was present in 83% of canine PC tumors. This mutation is infrequent in human CRPC. The next most frequent variant identified was present in 79% of prostate tumors, including three of four tumors that lack the BRAF V595E mutation. This variant has previously been identified in the COSMIC database of human somatic mutations, and overexpression of this protein is associated with human PC. Additionally, somatic mutations involving this gene were detected in 203 (10.6%) of 1916 human prostate tumors included in The Cancer Genome Atlas Program data set. This variant shows promise as a diagnostic and therapeutic target in both canines and humans with CRPC.

Funding Sources: Lineberger Comprehensive Cancer Center, Consortium for Canine Comparative Oncology

Genetics
Squamous cell carcinoma (SCC) of the oral cavity and skin is a common cause of death in captive North American snow leopards, accounting for over 33% of all reported cancers. Early studies suggest a link between SCC and precursor viral plaques. *Panthera uncia papillomavirus-1* (PuPV1) has been sequenced from oral plaques; however, a larger study population is needed to confirm this association. A different, yet unknown, papillomavirus is suspected to cause cutaneous plaques. The purpose of this study was to determine the prevalence of PuPV1 within oral plaques and to identify potentially novel papillomaviruses associated with cutaneous plaques. Samples of cutaneous (n=4) and oral (n=12) plaques were collected from archived biopsy and necropsy samples. PCR was performed using specific primers for PuPV1 as well as degenerate primers, enabling identification of potentially novel papillomavirus sequences. A putatively novel papillomavirus was detected within three (75%) cutaneous plaques. This novel sequence shared 76% sequence identity to the cat papillomavirus *Felis catus papillomavirus 2* (FcaPV2), which causes cutaneous viral plaques and has been implicated in development of cutaneous SCC. DNA specific for PuPV1 was detected in 83% of oral plaques, providing additional evidence of an association between PuPV1 and oral plaques. In the future, we aim to determine if these papillomaviruses are associated with cutaneous and oral SCC in captive Snow Leopards. Ultimately, identification of a causal viral agent in the development SCC in these animals will lay the foundation for development of prophylactic vaccines and targeted therapeutic strategies.

Subject Category: Infectious Disease
THE NOSE KNOWS: VALIDATION OF AN OLFACTORY TEST IN DOGS WITH AND WITHOUT NOSEWORK TRAINING

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Olfaction is a cognitive process used for communication and discrimination. Assessment of olfaction in dogs would be valuable to monitor sensory and cognitive decline associated with age. This has proved difficult, as untrained pet dogs rely primarily on vision during two-choice trials. This study aimed to validate a novel olfactory test using adult dogs with and without nosework training. Trials using hidden food rewards were conducted in light and darkness; dark conditions eliminated visual cues, requiring dogs to rely on olfaction. Percentage correct and latency to find the treat were recorded. We hypothesized that control and trained dogs would have a higher percentage correct in dark than light, and that trained dogs would have higher percentage correct overall than controls. Twenty control and 15 trained dogs completed the trials. Control and trained dogs performed similarly in light and dark conditions with no significant difference between the groups (t-test; light p=0.08; dark p=0.27). Across both groups, dogs performed significantly better in dark than light trials (p<0.001), with an average improvement of 18.9%. Trained dogs were not significantly faster during dark trials than controls (mean: 13.16s vs 16.63s). Results demonstrate that conducting trials in darkness encourages dogs to engage their noses to find hidden food treats. As no difference in percent correct was found between the groups, these findings establish a standard behavior of healthy adult dogs and may be used as a normative sample for investigation of sensory decline in dogs.

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