Poster #	Author (Classification-Mentor)	Title	Time
1	Aker, Savannah (NS - Lascelles)	DEMOGRAPHIC PARAMETERS, OWNER ASSESSMENTS, AND VETERINARY EXAM FINDINGS AS PREDICTORS OF DAILY ACTIVITY LEVELS AS MEASURED BY ACCELEROMETRY IN DOGS WITH OSTEOARTHRITIS	10:30 - 11:30
2	Browning, Matthew (VS - Kulkarni)	CpG-ADJUVANTED RECOMBINANT HVT-LT VACCINE PROVIDES PROTECTION AGAINST INFECTIOUS LARYNGOTRACHEITIS IN BROILER CHICKENS	12:00 - 1:00
3	Caldwell, Madison (VS/PhD - Blikslager/Ziegler)	NEONATAL ENTERIC GLIA ENHANCE INTESTINAL EPITHELIAL RESTITUTION IN VITRO FOLLOWING EXPOSURE TO STERILE COLONIC LUMINAL CONTENT OF MATURE BUT NOT NEONATAL PIGS	10:30 - 11:30
4	Cave, Ashley (VS - Lewbart)	MULTI-YEAR HEALTH ASSESSMENT OF BLUE-FOOTED BOOBIES (SULA NEBOUXII EXCISA) IN THE GALÁPAGOS ISLANDS	12:00 - 1:00
5	Chandra Deb, Liton (GS - Lanzas)	QUANTIFYING TRADE-OFFS BETWEEN THERAPEUTIC EFFICACY AND RESISTANCE DISSEMINATION FOR ENROFLOXACIN DOSE REGIMENS IN CATTLE.	10:30 - 11:30
6	Cheng, Xiao (GS - Cheng)	MICRONEEDLE PATCH DELIVERY OF PROTACS FOR ANTI-CANCER THERAPY	10:30 - 11:30
7	Cherukuri, Aswini (VS - Lewbart)	VEHICULAR TRAUMA PATTERNS OF CHELONIANS IN NORTH CAROLINA: SPATIAL ANALYSIS AND IDENTIFICATION OF HIGH RISK AREAS AND ROADS	12:00 - 1:00
8	Coleman, Caitlyn (VS - Schnabel)	INITIAL INVESTIGATION INTO THE EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR ON INTRASYNOVIAL TENOCYTES IN VITRO	12:00 - 1:00
9	Connard, Shannon (GS - Schnabel)	INITIAL INVESTIGATION INTO THE EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR ON INTRASYNOVIAL TENOCYTES IN VITRO	12:00 - 1:00
10	Craig, Sara (VS - Ziegler)	iDISCO HIGHLIGHTS POSTNATAL CHANGES IN ENTERIC GLIAL NETWORK DEVELOPMENT IN A COMPARATIVE PIG MODEL	10:30 - 11:30
11	Dillon, Megan (GS - Breen/Reiskind)	POPULATION GENETIC INSIGHTS INTO THE FREE-BREEDING DOG POPULATIONS AT CHERNOBYL	10:30 - 11:30
12	Edel, Margaret (UG - Metzler, LeGrand, Estes, Trivedi)	INTERPRETATION OF ETHOGRAM BEHAVIORS IN RESEARCH CANINES WITH INCREASED ENRICHMENT	10:30 - 11:30
13	Flynn, Jason (GS - Buchler)	THE EARLY CELL CYCLE IN CHYTRID FUNGI RESEMBLES EMBRYONIC CELL CYCLES	10:30 - 11:30
14	Gagliardi, Rachel (GS - Schnabel)	A NOVEL EQUINE IN VITRO MODEL OF OSTEOARTHRITIS UTILIZING FIBRONECTIN FRAGMENT STIMULATION.	12:00 - 1:00
15	Gin, Taylor (GS - Callahan)	FLEA-BORNE PATHOGENS IN FLEAS FROM NATURALLY INFESTED DOGS AND CATS IN PRIVATE HOMES IN FLORIDA	10:30 - 11:30
16	Henderson, Nichol (VS - Gilger)	OCULAR TOXICITY, DISTRIBUTION, AND SHEDDING OF INTRAVITREAL GENE THERAPY FOR UVEITIS	10:30 - 11:30
17	Hepworth, Emma (GS - Yoder)	INVESTIGATING HOW PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) SUPPRESS NEUTROPHIL FUNCTION	12:00 - 1:00
18	Hobbs, Kallie (GS - Sheats)	EX VIVO VALIDATION OF A NOVEL EXTRACORPOREAL THERAPY DEVICE FOR REMOVAL OF CYTOKINES IN HORSES	12:00 - 1:00

19	Howard, Jacob (VS - Len)	THE EFFECTS OF FSH AND P4 ON CANINE OOCYTE METABOLISM AND GENE EXPRESSION DURING IN VITRO MATURATION.	12:00 - 1:00
20	James, Rebekah (VS - Breen)	ASSESSMENT OF CHARACTERISTIC 'HOT-SPOT' GENE MUTATIONS IN HUMAN SOFT TISSUE SARCOMAS ACROSS MULTIPLE VETERINARY SPECIES	10:30 - 11:30
21	Johnson, Haley (VS - Shively)	ASSOCIATION BETWEEN CEREBROSPINAL FLUID BIOMARKERS OF ALZHEIMER'S DISEASE AND COGNITIVE PERFORMANCE IN VERVETS	10:30 - 11:30
22	Jolley, Ashlan (GS - Lanzas)	COMPANION ANIMAL ANTIMICROBIAL USE DATA FROM SMALL ANIMAL PRIVATE PRACTICES IN NORTH CAROLINA FROM 2019-2020	12:00 - 1:00
23	Joseph, Dani (GS - Mishra)	ROLE OF MAST CELLS IN ITCH AND INFLAMMATION IN A MOUSE MODEL OF ATOPIC DERMATITIS	10:30 - 11:30
24	Keefer, Maya (VS - Varner)	CLINICAL EVALUATION OF 3-SITE VERSUS 4-SITE DISTAL PARAVERTEBRAL BLOCKS IN STEERS UNDERGOING STANDING ABDOMINAL LAPAROTOMY	12:00 - 1:00
25	Korla, Praveen Kumar (PD - Birkenheuer)	TITLE: METAGENOMICS APPLICATIONS OF VECTOR BORNE DISEASES IN SMALL ANIMALS	10:30 - 11:30
26	Kornegay, Caroline (VS - Park)	PREDICTING OF SKIN FLAP PERFUSION AND VIABILITY USING INDOCYANINE GREEN (ICG) IN RATS	10:30 - 11:30
27	Kropf, Ashley (UG - Sheahan)	MODULATION OF CFTR FUNCTION USING AN IN VITRO MODEL OF EQUINE RECTAL EPITHELIUM	10:30 - 11:30
28	Livingston, Isabella (GS - Breen)	GENETICS AND GENOMICS AID IN IDENTIFICATION OF PARASITES FROM NON-NATIVE SPECIES IN AN ENDANGERED GALAPAGOS PINNIPED	12:00 - 1:00
29	Lorenz, Lizette (PD - Kaeser)	DIFFERENTIAL GENE EXPRESSION IN A SWINE MODEL FOR EOSINOPHILIC ESOPHAGITIS	12:00 - 1:00
30	Lunking, Vienna (VS - Oh)	CORNEAL LESIONS: COMPARING FEATURES SEEN ON ULTRASOUND BIOMICROSCOPY AND HISTOPATHOLOGY	12:00 - 1:00
31	Meredith, Bryanna K (VS Ostdiek/Langan/Luchins)	EVALUATION AND TIME AND COST SAVINGS ANALYSIS OF EXTENDED SANITATION INTERVAL OF CAGING AND ACCESSORIES AND AUTOMATED WATERING VALVES	12:00 - 1:00
32	Miranda Martinez, Ivonne (VS - Halleran)	PHENOTYPIC RESISTANCE OF ENTEROCOCCI OBTAINED FROM CATTLE FECES TO ERYTHROMYCIN AND VANCOMYCIN AFTER IN-FEED ADMINISTRATION OF TYLOSIN	12:00 - 1:00
33	Mojica Pérez, Julio (VS - Halleran)	NOVEL SAMPLING TECHNIQUES FOR ORAL PHARMACOKINETICS OF TYLOSIN	12:00 - 1:00
34	Mulder, Meg (VS - Hess)	VALIDATING GENE TARGETS FOR CANINE PERIPHERAL T-CELL LYMPHOMA IMMUNOTHERAPY	12:00 - 1:00
35	Neira Torres, Laura (VS - Foster)	ANTIBIOTIC CONCENTRATION IN THE PERITONEAL FLUID OF CATTLE	12:00 - 1:00
36	O'Neill, Mary (UG - Sheahan)	CHARACTERIZING ENTEROENDOCRINE CELLS IN HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION	12:00 - 1:00
37	Petersen, Jillian (VS - Sheahan)	DEVELOPMENT OF AN IN VITRO MODEL OF GLUCOSE STIMULATED GIP SECRETION IN EQUINE DUODENAL ORGANOIDS	10:30 - 11:30
38	Pierce, Hannah (UG - Harrison/Duke)	INVESTIGATION OF NEOPLASIA IN UNGULATES	10:30 - 11:30
39	Poisson, Lydia (VS/PhD - Gonzalez)	A NOVEL APPROACH USING SIDE POPULATION ANALYSIS TO IDENTIFY INTESTINAL STEM CELLS IN WILD-TYPE PIGS	12:00 - 1:00

40	Rabasco, Jordan (GS - Callahan)	CONTAMINATION LEVELS IN DIFFERING LENGTH DNA FRAGMENTS IN ILLUMINA SEQUENCING	12:00 - 1:00
41	Ramos Cabrera, Mara (VS - Gonzalez)	EQUINE PLACENTAL-DERIVED EXTRACT ENHANCES THE REGENERATIVE CAPACITY OF EQUINE INTESTINAL EPITHELIAL CELLS	12:00 - 1:00
42	Seda-Gómez, Fancyrette (VS - Gruber)	OPTIMIZING CELL BLOCKS AND STORAGE MEDIA CONDITIONS OF FINE NEEDLE ASPIRATES FROM CANINE LIVERS	10:30 - 11:30
43	Torres Machado, Nicole (VS - Gookin)	CHARACTERIZATION OF ORGANOIDS CULTURED FROM NORMAL AND MUCOCELE CANINE GALLBLADDERS	10:30 - 11:30
44	Wimbish, Candace (HO - Lynch)	PHARMACOKINETICS OF A CONTINUOUS INTRAVENOUS INFUSION OF HYDROMORPHONE IN HEALTHY DOGS	12:00 - 1:00
45	Yang, Yixuan (GS - Callahan)	BIOINFORMATICS ANALYSIS ON SHOTGUN SEQUENCING DATA FROM ANCIENT PARCHMENT	10:30 - 11:30
46	Yu, Junho (UG - Mishra)	G-PROTEIN COUPLED RECEPTOR 35 (GPR35) EXPRESSION AND FUNCTIONAL CHARACTERIZATION USING HISTOLOGICAL AND CELL-LINE BASED APPROACH	10:30 - 11:30

DEMOGRAPHIC PARAMETERS, OWNER ASSESSMENTS, AND VETERINARY EXAM FINDINGS AS PREDICTORS OF DAILY ACTIVITY LEVELS AS MEASURED BY ACCELEROMETRY IN DOGS WITH OSTEOARTHRITIS

Subject Category: Pain
Presenter: Savannah Aker (staff)

Christina Stevens[1], Savannah Aker[1], Erin Perry[1], Emily Haupt[1], Alejandra Mondino[2], Masataka Enomoto[1], Margaret E. Gruen[3,4], B. Duncan X. Lascelles[1,4,5,6]

csteven@ncsu.edu[1], saker@ncsu.edu[1], etperry2@ncsu.edu[1], eehaupt@ncsu.edu[1], amondin@ncsu.edu[2], menomot@ncsu.edu[1], megruen@ncsu.edu[3,4], dxlascel@ncsu.edu[1,4,5,6]
[1]Translational Research in Pain, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States
[2]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States
[3]Comparative Behavioral Research, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, United States
[4]Comparative Pain Research and Education Center, North Carolina State University, Raleigh, North Carolina, United States
[5]Thurston Arthritis Center, The University of North Carolina School of Medicine, Chapel Hill, North Carolina, United States
[6]Center for Translational Pain Research, Department of Anesthesiology, Duke University, Durham, North Carolina, United States

Accelerometry measures physical activity (PA) and is a validated objective measure of the impact of osteoarthritis (OA) pain in dogs and cats. However, several factors other than OA-pain can affect PA in dogs and relatively little is understood about the influence of these factors. Functional linear modeling (FLM) is an approach for analyzing and visualizing high-frequency longitudinal data (such as PA) and can be used to assess the influence of factors on activity patterns. This study aimed to investigate the effect of various factors on PA patterns in dogs with OA-pain using FLM. Ninety-nine client-owned dogs with radiographic and clinical evidence of OA were fitted with a collar-mounted activity monitor (Actigraph GT3X). Average vector magnitudes were recorded once per minute over 7 days and averaged to create 24-hour, per-minute activity profiles for each dog. Demographic information, owner completed OA clinical metrology instruments (CMIs: CBPI, LOAD) and veterinary examination findings (joint pain, muscle atrophy) were collected. Data were analyzed using FLM and a custom R package to evaluate the effect of each factor on 24-hour patterns of PA. At times of traditionally peak activity within a 24-hour period, dogs with OA-pain, higher age, CMI scores, joint pain, greater BCS and muscle atrophy all had decreased activity profiles. However, only age and hindlimb muscle atrophy had significant effects. Understanding what factors influence PA patterns in dogs with OA-pain, and how, will help refine the use of PA as an outcome measure in clinical pain studies.

CpG-ADJUVANTED RECOMBINANT HVT-LT VACCINE PROVIDES PROTECTION AGAINST INFECTIOUS LARYNGOTRACHEITIS IN BROILER CHICKENS

Matthew Browning: Veterinary Student
Carissa Gaghan, Isabel M. Gimeno, Ravi Kulkarni
mhbrowni@ncsu.edu, cegaghan@ncsu.edu, imgimeno@ncsu.edu, rrkulkar@ncsu.edu
Affiliation: NCSU CVM

Infectious laryngotracheitis (ILT) is an economically important disease of chickens. We have previously shown that in-ovo adjuvantation of recombinant herpesvirus of turkey-Laryngotracheitis (rHVT-LT) vaccine with CpG-oligonucleotides (ODN) can boost vaccine- induced responses in one-day-old broiler chickens. Here, we evaluated the protective efficacy of in ovo administered rHVT-LT+CpG-ODN vaccination against a wild-type ILT virus (ILTV) challenge at 28 days of age and assessed splenic immune gene expression as well as cellular responses. A chicken-embryo-origin (CEO)-ILT vaccine administered in water at 14-days of age was also used as a comparative control for protection assessment. Results showed that the birds vaccinated with rHVT-LT+CpG-ODN or the CEO vaccine showed significant protection against ILTV challenge and that the level of protection induced by both the vaccines was statistically similar. The protected birds were found to have a significantly upregulated expression of Interferon (IFN)γ or interleukin (IL)-12 cytokine genes. Furthermore, chickens vaccinated with rHVT-LT+CpG-ODN or CEO vaccine had significantly higher frequency of γδ T cells and activated CD4+ or CD8+ T cells, compared to unvaccinated-ILTV challenge control. Collectively, our findings suggest that CpG-ODN can be used as an effective adjuvant for rHVT- LT in ovo vaccination to induce protective immunity against ILT in broiler chickens.

Funding Source(s) if applicable: US POULTRY and EGG FOUNDATION
Subject Category: Infectious Disease

NEONATAL ENTERIC GLIA ENHANCE INTESTINAL EPITHELIAL RESTITUTION *IN VITRO* FOLLOWING EXPOSURE TO STERILE COLONIC LUMINAL CONTENT OF MATURE BUT NOT NEONATAL PIGS

Madison Caldwell (DVM/PhD student)

Laurianne Van Landeghem, Anthony Blikslager, Amanda Ziegler

mcaldwe2@ncsu.edu

NCSU CVM

Neonates exhibit significantly poorer outcomes from damage to the intestinal epithelium following ischemia. We recently discovered an epithelial restitution defect in ischemia-injured neonatal pig intestine that is rescued by application of homogenized juvenile mucosa, but the mechanism remains unclear. Enteric glial cells (EGC) promote epithelial restitution following injury via paracrine signaling and are abundant in the intestinal mucosa of juvenile but not neonatal pigs. In mice, postnatal maturation and maintenance of this EGC network is purportedly driven by colonization of gut microbiota. Therefore, we believe that changes to intestinal microbiota during weaning may play a key role in EGC maturation required for proper epithelial restitution. We hypothesized that treatment with mature (>6 weeks of age), but not neonatal (<3 weeks of age), luminal content would improve restitution of neonatal epithelial monolayers co-cultured with neonatal EGC. We compared the effect of sterile-filtered neonatal or mature luminal content addition to the apical chamber on scratch wound restitution in neonatal porcine IPEC-J2 monolayers in monoculture or co-culture with primary porcine neonatal submucosal EGC using a transwell system (n = 9). Our results support a significant effect of luminal content treatment in the presence of EGC (P=0.0085). Specifically, mature luminal content treatment enhanced IPEC-J2 wound closure in co-culture with EGC but not in monoculture (P=0.0156). These data suggest that EGC provide secretory signals to the intestinal epithelium in response to luminal content that promote repair. Future work aims to determine what is driving these differences in restitution by analyzing neonatal and mature luminal secretomes.

Funding sources: 5 T35 OD 11070-12, NIH K01 OD 028207, NIH P30 DK 034987, NIH-NICHD R01 HD095876, USDA-NIFA VMCG-0065, NCSU CVM VFPP
Subject category: Gastroenterology

MULTI-YEAR HEALTH ASSESSMENT OF BLUE-FOOTED BOOBIES (*SULA NEBOUXII EXCISA*) IN THE GALÁPAGOS ISLANDS

Ashley E. Cave (DVM/PhD student), Jacqueline R. Dillard, Catalina Ulloa, Juan-Pablo Muñoz-Pérez, Alice Skehel, Diane Deresienski, Ronald K. Passingham, Jason Castaneda, Gregory A. Lewbart, Carlos A. Valle.

NC State University, Raleigh, NC, USA (Dillard, Passingham, Lewbart, Deresienski), Universidad San Francisco de Quito (USFQ) and UNC at Chapel Hill (Ulloa, Muñoz-Pérez, Skehel, Valle),

Universidad San Francisco de Quito USFQ, Quito, Ecuador (Ulloa, Valle), University of the Sunshine Coast, Sippy Downs, Australia (Muñoz-Pérez)

The Galápagos blue-footed booby (*Sula nebouxii excisa*) is a sulid subspecies native to the Galápagos archipelago. Here we present physical examination, hematology, and blood chemistry results from 60 Galápagos blue-footed boobies that were captured by hand from their nests on North Seymour Island in June 2017 and July 2022. A portable blood analyzer (iSTAT) was used to obtain values in the field for hematocrit, hemoglobin, sodium, potassium, chloride, ionized calcium, total CO2, glucose, blood urea nitrogen, creatinine and anion gap for each bird. Blood lactate, total solids, packed cell volume, and blood smears were evaluated manually on site. A white blood cell differential was performed in 2017. The breeding status of each bird and the number of chicks in the nests were also recorded. Total CO2, blood urea nitrogen, ionized calcium, potassium, anion gap, hematocrit, and hemoglobin were all higher in 2022 than 2017. There were also more nests with chicks in them in 2022 than in 2017. Lactate, ionized calcium, hematocrit, and hemoglobin were all higher in females than in males, while blood urea nitrogen was higher in males than in females. These results provide a multi-year reference to the baseline health parameters in a free-living population of Galápagos blue-footed boobies that can be used to compare and monitor the health status of this species.

Research Grant:
This research was authorized by the Galápagos National Park Service and was conducted with support of the Heska Corporation, the Galápagos Academic Institute for the Arts and Sciences (GAIAS)-Universidad San Francisco de Quito (USFQ) and the Galápagos Science Center-USFQ/University of North Carolina-Chapel Hill.

Student Support:
NC State University Office of the Associate Dean for Research and Graduate Studies

Field of Research:
Wildlife conservation

Title: QUANTIFYING TRADE-OFFS BETWEEN THERAPEUTIC EFFICACY AND RESISTANCE DISSEMINATION FOR ENROFLOXACIN DOSE REGIMENS IN CATTLE.

Author: Liton Chandra Deb[1] (Graduate Student)

Co-Authors: Archana Timsina[1], ABM Shamim Ul Hasan[1], Suzanne Lenhart[2], and Cristina Lanzas[1*]

Email Address: lchandr@ncsu.edu, aneupan@ncsu.edu, ahasan5@ncsu.edu, slenhart@tennessee.edu, clanzas@ncsu.edu

Affiliation: [1] Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA
[2]Department of Mathematics, University of Tennessee, Knoxville, TN, 37996, USA

Abstract: Antimicrobials used in food-producing animals potentially increase the selection pressure on bacteria to become resistant, which can be transferred to humans. The study aims to evaluate the trade-offs between treatment effectiveness and cost and the dissemination of resistance for gut commensal bacteria (*E. coli*) and *P. multocida* bacteria. Three approved enrofloxacin dosing regimens were compared for their effects on bovine respiratory disease (BRD) treatment and resistance dissemination: 12.5 mg/kg, 7.5 mg/kg as a single dose, and 5 mg/kg as multiple doses. We developed a within-host differential equation model to illustrate the dynamics of antimicrobial drug concentration and bacterial populations in the host. The model was fitted to animal data of drug concentrations that describe the pharmacokinetic-pharmacodynamic parameters of enrofloxacin within a cattle host. The structural identifiability of the model was investigated before estimating the parameters' value. Our results indicated that high-dose scenarios increased treatment costs and bacterial resistance levels in the gut and lungs compared to multiple low-dose scenarios. A proposed scenario (7.5 mg/kg, two doses 24hrs apart) showed promising results with lower economic costs and reduced bacterial resistance for treating BRD in cattle. This model offers valuable insights into implementing effective strategies for sustainable and responsible antimicrobial practices in food animals by integrating critical factors influencing resistance development.

Funding Source: NIH R35GM134934

Primary Subject Area: Other (Population and Global Health)

MICRONEEDLE PATCH DELIVERY OF PROTACS FOR ANTI-CANCER THERAPY

Xiao Cheng, Graduate student
Ke Cheng, Faculty Mentor
Email: xcheng6@ncsu.edu
Affiliations: Department of Molecular Biomedical Sciences and Comparative Medicine Institute, North Carolina State University, Raleigh, North Carolina 27607, United States. Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States, and North Carolina State University, Raleigh, North Carolina 27606, United States.

Abstract: Proteolysis-targeting chimera (PROTAC) is an emerging technique for degrading disease-related proteins. However, most current PROTACs exhibit poor solubility and no organ targeting, which has hampered their druggability. Herein, we report direct and sustained delivery of PROTACs using microneedle patches to the diseased tissues. In this study, we use an estrogen receptor alpha (ERα) degrading PROTAC, ERD308, to treat ER-positive breast cancer. A pH-sensitive micelle, MPEG-poly(β-amino ester) (MPEG-PAE), is used to encapsulate ERD308 along with an FDA-approved CDK4/6 inhibitor, Palbociclib (Pal), before loading into biodegradable microneedle patches. One microneedle patch can achieve sustained drug delivery and release into deep tumors for at least 4 days, with over 87% of drugs retained in tumors. ERD308 released from the microneedle patches can sufficiently degrade ERα in MCF7 cells in vitro or in vivo. Co-administration of ERD308 and Palbociclib exhibits excellent efficacy by over 80% tumor reduction as well as a superb safety profile. Our work demonstrates the feasibility and proof-of-concept therapeutic potential of using microneedle patches to directly deliver PROTACs into tumors.

Funding sources: This work was supported by grants from the NIH (HL123920, HL137093, HL144002, HL146153, HL147357, and HL149940 to K.C.) and the American Heart Association (19EIA34660286 to K.C.).

Primary category: Biomedical engineering

VEHICULAR TRAUMA PATTERNS OF CHELONIANS IN NORTH CAROLINA: SPATIAL ANALYSIS AND IDENTIFICATION OF HIGH RISK AREAS AND ROADS

Aswini Cherukuri[1], veterinary student
Alexandra Sack[2], DVM, MPH, PhD and Gregory Lewbart[1], MS, DVM, DACZM, ECZM

acheruk@ncsu.edu

[1]NCSU CVM, [2]NCSU CVM Alumni

Vehicular trauma is the most common cause of chelonians presenting to the Turtle Rescue Team (TRT) at North Carolina State University College of Veterinary Medicine. Many of these chelonian species are declining throughout their range, and anthropogenic stressors are a major cause. This study aims to identify areas and roads that are at high risk for vehicular trauma through retrospective analysis of turtles presenting for vehicular trauma from 2005-2019. Statistical analysis of collision locations and descriptive analysis of high-risk areas and roads were conducted to determine factors associated with increased collisions and mortality. Geospatial factors examined include the proximity to water, protected areas, and TRT. Road attributes examined include road type, physical road characteristics, speed limit, and traffic level. Of the turtles that presented for vehicular trauma (n = 2577), 42% survived to be released and 53% died or were euthanized. Geocoded locations were obtained for 66.8% of incidents (n = 1723). Logistic regression of geocoded locations within 60 kilometers of TRT (n = 1462) showed the relationship of dying or being euthanized from vehicular trauma was not significantly associated with the measured geospatial factors and road attributes. This indicated a more in-depth analysis of the roads was needed. Ten high-risk areas and ten high-risk roads were identified based on the high spatial density of collisions. Through identification and analysis of high-risk areas and roads, management and mitigation measures can be implemented. The high rate of vehicular caused mortality demonstrates the need for collision prevention as a conservation priority.

Primary subject category: Other (wildlife medicine and management)

INITIAL INVESTIGATION INTO THE EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR ON INTRASYNOVIAL TENOCYTES *IN VITRO*

Caitlyn V Coleman; Veterinary Student
Shannon S Connard, Drew W Koch, Anna M Froneberger, Lauren V Schnabel
clveste2@ncsu.edu
NCSU CVM Department of Clinical Sciences, Raleigh, NC

In horses, tenosynovitis most commonly affects the digital flexor tendon sheath (DFTS). Injury to the deep and superficial digital flexor tendons (DDFT, SDFT) within the DFTS can result in the formation of intrasynovial adhesions, which carry a poor prognosis. Tissue plasminogen activator (TPA) is a commonly used anti-adhesive agent; however, its impact on tendon healing has yet to be investigated. The objectives of this study were to examine the effects of TPA, on equine DDFT- and SDFT-derived tenocyte 1) viability and proliferation, 2) gene and protein expression, and 3) migration *in vitro*. We hypothesized that TPA would not demonstrate cytotoxic effects on tenocytes or impair their proliferation capacity, migration ability, or gene expression. Briefly, DDFT- and SDFT-derived tenocytes were cultured in tenocyte media or tenocyte media containing clinically relevant doses of TPA. Cytotoxicity and proliferation were assessed using viability, population doubling, and proliferation assays. Gene expression will be evaluated using a multiplex immunoassay to quantify supernatant protein concentrations and NanoString nCounter Analysis System for transcriptional analysis. There were no significant effects of TPA on tenocyte viability or population doubling. Assays to evaluate tenocyte proliferation, gene expression, and migration are currently underway. Our initial findings suggest that TPA does not elicit any cytotoxic effects on tenocytes in vitro. The main limitation is that this study was performed in vitro. These findings will elucidate the effects of TPA on tenocytes in vitro and lay the groundwork to assess tendon healing, efficacy in adhesion prevention, and administration dose and frequency in vivo.

Research Grant: Fund for Orthopedic Research in honor of Gus and Equine athletes “F.O.R.G.E”
Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program
Subject: Clinical Medicine

INITIAL INVESTIGATION INTO THE EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR ON INTRASYNOVIAL TENOCYTES *IN* VITRO

Shannon Connard[1,2] – graduate student
Drew W. Koch[1,2], Caitlyn Coleman[1], Anna Froneberger[1], and Lauren V. Schnabel[1,2]

Email – ssconnar@ncsu.edu

Affiliations
[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
[2]Comparative Medicine Institute, North Carolina State University, Raleigh, North Carolina, USA

Abstract
In horses, tenosynovitis most commonly affects the digital flexor tendon sheath (DFTS). Injury to the deep and superficial digital flexor tendons (DDFT, SDFT) within the DFTS can result in the formation of intrasynovial adhesions, which carry a poor prognosis. Tissue plasminogen activator (TPA) is a commonly used antiadhesive agent; however, its impact on tendon healing has yet to be investigated. The objectives of this study were to examine the effects of TPA, on equine DDFT- and SDFT-derived tenocyte 1) viability and proliferation, 2) gene and protein expression, and 3) migration *in vitro*. We hypothesized that TPA would not demonstrate cytotoxic effects on tenocytes or impair their proliferation capacity, migration ability, or gene expression. Briefly, DDFT- and SDFT-derived tenocytes were cultured in tenocyte media or tenocyte media containing clinically relevant doses of TPA. Cytotoxicity and proliferation were assessed using viability, population doubling, and proliferation assays. Gene expression will be evaluated using a multiplex immunoassay to quantify supernatant protein concentrations and NanoString nCounter Analysis System for transcriptional analysis. There were no significant effects of TPA on tenocyte viability or population doubling. Assays to evaluate tenocyte proliferation, gene expression, and migration are currently underway. Our initial findings suggest that TPA does not elicit any cytotoxic effects on tenocytes *in vitro*. The main limitation is that this study was performed *in vitro*. These findings will elucidate the effects of TPA on tenocytes *in vitro* and lay the groundwork to assess tendon healing, efficacy in adhesion prevention, and administration dose and frequency *in vivo*.

Funding sources
Fund for Orthopedic Research in honor of Gus and Equine athletes "F.O.R.G.E"
Stipend support T32OD011130 Interdisciplinary Biomedical Research Training Program

Primary subject category – regenerative medicine

iDISCO HIGHLIGHTS POSTNATAL CHANGES IN ENTERIC GLIAL NETWORK DEVELOPMENT IN A COMPARATIVE PIG MODEL

Sara E Craig: DVM/PhD Student
Sophia P Jodka, Jack Odle, Laurianne Van Landeghem, Anthony T Blikslager, Amanda L Ziegler
sjerwin@ncsu.edu
NCSU CVM, NCSU CALS

Background
The glial network of the enteric nervous system is instrumental in intestinal repair, but is immature at birth. In mouse models, enteric glia are restricted to the submucosal and myenteric plexuses, and are driven to populate the lamina propria by changes in microbial populations at weaning. Our lab uses a comparative pig model, but early postnatal development of the enteric nervous glial network has not yet been described and measured in the pig.

Hypothesis
We hypothesized the density and distribution of glial cell subtypes would change within the early postnatal period in our comparative pig model.

Specific Aims
We aimed to measure the enteric glial network in our comparative pig model from birth to 21-days-of-age.

Methods
The immunolabeling-enabled three-dimensional imaging of solvent-cleared organs (iDISCO) technique was used to triple-stain full-thickness jejunum of 1-, 7-, 14-, and 21-day-old pigs against glial markers S100β, Sox10, and glial fibrillary acidic protein (GFAP). Samples were imaged with a light-sheet microscope and glial volumes were calculated in Imaris software.

Results
In the lamina propria, density by volume of GFAP+ glia decreases (P=0.1133) while S100β+ glia density increases (P=0.5326). The number of Sox10+ nuclei increase from 1 to 21 days of age (P=0.0147).

Conclusion
We believe this indicates GFAP is expressed in more mature glial cells and that S100β, a known inflammatory mediator, is participating in immune responses to colonizing bacteria while Sox10 marks the nuclei of progenitor glia. Understanding this early postnatal development will allow its modulation to accelerate maturation of repair mechanisms.

Funding: NIH T35, UNC CGIBD, USDA, NCSU CMI
Category: Gastroenterology

POPULATION GENETIC INSIGHTS INTO THE FREE-BREEDING DOG POPULATIONS AT CHERNOBYL

Megan N. Dillon[1,2]: Graduate student
Rachael Thomas[1], Tim A. Mousseau[3], Jennifer A. Betz[4], Norman J. Kleiman[5], Martha O. Burford Reiskind[2], Matthew Breen[1,6,7,8,9]
mdillon3@ncsu.edu; rthomas3@ncsu.edu; mousseau@sc.edu; drbetz@cleanfutures.org; njk3@cumc.columbia.edu; mbreiski@ncsu.edu; matthew_breen@ncsu.edu
[1]Department of Molecular Biomedical Sciences, NCSU CVM; [2]Department of Biological Sciences, NCSU; [3]Department of Biological Sciences, University of South Carolina; [4]Visiting Veterinarians International; [5]Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University; [6]Comparative Medicine Institute, NCSU; [7]Center for Human Health and the Environment, NCSU; [8]Cancer Genetics, UNC Lineberger Comprehensive Cancer Center, University of North Carolina; [9]Duke Cancer Institute, Duke University

ABSTRACT:
In 1986, an explosion at the Chernobyl Nuclear Power Plant released hundreds of tons of radioactive debris that contaminated the surrounding environments. In addition to the radioactive materials, the area surrounding the power plant was further contaminated by subsequent remediation efforts which introduced heavy metals, organics, and other hazardous compounds. While the Chernobyl Nuclear Power Plant is the site of the world's largest nuclear disaster and produced an extremely unique environment, there are 1,877 sites in the United States that the Environmental Protection Agency recognizes as superfund sites, defined as a polluted location that requires long-term cleanup. These areas can contain harmful chemical and environmental toxicants, such as lead, arsenic, and mercury, and pose a threat to local inhabitants. Many species of wildlife still inhabit Chernobyl Exclusion Zone (CEZ) where they have been continuously exposed to the harmful environment for potentially many generations. Our research focuses on unowned, semi-feral dogs living within the CEZ: one population living around the Chernobyl Nuclear Power Plant and one living 16 km away in Chernobyl City. By studying their genetics, we can better predict how humans and wildlife living in similarly harsh environments may be impacted. Through genetic analyses of these semi-feral dogs, we have identified a striking level of genetic diversity between a population at the power plant and other, outside populations, and evidence suggestive of directional selection. Though once domestic, these now free-breeding dogs will provide insights towards the genetic response of exposed communities to environmental contaminants.

Funding Sources: Triangle Center for Evolutionary Medicine Graduate Student Award (MND); Cancer Genomics Fund (MB); Clean Futures Fund, SPCA International, Samuel Freeman Charitable Trust, and the Office of Research and the South Carolina Honors College at USC (TAM)
Primary subject category for presentation: Genetics

INTERPRETATION OF ETHOGRAM BEHAVIORS IN RESEARCH CANINES WITH INCREASED ENRICHMENT

Margaret Edel
Undergraduate
Lexi Roof[1], Alana Boone[1], Jess Schisnky[1], Marnie Metzler[2], Jessie LeGrand[2], Jenny Estes[2], Shweta Trivedi[3]
maedel@ncsu.edu, laroof@ncsu.edu, jlschins@ncsu.edu, mgsilver@ncsu.edu, jlegran@ncsu.edu, jmestes2@ncsu.edu, strived@ncsu.edu
NCSU Canine College Internship[1], NCSU CVM Lab Animal Resources[2], NCSU Animal Science Department[3]

Animal welfare has become a crucial concern in recent years, with public opinion increasingly against the use of animals in research. This study aims to demonstrate that the welfare of dogs used in laboratory research can be maintained and that they exhibit natural behaviors comparable to household canines when provided with appropriate enrichment. In a stock dog colony comprising 22 dogs used in student teaching labs and non-invasive research studies, each dog was given additional social and environmental enrichment, and their natural behaviors were observed and recorded. An ethogram was created to document their primary behaviors, which was then applied to data collection to identify welfare concerns and the effects of positive reinforcement conditioning on these behaviors. The results demonstrate that providing appropriate enrichment can significantly influence the expression of behavior and contribute to better animal welfare in research settings.

Other

THE EARLY CELL CYCLE IN CHYTRID FUNGI RESEMBLES EMBRYONIC CELL CYCLES

Jason Flynn (Graduate Student)
Edgar M. Medina, Yukun Jennifer Zhang, Nicolas E. Buchler
jaflynn2@ncsu.edu
Affiliations: CBS, NCSU CVM, CMI, CMMTP

Abstract: Cell cycle coordination of cell growth and division shows diversity between cell types and across kingdoms. For example, somatic cells need to grow during the gap phases between DNA replication and mitoses, and key cell cycle genes are transcribed “just-in-time” prior to an upcoming cell cycle phase. At the other extreme, embryos are large pre-grown cells packed with maternally supplied transcripts, and the early cell cycles in embryos have no gap phase and do not exhibit “just-in-time” transcription. To better understand the evolution of the cell cycle in fungi and animals, our lab works with a chytrid fungus, *Spizellomyces punctatus* (*Sp.*), an early-diverging fungal lineage that has features of both fungi and an animal-like ancestor. We used RNA-sequencing and timelapse fluorescence imaging to measure transcription and key cell cycle events during the chytrid life cycle. Similar to embryos, Sp. does not exhibit “just-in-time” transcription during its early cell cycle, most likely due to a supply of maternally supplied transcripts. This includes the transcription of a mitotic cyclin, whose abundance is known to oscillate and drive cells into mitosis. Using timelapse imaging, I will show that fluorescently tagged mitotic cyclin oscillates at the protein level. However, unlike embryonic cells, the early *Sp.* cell continues to grow and its period shortens over time. Characterization of the cell cycle in chytrid fungi aids in the understanding of cell cycle evolution and its differences between animals and fungi.

Funding Source: Comparative Molecular Medicine Training Program (CMMTP) [NIH T32GM133393]
Primary Subject Category: Cell Biology

A NOVEL EQUINE IN VITRO MODEL OF OSTEOARTHRITIS UTILIZING FIBRONECTIN FRAGMENT STIMULATION.

Rachel Gagliardi[1,2]**:** graduate student

Drew W. Koch[1,2], Richard Loeser[3,4], Lauren V. Schnabel[1,2,4]

Email: rgaglia@ncsu.edu, dwkoch@ncsu.edu, richer_loeser@med.unc.edu, lvschnab@ncsu.edu

Affiliations:
[1]Department of Clinical Sciences, College of Veterinary Medicine, NC State University, Raleigh, NC
[2]Comparative Medicine Institute, NC State University, Raleigh, NC
[3]Division of Rheumatology, Allergy and Immunology, UNC, Chapel Hill, NC
[4]Thurston Arthritis Research Center, UNC, Chapel Hill, NC

Abstract:
Osteoarthritis (OA) is a debilitating disease that impacts millions of individuals. A significant hindrance to therapeutic discovery is the limitations of current *in vitro* models available for the study of OA. Current models utilize lipopolysaccharide (LPS) or interleukin-1-beta (IL-1β) to stimulate synoviocytes and chondrocytes, to induce an OA-like phenotype. Naturally occurring OA is documented to be a disease of chronic low-level inflammation that leads to dysregulation of normal cellular function within the joint. However, current models that utilize LPS and IL-1β invoke an inconsistent, intense, and transient inflammatory response. We hypothesized that utilizing fibronectin fragments (FN-f) *in vitro* to stimulate synoviocytes and chondrocytes both in monolayer and co-culture may result in a model that more closely mimics naturally occurring OA. Fibronectin is a component of the extracellular matrix and becomes fragmented during OA, which is believed to contribute to OA pathogenesis. To induce OA in our model, cells were stimulated with FN-f for 18h and RNA was isolated at the conclusion of stimulation and 24h post-stim. Gene expression of a select set of genes that are widely documented to be altered in an OA disease state was analyzed using a Nanostring nCounter. Several key genes that are altered in OA, such as IL-6, CXCL6, CCL2, MMP3 and MMP13, were also significantly impacted by our FN-f stimulation model. Further analysis to fully characterize the OA phenotype resulting from FN-f stimulation is currently underway. Future studies will aim to compare our FN-f model to traditional LPS and IL-1β models.

Funding Support: 2022 NCSU CVM Intramural Research Grant (LVS and RG), R37 AR049003 (RL)**;** NCSU GAANN Biotech Fellowship

Subject Category: Other

FLEA-BORNE PATHOGENS IN FLEAS FROM NATURALLY INFESTED DOGS AND CATS IN PRIVATE HOMES IN FLORIDA

Taylor E. Gin (graduate student)

Trey Tomlinson, Grace Wilson, Amiah Gray, Cameron Sutherland, Kamilyah Miller, Krista Li, Michael Canfield, Brian Herrin, Erin Lashnits, Benjamin J. Callahan

tegin@ncsu.edu
bcallah@ncsu.edu

Kansas State University College of Veterinary Medicine, Manhattan, KS (Wilson, Tomlinson, Gray, Sutherland, Miller, Herrin)

University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI (Li, Lashnits)

North Carolina State University College of Veterinary Medicine, Raleigh, NC (Gin, Callahan)

Animal Dermatology South, 7741 Congress St, New Port Richey FL 727-807-3240 (Canfield)

Background: The cat flea, *Ctenocephalides felis*, is the most common ectoparasite of dogs and cats, and can transmit a variety of pathogens including zoonotic *Bartonella* and *Rickettsia* species. The risk factors underlying transmission of these pathogens are incompletely elucidated.

Objective: Describe the flea-borne pathogens of fleas from owned cats and dogs and determine associations between flea pathogen carriage and pet and household characteristics.

Animals: 32 homes in west central Florida with flea infestations, including 40 cats and 8 dogs.

Methods: Fleas on each cat and dog were counted using a standardized procedure, then collected; fleas in the home were also counted using overnight intermittent light traps, then collected. A survey was used to gather demographic and household information as potential explanatory variables. Fleas were pooled by animal and tested using 16S-rRNA next generation sequencing. Associations between the presence of *Bartonella* and *Rickettsia* spp. in fleas with potential explanatory variables were assessed using mixed effects modeling.

Results: There were 272 fleas collected from 40 cats in 31 homes, and 98 fleas from 8 dogs in 7 homes. Pathogens in fleas and the proportion of cats and dogs with fleas infected with each pathogen will be presented. Associations between the presence of *Bartonella* and *Rickettsia* spp. and potential explanatory variables, including host demographics, home characteristics, and geographic location, will be shown.

Conclusions: This study evaluated flea-borne pathogens in fleas from owned pets in their homes, reflecting potential flea-borne disease exposures for pets and pet owners with limited access to veterinary care.

Funding
Study was funded by Elanco Animal Health. Research Grant: Evaluation of an oral lotilaner product Control Flea Populations on Naturally Infested Cats in Private Residences in Tampa FL 2022 - Post-Marketing Agreement.

Taylor Gin is a PhD student partially funded by an NIH T32 grant.

Primary Subject Category: Infectious Disease

OCULAR TOXICITY, DISTRIBUTION, AND SHEDDING OF INTRAVITREAL GENE THERAPY FOR UVEITIS

Nichol Henderson, Veterinary student
Kimberly A. S. Young, Jacklyn H. Salmon, Matthew L. Hirsch, Lauren V. Schnabel, & Brian C. Gilger
nmhender@ncsu.edu
NCSU CVM

Non-infectious uveitis (NIU) is a painful, recurrent disease that affects ~25% of horses. Current treatments are non-specific and long-term use is accompanied by serious adverse effects. Long-term treatment is needed to prevent vision loss, so novel therapeutics with prolonged action and fewer adverse effects are desired. Adeno-associated virus (AAV) gene therapy was previously explored in rats to elicit the immune tolerance mechanisms of equine Interleukin 10 (eqIL10). This study aimed to evaluate ocular toxicity, distribution of viral transduction, and viral shedding following a single intravitreal (IVT) injection of AAV8-eqIL10 in normal horses. Each horse received an IVT injection in both eyes with either a balanced salt solution (n=1), a low dose of AAV8-eqIL10 ($3.75x10^{11}$ vg, n=2), or a high dose of AAV8-eqIL10 ($3.75x10^{12}$ vg, n=2). Ophthalmic examinations (OEs) were performed on days 0, 1, 3, 7, and weekly thereafter until euthanasia at approximately day 90 post IVT injection. Viral shedding patterns will be determined using blood, tear, urine, and fecal samples. Ocular histology will be performed for signs of toxicity and vector biodistribution on selected ocular tissues and organs will be examined. Serum antibody titers will be run on blood from days 0, 28, and 84. Results of OEs did not indicate a chronic inflammatory response in any treatment group. Shedding, distribution, and toxicity data are still undergoing analysis. While this data is required for a thorough safety assessment of AAV8-eqIL10 IVT injections, the low OE scores support this as a potential treatment for NIU.

Funding from North Carolina Biotechnology Center Translational Research Grant
Subject category: Clinical Medicine

INVESTIGATING HOW PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) SUPPRESS NEUTROPHIL FUNCTION

Emma M.W. Hepworth[1,2,3], Graduate Student

Co-authors: Ashley M. Connors[1,2,3], Drake W. Phelps[1,2], and Jeffrey A. Yoder[1,2,3,4]

ewhepwor@ncsu.edu, aconnor@ncsu.edu, phelpsd23@ecu.edu, jayoder@ncsu.edu

Affiliations: [1]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University; [2]Center for Environmental and Health Effects of PFAS, [3]Toxicology Program, [4]Center for Human Health and the Environment, North Carolina State University.

Abstract:

Per- and polyfluoroalkyl substances (PFAS) are widespread and persistent pollutants and can be detected in the serum of an overwhelming majority of people in the U.S. There is substantial evidence that PFAS alter immune function. Two particular PFAS, perfluorohexanoic acid (PFHxA) and ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX), have been found to suppress the neutrophil respiratory burst, a critical process in the innate immune response. However, it is not yet known which aspect of neutrophil biology the PFAS are disrupting. One possible explanation is that PFAS exposure is altering cellular metabolism, interfering with the ability of the neutrophils to produce the reactive oxygen species (ROS) necessary to perform the respiratory burst. Compared to some other innate immune cells, the characteristic metabolic profile of neutrophils is primarily glycolytic, in part because glycolysis supplies the cells with the burst of energy necessary to rapidly produce ROS. We are performing real-time cell metabolic analyses with an Agilent Seahorse Analyzer to investigate if PFAS-exposed neutrophil-like HL-60 cells have altered cellular metabolism. We will present preliminary findings from cells exposed to GenX for 24 or 96 hours that were then evaluated using an Induced Glycolytic Rate Assay, where basal glycolysis is measured prior to inducing the respiratory burst and resulting changes in glycolytic rate are measured. Uncovering PFAS-induced changes in the metabolism of neutrophils will improve our understanding of the mechanisms behind PFAS suppression of immune cell function and how this can contribute to increased susceptibility to infection.

Funding sources: NIH P42-ES031009, NIH T32-ES007046
Primary subject category: Immunology

EX VIVO VALIDATION OF A NOVEL EXTRACORPOREAL THERAPY DEVICE FOR REMOVAL OF CYTOKINES IN HORSES

Kallie Hobbs, DVM,MS, DACVIM -Graduate Student : kjhobbs@ncsu.edu

Megan Burke, DVM, DACVS :mjburke3@ncsu.edu

Yu Ueda, DVM, PhD, DACVECC : yueda@ncsu.edu

Katie Sheats-DVM, PhD, DACVIM : katiesheats@gmail.com

Funding: Cytosorbents and CVM Intramural grant

Introduction:

Plasma cytokine adsorption has shown benefit as an adjunctive therapy in human sepsis, but has yet to be investigated in equine sepsis. For this project, we hypothesized that *ex vivo* filtration with a novel cytokine adsorption device (VetResQ®) would significantly reduce 1. Equine cytokine concentrations in LPS stimulated equine plasma, and 2. Plasma concentrations of common medications administered *in vivo*.

Methods:

Four liters of heparinized whole blood was collected from healthy adult horses (n=7) and stimulated with LPS (100 ng/mL) for six hours (37°C.) Plasma was harvested through centrifugation and then filtered through a VetResQ® cytokine adsorption device using an extracorporeal blood pump circuit. Samples were collected at 10 timepoints for multiplex cytokine analysis (Millipore®). Chemistry analysis was performed before and after filtration. One healthy adult horse received potassium penicillin (22,000 IU/kg), gentamicin (6.6 mg/kg) and flunixin meglumine (1.1 mg/kg). Two liters of heparinized whole plasma was collected (30 minutes) and filtered through the VetResQ® cytokine adsorption device for 6 hours. Drug concentrations before and after filtration were determined by HPLC. Pre vs. post filtration sample concentrations were analyzed by Student's t test using Graphpad Prism 9.0 ($p<0.05$).

Results:

Filtration of LPS-stimulated equine plasma (n=5) for 6 hours resulted in significant mean reductions in IL-10, IL-5, IL-8, TNF-a, and IL-1β. Albumin and drug concentrations are significantly reduced by filtration.

Conclusions:

Ex vivo filtration with VetResQ® significantly reduced plasma concentrations of equine cytokines from LPS-stimulated whole blood. Additional research is underway to determine potential impacts of cytokine adsorption on other relevant patient parameters.

THE EFFECTS OF FSH AND P4 ON CANINE OOCYTE METABOLISM AND GENE EXPRESSION DURING IN VITRO MATURATION.

Jacob Howard (Veterinary Student)

Jose Len MVZ MS PhD

jmhowar5@ncsu.edu

jalenyin@ncsu.edu

NCSU CVM

In vitro maturation (IVM) of the canine oocyte is the foremost limiting factor of successful *in vitro* embryo production (IVEP) for the canine model. Poor comprehension of canine oocyte metabolism has contributed to low maturation rates *in vitro*. This project is investigating the effects of common hormones used during oocyte *in vitro* maturation on the metabolism and gene expression of the oocytes and cumulus cells (CCs). We hypothesize that supplementing the maturation medium with FSH, P4, or a combination of both hormones will increase the metabolism of the canine oocytes and stimulate the expression of different maturation related genes. Grade 1 cumulus-oocyte complexes (COCs) were selected from ovaries donated from a local rescue. COCs were randomly assigned into the following treatment groups: control, FSH, P4, and FSH/P4. COCs were incubated at 38.5°C in a 5% CO2 atmosphere. Metabolism was assessed using extracellular flux analysis at 24, 48, and 72 hours. After 72 hours, COCs were denuded and oocytes and CCs preserved for future gene expression using the nCounter system. The effect of hormonal supplementation on COCs metabolism and gene expression will be analyzed using a general linear model. Early results suggest that regardless of supplementation in the maturation medium, canine COCs metabolism increase during *in vitro* maturation The FSH only treatment group shows the most increased metabolic rate on average. More trials need to be performed and statistical analysis used before any true conclusions can be made about this information.

We would like to acknowledge the NC State University Equine Health Program and Fund for Discovery for their gracious funding. We would also like to acknowledge Saving Grace Animal Rescue and the North Carolina State University College of Veterinary Medicine for their continuing support.

Primary subject category: canine reproduction

POSTER PRESENTATION

ASSESSMENT OF CHARACTERISTIC 'HOT-SPOT' GENE MUTATIONS IN HUMAN SOFT TISSUE SARCOMAS ACROSS MULTIPLE VETERINARY SPECIES

Rebekah James – veterinary student

Isabella Livingston, Leigh Duke, Hannah Pierce, Gregory Lewbart, Tara Harrison, Matthew Breen

rjames2@ncsu.edu
mbreen3@ncsu.edu

Affiliations: NCSU CVM

Funding: Boehringer Ingelheim Veterinary Scholars Program, the Cancer Genomics Fund

Category: Genetics, Oncology

Soft tissue sarcomas (STS) are a collection of cancers affecting many veterinary species. The study of recurrent gene mutations in cancers is vital in understanding the drivers of disease, as well as the identification of therapeutic targets and prognostic indicators. In human STS, recurrent mutations have been identified in several genes, including *TP53*, *CDKN2A*, *PIK3CA, and PTEN*. The incidence of these mutations has led to them being termed 'hot-spot' events. While the mutational status of these genes has been investigated in some companion animals (primarily the domestic dog and cat) there is a lack of data for exotic pets and zoologic species. The Exotic Animal Medicine Service (EAMS) at NC State University hosts a collection of tumor biopsies from a range of species. In this study, histopathology confirmed cases of STS in multiple exotic species, including *Cebus sapajus* (Capuchin monkey), *Alces alces* (moose), *Daubentonia madagascariensis* (aye-aye), *Crotalus horridus* (timber rattlesnake), and *Oryctolagus cuniculus* (rabbit), were investigated for the presence of signature hot-spot mutations. Tumor and matched tissues were used as the source of genomic DNA. Known genome sequence data for each species (or the closest phylogenetic relative), accessible in the UCSC Genome Browser, were used to design custom PCR primers to amplify target regions of interest. PCR amplicons of both tumor and matched normal genomic DNA for each species were then sequenced and the paired data compared to identify the presence of somatic variants. Results (pending) indicated that 'hot-spot' mutations associated with human STS **were/were not** detected in the exotic species investigated.

ASSOCIATION BETWEEN CEREBROSPINAL FLUID BIOMARKERS OF ALZHEIMER'S DISEASE AND COGNITIVE PERFORMANCE IN VERVETS

Haley N. Johnson, veterinary student

Brett M. Frye, Thomas C. Register, Courtney L. Sutphen, Jacob D. Negrey, and Carol A. Shively

hnjohns2@ncsu.edu

Department of Pathology, Section on Comparative Medicine, Wake Forest School of Medicine, Winston-Salem, North Carolina.

Alzheimer's disease (AD) presents with distinct changes to cerebrospinal fluid (CSF) composition as well as cognitive decline. Determining the relationship between changes in CSF constituents and cognition can be difficult in clinical settings. Vervet monkeys (*Chlorocebus aethiops sabaeus)* are well-established models of early AD-like neuropathology, and thus provide opportunities to examine this relationship. Cognitive performance and CSF markers of AD were evaluated in a sample of nineteen known aged female vervets (ages: 10-29y, mean=20.1y), representative of middle-aged to elderly humans, living in large social groups at Wake Forest University. Our first goal was to determine the impact of age on two AD-related CSF biomarker ratios- phosphorylated tau to beta amyloid 1–42 (pTau181:$A\beta_{42}$) and $A\beta_{42}$ to $A\beta_{40}$ ($A\beta_{42}$:$A\beta_{40}$). We next examined the correlations between CSF biomarkers and cognitive performance in a maze test of executive function (Wake Forest Maze Test) and an assessment of working memory (Delayed Response Task), while controlling for the effects of age. We found that pTau181:$A\beta_{42}$ significantly increased with age ($p<0.05$), whereas $A\beta_{42}$:$A\beta_{40}$ was unrelated to age ($p>0.80$). Partial Pearson correlations did not reveal significant relationships between contemporaneous CSF biomarker values and measures of cognitive performance. These preliminary results indicate that vervets may recapitulate aging-related changes in CSF biomarkers that are relevant to AD. However, in this relatively small cross-sectional study we did not find evidence of an age-independent relationship between cognitive function and CSF Aβ and p-tau peptides. Longitudinal analyses are required to determine the temporal associations between these variables.

Research Grants: T35OD010946, P40OD010965, UL1TR001420, R24AG073199

NIH T35 Veterinary Student Summer Research Fellowship 2022

Primary Subject: Neurosciences

COMPANION ANIMAL ANTIMICROBIAL USE DATA FROM SMALL ANIMAL PRIVATE PRACTICES IN NORTH CAROLINA FROM 2019-2020

Ashlan Jolley: Veterinary/Graduate Student
William Love, Erin Frey, Cristina Lanzas
aljolley@ncsu.edu, wjlove@ncsu.edu, erin_frey@ncsu.edu, clanzas@ncsu.edu
NCSU CVM

Antimicrobial resistance (AMR) is recognized as a leading threat to public health worldwide. Understanding antimicrobial use (AMU) as a prominent driver of resistance development in microbial pathogens is critical to informing effective intervention strategies. The majority of AMU research in the United States has traditionally been focused on use in humans and food animals. However, with almost 140 million dogs and cats owned by Americans in 2018, companion animals represent a significant source of AMU that is often overlooked. In collaboration with IDEXX Laboratories, Inc., North Carolina State University (NCSU) acquired companion animal AMU data from 390 private small animal practices in North Carolina (NC). 819,180 AMU records for oral (53%), injectable (13%), and topical (26%) agents were extracted from the practice Patient Information Management Systems (PIMS) from 2019-2020. All formulations–including those with an unclassified route of administration (9%)–were combined for analysis. Repeated entries representing multiple doses within an individual course were consolidated into single records. Fluoroquinolones (16%), aminoglycosides (14%), penicillin combination products (13%), nitroimidazoles (9%), and third-generation cephalosporins (9%) were the most common drug classes prescribed overall. Of the 651,335 canine records, the most used drugs were oral amoxicillin-clavulanate (11%), metronidazole (9%), cephalexin (7%), cefpodoxime (5%), and topical gentamicin (7%). In the 167,845 feline records, oral amoxicillin-clavulanate (15%), metronidazole (6%), amoxicillin (6%), injectable cefovecin (13%), and topical tobramycin (4%) were the most frequently used. These results provide an introductory look into companion animal AMU across North Carolina.

Funding Sources: US Food and Drug Administration (#FDA-U01FD007057)

Pharmacology

ROLE OF MAST CELLS IN ITCH AND INFLAMMATION IN A MOUSE MODEL OF ATOPIC DERMATITIS

Dani Joseph
graduate student
Dr. Santosh Mishra
djoseph2@ncsu.edu
Functional Genomics Graduate Program, Department of Molecular Biomedical Sciences, NCSU CVM.

Atopic dermatitis (AD) is an allergic skin disease characterized by dehydrated, scaly, thickened skin and severe pruritus (or itch) with limited effective drug options. Although an increase in mast cells (MC) has been shown in the early to late stages of AD development, a critical effector immune cell in AD, which secretes cytokines/proteins involved in inflammation and itch in human and preclinical models. However, this key immune cell relevance in AD-associated symptoms remains elusive. The work explores a possible functional connection between MC number and AD symptoms such as itch and skin thickness (direct measure of inflammation) in a preclinical model. Here, we used an MC903-induced mouse model of AD and analyzed skin tissues for histochemical analysis to characterize its correlation with AD-associated symptoms such as itch and inflammation. We found a significant increase in MC numbers during the AD progression. There is a linear increase in MC number which is correlated with skin thickness and pruritus in AD. The Spearman ranked correlation coefficient between MC, and number and itch behavior MC number and skin thickness are significant. These results indicate that the MC generates and maintains AD-related pruritus and skin inflammation in mouse model of AD. Future work will be essential to identify mechanisms that regulate MC in AD to help develop potential therapeutics.

NCSU, CMI, NIH R01 AR079713

Neuroscience, Immunology, itch

CLINICAL EVALUATION OF 3-SITE VERSUS 4-SITE DISTAL PARAVERTEBRAL BLOCKS IN STEERS UNDERGOING STANDING ABDOMINAL LAPAROTOMY

Maya K. Keefer, Veterinary Student
Margaret A. Mooring, Jennifer Halleran, Derek Foster, Kelley M. Varner

Affiliations: North Carolina State University College of Veterinary Medicine

Standing laparotomies are routine veterinary interventions in bovine practice in the USA. They are commonly performed for abdominal exploratory surgeries, correction of displaced abomasums, and cesarean-sections. It is estimated that over 500,000 cattle will undergo standing laparotomy for one of these conditions in the US this year.
Distal paravertebral blocks are one of the described techniques for generating paralumbar fossa anesthesia in cattle. This block aims to desensitize T13, L1, and L2 spinal nerves as the nerve roots cross L1, L2, and L4 transverse processes. Some practitioners report improved perceived efficacy when distal paravertebral (DPV) blocks are also performed at the L3 process. Our study aimed to discover if four injection sites resulted in improved block success, surgical condition scores, and improved postoperative pain scores in steers undergoing standing laparotomy when compared to the traditional three-site injection technique. We hypothesized that four-site distal paravertebral would result in improved surgical conditions and lower pain scores following laparotomy.
Two groups of 6 steers (n=12) were randomly assigned to one of two treatment groups (3DPV and 4DPV) and a blinded researcher was provided with the test solution in labeled syringes that corresponded with injection sites. The 4DPV group had 2 mg/kg 2% lidocaine injected at each site. In the 3DPV group, an equivolume of 0.9% NaCl was administered at the L3 process. Physiologic data, reaction scores, and surgical scores were collected at predefined surgical events. Post-operative pain scores and physiologic data were collected at predetermined time points for 24 hours following surgery.

Funding Source: We thank FARAD for their support of this research.
Subject category: Clinical Medicine

TITLE: METAGENOMICS APPLICATIONS OF VECTOR BORNE DISEASES IN SMALL ANIMALS

Author name: **Praveen Kumar Korla (Post-Doc)**

Co-authors: Henry Marr, Michael Karounos and **Adam Birkenheuer***

Email: pkkorla@ncsu.edu, ajbirken@ncsu.edu

Department of clinical sciences. CVM, NC State University

Abstract

Background

Identification of pathogens in clinical samples remains challenging. Additionally, few genome sequences of veterinarian pathogens have been characterized. This absence of genome sequence information hinders our ability to design pathogen specific assays. Metagenomics determines the genome sequences of multiple organisms from a single sample. We hypothesized that a metagenomics approach could identify and reconstruct genomes of the pathogens from clinical samples.

Materials and methods

We extracted total DNA from four whole blood samples from animals with known infections (200ul without using any special preparation methods). These samples had a host to pathogen DNA ratios ranging from 1.56 to 13,473. We submitted extracted DNA for Next generation sequencing (Illumina Miseq). The resulting sequences were run through three pipelines to identify and assemble pathogens sequences. Assembles contigs were compared to reference genomes and GenBank nr database.

Results

Metagenomic approaches successfully identified partial genome sequences from all four clinical samples. The percentage of reads that mapped to pathogen sequences ranged 48.49% to 0.09%. The assembled pathogen sequences had genome coverages ranging from 99.9% to 5.7%.

Conclusion: A metagenomic approach can be used to both identify the presence of pathogens and can recover near complete genome sequences from samples with high parasitemia.

Primary subject category for presentation: Infectious diseases

PREDICTING OF SKIN FLAP PERFUSION AND VIABILITY USING INDOCYANINE GREEN (ICG) IN RATS

Dr. Karen Park (Assistant Professor, DACVS), Caroline Kornegay (veterinary student)
kmpark2@ncsu.edu, cfkorneg@ncsu.edu
North Carolina State University College of Veterinary Medicine
Clinical Medicine

Skin flaps are commonly used in veterinary medicine to cover large skin defects for wound closure or extensive tumor resection. Post-operative complications with skin flaps have been reported to be as high as 89% in dogs and cats (Aper et al, 2003; Field et al, 2015). Necrosis of the skin flap has been described as a common complication and is associated with subsequent failure of the flap with skin loss. Near-infrared light fluorescence (NIRF) using indocyanine green (ICG) is an emerging technique that can allow for intra-operative assessment of the vascularity of the skin flap and has potential to predict complications and decrease patient morbidity. Our goals are to evaluate the use of NIRF/ICG on a skin flap using a rat model at multiple points during surgery and post-operatively to define skin flap perfusion based on the visualized degree of fluorescence compared to the gross appearance of the skin flap. Rats will be placed under general anesthesia for the procedure and will be divided into two different groups. ICG/NIRF will be used to assess and create a skin flap over the dorsum of the rat. All flaps will be imaged daily for 10 days following the procedure to assess flap viability and correlate the degree of ICG/NIRF fluorescence with the gross appearance of the skin. Data collected from this study will be used to aid in intra-operative skin flap creation and contribute to identifying the viability of the skin flap to help minimize post-operative complications and subsequent patient morbidity.

MODULATION OF CFTR FUNCTION USING AN IN VITRO MODEL OF EQUINE RECTAL EPITHELIUM

Ashley Kropf, undergraduate
Lara Madding, Breanna Sheahan
aakropf@ncsu.edu, lmaddin@ncsu.edu, bjsheaha@ncsu.edu
College of Agriculture and Life Sciences, NC State University; NCSU CVM

Diarrhea is a frequent occurrence in equine colitis patients, and can result in severe electrolyte imbalance, dehydration, and death. Current treatments include replacement of ongoing fluid losses, but no therapeutics directly target the underlying mechanism of secretory diarrhea. The cystic fibrosis transmembrane conductance regulator (CFTR) channel is an anion channel found on the apical membrane of epithelial cells, where it secretes chloride and subsequently water into the lumen of the intestine. Excess activation of CFTR results in secretory diarrhea. Therefore, transient inhibition of CFTR is a promising anti-diarrheal therapy for equine colitis. We hypothesized that equine rectal organoids grown from rectal biopsies can serve as an in vitro model of CFTR modulation. Rectal biopsies from healthy horses with no evidence of gastrointestinal disease (n=7), and post-mortem rectal biopsies from horses euthanized due to severe diarrhea were obtained (n=3). Biopsies were used to culture 3D intestinal organoids. Equine rectal organoid CFTR function was modulated with CFTR activators and inhibitors. Organoid swelling was quantified as a proxy of CFTR responsiveness. Forskolin (Fsk) was used to activate the CFTR channel after treatment with inhibitors proven effective in human models such as CFTRinh-172, GlyH-101, and (R)-BPO-27. In equine rectal organoids, CFTRinh-172 was not effective at inhibiting CFTR function. GlyH-101 caused significant cytotoxicity. (R)-BPO-27 was effective at inhibiting CFTR function at multiple concentrations without cytotoxicity. Further studies will focus on the role of CFTR in clinical cases and (R)BPO-27's efficacy in limiting equine secretory diarrhea.

CVM Intramural Grant 2022-2023 (Sheahan B), Summer Undergraduate Research Award (Kropf A)
Gastroenterology

GENETICS AND GENOMICS AID IN IDENTIFICATION OF PARASITES FROM NON-NATIVE SPECIES IN AN ENDANGERED GALAPAGOS PINNIPED

Isabella Livingston[1,7], Graduate Student

Taylor M. Gregory[2,3], Eleanor C. Hawkins[2], Andrea Loyola[4], Ashley Cave[2], Shelly L. Vaden[2], Diane Deresienski[2,5], Matthew Breen[7], Marjorie Riofrio-Lazo[5,6], Greg A. Lewbart[2,5], Diego Paez-Rosas[5,6]
Email: igliving@ncsu.edu

[1]Genetics and Genomics Graduate Program, North Carolina State University, Raleigh, North Carolina 27607, USA; [2]Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina 27607, USA; [3]Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, Texas 76110, USA; [4]Dirección Parque Nacional Galápagos, Departamento de Ecosistemas, Isla Santa Cruz EC200350, Islas Galápagos, Ecuador; [5]Galapagos Science Center, USFQ & UNC-Chapel Hill, Av. Alsacio Northia, Isla San Cristóbal EC200150, Islas Galápagos, Ecuador; [6]Universidad San Francisco de Quito (USFQ), Colegio de Ciencias Biológicas y Ambientales, Isla San Cristóbal EC200150, Islas Galápagos, Ecuador; [7]Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina 27607, USA.

Subject: Genetics/Infectious Disease

Darwin's discovery of the Galapagos revolutionized our understanding of the natural world. Since then, the Galapagos have remained one of the most preserved natural habitats. Despite this, the archipelago faces growing threats as a result of anthropogenic impacts, including introduced species. These species, including companion animals like dogs, are a primary cause of biodiversity loss through competition, predation, and infectious disease. Companion animals roam freely on occupied islands and have unrestricted interaction with wildlife. Historically, introductions of new species to the archipelago has been limited due to its geographic isolation, so native life cannot adapt to new pressures and possess an increased vulnerability to disturbances. Further, they likely evolved without many pathogen exposures; thus, interaction with introduced species can cause disease spillover, a catastrophic threat to the archipelago. In May of 2022, I, along with members of NCSU's CVM, traveled to the Galapagos to conduct health assessments for a characteristic species, the Galapagos sea lion. We performed health assessments and collected samples from 28 sea lions. We tested blood from each with a commercial heartworm test kit on site and documented the presence of canine heartworm in this species for the first time. We validated this finding with genomic assays for identifying filarial nematode DNA in sea lion whole blood. With our assays, we are exploring the prevalence of parasites in these pinnipeds. This study will provide context for the conservation of a Galapagos pinniped and innovative measures needed to mitigate the detrimental impacts of introduced species on native wildlife.

DIFFERENTIAL GENE EXPRESSION IN A SWINE MODEL FOR EOSINOPHILIC ESOPHAGITIS

Lizette M. Lorenz[1] postdoc- Research Assistant Professor
David Brodsky[1], Anthony Blikslager[1], Evan Dellon[2], Scott Laster[3], Celine Chen[4], Harry Dowson[4], Tobias Kaeser[1,5]
lmlorenz@ncsu.edu, dmbrodsk@ncsu.edu, evan_dellon@med.unc.edu, atbliksl@ncsu.edu, smlaster@ncsu.edu, celine.chen@usda.gov, harry.dawson@usda.gov, Tobias.Kaeser@vetmeduni.ac.at

[1] NCSU CVM, [2] UNC Chapel Hill, [3] NCSU Biological Sciences, [4] USDA, [5] Vetmeduni Vienna

Abstract: Food allergy (FA) is an increasing worldwide health problem that affects children and adults. The US spends around $25B in direct health cost and despite the extensive research efforts, there is still no cure. FA in some cases can become a chronic immune mediated inflammatory condition which can be seen in the case of eosinophilic esophagitis (EoE) where the reaction to foods causes severe inflammation in the esophagus. None of the available treatments provide full remission. Therefore, it is urgent to investigate the causes and mechanisms that lead to EoE and FA in general. In our lab we have established the pig as a large animal model to study EoE. The pig combines high biological relevance based on their similarities to humans with a well-equipped immunological toolbox to study relevant disease-related immune responses. We have used Hen Egg White Protein (HEWP) along with an adjuvant to induce EoE in our pigs. In our preliminary animal trials, we have observed the onset of inflammation in the esophagus and clinical symptoms. The derived RNAseq results from these trials show that there is a clear upregulation of esophageal immune expression of certain RNAs in our EoE pigs vs. controls. We found a differential expression of C3, CXCL8, RARRES1, CXCL6, MUC5AC, CXCL1, and SAA3 genes. We believe these molecules play a significant role in the onset and pathogenesis of EoE. In future studies we plan to corroborate the expression of these genes with platforms for Spatial Transcriptomics and immunofluorescence.

Funding for this project was provided by the CMI and NIH R21 grants.
Subject category: Immunology, Gastroenterology

CORNEAL LESIONS: COMPARING FEATURES SEEN ON ULTRASOUND BIOMICROSCOPY AND HISTOPATHOLOGY

Vienna Lunking – Veterinary Student
Danielle Meritet, Brian Gilger, Annie Oh
vmlunking@ncsu.edu
Affiliation: NCSU CVM

Ultrasound biomicroscopy (UBM) uses high frequency ultrasound to conduct detailed non-invasive imaging of the cornea and anterior segment. Ophthalmic pathologies affect the echogenicity and appearance of the scan, though the precise changes are unknown. Therefore, the purpose of this study was to compare the characteristics of corneal and anterior segment lesions on UBM to histopathology. We hypothesized that pathological processes which add to cellular and structural composition, such as white blood cells or fibrin, result in hyperechogenicity, while fluids relatively low in cells, like aqueous humor, appear dark. Enucleated eyes from 18 canine and equine patients with various forms of corneal pathology were included in the study, along with two controls. Longitudinal UBM scans in line with the observed lesion were taken dorsally, axially, and ventrally; a histopathology slide of the same cross-section was prepared. Images were compared. Our results supported our hypothesis, with inflammatory processes causing increased hyperechogenicity seen in all affected layers of the cornea. Neovascularization could often be identified as hyperechoic areas within the stroma dotted with hypoechoic vessel lumens. Corneal hemorrhage and hyphema presented with increased brightness, likely due to the mix of inflammatory cells also present. While areas of pathology were identifiable on UBM, different types of cellular infiltrates were not distinguishable, and borders between separate disease features seen on histopathology were not necessarily apparent on UBM if abutted tissues shared similar reflectivity. When used in conjunction with a thorough ophthalmic exam, UBM is a clinically relevant tool for localizing and identifying corneal pathologies.

Funding sources: NIH T-35 Grant
Primary subject category: Clinical Medicine

EVALUATION AND TIME AND COST SAVINGS ANALYSIS OF EXTENDED SANITATION INTERVAL OF CAGING AND ACCESSORIES AND AUTOMATED WATERING VALVES

Bryanna K Meredith (veterinary student)
Bridget M Clancy, Allison M Ostdiek, George P Langan, Kerith R Luchins
bkmeredi@ncsu.edu
University of Chicago: Animal Resources Center, NCSU CVM

Although the Guide suggests changing rodent cage accessories every 2 weeks, it states that “decreased sanitation frequency may be justified if the microenvironment in the cages…is not compromised”. Increased use of rodent individually ventilated cages (IVCs) has led to investigation of extended cage sanitation intervals. The purpose of this study was to evaluate extended sanitation intervals of cage accessories (automated watering valve, wire bar lid, and filter top) of mouse IVCs at the University of Chicago. We hypothesized that there would be no significant difference in relative light units (RLUs) measured by ATP luminometry of cage accessories at control time 14 days compared to each time interval: 28 days, 56 days, and 84 days. We extended the study for automated watering valves to 168 days. We also hypothesized that a time-and-motion study assessing a sanitation interval of 84 days for all components would result in significant time and cost savings. A total of 24 cages containing 4 or 5 mice each were used for swabbing of cage accessories. There were no significant differences ($P > 0.05$) between 14 days and each other time point for all cage accessories. Additionally, the time and cost savings analysis found that extending the sanitation interval of cage accessories to once every 86 days for a mouse census of ~22,000 cages would save ~7,000 technician hours annually for a total labor cost savings of ~$240,000. Extended cage change of rodent accessories is a feasible alternative that decreases workload of animal care staff without compromising sanitation.

Funding Source: ASLAP Foundation
Category: Other

PHENOTYPIC RESISTANCE OF ENTEROCOCCI OBTAINED FROM CATTLE FECES TO ERYTHROMYCIN AND VANCOMYCIN AFTER IN-FEED ADMINISTRATION OF TYLOSIN

Ivonne M. Miranda Martinez, Veterinary Student

Julio A. Mojica Perez, Madelyn Schwartz, Laura Neumann, Derek Foster, and Jennifer L. Halleran

immirand@ncsu.edu, jamojica@ncsu.edu, mschwar6@ncsu.edu, lmneuman@ncsu.edu, dmfoster@ncsu.edu, jlhaller@ncsu.edu

North Carolina State University, College of Veterinary Medicine.

Tylosin is an oral antibiotic administered during the finishing period in steers to prevent liver abscesses. Subtherapeutic drug concentrations of tylosin in the gut may exert selective pressure on cattle microflora, promoting resistance to antibiotics of critical importance in human medicine. Our objective is to characterize phenotypic resistance of enterococci obtained from cattle feces against erythromycin and vancomycin after in-feed tylosin administration. We hypothesize that resistance to the antibiotics being tested will increase after tylosin administration but rebound back to baseline at the end of the study period. For this study, 6 steers were dosed once daily for three days with in-feed tylosin, and fecal samples were taken daily for a period of 7 days. Feces were serially diluted and plated on Nutrient agar (positive control), m-Enterococcus agar, and m-Enterococcus agar infused with erythromycin (8µg/ml) and vancomycin (32µg/ml), with antibiotic concentrations in the agar being the human resistance breakpoint. Plates were incubated at 37°C for 48 hours. Colony growth was observed and quantified. We expect that the prevalence of erythromycin- and vancomycin-resistant enterococci will increase after administration of tylosin and will return to baseline at the end of the study. Oral administration of tylosin may promote cross-selection of resistant bacteria, leading to macrolide and glycopeptide inefficacy against enterococci and other infectious bacterial species. Although antimicrobial resistance (AMR) can be obtained from multiple sources, establishing an appropriate dosing regimen for tylosin therapy may help reduce the number of resistant bacteria shed into the environment and subsequently, reduce the public health risk.

Funding: Start up-FARAD, Fund for Discovery, NIH T35OD011070 Interdisciplinary Biomedical Research Training Program
Category: Infectious Disease

NOVEL SAMPLING TECHNIQUES FOR ORAL PHARMACOKINETICS OF TYLOSIN

Julio A. Mojica Pérez, veterinary student

Ivonne Miranda Martínez, Madelyn Schwartz, Laura Neumann, Danielle Mzyk, Linda Dillenbeck, Derek Foster, Jennifer Halleran

jamojica@ncsu.edu, immirand@ncsu.edu, mschwar6@ncsu.edu, lmneuman@ncsu.edu dalindqu@ncsu.edu, lmsjalan@ncsu.edu, dmfoster@ncsu.edu, jlhaller@ncsu.edu,

North Carolina State University, College of Veterinary Medicine

Tylosin is an in-feed antimicrobial used to reduce the incidence of liver abscesses in beef cattle. There are no studies investigating oral tylosin pharmacokinetics. This study aims to understand the pharmacokinetics of orally administered tylosin at the FDA approved labelled dose. While there are reports documenting whole liver tissue residues of tylosin, these are terminal studies and may not reflect accurate tissue concentrations. Collecting portal blood after gastrointestinal absorption may be as close to the site of action to assess accurate tylosin concentrations. Our goal was to test a novel technique for a more accurate drug concentration determination, while not sacrificing the animals. We hypothesize that tylosin concentration will be greater in the portal vein when compared to the peripheral circulation. The animals used were 6-7-month-old crossbred steers. To find a significant difference in drug concentrations between sample sites, a statistical power calculation determined the sample size of 6 steers. Under sedation and with the use of a local block, the portal vein was catheterized utilizing ultrasound as guidance. Steers were administered tylosin orally daily for three days in their feed. Portal and jugular vein blood samples were collected at periodic intervals to measure and compare tylosin concentration via high-performance liquid chromatography analysis. The portal vein catheterization method was successful in 100% of the steers and remained functional for at least 7 days after placement. The described technique has significant potential for future scientific investigations for determining medication concentrations in the liver.

Funding: Start-up/FARAD

NC State University Herbert Benjamin Endowment

Category: Pharmacology

VALIDATING GENE TARGETS FOR CANINE PERIPHERAL T-CELL LYMPHOMA IMMUNOTHERAPY

Megan Mulder, Veterinary Student
Jennifer Holmes and Paul Hess
mkmulder@ncsu.edu, jcholmes@ncsu.edu, prhess@ncsu.edu

North Carolina State University - College of Veterinary Medicine

Canine peripheral T-cell lymphoma (PTCL) is a deadly cancer that responds poorly to chemotherapy alone. Vaccine-based immunotherapy may help eliminate residual chemoresistant cells, improving survival. A vaccine's most critical component is an antigen, a protein that T-cells must "see" for target cell recognition. Valuable cancer vaccine antigens must be 1) present in multiple patients' cancers; 2) absent in normal tissues (no autoimmunity); and 3) absent in thymic epithelial cells (TECs) to avoid deletion of responding T-cells, nullifying vaccine efficacy. PTCL turns on genes called cancer-testis antigens (CTA) which are present in germ cells but silenced in somatic tissue. Using RNAseq and qPCR, we identified five CTAs that fulfill the first two criteria of a valuable cancer vaccine antigen. Our goal was to determine which of those also met the third. Using endpoint RT-PCR, we performed low-resolution screening in bulk prenatal thymus (0.5% TECs, 99.5% thymocytes). For negative CTAs, re-screening at higher resolution (enriched TECs) would be needed, but TECs have never been isolated in dogs. We hypothesized that canine TECs could be enriched by adapting a murine "panning" method, increasing the probative power of endpoint PCR in meeting the last criterion. All five CTAs were absent in bulk prenatal thymus cDNA and need higher resolution TEC-level screening. Because thymus is scarce, surrogates for TECs and thymocytes were used in panning development. Our panning successfully depleted 83% of "thymocytes" from the model single-cell population, suggesting the potential for significant enrichment of TECs in thymic cell suspensions.

Student funding: National Institutes of Health T35OD011070 Interdisciplinary Biomedical Research Training Program
Additional funding: The Shadow Whatley Research Fund; Fund for Discovery

Category: Oncology

ANTIBIOTIC CONCENTRATION IN THE PERITONEAL FLUID OF CATTLE

Laura C. Neira Torres, veterinary student

Laura Neuman, Danielle Myzk, Jennifer Halleran, Timo Prange, and Derek Foster

lcneirat@ncsu.edu , dalindqu@ncsu.edu, jlhaller@ncsu.edu, lmneuman@ncsu.edu,

tprange@ncsu.edu, and dmfoster@ncsu.edu

North Carolina State University, College of Veterinary College

Intraperitoneal administration of antibiotics is commonly used in bovine surgery to prevent postoperative complications. Though ampicillin is labeled for intramuscular injection, administering this antibiotic intraperitoneal is common in bovine surgery. The objective of this study is to compare abdominal drug concentrations when ampicillin is administered through intraperitoneal or intramuscular route. Our hypothesis is that administering ampicillin intramuscularly will better achieve pharmacokinetic/ pharmacodynamic targets than intraperitoneal administration. 12 six month old crossbred dairy steers had standing surgery performed. Prior to closure of the abdominal wall, they were randomly allocated to receive 11 mg/kg of ampicillin intraperitoneally or intramuscularly. At surgery, ultrafiltration probes were placed in colon, ileum, peritoneal cavity, and incision. Ultrafiltrate and blood samples were collected for 48 hours. Seven days after the surgery, the steers were euthanized, and tissues were collected to assess drug residues. All the probes placed collected samples 75% of the time during the study. The location with the highest amount of success rate was the ileum with 80% of the samples collected. The locations with fewest samples collected were the peritoneum and colon with 70% success rate. Analysis of drug concentrations is pending. During the study, the animal behavior affected the duration of peritoneal probe sampling. Most of the ultrafiltration probes were functional during the entirety of the study making this method successful for assessment of peritoneal drug concentrations.

Funding: FARAD / USDA-NIFA/ NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Category: Pharmacology

CHARACTERIZING ENTEROENDOCRINE CELLS IN HORSES WITH PITUITARY PARS INTERMEDIA DYSFUNCTION

Mary O'Neill (mkoneill@ncsu.edu; NCSU CALS)
Dr. Breanna Sheahan (bjsheaha@ncsu.edu; NCSU CVM)

Pituitary Pars Intermedia Dysfunction (PPID) is a progressive equine disease of the pituitary gland and is the most common endocrine disease of geriatric horses. PPID is associated with hyperinsulinemia which may drive the development of laminitis, a painful and life-threatening disease. Insulin secretion after a meal is augmented by incretins, which are produced by enteroendocrine cells (EECs) in the intestine. Therefore, EECs could be a therapeutic target to reduce laminitis risk in PPID-affected horses. Our objective was to characterize EECs and incretin expression in intestinal tissues from young horses, PPID+ aged horses, and PPID- aged horses.We collected jejunal and rectal tissue from horses presented to NC State Equine Hospital after euthanasia with client consent. Rectal biopsies were also obtained from healthy horses in the NC State Research Herd. PPID status was determined by ACTH concentration or visual assessment. Jejunal and rectal EEC number was determined by Chromogranin A immunofluorescence. Gene expression was performed on rectal epithelium for the following: *beta actin*, *chromogranin A* (EECs), and *glucagon* (propeptide of GLP-1, an incretin). There was no difference in the number of EECs. There was more variation in the number of EECs with increasing age but no specific trend was identified. Rectal *chromogranin A* mRNA expression was not different between groups. However, rectal *glucagon* mRNA expression was significantly lower in PPID+ horses compared to young horses or age-matched PPID- horses. These results suggest that while the number of EECs present may not be different in PPID+ horses, their composition (incretins) may differ.

Funding: CVM Intramural Grant 2022-2023 (Breanna Sheahan); Office of Undergraduate Research Award (Mary O'Neill)
Primary Subject Category: Gastroenterology

DEVELOPMENT OF AN IN VITRO MODEL OF GLUCOSE STIMULATED GIP SECRETION IN EQUINE DUODENAL ORGANOIDS

Jillian Petersen: Veterinary Student
Lara Madding, Breanna Sheahan
jmpeter7@ncsu.edu, lmaddin@ncsu.edu, bjsheaha@ncsu.edu
NCSU CVM, Raleigh, NC

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin secreted by duodenal epithelium that regulates insulin secretion and inhibits gastrointestinal motility. Thus, GIP dynamics may be relevant to horses with equine metabolic syndrome and/or postoperative colic horses suffering from ileus. Mechanisms of GIP secretion can be interrogated by comparing glucose-stimulated in vivo secretion to in vitro secretion. The purpose of this study was to develop a platform for assessment of GIP secretion in equine duodenal organoids. An oral sugar test (OST) was performed on healthy horses (n=11) to determine in vivo GIP secretion. Plasma [GIP] and serum [insulin] were measured via ELISA. Plasma [GIP] increased after the oral sugar test. Plasma [GIP] was highest in horses that were insulin resistant (IR). This suggests that GIP may influence insulin concentrations in IR horses. To study in vitro secretion, endoscopically-guided duodenal biopsies were obtained from the same horses after the OST to produce duodenal organoids. Organoids were incubated in control, glucose, or maximally-stimulated solutions with a DPP4 inhibitor for 2 hours to stimulate GIP secretion. Organoid secretion [GIP] was at or below the detection limit of the assay. It is unknown whether the lack of GIP present in the organoid secretion is due to buffer dilution, low cell concentration in the cultures, or some other factor. Optimization is ongoing to enhance GIP secretion. Once optimized, future studies will compare GIP secretion in vivo and in vitro between patient populations of interest (post-operative colics or horses with equine metabolic syndrome) and healthy horses.

Funding Sources:
NIH T35 Interdisciplinary Biomedical Research Training Program (Petersen)
Dr. Sheahan's start up funds
NCSU CVM Intramural grant 2022 (Sheahan)
Fund for Discovery

Subject Category: Gastroenterology

INVESTIGATION OF NEOPLASIA IN UNGULATES

Hannah Pierce, Undergraduate
Leigh Duke, Tara Harrison
hkpierce@ncsu.edu
NCSU CVM

Many species of ungulates are affected by neoplasia. An investigation was done to evaluate the types of neoplasia most commonly found in ungulates. Cases of histologically confirmed neoplasia from published literature and records from the Exotic Species Cancer Research Alliance's (ESCRA) database (with data from zoos, museums, and institutions across the globe) were compared across age, sex, species, diagnosis, tumor behavior, treatment, and survival. There were 131 cases found in published literature, and 164 cases in the ESCRA database for a total of 295 animals included in this study. Results from both the literature and the database indicated that giraffe (21% and 5% of cases respectively) and rhinoceros (24% and 10% of cases respectively) were the most commonly affected by neoplasia. Zebra were also found to be commonly affected by neoplasia in the ESCRA database (44% of cases). Both the literature and database found that sarcoids and squamous cell carcinomas were the two most common neoplasias. Continued comparison of this data will improve evaluation of risk factors for specific species as well as improve health and treatments of ungulates.

Funded by the NIH NCI Division of Cancer Biology
Subject Category: Oncology

A NOVEL APPROACH USING SIDE POPULATION ANALYSIS TO IDENTIFY INTESTINAL STEM CELLS IN WILD-TYPE PIGS

Lydia Poisson: Combined Degree Student
Caroline McKinney-Aguirre, John M. Freund, and Liara M. Gonzalez

lpoisso@ncsu.edu, camckinn@ncsu.edu, jmfreun2@ncsu.edu, lmgonza4@ncsu.edu
Department of Clinical Sciences, NCSU CVM, Raleigh, NC

Severe intestinal ischemia is an emergency diagnosis in human and veterinary medicine, with mortality rates reaching 80%. Improved animal models are needed to study the reparative processes driven by intestinal stem cells (ISCs). Pigs closely approximate human anatomy and physiology, but methods to study porcine ISCs (pISCs) are limited: Most require fixation, precluding *in vitro* work, or use of a single-available transgenic model. Thus, we sought to implement an alternative method for identifying pISCs. Side population (SP) analysis has proven effective in identifying mouse and human ISCs via flow cytometry (FCM). SP functions by using cell-permeable dye, DyeCycle Violet (DCV), that is pumped out of cycling cells, such as ISCs, via ABC transporters. In contrast, non-cycling cells (*e.g.*, epithelial cells) retain DCV, thereby fluorescing as a separate population upon FCM analysis. Verapamil, a Ca^{2+} channel blocker, is used as a control to inhibit the ABC transporters' efflux of DCV. Cell surface markers CD45 (leukocytes) and CD166 (goblet, Paneth cells) are used to assess SP heterogeneity. We employed chemical and mechanical dissociation to produce a single-cell suspension containing a SP upon FCM analysis. Consistent with cycling cells, verapamil incubation prevented this SP. Subsequent analyses will include CD45 and CD166. Future directions will implement FACS for downstream analyses, including 3D ISC culture and novel surface marker identification. These data represent the first description of SP in a porcine intestinal model. The ability to isolate pISCs will broaden our understanding of these cells to potentially leverage their regenerative capacity to develop novel therapies.

Research Funding: U.S. Department of Defense PR181265

Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Primary Subject Category: Cell Biology

Title: CONTAMINATION LEVELS IN DIFFERING LENGTH DNA FRAGMENTS IN ILLUMINA SEQUENCING

Author: **Jorden Rabasco,** graduate student

Co-authors: Teresa Tiedge, Benjamin Callahan

Email: jrabasc@ncsu.edu

Affiliations: NCSU CVM, PreMiEr

Currently low-biomass microbial communities can not be accurately measured in a consistent manner by microbiome analysis techniques. These techniques were established to measure high-biomass environments and when applied to low-biomass environments can lead to rampant contamination. The true scope of the issue comes into focus when considering how many low-biomass microbial environments there are near humans on a day-to-day basis, particularly those in the built environment. These areas, while typically low in biomass, can influence human health and therefore there is a concerted effort to discover novel methodologies designed specifically for low-biomass environments. To this end, this research seeks to investigate the effect of different length DNA template on contamination levels in Illumina based sequencing. In this study, three differing length DNA insert fragments will be generated from serially diluted, known stock solutions. The fragments will then be used as template in Illumina based sequencing and the resulting reads will be resolved to ASVs and taxonomically classified via the DADA2 pipeline. The percentage of contaminant ASVs per sample will be calculated based on the expected taxa from the original known sample. This presentation will feature the results of a pilot study conducted to assess the viability of the experimental design and ascertain an initial insight into whether choice of DNA template length can modulate the level of contamination in Illumina based sequencing.

Funding: PreMiEr

Category: Other (Microbiome)

EQUINE PLACENTAL-DERIVED EXTRACT ENHANCES THE REGENERATIVE CAPACITY OF EQUINE INTESTINAL EPITHELIAL CELLS

Mara P. Ramos Cabrera, Veterinary Student

Caroline McKinney-Aguirre, Liara Gonzalez

mpramosc@ncsu.edu, camckinn@ncsu.edu, lmgonza4@ncsu.edu

NCSU CVM

Colic is the most common presenting complaint and the leading cause of death in horses aside from old age. Severe manifestations of colic cause intestinal hypoxia resulting in neutrophilic influx, endotoxemia, and epithelial cell injury. Despite the substantial danger of colic-induced hypoxic intestinal injury, therapeutic options are limited. Placental-derived therapeutics have been demonstrated to improve intestinal health by supporting the stem cell niche in mouse epithelial disease. We aimed to determine if equine acellular placental-derived extract (PE) would have similar therapeutic effects on epithelial cell proliferation and barrier repair after intestinal injury. We hypothesized that PE would promote intestinal epithelial cell repair in an equine in vitro injury model. To test this, monolayers were derived from banked jejunal enteroids obtained from full-thickness biopsies. Once confluent, monolayers were subjected to 4 hours of 1% hypoxia followed by a scratch assay. Experimental wells were treated with three doses of PE (0.05mg/mL, 0.075mg/mL, 0.15mg/mL) or bovine serum albumin as control (BSA, 0.075mg/mL). To assess the effect of PE on epithelial healing, time to defect closure, changes in transepithelial electrical resistance (TEER) after closure, and changes in proliferative cell biomarker expression were evaluated. Preliminary results show that PE significantly accelerates epithelial wound closure at 6H after injury (BSA: 33%, 0.075mg/mL: 77%, $p<0.0001$). However, TEER data shows no significant contribution to tight junction recovery. The use of PE to improve epithelial restitution could be a clinically effective treatment in colic cases with intestinal hypoxia and injury.

Funding sources: NC State University College of Veterinary Medicine, Fund for Discovery, North Carolina Horse Council

Primary subject category for presentation: Clinical Medicine, Gastroenterology, Regenerative Medicine

OPTIMIZING CELL BLOCKS AND STORAGE MEDIA CONDITIONS OF FINE NEEDLE ASPIRATES FROM CANINE LIVERS

Francyrette A. Seda Gómez, veterinary student
Jessica Lambert, Alexander Tufano, Erika Gruber
fasedago@ncsu.edu, atufano@ncsu.edu, ejgruber@ncsu.edu

North Carolina State University, College of Veterinary Medicine

Fine needle aspiration (FNA) is crucial in identifying and differentiating types of cancer in dogs. Similar to small biopsies, cell blocks (CBs) prepared from freshly collected aspirates have been found to provide sufficient material for microscopic evaluation of tumors. To make CBs more accessible to veterinarians and patients beyond academic institutions, our aim was to determine if CBs from aspirates stored at 4°C for up to 72 hours would be comparable to those made immediately after collection. We also investigated if protein in the storage medium could improve sample quality. FNAs were collected from the livers of recently euthanized healthy dogs. Aspirated material was expelled into tubes containing Hanks buffered saline solution (HBSS), 100% fetal bovine serum (FBS), 10% FBS in HBSS, or 5% bovine serum albumin (BSA) in HBSS. At 0h, 24h, and 72h, tubes were centrifuged, and the supernatant was replaced with 50µL of 3% agarose gel to create the CB. For microscopic analysis, the CBs were fixed in 10% neutral buffered formalin for paraffin embedding, sectioning, hematoxylin & eosin staining. Preliminary results suggest that storing aspirated liver in HBSS for 24 and 72 hours decreases cellularity and lessens intact morphology. Improved cellularity and morphology were observed in aspirates stored in 100% FBS, 10% HBSS, and 5% BSA. Hepatic aspirates stored in media containing either BSA or FBS retain morphologic features suitable for incorporation into CBs for microscopic evaluation.

Research Grant: NCSU Population Health and Pathobiology Faculty
Student Support: NIH T35 OD011070 Interdisciplinary Biomedical Research Training Program

Category: Clinical Pathology

CHARACTERIZATION OF ORGANOIDS CULTURED FROM NORMAL AND MUCOCELE CANINE GALLBLADDERS.

Nicole K. Torres Machado, veterinary student
Jennifer Holmes, Jody L. Gookin
nktorres@ncsu.edu, jcholmes@ncsu.edu, jody_gookin@ncsu.edu

North Carolina State University, College of Veterinary Medicine.

Mucocele formation results from the accumulation of thick and dehydrated mucus within the canine gallbladder often leading to gallbladder rupture. Surgical removal of the gallbladder can be curative but carries a high mortality rate. The cause of mucocele formation is unknown and there are currently no models to study disease pathogenesis. The cystic fibrosis transmembrane conductance regulator (CFTR) is an epithelial chloride channel responsible for hydration of gallbladder mucus. Prior studies of CFTR knockout laboratory animals demonstrate a gallbladder pathology similar to dogs with mucocele formation. The objective of our study was to establish an organoid culture model to investigate CFTR in healthy and mucocele gallbladders. We hypothesized that organoids cultured from dogs with mucocele formation would have abnormal CFTR expression and/or secretory function. Organoids were cultured from healthy and mucocele gallbladder mucosa using methods established for intestinal epithelium. Immunofluorescence imaging was performed to visualize CFTR expression. Organoid swelling in response to cAMP agonists was measured to quantify epithelial chloride secretion. Healthy gallbladder organoids express CFTR and swell in response to CFTR stimulation. Mucocele organoids lacked CFTR expression but retained the ability to swell in response to CFTR stimulation. Results suggest that mucocele gallbladder organoids may be capable of cAMP-mediated secretion that is independent of CFTR expression. Further studies to determine the dependence of mucocele organoid swelling on CFTR activity are needed. Discovery of alternative pathways for secretion by mucocele organoids could provide treatment targets to promote mucus hydration in dogs with gallbladder mucocele formation.

Funding: National Institutes of Health Interdisciplinary Biomedical Research Training Program T35-T35OD011070
AKC Canine Health Foundation #03145
Fund for discover

Category: Gastroenterology

PHARMACOKINETICS OF A CONTINUOUS INTRAVENOUS INFUSION OF HYDROMORPHONE IN HEALTHY DOGS

Author: **Candace Wimbish, DVM,** Category: House Officer
Co-Authors: Alex Lynch, BVSc(Hons) DACVECC MRCVS, NCSU CVM, amlynch3@ncsu.edu
Kristen M. Messenger, DVM, PhD, DACVAA, DACVCP, NCSU CVM, kmmessen@ncsu.edu
Heather Knych, DVM, PhD, DACVCP, UC Davis School of Veterinary Medicine, hkknych@ucdavis.edu
Mark Papich, DVM, MS, DACVCP, NCSU CVM, mark_papich@ncsu.edu
Subject Category: Pharmacology

Hydromorphone is common IV bolus analgesia in dogs yet is associated with adverse effects. A continuous infusion may mitigate these effects however, the pharmacokinetics of this regimen has not been described. Recommended dosing protocols are based upon simulations following bolus data. The objective of this study was to describe the pharmacokinetics of hydromorphone in dogs receiving an IV bolus followed by an IV infusion.

Six purpose-bred dogs were used in a prospective study. Each dog received an IV bolus of hydromorphone (0.1 mg/kg) followed by an infusion (0.01 mg/kg/hr) for a 48-hour period. Blood samples were collected at sixteen points between 0-58 hours relative to bolus administration. Plasma concentrations were analyzed by high pressure liquid chromatography. Pharmacokinetic estimates were obtained with compartmental methods.

The data was fit using a two-compartment IV bolus/infusion model with first order elimination. At the end of the infusion, plasma hydromorphone concentrations were 6.8 (5.5-19.6) ng/mL. Following IV administration, total body clearance was 30.4 (19.8-36.7) mL/min-1/kg-1; volume of distribution at steady state was 4.5 (3.2-7.8) L/kg-1. Elimination half-life was 0.8 (0.6-1.0) hr. The distribution half-life was 0.7 (0.6-1.0) hr and the terminal elimination half-life was 11.2 (7.6-24.3) hr.

An IV bolus of 0.1 mg/kg, followed by an IV infusion of 0.01 mg/kg/hr maintains steady-state concentrations between approximately 8–12 ng/mL, with a rapid elimination once the infusion is discontinued. This suggests 0.01 mg/kg/hr is sufficient to maintain therapeutic concentrations. Previous dosing recommendations of 0.03 mg/kg/hr will likely result in plasma concentrations exceeding the target for hydromorphone.

Bioinformatics Analysis on Shotgun Sequencing Data from Ancient Parchment

Yixuan Yang, graduate student
Matthew Teasdale, Matthew Collins, Melissa Scheible, Rachael Thomas, Kelly Meiklejohn, Timothy Stinson, Benjamin Callahan
yyang55@ncsu.edu, matthew.teasdale@palaeome.org, matthew@palaeome.org, mkscheib@ncsu.edu, rthomas3@ncsu.edu, kameikle@ncsu.edu, bcallah@ncsu.edu

Bioinformatics Research Center, North Carolina State University
College of Veterinary Medicine, North Carolina State University
Department of Archaeology, University of Cambridge

Abstract

Parchment, widely used for information recording in ancient times, contains a lot of biological information on its surface that has not been explored yet. Understanding this information may provide novel insights or bring new evidence to various fields of study. Here, we use a bioinformatics method to compare the shotgun sequencing data from parchment surface to the genome DNA of different animal species, aiming to determine the material origin of the parchment. By using the Basic Local Alignment Search Tool (BLAST), we determined the material of four out of ten parchment samples. The remaining samples are not yet identified, likely due to factors such as sampling method and DNA degradation.

NCSU Research and Innovation Seed Funding Program

Bioinformatics, Archaeology

G-PROTEIN COUPLED RECEPTOR 35 (GPR35) EXPRESSION AND FUNCTIONAL CHARACTERIZATION USING HISTOLOGICAL AND CELL-LINE BASED APPROACH

Junho Yu[1] Category: Undergraduate

Alla D. Lyfenko[2], Josh Wheeler[2], Santosh K. Mishra[2]

[1]North Carolina State Univ., Raleigh, NC; [2]Mol. Biomed. Sci., NCSU CVM, Raleigh, NC

Junho Yu: jyu29@ncsu.edu
Alla D. Lyfenko: adlyfenk@ncsu.edu
Josh Wheeler: jjwheel2@ncsu.edu
Santosh K. Mishra: skmishra@ncsu.edu

Abstract:
Itch is a major symptom of cutaneous diseases such as psoriasis and atopic dermatitis. Still, the mechanisms behind these debilitating conditions remain unknown, especially receptors that transduce itch signaling via these peripheral afferents innervating skin and send signals to the central nervous system. Earlier, we and others have reported an expression of G-protein Coupled Receptor 35 (GPR35) in the Dorsal Root Ganglia (DRG) sensory neurons, but their expression profile and function were less known. We used a transgenic line, immunological and cell-based assays to examine GPR35 expression and its function. DRGs from C57BL/6J and a transgenic line that expresses the fluorescent red protein (tdTomato) in the GPR35-promoter were collected under various fixation conditions and sectioned onto glass slides for immunohistological analysis in combination with different pruriceptive markers.

Further, we examined the functional role of GPR35 using a heterologous expression system. We utilized Calcium Imaging to visualize the changes in the calcium concentration of HEK293 cells transfected with GPR35, TRPA1, and GPR35+TRPA1 and control (empty vectors) using agonists specific for receptor activation. In summary, we showed that GPR35 colocalized with TRPA1 and TRPV1-expressing neurons. We found increased calcium transients in cells co-transfected with GPR35 and TRPA1 compared to control in response to specific agonists. Our findings suggest a possible involvement of GPR35 in sensory transduction and may act as a potential therapeutic target.

Funding Sources/Grant:
NIH T34 GM131947
NIH R01 AR079713

Primary Subject Category: Neurosciences