Poster #	Presenter - last, first (mentor)	Poster Presentations	Time
1	B. de Oliveira, Ana (HO - Silverstein Metzler)	THE BEST NEST MAKES FOR THE BEST REST – BEHAVIORAL ASSESSMENT OF MOUSE BEDDING PREFERENCE	10:30 am - 12:00 pm
2	Baek, Leia (US - Breen)	MERCURY AND SELENIUM EXPOSURE IN GALAPAGOS (ZALOPHUS WOLLEBAEKI) AND CALIFORNIA (ZALOPHUS CALIFORNIANUS) SEA LIONS	10:30 am - 12:00 pm
3	Baldeon, Mary (PD - Knazovicky)	ARTHROSCOPIC INTRA-ARTICULAR PRESSURES OF THE SHOULDER AND STIFLE	10:30 am - 12:00 pm
4	Bellina, Meredith (VS - Lewbart / Armwood)	*MICROPTERUS NIGRICANS* ADOMAVIRUS-1 IN NEOPLASTIC AND NON-NEOPLASTIC MELANOPHORE PROLIFERATIONS IN LARGEMOUTH BASS (*MICROPTERUS NIGRICANS*)	11:30 am- 1:00 pm
5	Borys, Kristina (GS - Brooks)	BMP2 IS REQUIRED FOR CELL REMODELING IN CRANIAL NEURAL TUBE CLOSURE	10:30 am - 12:00 pm
6	Bradley, Tyneisha (GS - Clinton)	GENETIC ANALYSES OF CYTOCHROME P450 AND IMMUNE-RELATED GENES TO UNDERSTAND VARIABILITY IN METABOLIC FUNCTION AND ANTIBIOTIC RESISTANCE IN AFRICAN AMERICANS	10:30 am - 12:00 pm
7	Brake, Abbey (VS - Kulkarni)	ANTIMICROBIAL AND IMMUNOLOGICAL CHARACTERIZATION OF CHICKEN LACTOBACILLUS STRAINS	11:30 am- 1:00 pm
8	Brannon-Vaughan, Embrea (VS -	Enabling Tumor-Specific Drug Delivery by Targeting the Warburg Effect of Cancer	11:30 am- 1:00 pm
9	Carrillo, Joshua (VS - Mariani)	3D PRINTING SURGICAL DRILL GUIDES FOR SPINAL STABILIZATION SURGERY	11:30 am- 1:00 pm
10	Chan, Pok (S - Kulkarni)	CLOSTRIDIUM PERFRINGENS INDUCES HOST INFLAMMATORY RESPONSE VIA SUPPRESSING REGULATORY T CELL RESPONSE IN CHICKENS	10:30 am - 12:00 pm
11	Cole, Kevin (UG - Bayless)	DNASE I-LOADED FIBRIN-SPECIFIC NANOGELS FOR TREATMENT OF IMMUNOTHROMBOSIS	10:30 am - 12:00 pm
12	Copeland, Carson (VS - Schaaf / Cline)	CHARACTERIZATION OF DELAYED RADIATION-INDUCED KIDNEY INJURY IN RHESUS MACAQUES	11:30 am- 1:00 pm
13	Copper, Keeley (VS - Sheahan)	THE EFFECTS OF CLOSTRIDIOIDES DIFFICILE TOXIN B ON EQUINE COLONIC MONOLAYERS	11:30 am- 1:00 pm
14	Creamer, Maggie (PD - Lascelles)	PAIN AND PLEASURE: FACTORS CONTRIBUTING TO ANHEDONIA SCALE RESPONSES AND CORRELATION WITH A NOVEL BEHAVIOR TEST IN DOGS	10:30 am - 12:00 pm
15	Croy, Hannah (GS - Eells)	SARS-COV-2 RESPIRATORY CONFINEMENT DOES NOT EXACERBATE MPTP-INDUCED NIGROSTRIATAL DEGRADATION	10:30 am - 12:00 pm
16	Dausch, Kaelyn (VS - Gilger)	OCULAR PARAMETERS RELATED TO DRUG DELIVERY IN MARINE MAMMALS	11:30 am- 1:00 pm
17	Davenport, Hannah (VS - Rahe)	ESTABLISHMENT AND CHARACTERIZATION OF PORCINE LYMPHOMA CELL LINES	11:30 am- 1:00 pm

18	Dedes, Katarina (VS - Harrison)	RETROSPECTIVE REVIEW OF NEOPLASIA CASES IN NON-DOMESTIC FELIDS	11:30 am-1:00 pm
19	Driggers, Jacobs (GS - Fletcher)	THE HOST ASSOCIATED GLYCAN CHONDROITIN SULFATE PROMOTES GROWTH OF FUSOBACTERIUM NUCLEATUM	10:30 am - 12:00 pm
20	Enomoto, Hiroko (S - Lascelles)	DIRECT ASSESSMENT OF INTESTINAL PERMEABILITY AS A RISK FACTOR FOR MULTIPLE JOINT OSTEOARTHRITIS IN COHORTS OF HUMANS AND PET DOGS	10:30 am - 12:00 pm
21	Fares, Abdelhamid (GS - Gimeno)	EFFECT OF MDV-1 VACCINES ADMINISTERED ALONE OR WITH HVT ON THE DEVELOPMENT OF THE CHICKEN EMBRYO IMMUNE SYSTEM	10:30 am - 12:00 pm
22	Ferrans, Morgan (PD - Lascelles / Gruen)	EXPLORING AN ADAPTED PAIN CATASTROPHIZING PARADIGM FOR USE IN THE NATURALLY OCCURRING CANINE MODEL OF OSTEOARTHRITIS	10:30 am - 12:00 pm
23	Gaghan, Carrisa (GS - Kulkarni)	*IN VITRO* CHARACTERIZATION OF TURKEY *LACTOBACILLUS STRAINS* POSSESSING IMMUNOMODULATORY AND ANTI-*CLOSTRIDUM SEPTICUM* PROPERTIES	10:30 am - 12:00 pm
24	Gallagher, Mary Kate (VS - Van Landeghem)	Investigating the Role of Glial Cells In Chemoresistance In Pancreatic Cancer	11:30 am-1:00 pm
25	Glass, Reagan (VS - LI)	Hemin as the mitochondrial driver of procoagulant platelet phenotypes in dogs with immune-mediated hemolytic anemia	11:30 am-1:00 pm
26	Gonzalez, Josue (VS - Stern)	QUANTIFYING FIBROFATTY MYOCARDIAL INFILTRATION ACROSS DOG CARDIAC DISEASE PHENOTYPES	11:30 am-1:00 pm
27	Goodnight, Beckett (VS - Meiklejohn)	Optimizing DNA Sequencing From Items In the Illegal Leather Trade	11:30 am-1:00 pm
28	Greene, Curtis (VS - Gruen)	PRELIMINARY QUANTIFICATION OF FUNCTIONAL STRENGTH IN NON-FRAIL DOGS	11:30 am-1:00 pm
29	Grubbs, Summer (VS - Olby)	NEUROLOGIC SIGNS IN DOGS FOLLOWING BEDINVETMAB (LIBRELA™) ADMINISTRATION: A SURVEY OF NEUROLOGISTS	11:30 am-1:00 pm
30	Hall, Amber & Nathan (US - Lewis)	EVALUATION OF MEDICAL INFRARED THERMOGRAPHY AS A SCREENING TOOL IN DOGS WITH OSTEOARTHRITIS	10:30 am - 12:00 pm
31	Harrison, Helena (VS - Harrison)	EVALUATION OF NEOPLASIA ACROSS BEAR SPECIES	11:30 am-1:00 pm
32	Henderson, Madison (VS - Eells)	QUANTIFICATION OF NEUROINFLAMMATION AND DOPAMINE NEURON SURVIVAL IN A SARS-COV-2	11:30 am-1:00 pm
33	Hepworth, Emma (GS - Yoder)	DEVELOPING A HIGH-THROUGHPUT MULTI-PARAMETER CHEMICAL SCREEN FOR PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) USING ZEBRAFISH AND A MULTI-CAMERA ARRAY MICROSCOPE	10:30 am - 12:00 pm

34	Heywood, Lilly (GS - Sheahan)	TRANSCRIPTOMIC ALTERATIONS IN EQUINE COLONIC MONOLAYERS EXPOSED TO C. DIFFICILE TOXIN B	10:30 am - 12:00 pm
35	Horta, Giovanna (UG - Nascone Yoder)	MORPHOLOGICAL AND MOLECULAR EFFECTS OF JUN-N-TERMINAL KINASE (JNK) ON LEFT-RIGHT ASYMMETRY IN THE XENOPUS STOMACH	10:30 am - 12:00 pm
36	Hughes, Emma (UG - Eells)	SARS-CoV-2 AGE-COORELATED EXACERBATION OF MIDBRAIN DOPAMINE DAMAGE	10:30 am - 12:00 pm
37	Huston, Danielle (S - Lascelles)	A NONINFERIORITY TRIAL EVALUATING THE EFFICACY OF BEDINVETMAB COMPARED TO GRAPIPRANT FOR OSTEOARTHRITIS-PAIN IN DOGS USING FORCE PLATE GAIT ANALYSIS	10:30 am - 12:00 pm
38	Im, Sekwon (VS - Hess)	INVESTIGATING CANCER-TESTIS ANTIGENS AS IMMUNOTHERAPY TARGETS FOR PERIPHERAL T-CELL LYMPHOMA	11:30 am- 1:00 pm
39	Jaen, Xochilt - (UG - Theriot)	CLOSTRIDIOIDES DIFFICILE TOXINS ALTER HOST BILE ACID AND CHOLESTEROL METABOLISM GENE EXPRESSION IN THE COLONIC EPITHELIUM	10:30 am - 12:00 pm
40	Jenkins, Hakeem (S - Gamsjäger)	AN INVESTIGATION OF WATER MIXING TEMPERATURE AND ITS IMPACT ON IMMUNOGLOBULIN G CONCENTRATION IN BOVINE COLOSTRUM REPLACER PRODUCTS	10:30 am - 12:00 pm
41	Kabakchieva, Gabriella (VS - Dembek)	ARGININE VASOPRESSIN STIMULATION FOR DIAGNOSING RELATIVE ADRENAL INSUFFICIENCY IN HORSES	11:30 am- 1:00 pm
42	Kim, Keon (PD - Her)	Assessment of the prognostic value of the respiratory rate–oxygenation index in predicting early need for mechanical ventilation in dogs treated with high-flow nasal cannula oxygen therapy	10:30 am - 12:00 pm
43	Ku, Isabel (VS - Gilger)	ASSESSMENT OF OCULAR TOXICITY FOLLOWING CORNEAL APPLICATION OF COLD ATMOSPHERIC PLASMA IN RABBITS USING MULTIMODAL IMAGING	11:30 am- 1:00 pm
44	Leber, Meghan (VS - Birkenheuer)	REEVALUATING DOMESTIC CATS AS RESERVOIR HOSTS: PREVALENCE OF CYTAUXZOON FELIS SEROREACTIVITY IN QPCR-NEGATIVE SAMPLES	11:30 am- 1:00 pm
45	Lee, Hannah (GS - Guilluy)	GEF-H1 DRIVES CANCER CELL CONTRACTILITY AND MIGRATION IN CONFINED ENVIRONMENTS	10:30 am - 12:00 pm
46	Lierz, Sydney (VS - McKinney-Aguirre)	OPTIMIZING EQUINE INTESTINAL VIABILITY AND INJURY ASSESSMENTS UTILIZING AN EX VIVO PERFUSION PLATFORM	11:30 am- 1:00 pm
47	Livingston, Isabella (GS - Breen)	CROSS-SPECIES EXOME SEQUENCING REVEALS RECURRENT GENOMIC ALTERATIONS IN CALIFORNIA SEA LION (ZALOPHUS CALIFORNIANUS) UROGENITAL CARCINOMA	10:30 am - 12:00 pm
48	Long, Kylee (VS - Li)	EVALUATION OF HYPERCOAGULABILITY, AND PLATELET ACTIVATION IN CATS WITH DIFFERNT MANIFESTATIONS OF CONGESTIVE HEART FAILURE	10:30 am - 12:00 pm

49	Lopez Cruz, Carla (GS - Gonzalez)	APPLICATION OF A FRAMEWORK TO MITIGATE THE RISK OF SURGICAL SITE INFECTION AFTER EXPLORATORY CELIOTOMY IN HORSES: A RETROSPECTIVE STUDY.	10:30 am - 12:00 pm
50	MacDonald, Sydney (VS - Lewbart)	A COMPREHENSIVE STUDY ON BLOOD CHARACTERISTICS AND HEALTH IN WILD-CAUGHT MAHI-MAHI *(CORYPHAENA HIPPURUS)*	11:30 am- 1:00 pm
51	MacFarlane, Carley-Martin (VS - Her)	COMPARATIVE ASSESSMENT OF HEMOPERFUSION DEVICES FOR CARPROFEN REMOVAL IN IN VITRO AND EX VIVO MODELS	11:30 am- 1:00 pm
52	Marchal, William (VS - Halleran)	DEVELOPMENT OF AN IN VIVO CAPRINE MODEL FOR STAPHYLOCOCCUS AUREUS MASTITIS IN WOMEN	11:30 am- 1:00 pm
53	Martinez, Margaret (S - Qurollo)	DETECTION OF A NOVEL HEPATOZOON IN THE UNITED STATES DURING LARGE-SCALE FELINE VECTOR-BORNE ORGANISM PCR PREVALENCE STUDY	10:30 am - 12:00 pm
54	McManus, Sydney (VS - Love)	NO SAMPLE LEFT BEHIND: QUALITY IMPROVEMENT PROJECT IN LABORATORY SAMPLE COLLECTION AND PROCESSING AT A VETERINARY TEACHING HOSPITAL	11:30 am- 1:00 pm
55	Meier, Jeffreey (VS - Sano)	OPTIMIZATION OF MACROPHAGE REPORTING ASSAY FOR CATEGORIZING MACROPHAGE RESPONSE TO INSPIRE THERAPY	11:30 am- 1:00 pm
56	Miller, Cary (VS - Schnabel)	PREVALENCE OF SUBCLINICAL LAMINITIS IN WORKING SPORT HORSES	11:30 am- 1:00 pm
57	Mosley, Quiana (GS - Lopez Soto)	Characterizing Alternative Splicing Networks in Somatosensory Neurons	10:30 am - 12:00 pm
58	Nikolov, Damia - (US - Theriot)	CHARACTERIZATION OF A BILE SALT HYDROLASE FROM COMMMENSAL BACTERIUM CLOSTRIDIUM HIRANONIS	10:30 am - 12:00 pm
59	Odegard, Natasha (VS - Meiklejohn)	DESIGNING AND OPTIMIZING AN STR PANEL FOR TURKEY INDIVIDUALIZATION TO SUPPORT THE NC WILDLIFE RESOURCES COMMISSION	11:30 am- 1:00 pm
60	O'Neill, Mary (VS - Sheahan)	THE IMPACT OF CHOLINE SUPPLEMENTATION ON CELL PROLIFERATION AND BARRIER FUNCTION OF EQUINE COLONIC EPITHELIUM	11:30 am- 1:00 pm
61	O'Quinn, Aubrey (US - Bayless)	EFFECTS OF ANTI-THROMBOTICS ON NET-MEDIATED CLOTTING IN HORSES	10:30 am - 12:00 pm
62	Palyvou, Efstathia (PD - Nolan)	ATR INHIBITION COMBINED WITH RADIATION THERAPY FOR THE TREATMENT OF FELINE ORAL SQUAMOUS CELL CARCINOMA	10:30 am - 12:00 pm
63	Pimplapure, Anuja (GS - Sharma)	FUNCTIONAL CHARACTERIZATION OF GLYCANS ON HIV-1 ENVELOPE GLYCOPROTEIN TRIMER	10:30 am - 12:00 pm
64	Rekha, KC (GS - Saini)	RESOLUTION KINETICS OF MIXED ALLERGENS-INDUCED ALLERGIC AIRWAY INFLAMMATION AND AIRWAY REMODELING	10:30 am - 12:00 pm
65	Ren, Jiayi (GS - Suryawanshi)	INHIBITION OF GLYCOGEN SYNASE KINASE 3β (GSK3β) SUPPRESSES HSV-1 REPLICATION IN EPITHELIAL CELLS	10:30 am - 12:00 pm

66	Rexroat, Elizabeth (VS - Ghashghae)	FROM BEHAVIOR TO CIRCUITS: UNCOVERING THE EMERGENCE OF VALUE PERCEPTION IN THE DEVELOPING MOUSE BRAIN	11:30 am-1:00 pm
67	Ripper, Mackenzie (VS - Crespo)	COMPARATIVE EVALUATION OF RADIOGRAPHIC AND ULTRASONOGRAPHIC IMAGING IN PULLETS	11:30 am-1:00 pm
68	Roberts, Natalie (GS - Koci)	DIETARY MODULATION OF GUT MICROBIOTA ALTERS SALMONELLA COLONIZATION AND DIVERSITY IN CHICKENS SUPPLEMENTED WITH PROBIOTICS AND PREBIOTICS	10:30 am - 12:00 pm
69	Rosario, Fabiola (VS - Schnabel)	ALPHA2EQ DOWNREGULATES INFLAMMATORY GENE EXPRESSION IN EQUINE SYNOVIAL FIBROBLASTS IN VITRO	11:30 am-1:00 pm
70	Schmidt, Luis (HO - Her)	EVALUATION OF THE OXYGEN SATURATION INDEX (OSI) AS A PREDICTOR OF OUTCOME AND SURROGATE FOR OXYGENATION INDEX (OI) IN DOGS UNDERGOING MECHANICAL VENTILATION	10:30 am - 12:00 pm
71	Stalford, Katherine (VS - Birkenheuer)	INVESTIGATION OF VECTOR-BORNE AND RELATED DISEASES IN THE HOOFSTOCK SPECIES OF THE SAN DIEGO ZOO SAFARI PARK	11:30 am-1:00 pm
72	Stern, Michelle (VS - Lascelles)	ADVANCING CANINE BEHAVIORAL RESEARCH: DEVELOPMENT AND VALIDATION OF OWNER-COMPLETED QUESTIONNAIRES FOR ASSESSING ANHEDONIA IN DOGS	11:30 am-1:00 pm
73	Stonemetz, Brooke (VS - Guilluy)	ANALYZING THE ROLE OF HISTONE H3 METHYLATION DURING Hydra vulgaris REGENERATION	11:30 am-1:00 pm
74	Stywall, Akiya (VS - Olby)	Stride length in relation to age and owner reported cognitive performance in aging dogs	11:30 am-1:00 pm
75	Thonen-Fleck, Connor (VS - Pierce / Stern)	INVESTIGATING THE GENETIC ORIGIN OF COR TRIATRIATUM SINISTER IN THE DOMESTIC CAT	11:30 am-1:00 pm
76	Tiffinger, Kornelia (GS - Lascelles)	THE COMPARISON IN PHARMACOKINETICS AND ADVERSE EFFECTS OF SINGLE SUBCUTANEOUS EXTENDED-RELEASE BUPRENORPHINE (ETHIQA XR®) BETWEEN INTACT FEMALES AND INTACT MALES OF HEALTHY BEAGLES	10:30 am - 12:00 pm
77	Tomblin, Emily (VS - Gruber)	TOLL-LIKE RECEPTOR AGONISTS INDUCE CANINE OSTEOSARCOMA CELL DEATH	11:30 am-1:00 pm
78	Tran, Becky (VS - Crespo / Kulkarni)	INTERACTION OF AVIAN MACROPHAGE WITH ENTEROCOCCUS CECORUM AND ANTIBACTERIAL EFFECTS OF PROBIOTIC LACTOBACILLUS BACTERIA	11:30 am-1:00 pm
79	Trull, McKenna (US - Suryawanshi)	EXTENDING IFN-λ RELEASE AT THE CORNEAL SURFACE USING A NOVEL HYDROGEL FORMULATION SUPPRESSES CORNEAL IMMUNOPATHOLOGY AFTER OCULAR HSV-1 INFECTION	10:30 am - 12:00 pm

80	Turner, Abby (VS - Jeong)	DEFINING THE HISTOPATHOLOGIC AND MOLECULAR CHARACTERISTICS OF FELINE EXOCRINE PANCREATIC CARCINOMA	11:30 am-1:00 pm
81	Tyagi, Aakriti (S - Kulkarni)	INVESTIGATING IMMUNOLOGICAL AND PATHOLOGICAL CHANGES IN LAYER CHICKENS AFFECTED WITH OVARIAN ADENOCARCINOMA	10:30 am - 12:00 pm
82	Tysver, Ariel (VS - Sano)	Optimization of Cell Transfection with Electroporation in 3D Culture	11:30 am-1:00 pm
83	Vaughan, Haley (VS - Mzyk)	PHARMACOKINETICS OF INTRAVENOUS AMPICILLIN SODIUM IN HOSPITALIZED CALVES	11:30 am-1:00 pm
84	Wells, Mary (VS - Gilger)	TOLERABILITY, TOXICITY, AND PHARMACOKINETICS OF EPISCLERAL SUSTAINED RELEASE IMPLANTS CONTAINING TACROLIMUS AND RAPAMYCIN IN NEW ZEALAND WHITE RABBITS	11:30 am-1:00 pm
85	Williams, Abigail (US - Goya-Jorge)	NORMOTHERMIC MACHINE PERFUSION ENABLES REPERFUSION INJURY ASSESSMENT AND EX VIVO SIMULATION OF INTESTINAL TRANSPLANTATION	10:30 am - 12:00 pm
86	Wright,Abbie (S - Nascone-Yoder) / Hinnant, Taylor (PD - Nascone-Yoder)	The Xenopus intestine exhibits left-right asymmetry prior to gut looping events	10:30 am - 12:00 pm
87	Zsofia, Vigh (GS - Her)	BACLOFEN CLEARANCE ACROSS MULTIPLE EXTRACORPOREAL THERAPIES: AN EX VIVO EVALUATION	10:30 am - 12:00 pm

THE BEST NEST MAKES FOR THE BEST REST – BEHAVIORAL ASSESSMENT OF MOUSE BEDDING PREFERENCE

Ana Carolyne B. de Oliveira house officer

Sina Mahs, Rebecca Michelle Heidenfelder, Brandon Whipple, Marnie Silverstein Metzler

aborges2@ncsu.edu, selilhol@ncsu.edu, rmheinden@ncsu.edu, jbwhippl@ncsu.edu, mgsilver@ncsu.edu

919-650-0618

NCSU CVM LAR

Mice are the most commonly used research animal model and their housing conditions significantly influence their health and well-being. This study aimed to identify the bedding substrate and nesting material preferred by research mice.Three cages of 3 mice/cage for each sex were randomly assigned to each of the following treatment groups: EOC Only (Enrich-o-Cob), EOC + CB (EOC plus cardboard), EOC + N (EOC plus Nestlet), EOC + CP (EOC plus crinkle paper), BOC + N (Bed-o-Cob plus Nestlet), and BOC + CP (Bed-o-Cob plus crinkle paper) . To assess the impact of bedding and nesting materials on nesting behaviors, nest quality and nest base incorporation NBI) were scored at 1-week after cage set-up, on the day of cage change, the morning after cage change, and 1-week after cage change. Behavior was recorded at 24, 48, and 72 hours to assess interaction with the enrichment material. Data was analyzed by mixed models ANOVA. EOC+CP and BOC+CP groups had higher nest scores and nest base incorporation at all time points compared to EOC only, EOC + CB, EOC+N and BOC+N groups. All groups had no significant differences in cage change time.These findings will be of value for NCSU husbandry program to increase rodents' well-being and decrease behavioral reports.

Basic research

Other - Laboratory Animal Behavior

MERCURY AND SELENIUM EXPOSURE IN GALAPAGOS (ZALOPHUS WOLLEBAEKI) AND CALIFORNIA (ZALOPHUS CALIFORNIANUS) SEA LIONS

Ashley E. Cave[1,2], **Leia Baek (undergraduate)[1],** Norman Kleiman[3], Jana Zilkha[3], Isabella Livingston[1], Taylor Gregory[4], Eleanor Hawkins[2], Shelly Vaden[2], Kathrin Schilling[3], Ronald Glabonjat[3], Marjorie Riofrío-Lazo[5,6], Diane Deresienski[2,5], Gregory A. Lewbart[2,5], Anthony J. Orr[7], Alissa C. Deming[8], Diego Páez-Rosas[5,6,9,10], Matthew Breen[2]

[1]Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina 27607, USA; [2]Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina 27607, USA; [3]Department of Environmental Health Sciences, Columbia University Mailman School of Public Health, New York, New York, 10032, USA; [4]Greensboro Science Center, Greensboro, North Carolina 27455, USA; [5]Galapagos Science Center, USFQ & UNC-Chapel Hill, Av. Alsacio Northia, Isla San Cristóbal EC200150, Islas Galapagos, Ecuador; [6]Universidad San Francisco de Quito (USFQ), Colegio de Ciencias Biológicas y Ambientales, Isla San Cristóbal EC200150, Islas Galapagos, Ecuador; [7]Marine Mammal Laboratory, Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98115, United States; [8]Department of Conservation Medicine and Science Pacific Mammal Center, Laguna Beach, California, 92651, USA; [9]Fundación Conservando Galápagos, Galapagos Conservancy. Isla Santa Cruz EC200350, Islas Galápagos, Ecuador.[10]Dirección Parque Nacional Galapagos, Departamento de Ecosistemas, Isla Santa Cruz EC200350, Islas Galapagos, Ecuador.

Subject Category: Other (One Health)

Trace element contamination poses a potential threat to marine ecosystems, particularly top predators, where these elements can bioaccumulate. Exposure to elevated levels of mercury has been associated with cognitive deficits, developmental delays, and miscarriages. Effects like these are concerning for biodiversity in general, but they can worsen when they threaten endangered species. This study investigates selenium (Se) and mercury (Hg) concentrations, and Hg:Se molar ratios in two otariiid species: Galapagos sea lions (GSL, *Zalophus wollebaeki*) and California sea lion (CSL, *Zalophus californianu*). Fur samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to determine trace element concentrations. Washed fur was analyzed to assess systemic exposure, and unwashed fur was analyzed to assess environmental levels. White blood cell (WBC) differentials were also evaluated to explore relationships with trace element exposure. GSL exhibited higher mercury and selenium concentrations than CSL in both washed and unwashed fur, potentially reflecting local volcanic activity in the Galapagos archipelago. Hg and Hg:Se ratios were positively associated with length in washed fur samples (value), and Se was significantly higher in females than males (value). This work serves as pilot data in understanding trace element exposure in these two species; however, more research is needed to monitor their sources of exposure and potential health effects. Understanding interspecific and geographic differences in trace element bioaccumulation provides valuable insight into marine ecosystem health and potential risks to both wildlife and human populations that utilize marine resources.

Funding sources: AEC was supported by an NIH Interdisciplinary Biomedical Research Training Program (T35OD011070). Analysis was supported with funds from the NC State Cancer Genomics Fund (MB). The Galapagos sea lion sampling was supported by the POA-GSC of the San Francisco de Quito University and the POA-Grant of the Galapagos Conservancy.

ARTHROSCOPIC INTRA-ARTICULAR PRESSURES OF THE SHOULDER AND STIFLE

Baldeon, M.A.[1]: Post-doc
Knazovicky, D.[2]
Email: mabaldeo@ncsu.edu; dknazov@ncsu.edu
Mobile: 240-988-0426
Affiliations: [1] NCSU Translational Pain in research laboratory [2]NCSU CVM

INTRODUCTION:

Arthroscopy reduces morbidity and has superior sensitivity and specificity[2] when compared to arthrotomy. The risks of arthroscopy include fluid extravasation and capsular plastic deformation. The yield point of a human joint capsule without rupture is 170 mmHg, a pressure demonstrated during stifle arthroscopy. This study investigated the joint pressure (IAP) upon saline distention and the differences in pump pressures and IAP in both the canine shoulder and stifle.

MATERIALS & METHODS:

This study used fresh canine cadavers. A 3.0 mm arthroscope was used and IAP measured with an 18G needle connected to anesthetic invasive blood pressure monitor. Readings were obtained (1) unadulterated joint (2) 10 mL saline injection (3) every minute thereafter. After two readings the pressure was increased by 10 mmHg between 30-100 mmHg. The pressure was decreased to 80, 50, and 30 mmHg. While decreasing, stifle joint IAPs were measured at 130° and 90°.

RESULTS:

Saline distention IAPs in the stifle were lower than the shoulder joint with one exception. All fluid pump setting pressures were significantly lower than measured IAP ($P = <0.0001$) by approximately 20 mmHg. Pressure variability was smaller for shoulders than stifles. Standard deviation increased with increased pressures in the stifle.

DISCUSSION/CONCLUSION:

Saline distention of the joint should be done with minimal fluid. Pump pressures can be expected to exceed IAP by a significant amount. At lower pump pressures the difference between pump pressures and IAP is smaller than at higher pump pressures. Increased pump pressure did not improve visibility.

Funding: NC state startup funds were used for purchases of materials
Clinical research
Subject Category: Clinical medicine

MICROPTERUS NIGRICANS ADOMAVIRUS-1 IN NEOPLASTIC AND NON-NEOPLASTIC MELANOPHORE PROLIFERATIONS IN LARGEMOUTH BASS (*MICROPTERUS NIGRICANS*)

Meredith Bellina[1]; Veterinary Student
Chareerut Phruksaniyom[1], R. Kent Passingham[2], Gregory Lewbart[2], Abigail Armwood[1]
mlbellin@ncsu.edu, 704-254-3201

[1]Department of Population Health and Pathobiology, NCSU CVM, Raleigh, North Carolina, USA
[2]Department of Clinical Sciences, NCSU CVM, Raleigh, North Carolina, USA

Adomaviruses, family *Adomaviridae*, are a recently discovered family of circular double-stranded DNA viruses, related to papillomaviruses, polyomaviruses, adintoviruses, and adenoviruses. Smallmouth and largemouth bass infected with adomaviruses frequently display non-neoplastic cutaneous hyperpigmented lesions. The Armwood lab recently associated *Micropterus nigricans* adomavirus 1 (MnA-1) with malignant melanophoromas in largemouth bass. The study objectives were to classify largemouth bass adomavirus (MnA-1)-associated lesions genetically and cellularly, and to identify correlations between HPMLs and malignant melanophoromas. The hypothesis stated that MnA-1 is associated with non-neoplastic cutaneous melanocytic lesions in largemouth bass, which can transform to melanocytic neoplasia as evidenced by histology, immunohistochemistry, PCR, and transmission electron microscopy. Histologic examination revealed non-neoplastic proliferations of melanocytes within the dermis and epidermis in 100% (7/7) of fish, with proliferation to malignant melanophoroma in 14% (1/7) of fish. Chronic inflammation composed of lymphocytes and few eosinophilic granular cells were identified in 43% (3/7) cases, including the melanophoroma. Melanophores were immunoreactive for PNL2 in 50% (1/2) of cases and immunonegative for MelanA in one case. MnA-1 was detected in 100% (7/7) of largemouth bass lesions using PCR and confirmed with Sanger sequencing. Smallmouth bass adomaviruses, MdA-1 and MdA-2, were not detected. Transmission electron microscopy did not reveal virions in melanocytes or epidermal cells, potentially due to late stage infection or low numbers of virions present. Study findings will advance the understanding of adomaviral pathogenesis and lesion progression to malignant melanophoromas.

Research Grant: Armwood Start-Up Funds

Student Support: Boehringer-Ingelheim Veterinary Scholars Program

Basic Research

Primary Subject Category: Infectious Disease

BMP2 IS REQUIRED FOR CELL REMODELING IN CRANIAL NEURAL TUBE CLOSURE

Kristina A. Borys (graduate student)
Eric Brooks

The neural tube is a critical early embryonic structure in the development of the central nervous system. Formation of the neural tube occurs through neural tube closure (NTC), a sheet of neuroepithelial cells elevate and fuse at their dorsal edges to create a closed, hollow tube. Failure to close efficiently results in neural tube defects (NTD) that lead to structural birth defects and lethality, in humans NTD's impact ~1 in 2000 pregnancies. Defects occur through the dysregulation of morphogen signaling and the subsequent cellular behavioral changes. Bone morphogenic proteins (BMP) are an essential group of developmental morphogens and during cranial NTC Bmp2 is required for closure. However, the cellular mechanisms that are dysregulated in the absence of Bmp2 is unknown. A transgenic mouse model was utilized to create a global loss of Bmp2 (Bmp2fn;Sox2-Cre) and embryos were collected at various time points across neural tube closure. The apical surface of the neuroectodermal cells were visualized using phalloidin staining's to measure changes in apical constriction and orientational changes of the neuroectoderm. No changes in apical constriction were found between Bmp2 and wildtype groups. In the Bmp2 we found that a complete randomization of cells at the start and midway point of NTC. Effective orientational rearrangement of cells relies on patterned polarization, which implicates Bmp2 as an essential morphogen in the polarization of the neuroectoderm during NTC.

Email Address: kaborys@ncsu.edu
Mobile: 315-559-3365
Developmental Genetics

GENETIC ANALYSES OF CYTOCHROME P450 AND IMMUNE-RELATED GENES TO UNDERSTAND VARIABILITY IN METABOLIC FUNCTION AND ANTIBIOTIC RESISTANCE IN AFRICAN AMERICANS

Author: [1,2]**Tyneisha L. Bradley (Graduate Student)**

Co-Author/Faculty Mentor: [2]Dr. Carter Clinton

Email: tlbradl3@ncsu.edu, ckclinto@ncsu.edu

Mobile: 803-293-6809

Affiliation(s): [1]NCSU CVM, [2]NCSU COS

Abstract:

Cytochrome P450 (CYP) enzymes and immune response genes represent two deeply conserved systems mediating human adaptation to environmental pressures, xenobiotic metabolism, and immune defense. Genetic diversity within these loci reflects histories of selection, migration, and admixture, yet their combined influence on health variation remains underexplored, particularly among African Americans. This study integrates population and functional genomics to investigate variation in CYP (CYP2D6, CYP2C9, CYP2C19, CYP1A1, CYP1B1) and immune-related genes (IL6, TLR1, TLR4, CCR5, NOD2) across African American (AA), African (AFR), and European (EUR) populations using comparative data from the 1000 Genomes Project and whole-genome sequencing (WGS) of saliva-derived DNA collected in partnership with North Carolina African American descendant communities. Haplotype and population differentiation analyses (±100 kb) conducted using PLINK, VCFtools, and FSTest revealed substantial interpopulation differences, with TLR1 and CYP1B1 showing the highest haplotype diversity (**TLR1**—AA: 37, AFR: 31, EUR: 4; **CYP1B1**—AA: 29, AFR: 32, EUR: 18). Comparative population genomic scans using selscan and Weir & Cockerham FST metrics identified strong differentiation signals, with the highest values observed for TLR1 (0.020 AFR:AA; 0.102 AFR:EUR; 0.092 EUR:AA). Experimental assays will evaluate how adaptive CYP variants identified from saliva-derived DNA influence antibiotic metabolism and resistance by measuring enzyme activity, substrate specificity, and metabolite profiles using recombinant variant expression, bacterial growth inhibition models, and LC–MS-based metabolic profiling. This work advances understanding of how adaptive genetic diversity in metabolic and immune pathways contributes to differential drug efficacy and antibiotic resistance risk, informing precision medicine in underrepresented populations.

Funding Source: North Carolina State University Department of Biological Sciences

Research Type: Basic Research

Primary Subject Category: Genetics, Other

ANTIMICROBIAL AND IMMUNOLOGICAL CHARACTERIZATION OF CHICKEN *LACTOBACILLUS* STRAINS

Abbey Brake[1]; Veterinary Student
Carissa Gaghan[1], Aakriti Tyagi[1], Ravi Kulkarni[1]
Ambrake3@ncsu.edu

Affiliations
[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States

Abstract
Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an economically important bacterial disease of chickens.

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an economically important bacterial disease of chickens. In the current era of growing poultry without antibiotics, NE incidences in chicken flocks are on the rise. To develop novel *Lactobacillus*-based probiotics for NE control in poultry as antibiotic alternative, this work was aimed at isolation, identification and characterization of lactobacilli possessing anti-*C. perfringens* and anti-inflammatory properties in-vitro. A total of 60 fecal/intestinal samples collected from 3-week-old heathy broilers were analyzed to isolate *Lactobacillus* colonies followed by 16s rRNA sequencing. About 53 sequences had a definitive *Lactobacillus* species ID and 13 *Lactobacillus* strains, representing all the species, were further tested for their anti-*C. perfringens* property using agar-well diffusion assay. The pH-neutralized cell-free supernatants from all 13 strains displayed zones of inhibition but with varying degrees of antimicrobial effects. Additionally, a bacterial coculture assay to determine the direct *C. perfringens* inhibitory effect of selected strains, NCKL-11C, 45C and 49B, showed marked antimicrobial effects. Furthermore, avian macrophages stimulated with these 3 strains showed that Str. 45C and 49B induced anti-inflammatory responses, as determined by downregulated IL-1β and upregulated IL-10 cytokine expression coupled with increased cellular production of Nitric Oxide. Collectively, our work showed that in-vitro isolation, identification and screening methods employed for chicken lactobacilli can yield selection of certain *Lactobacillus* strains possessing superior anti-inflammatory and antimicrobial effect against *C. perfringens*.

Funding sources
Stern Translation Cardiac Genetics and Pharmacogenomics Laboratory Fund

3D PRINTING SURGICAL DRILL GUIDES FOR SPINAL STABILIZATION SURGERY

Joshua Carrillo- Veterinary student
Christopher Mariani, Ola Harrysson
Jmcarril@ncsu.edu, Clmariani@ncsu.edu, Oaharry@ncsu.edu
828-782-1885
NCSU CVM (Carrillo, Mariani)
NCSU ISE* (Harrysson)

Placement of surgical implants is frequently required in dogs with various spinal disorders but there is a substantial risk of iatrogenic injury to neurovascular structures bordering the implant site. Patient specific, 3D printed drill guides (3DPDG) have been evaluated as a method of constraining implant trajectories and improving safety. However, several important practical questions regarding this process remain, including 3DPDG printing times (important for emergent cases), effects of surgical sterilization on guide integrity and comparisons of the accuracy of 3DPDG-assisted implant placement versus traditional freehand placement. This study was designed to answer some of these questions.

Using computed tomographic images of a canine thoracic spine, we created 3DPDG using additive manufacturing software (Materialise) and several 3D printers (Formlabs V3 and Rapidshape D50+). The 3DPDG were designed with trajectories targeting the pedicles of T8-13. Select guides were sterilized using either hydrogen peroxide or steam autoclave techniques and a surface scanner was used to compare guide surfaces pre- and post-sterilization. 3D-printed biomodels of the spine were then created using a resin combination that mimics bone. These biomodels were used to evaluate freehand versus 3DPDG-assisted creation of drill tracts by three surgeons of varied experience (novice, intermediate, expert).

Print times for the 3DPDG created with the Rapidshape printer were much faster than the Formlabs. Both hydrogen peroxide and steam autoclave sterilization had minimal effects on the surface contours of the 3DPDG printed with either printer. Analysis of the freehand versus 3DPDG-assisted drill tracts using the biomodels is ongoing.

Research Grant: Jack Dale Brown, Sr. and Jack Dale Brown, Jr. Small Companion Animal Research Endowment

Student Support: Veterinary Scholars Program

Clinical Research

Biomedical Engineering, Neurosciences, 3D Printing
ISE* -Industrial and Systems Engineering

CLOSTRIDIUM PERFRINGENS INDUCES HOST INFLAMMATORY RESPONSE VIA SUPPRESSING REGULATORY T CELL RESPONSE IN CHICKENS

Pok Man Chan (Staff)

Carissa Gaghan, Abigail Armwood, Ravi Kulkarni

pchan3@ncsu.edu, cegaghan@ncsu.edu, arwisnet@ncsu.edu, rrkulkar@ncsu.edu

NCSU CVM

Host immune responses during *Clostridium perfringens*-induced necrotic enteritis (NE) in chickens are poorly understood. The present work used an experimental NE model of broiler chickens to investigate responses of conventional and regulatory CD4+, TCRγδ+ and IgM+ B cells in cecal tonsil (CT) tissues, along with evaluating pathological changes and immune gene expression in duodenum, jejunum and CT tissues. Results showed that the infected chickens had reduced ($P=0.0524$) body weight gain and increased ($P<0.05$) NE severity as indicated by gross and histopathology lesions in the small intestine. Immunophenotyping analysis of CT cells revealed a reduction ($P<0.05$) in the frequencies of regulatory CD4+CD25+ and TCRγδ+CD25+ cells, and an increased ($P<0.05$) IgM+ B cell frequency compared to uninfected control. Gene expression analysis found infected birds had higher ($P<0.05$) transcription of IL-1β in all tissues compared to control. Additionally, in the CT and jejunum, an increased ($P<0.05$) IL-6 and/or IFN-γ, and a decreased ($P<0.05$) CD25 gene expression in the infected group were observed. Furthermore, an increased infiltration of PAX5+ B cells in locally infected mucosal tissues was observed by immunohistochemical analysis. Collectively, these results suggested that virulent *C. perfringens* seems to induce inflammatory mucosal and lymphoid responses via augmenting the expression of pro-inflammatory cytokines, while suppressing CD4+CD25+ regulatory cell responses. These findings may have key implications in further studying NE pathogenesis and immunity, and in finding effective non-antibiotic disease control measures. Future work related to the functional characterization of TCRγδ+CD25+ cells in the context of NE in chickens is also needed.

Basic Research

Subject Category: Immunology

DNASE I-LOADED FIBRIN-SPECIFIC NANOGELS FOR TREATMENT OF IMMUNOTHROMBOSIS

Kevin Cole, II (undergraduate)
Ethan Pozy, Dr. Megan Palmisano, Dr. Rosemary Bayless, Dr. Ashley Brown
Email Addresses: kmcole4@ncsu.edu, epozy@ncsu.edu, mgpalmis@ncsu.edu, rlbayles@ncsu.edu, aecarso2@ncsu.edu
Mobile #: (336) 979-0445
Affiliations:
Department of Biological Sciences, North Carolina State University
Department of Molecular Biomedical Sciences, NCSU CVM
Comparative Medicine Institute, North Carolina State University
Lampe Joint Department of Biomedical Engineering, University of North Carolina at Chapel Hill and North Carolina State University
Department of Material Science and Engineering

Abstract:
Sepsis and systemic inflammation increases the risk of immunothrombosis, a process where activated white blood cells drive coagulation. Neutrophils, a critical component of the innate immune system, release neutrophil extracellular traps (NETs), composed of nuclear DNA that promote thrombosis and contribute to resistance in clot degradation. This study explores the use of fibrin-specific nanogels (FSNs) dual-loaded with tissue plasminogen activator (tPA), a fibrinolytic agent, and deoxyribonuclease I (DNase), which degrades DNA in NETs, as a potential treatment for neutrophil-mediated immunothrombosis.

Core-shell gels were started with a core gel synthesized using 10% Bis and 90% NIPAM. A shell composed of 93% NIPAM, 2% Bis, and 5% acrylic acid was synthesized on top of the cores. Core-shell gels were functionalized with df5 antibody fragments via N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, N-hydroxysuccinimide chemistry, and loaded to release tPA and DNase concentrations of 3 mg/mL and 3 or 30 U/mL, respectively. Plasma from six healthy horses was used to create *ex vivo* fibrin clots with and without neutrophils that were isolated and stimulated with Phorbol 12-myristate 13-acetate to induce NET release. A plate-based clot absorbance assay was performed with FSNs added either during clot formation (endogenously) or after (exogenously).

Results showed that stimulated neutrophils reduced clot degradation by tPA-loaded FSNs. However, adding DNase restored degradation rates to levels comparable to conditions without neutrophils. DNase at 3 U/mL significantly improved fibrinolysis. These findings suggest that DNase-loaded FSNs effectively counteract NET-mediated resistance to fibrinolysis, offering a targeted potential approach to treat immunothrombosis in septic patients.

Funding Source: American Heart Association
Research: Basic Research
Subject Category: Biomedical Engineering

CHARACTERIZATION OF DELAYED RADIATION-INDUCED KIDNEY INJURY IN RHESUS MACAQUES

Carson Copeland[1]: Veterinary Student
John Olson[2], Eric Cohen[3], George Schaaf[2], J. Mark Cline[2]
cncopela@ncsu.edu

[1]North Carolina State University College of Veterinary Medicine, Raleigh, NC; [2]Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC; [3]Department of Medicine, New York University Grossman School of Medicine, New York, NY

The Wake Forest Radiation Late-Effects Cohort (RLEC) has a unique, long-term repository of bloodwork and imaging data for irradiated rhesus macaques (*Macaca mulatta*). The RLEC exhibits a multimorbid phenotype with many effects, including kidney injury, manifesting 3-10 yr after irradiation. The subset of the RLEC used for this retrospective study consists of 26 animals (18 male, 8 female) aged 5.4-10.1 yr (mean 7.9 yr). This group was partial-body irradiated at 2.9-5.1 yr (mean 3.8 yr) with 5% bone marrow sparing at doses of 9-10 Gy (mean 9.7 Gy). These macaques have been under observation for up to 5 yr (mean 3.1 yr) with annual CT scans and semi-annual serum chemistry. They were compared to 7 age-matched unirradiated controls aged 4.8-11.9 yr (mean 7.4 yr). Kidney function post-irradiation was assessed with serum BUN and creatinine, estimated glomerular filtration rate (eGFR), and CT quantification of renal cortical fibrosis. Renal cortical fibrosis was quantified with a previously published method using TeraRecon software. Briefly, mean densities of renal cortex ROIs hand-drawn on coronal CT images were collected in triplicate and averaged. We hypothesized that BUN, creatinine, and renal cortical density would be higher and eGFR would be lower in irradiated animals compared to controls. Irradiated animals had higher BUN ($p=0.004$) and a trend for higher Cr ($p=0.072$) than unirradiated controls. eGFR was lower in irradiated animals compared to controls ($p<0.0001$). Unexpectedly, cortical density was lower in irradiated animals ($p=0.018$). Further research is required to determine the mechanisms of decreased cortical density in irradiated animals.

Funding Sources: This project was supported by NIH/NIAID grants U01 AI150578, U19 AI67798, and T35 OD010946.

Research Type: Basic

Primary Subject Category: Other

THE EFFECTS OF CLOSTRIDIOIDES DIFFICILE TOXIN B ON EQUINE COLONIC MONOLAYERS

Keeley Copper[1]**;** Veterinary Student
Lilly M B Haywood[1], Casey Theriot[1], Breanna J Sheahan[1]
Kacopper@ncsu.edu | 910-639-3572

Affiliations
[1]NCSU, CVM, Raleigh, North Carolina, USA

Abstract

Clostridioides difficile is a major cause of equine enterocolitis, increasing diarrhea-associated mortality 2.7-fold. Its role in nosocomial infections across multiple species highlights its clinical relevance and conserved pathogenic mechanisms. The objective of this study was to examine the effects of *C. difficile* toxin B (TcdB) on equine right dorsal colon (RDC) monolayer cultures. Specifically, we tested the impact of the toxin on 1) barrier permeability, and 2) the structure of tight junction protein Claudin 4. We hypothesized that the application of TcdB to monolayer cultures would increase cell permeability, and degrade the structure of tight junction proteins. RDC monolayers from healthy horses were exposed to TcdB both apically and basally for either 8 hours (n=5) or 24 hours (n=5). Barrier permeability was measured over time via transepithelial electrical resistance (TEER) and further evaluated with a FITC-dextran (4kDa) assay. In situ immunofluorescence was performed using Claudin-4 antibody to label tight junctions. Monolayer cellular structure was compared between control and experimental groups with Hematoxylin and Eosin staining. Preliminary findings revealed that TcdB application increases barrier permeability, disrupts Claudin-4 tight junction structure, and causes cytotoxicity. Eight hours of exposure was less cytotoxic than 24 hours, with higher TEER values and a lower flux of FITC-dextran. Findings from this study will enhance the understanding of *C. difficile* TcdB pathogenicity in horses and lay the groundwork for establishing an in vitro model to identify potential therapies.

Funding Sources
Foundation for the Horse Innovation and Discovery Award (Sheahan B)
Morris Animal Foundation Fellowship (Haywood L)

Subject Category
Basic Research - Equine Gastroenterology

PAIN AND PLEASURE: FACTORS CONTRIBUTING TO ANHEDONIA SCALE RESPONSES AND CORRELATION WITH A NOVEL BEHAVIOR TEST IN DOGS

Maggie Creamer[1,2]: Postdoc
Michelle Stern[1], B. Duncan X. Lascelles[2,3,4,5], Margaret Gruen[1,3]
mlcreame@ncsu.edu [919-802-4864]; mstern@ncsu.edu; Duncan_lascelles@ncsu.edu; megruen@ncsu.edu

[1]Comparative Behavioral Research and Thinking Pets Program, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[2]Translational Research in Pain, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[3]Comparative Pain Research and Education Centre, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[4]Center for Translational Pain Research, Department of Anesthesiology, Duke University, Durham, NC, United States
[5]Thurston Arthritis Center, UNC, Chapel Hill, NC, United States

Chronic pain from osteoarthritis is associated with symptoms of depression in humans, including anhedonia, the inability to experience pleasure. Dogs with osteoarthritis may similarly experience anhedonia. To assess anhedonia in dogs, we developed two novel questionnaires: the Dimensional Anhedonia Ratings Scale for dogs (DARS-d), adapted from the validated scale in humans, and the Canine ANhedonia Scale (CANS). Questionnaires were distributed digitally together with questions about dogs' demographics and environment. We also created a novel behavior test in which dogs could engage with two pleasurable experiences: owner affection and kibble consumption. We collected 146 questionnaire responses and conducted the behavior test with nineteen dog-owner pairs. Preliminary analyses included non-parametric tests for each demographic and environmental variable against questionnaires, and Pearson correlations for questionnaire and behavior measures; p-values were adjusted for multiple comparisons. Contrary to predictions, DARS-d nonsocial ($p<0.1$, trend) and total scores ($p<0.05$) were higher (less anhedonic) for dogs reported to be experiencing pain. Dogs scoring higher on the CANS ($r=0.91$, $p<0.001$) and DARS-d ($r=0.66$, $p<0.05$) consumed more kibble, although all but three dogs ate every kibble offered. Dogs scoring higher on the nonsocial DARS-d subscale spent less time near their owners during the behavior test ($r=-0.60$, $p<0.05$). These results offer some evidence of criterion validity in DARS-d scores when compared with behavior measures, although the assumption that pain would reduce these scores was not supported. Tracking DARS-d scores over osteoarthritis progression or with pain treatment will offer more insight into how chronic pain interacts with measures of anhedonia in dogs.

Funding statement: Research reported in this abstract was supported by NIAMS, NIH, under Award Number R01AR085335. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Clinical Research; Pain

SARS-COV-2 RESPIRATORY CONFINEMENT DOES NOT EXACERBATE MPTP-INDUCED NIGROSTRIATAL DEGRADATION

Hannah Croy: Graduate Student
Emma Hughes, Madison Henderson, Dr. Jeffrey Eells
hecroy@ncsu.edu , elhughe2@ncsu.edu , mmhende2@ncsu.edu , jbeells@ncsu.edu
(724)-766-6557
NCSU CBS, NCSU CVM

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has demonstrated neurotropism and infiltration of the blood brain barrier (BBB), suggesting its possible role in accelerating neurodegenerative diseases, particularly Parkinson's Disease (PD). PD is characterized by the depletion of dopamine neurons (DN) in the substantia nigra pars compacta (SNpc) and the presence of muscle rigidity, declined motor coordination, and tremors. 10-15 years prior to a clinical diagnosis, during the prodromal stage, patients experience symptoms such as depression, muscle weakness, hyposmia, and ageusia, which overlap with symptoms observed in patients with post-acute sequalae of COVID-19 (PASC), where residual symptoms remain 6 weeks post infection. Previous studies have shown infiltration of the SNpc during acute infection, however, the long-term sensitivity of DNs in the absence of BBB disruption remains unknown. To investigate whether peripheral SARS-CoV-2 infection sensitizes SNpc DNs to degeneration, we utilized a mouse model expressing human ACE2 selectively in respiratory epithelium via intranasal administration of a modified AAV2/7-hACE2 vector, which enables respiratory confinement of the virus. Mice were then exposed to SARS-CoV-2 or mock treatment and subsequently given MPTP, a mitochondrial complex I inhibitor known to induce PD-like pathology. Quantification of TH positive neurons in the SNpc across Bregma levels -2.54 to -3.64 revealed no significant difference in DN loss between SARS-CoV-2 MPTP and Mock MPTP groups. These findings suggest that in the absence of direct neuroinvasion or BBB infiltration, peripheral SARS-CoV-2 infection does not exacerbate MPTP-induced nigrostriatal degeneration.

Funding: n/a or NCSU Start-Up
Basic Research
Neurosciences

OCULAR PARAMETERS RELATED TO DRUG DELIVERY IN MARINE MAMMALS

Kaelyn E. Dausch[1]; Veterinary Student
Carmen MH. Colitz[2], Jacklyn H. Salmon[1], Craig A. Harms[1,3], Victoria G. Thayer[1,3,4], Brian C. Gilger[1]
kedausch@ncsu.edu, (919-623-6143)

Affiliations
[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
[2]All Animal Eye Care, Inc, Jupiter, Florida, USA
[3]Center for Marine Sciences and Technology, North Carolina State University, Morehead City, North Carolina, USA
[4]Jennette's Pier, Nags Head, North Carolina, USA

Abstract
Pinniped and cetacean keratopathy (PCK) are the most prevalent ocular abnormalities in pinnipeds and cetaceans under professional care, especially bottlenose dolphins (*Tursiops truncatus*) and California sea lions (*Zalophus californianus*). PCK results in the scarring and opacity of a normally clear cornea, which can cause blindness. Ocular drug-eluting implants have been used in treating PCK. However, the limited published literature on ocular anatomy has hindered the development of implants specifically designed for marine mammals. This study aims to provide a comprehensive dataset on marine mammal ocular parameters, specifically for bottlenose dolphins and California sea lions. It is hypothesized that these data will enhance the understanding of anatomy required for determining effective tissue drug distribution. Ultrasonography was used to image and measure ocular structures, and then the eyes were dissected, and tissue size, volumes, and weights of ocular tissues were measured. This data will then be analyzed separately for males and females, as well as combined for both species. Preliminarily, California sea lions seem to have a larger globe and heavier corneas and uveal tracts, while bottlenose dolphins have a thicker sclera supporting the back of their eye. More samples are required, but members of the Southeast and Western Regional Marine Mammal Stranding Networks are providing samples as they become available. The establishment of normal reference intervals for ocular tissue in marine mammals provides a baseline for exploring future therapies to treat PCK, as well as a more solid foundation for the understanding of the physiology of marine mammal vision.

Funding Source: Office of Naval Research Project ID 538558

Clinical Research

Primary Subject Category: Ophthalmology

ESTABLISHMENT AND CHARACTERIZATION OF PORCINE LYMPHOMA CELL LINES

Hannah Davenport[1] - veterinary student
Kelly-Grace Keen[1], Sitka Eguiluz-Hernandez[1], Michael Rahe[1]
hdavenp@ncsu.edu
757-752-0085

Affiliations
[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Subject category: Immunology – Basic research

Six-month-old market weight pigs are often identified at slaughter due to gross findings of lymphoma. However, very little is known about the characteristics or cause of this neoplasia in such young animals. The objective of this study was to establish a well characterized porcine lymphoma cell line that will allow for enhanced investigation of lymphoma as well as the porcine immune system. At the time of slaughter, the spleen and parotid lymph nodes were collected from three pigs with suspected lymphoma. Single cell suspensions were created for culture and analysis. Isolated neoplastic lymphoid cells from each pig were grown and passaged in cell culture with doubling rates of approximately 48-72 hours. Surface marker staining and flow cytometry indicated the presence of primarily B-cells with rare suspected T-cells in proliferating populations. Fluorescence-activated cell sorting separated these two populations into 96-well plates to propagate distinct monoclonal cell lines. Following successful culture, flow cytometry will be used to confirm and further phenotype monoclonal populations. Further characterization of the cell lines with RNA sequencing can be performed to reveal gene expression patterns and search for potential endogenous retroviruses. Collectively, this work has the potential to be valuable in advancing the understanding of porcine lymphoma while also developing additional tools that may aid in the translational research of cellular immunology and oncology.

Funding sources: NC State University Fluoroscience Endowment, Rahe Startup

RETROSPECTIVE REVIEW OF NEOPLASIA CASES IN NON-DOMESTIC FELIDS

Katarina Dedes[1]**;** Veterinary Student
Leigh Duke[1,2], Brigid Troan[1,2,4], Lisa Abegglen[2,3], Amy Boddy[2,5], Scott H. Harrison[6], Tara M. Harrison[1,2]
kmdedes@ncsu.edu, 856-834-9423

Affiliations
[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
[2]Exotic Species Cancer Research Alliance (ESCRA)
[3]Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah, USA
[4]North Carolina Zoo, Asheboro, North Carolina, USA
[5]Department of Anthropology, University of California, Santa Barabara, California, USA
[6]Department of Biology, North Carolina Agriculture and Technical State University, Greensboro, North Carolina, USA

Abstract
Improved medicine for zoological species has advanced the diagnosis and treatment of neoplasia in non-domestic felids. This study aimed to identify the prevalence and risk factors of neoplasia across felid species using medical records from an online database (Exotic Species Cancer Research Alliance (ESCRA)) and published literature. We evaluated information including tumor type, malignancy, metastasis, species, sex, and treatment. Of the 206 ESCRA cases, the most prevalent species were tigers (22.8%), lions (18.5%), and clouded leopards (15.0%). The most common neoplasms were carcinoma (24.3%) and lymphoma/leukemia (21.4%). Overall, 30.1% of tumors were benign and 69.9% were malignant, with similar cancer prevalence in males (32.0%) and females (31.6%). Among 736 published cases, tigers (32.3%), lions (22.8%), and leopards (9.6%) were most frequently observed. Carcinomas (40.0%) and sarcomas (8.1%) predominated. Of these, 32.2% were benign, 65.6% were malignant, and 2.2% were of unknown malignancy. Females showed a higher prevalence of neoplasia (36.6%) than males (23.6%). Overall, animals with benign tumors were significantly more likely to survive than those with malignant ones. Carcinomas were associated with higher mortality, while melanomas and papillomas correlated with improved survival. Females in the database were more likely to die than males. These findings enhance our understanding of cancer in non-domestic felids and can inform improved surveillance, diagnosis, and treatment protocols to support conservation and welfare of these species.

Clinical Research
Funding Source: NC State University Fluoroscience Endowment
Primary Category of Research: Other (Oncology/Zoological)

THE HOST ASSOCIATED GLYCAN CHONDROITIN SULFATE PROMOTES GROWTH OF FUSOBACTERIUM NUCLEATUM

Jacob Driggers – graduate student

Dr. Joshua R. Fletcher

jhdrigge@ncsu.edu

(919) 880-5816

Affiliations: NCSU CVM

Abstract: Poster Presentation

Colorectal cancer (CRC) is among the most frequently diagnosed cancers with a concerning increase in diagnoses among young people. Annually, 1.8 million new cases with over 800,000 deaths worldwide are attributed to colorectal cancer. Microbiome analyses have identified the putative oncobacterium *Fusobacterium nucleatum* in abundance in polymicrobial biofilms on the surface of CRC tumors. *F. nucleatum* in the gut promotes tumorigenesis in mouse models of CRC, although the underlining mechanisms driving tumorigenesis are unknown. An aspect of cancer biology is increased chondroitin sulfate (CS) proteoglycan abundance on the surface of CRC tumors, which are available as a nutrient source to gut microbes. We sought to investigate the impact of CS on *F. nucleatum* growth and metabolism in a chemically defined media. We determined that chondroitin sulfate increased its growth and significantly altered its transcriptome. CS caused upregulation of genes associated with carbohydrate import and metabolism, transcriptional regulation, and virulence. Genes linked to hydrogen sulfide production, a known carcinogen, were also upregulated when *F. nucleatum* was grown in CS. We conclude that *F. nucleatum* utilizes CS as a carbon source and this influences the expression of virulence genes. In future work, we will make mutants in genes potentially associated with CS acquisition and metabolism to confirm their functions. In parallel, we will use CRISPR to construct *XYLT2* deletions in cancer cell lines to abolish CS biosynthesis. These cells will be infected with *F. nucleatum* to determine if the change in the cell glycome impacts its ability to attach, invade, and stimulate inflammation.

Basic Research

Subject: Infectious Disease

DIRECT ASSESSMENT OF INTESTINAL PERMEABILITY AS A RISK FACTOR FOR MULTIPLE JOINT OSTEOARTHRITIS IN COHORTS OF HUMANS AND PET DOGS

Hiroko Enomoto[1], Staff

Masataka Enomoto[2], Liubov Arbeeva[3], M. Andrea Azcarate-Peril[4], Virginia B. Kraus[3,5], Richard F. Loeser[3,6], Yvonne M. Golightly[3,7], Amanda E. Nelson[3,6], B. Duncan X. Lascelles[2], henomot@ncsu.edu, menonot@ncsu.edu, Liubov_arbeeva@med.unc.edu, azcarate@med.unc.edu, kraus004@duke.edu, Richard_loeser@med.unc.edu, ygolightly@unmc.edu, amanda_nelson@med.unc.edu, dxlascel@ncsu.edu

[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC; [2]Translational Research in Pain and Comparative Pain Research and Education Center, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC USA; [3]Thurston Arthritis Research Center, University of North Carolina, Chapel Hill, NC, USA; [4]Center for Gastrointestinal Biology and Disease (CGIBD), Department of Medicine, Division of Gastroenterology and Hepatology, School of Medicine, UNC Microbiome Core, University of North Carolina, Chapel Hill, NC; [5]Duke Molecular Physiology Institute, Duke University, Durham, NC USA;[6]Division of Rheumatology, Allergy and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [7]College of Allied Health Professions, University of Nebraska Medical Center, Omaha, NE, USA

Abstract

Factors that promote development and worsening of radiographic multiple joint osteoarthritis (rMJOA), defined as involvement of 3+ unique appendicular joint sites, remain unclear. However, increased intestinal permeability (IP) has been proposed as a contributing risk factor. The aim of this study was to determine cross-sectional associations between directly measured IP (through measurement of ingested and excreted sugars in human and measurement of iohexol in dogs) and rMJOA in community cohorts of humans and pet dogs. For IP assessment in humans, 96 patients (21%: had rMJOA) from the Johnston County Health Study ingested 10g lactulose and 5g 12C-mannitol, and sugars excreted in urine were measured at baseline, 3 and 5 hours by using high-performance liquid chromatography (HPLC)–tandem mass spectrometry. Lactulose:mannitol ratio (L:M ratio) was calculated. IP in 110 pet dogs (22% had rMJOA) was assessed by measuring iohexol serum concentrations at baseline, 1, 2 and 4 hour post oral dosing of iohexol at 2.0 mL/kg. The L:M ratio and serum iohexol were compared with and without MJOA and by number of sites with rOA. No associations were observed between increased IP and rMJOA in humans or pet dogs, likely due to limited sample size and cross-sectional data. However, there were significant correlations of IP with lipopolysaccharide and age in human participants with rMJOA. IP assessed using L:M ratio at 5 hours for humans, or total time-integrated serum iohexol levels at 4 hours in dogs was increased when four or more joint sites were involved with OA.

Category: Pain, Clinical research, Funding: This work was funded in part by: R01AR080733; P30AR072580; K24AR081368; U01DP006266; UNC Thurston Arthritis Research Center. We wish to thank the staff and participants in both cohorts, without whom this work would not be possible.

CLOSTRIDIOIDES DIFFICILE TOXINS ALTER HOST BILE ACID AND CHOLESTEROL METABOLISM GENE EXPRESSION IN THE COLONIC EPITHELIUM

Xochilt M. Espinoza Jaen: Undergraduate researcher
Stephanie A. Thomas, Abigail S. Gancz, Casey M. Theriot
xmespino@ncsu.edu
252-214-2016
Basic Research
NCSU CVM, Department of Population Health and Pathobiology, Raleigh, NC
Gastroenterology and or Infectious Diseases

Clostridioides difficile infection (CDI) is a significant public health threat, underscoring the urgent need for novel therapeutics. The nuclear receptor Farnesoid X receptor (FXR) is a promising target, as it regulates bile acid homeostasis by downregulating bile acid synthesis genes and thereby promoting colonization resistance against *C. difficile.* However, how CDI toxins affect FXR signaling and whether host-specific factors influence this response remains unclear. To investigate this, primary colonic epithelial cells derived from three human donors were exposed to *C. difficile* toxins (TcdA, TcdB and TcdA+TcdB). RNA was extracted, converted to cDNA and analyzed by qRT-PCR to assess expression of genes *NR1H4* (FXR), *FGF19*, *NR0B2* (SHP), *FABP6* and *Slc10a2* (ASBT). Gene expression data was normalized to internal expression control, *GAPDH*, followed by normalization of treatment groups to the no treatment control. Statistical analysis using the Shapiro–Wilk test and an unpaired T test ($p < 0.05$) identified significant gene expression changes in two of the three donors. However, linear mixed-effects modeling revealed no overall toxin exposure effect across donors. In contrast, analysis of variance (ANOVA) indicated significant donor-specific differences in toxin responses, suggesting that individual hosts exhibit distinct patterns of bile acid regulation following toxin exposure. These results suggest that *C. difficile* toxins may differentially affect FXR-mediated bile acid regulation depending on host-specific factors. Future studies with additional donors and multi-omic approaches will be needed to determine how this variability influences CDI progression and therapeutic response, and to further elucidate mechanisms linking FXR activity, bile acid metabolism and colonization resistance.

EFFECT OF MDV-1 VACCINES ADMINISTERED ALONE OR WITH HVT ON THE DEVELOPMENT OF THE CHICKEN EMBRYO IMMUNE SYSTEM

Abdelhamid Fares[1] (Graduate Student)

Raveendra Kulkarni[1], Nagwa Khaled[1], Deanna Emanuel[1], Christa Goodell[2], William Stanley[2], Isabel Gimeno[1]

Emails: amfares@ncsu.edu, imgemino@ncsu.edu

Mobile: (984)810-6372

Affiliations: [1]NCSU CVM, [2]Boehringer Ingelheim

In previous studies, we have demonstrated that HVT hastens immunocompetence of chickens when administered in ovo. HVT immunostimulant effect is stronger than that of known adjuvants such as poly I:C. Preliminary studies have also shown that in ovo administration of CVI988 had minor to no adjuvant effect, but it enhanced the transcription of various cytokines when administered together with HVT. In the present study, we have evaluated how in ovo administration of two MDV-1 vaccines (CVI988 and CVI-LTR), alone or in combination with HVT, affects the percentage of activated T cells and macrophages in one-day-old chickens. Our results confirm that HVT strongly activates T cells more efficiently than either CVI988 or CVI-LTR. However, administration of CVI-LTR together with HVT had the strongest immunostimulant effect on activation of CD4+ and CD8α+ T cells, significantly higher than HVT alone. In addition, CVI-LTR and CVI-LTR + HVT, but not other treatments, increased the proportion of γδT CD8αα cells that have been related to cytotoxic ability. A decrease in CD8β and activated CD8β T cells was found in the group vaccinated with CVI-LTR but not in the group vaccinated with CVI-LTR + HVT. Also, the combination of HVT with other vaccines affected vaccine tissue tropism, and the thymus is a target organ for all vaccines. So, our results conclude that administration of CVI-LTR with HVT significantly enhances the adjuvant effect of HVT and can render chickens more immunocompetent at hatch.

Basic research.

Infectious Disease

EXPLORING AN ADAPTED PAIN CATASTROPHIZING PARADIGM FOR USE IN THE NATURALLY OCCURRING CANINE MODEL OF OSTEOARTHRITIS.

Morgan Ferrans (Postdoc)
Liubov Arbeeva, Rachel Caddiell, B. Duncan X. Lascelles, Margaret Gruen
mgferra2@ncsu.edu
(410) 599-5548

Comparative Behavioral Research and Thinking Pets Program, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
Translational Research in Pain, Department of Clinical Sciences, NCSU CVM
Thurston Arthritis Center, UNC Chapel Hill
Comparative Pain Research and Education Centre, Department of Clinical Sciences, NCSU CVM
Center for Translational Pain Research, Department of Anesthesiology, Duke University,

Funding sources: NIAMS, NIH, Award Number R01AR085335
Research Type: Clinical
Subject Category: Pain

Pain catastrophizing, a maladaptive expectation of pain, is linked to poor health outcomes across multiple chronic pain conditions, including osteoarthritis (OA) in humans. Dogs with naturally-occurring OA are considered a good translational model of the human OA condition; however, it is unknown if pain catastrophizing occurs and influences health outcomes in dogs. Quantitative sensory testing (QST) provides a semi-objective framework to assess pain sensitivity and anticipation by measuring latency to withdraw from a hot thermal stimulus. Previous canine catastrophizing paradigms we have explored required dogs to learn associations between a color and location of a testing mat with the presence/absence of heat, involving cognitive processing and associative learning. Here, we report early work using a refined QST paradigm with reduced learning demands. After five acclimation trials, dogs experience ten noxious heat-stimulus trials to build pain expectation. In subsequent test trials, latency to withdraw from a neutral-temperature probe allows for assessment of the extent to which they expect pain (catastrophize) based on prior experience.

Pilot data (n=10; 8 healthy, 2 OA) demonstrate feasibility and differences in withdrawal latency across phases. Preliminary analysis used functional principal components analysis to explore latency profiles following heat exposure, and future work will use trajectory modeling to identify probability-based groupings of latency responses. Our pilot data suggest this methodology offers a practical approach to characterize pain catastrophizing in companion dogs, and present a statistical approach appropriate for future modeling. Further work will compare healthy and OA-pain status and relate pain catastrophizing to analgesic response outcomes.

Title: *IN VITRO* CHARACTERIZATION OF TURKEY *LACTOBACILLUS* STRAINS POSSESSING IMMUNOMODULATORY AND ANTI-*CLOSTRIDUM SEPTICUM* PROPERTIES

Author: **Carissa Gaghan**[1] Category: Graduate Student
Co-Authors: Dr. Anil Thachil[2], Dr. Rodolphe Barrangou[3], Dr. Sarah O'Flaherty[3], Dr. Ravi Kulkarni[1]
Email Addresses: cegaghan@ncsu.edu, anil.thachil@ncagr.gov, rbarran@ncsu.edu, sjoflahe@ncsu.edu, ravi_kulkarni@ncsu.edu
Mobile Number: 860-682-4341
Affiliations: [1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University
[2]Bacteriology and Mycology Division, Rollins Animal Disease Diagnostic Laboratory
[3]Department of Food Bioprocessing and Nutrition Sciences, North Carolina State University

Abstract:
Clostridial Dermatitis (CD), caused by *Clostridium septicum*, is an economically important disease of turkeys, especially in flocks raised without antibiotics. Unfortunately, there are no effective non-antibiotic means currently available to prevent CD in turkeys. In the present study, we aimed at identifying *Lactobacillus* isolates of turkey origin possessing superior immunomodulatory effects to be selected for development of a probiotic-based vaccine. Following isolation of 16 *Lactobacillaceae* strains of various species from healthy turkey intestines, an *in-vitro* avian macrophage assay was used to treat cells with the 16 strains followed by measuring expression of immune genes (IL-1β, IL-6, IL-10, TGFβ) by real-time PCR, MHC-II (antigen-presenting molecule) expression by Flow Cytometry, and Nitric Oxide (NO) production by Griess assay. The results indicated that strains NCKL-11, 22, and 32 showed superior immunomodulatory properties due to an upregulation of immunoregulatory cytokine expression (IL-10, TGFβ) or decreased expression of IL-1β and/or IL-6 when compared to the untreated control. All strains induced increased MHC-II surface expression compared to the control. Furthermore, NO production was higher in cells receiving NCKL-1, 2, 6, 22, and 41 compared to the control. Finally, in determining anti-*C. septicum* property NCKL-22 showed the most significant reduction compared to other isolates. Collectively, our results indicate that NCKL-22 (*L. reuteri*), 11 (*L. salivarius*), and 1 (*L. saerimneri*) possess superior immunomodulatory properties and further work to clone a protective antigen of *C. septicum*, the Non-Toxic Alpha Toxin Domain-2 (ntATX-D2) into these strains is currently underway to develop a probiotic vaccine against CD in turkeys.

Funding Sources: USDA-NIFA
Research: Basic
Primary Subject Category: Immunology

INVESTIGATING THE ROLE OF GLIAL CELLS IN CHEMORESISTANCE IN PANCREATIC CANCER

Mary Kate Gallagher - Veterinary Student, Class of 2028
Elyse Wood, Chloé Mariant, Jason Frye, Laurianne Van Landeghem, PhD
mkgalla2@ncsu.edu
(732)-759-5253
NCSU CVM, NCSU Department of Molecular and Biomedical Sciences

Pancreatic ductal adenocarcinoma is an aggressive disease with patients often presenting with metastatic disease at diagnosis. This limits treatment to systemic chemotherapy and is complicated by the tumor microenvironment and high rates of chemoresistance. The complex interactions between cancer cells, cancer associated fibroblasts, and nerves in the TME may be mediating this resistance. In up to 95% of PDAC tumors, there is evidence of perineural invasion, when tumor cells infiltrate the surrounding nerves. Recent studies have investigated the role of Schwann cells, the principal glial cell of the PNS, in PDAC metastasis, but there is a lack of research on the role of glial cells in chemoresistance. Previous research shows an increased expression of Follistatin-like 3 in colorectal cancer, and current research in our lab is investigating the role of *FSTL3* and chemoresistance in CRC. The objective of this study was to investigate *FSTL3* expression in Schwann cells after chemotherapy treatment. We hypothesized that Schwann cells upregulate *FSTL3* expression after chemotherapy, leading to chemoresistance. Single-cell RNA sequencing data from publicly available datasets confirmed an increase in *FSTL3* expression in Schwann cells post-chemotherapy. To investigate this correlation, we treated S16 cells with high dose 5-FU and quantified *FSTL3* expression by RT-PCR. Preliminary results revealed an increase in *FSTL3* expression after 5-FU compared to control. We also created a protocol to isolate and culture pancreatic Schwann cells from mice to test our hypothesis in primary cells. Findings from this study will help determine how Schwann cells may contribute to chemoresistance in PDAC.

Research grant: R01CA270462
Student support: Stern Translation Cardiac Genetics and Pharmacogenomics Laboratory Fund
Basic research, Gastroenterology

Hemin as the mitochondrial driver of procoagulant platelet phenotypes in dogs with immune-mediated hemolytic anemia

Reagan Glass[1]; Veterinary Student
Avanlene Tan[2], Kylee Long[1], Meg Shaverdian[3], Lunden Simpson[1], Ronald H. L. Li[1]

rbglass@ncsu.edu

[1]Department of Clinical Sciences, NC State CVM, Raleigh, North Carolina
[2]Small Animal Specialist Hospital, Sydney, New South Wales, Australia
[3]Department of Surgery, University of California, San Francisco, San Francisco, California

Canine immune-mediated hemolytic anemia (IMHA) is an autoimmune disease in which red blood cells are destroyed by the host's immune system, leading to systemic hypercoagulability and thromboembolism. Our previous research identified procoagulant platelets in dogs, driven by exogenous heme molecules and platelet agonists like thrombin. However, the exact priming mechanisms by hemin are unclear. We hypothesized that hemin lowers the procoagulant threshold of platelets by potentiating mitochondrial membrane potential (Δψm) loss through mitochondrial reactive oxygen species (mROS) generation. To measure Δψm, platelets from six healthy dogs were loaded with tetramethylrodamine methyl ester (TMRM) before hemin treatment in the presence or absence of thrombin, and convulxin or collagen. To evaluate cytosolic and mitochondrial ROS, platelets were incubated with 2',7'-dichlorodihydrofluorescein diacetate and Mito-Superoxide Indicator, respectively and analyzed by flow cytometry at 0, 3, and 5 minutes after activation. Hemin potentiated platelet Δψm loss upon treatment with thrombin and collagen/convulxin ($p<0.05$). Hemin alone did not induce cytosolic ROS, but it augmented agonist-mediated ROS generation across all time points ($p<0.05$). Furthermore, cytosolic ROS was preceded by instantaneous mitochondrial ROS generation driven primarily by hemin in the presence of strong platelet agonists. Our next step is to delineate this priming mechanism of hemin in platelets by determining if inhibition of mitochondrial ROS driven by hemin can prevent Δψm loss and procoagulant platelet formation. Results of this data will provide necessary insight into the mechanism of hypercoagulability in IMHA dogs in order to develop an effective thromboprophylactic therapy.

Funding Source:
NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Cell Biology and Immunology

QUANTIFYING FIBROFATTY MYOCARDIAL INFILTRATION ACROSS DOG CARDIAC DISEASE PHENOTYPES

Type of research: Basic research

Primary subject category: Clinical medicine

Josue Gonzalez[1], veterinary student
Panchan Sitthicharoenchai, Victor N. Rivas, Sandra M. Losa, Joshua A. Stern
[1]Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
jgonzal9@ncsu.edu jastern@ncsu.edu

Abstract

Myocardial fibrofatty infiltration (FFI) is defined by the accumulation of fibrosis and adipocytes in the myocardium, secondary to myocyte apoptosis and/or progressive proteomic dysregulation. It is largely considered a pathognomonic diagnostic criterion for arrhythmogenic right ventricular cardiomyopathy (ARVC) in histopathology. However, previous studies suggest that FFI is a nonspecific histopathologic finding present in other cardiac pathologies, particularly dilated cardiomyopathy (DCM). This study aimed to evaluate myocardial FFI specificity as a diagnostic marker for ARVC by comparing the degree of FFI across the most common cardiac disease phenotypes in dogs. We hypothesized that FFI is a canonical myocardial remodeling process in response to altered underlying hemodynamic and/or myocardial states, rather than an exclusive feature of ARVC. 2015-2024 necropsy records of NC State University canine patients were queried for specific cardiac diseases (ARVC, DCM, subvalvular aortic stenosis, mitral valve dysplasia, patent ductus arteriosus, pulmonic stenosis, tricuspid valve dysplasia, and myxomatous mitral valve degeneration). Following confirmation of both clinical and gross pathological diagnosis of disease, slides from formalin-fixed paraffin-embedded cardiac tissues were stained with H&E and Masson's Trichrome. Whole slide scanning was performed, and 10 randomly selected fields were analyzed to quantify FFI for each sample. Histologic quantification of FFI is currently in progress. Ultimately, we aim to highlight the specificity of myocardial FFI across eight cardiac diseases and determine the potential need for expanded diagnostic criteria in the post-mortem diagnosis of ARVC.

Funding Source

Stern Translation Cardiac Genetics and Pharmacogenomics Laboratory Fund

OPTIMIZING DNA SEQUENCING FROM ITEMS IN THE ILLEGAL LEATHER TRADE

Beckett Goodnight[1]; Veterinary Student
Natasha Odegard[1], Melissa Scheible[2], and Dr. Kelly Meiklejohn[2]
bgoodni@ncsu.edu
919-538-3018

Affiliations
[1]College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
[2]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Abstract
Leather products can be derived from mammal, reptile, and even fish skins then furnished into items like boots, handbags and belts. Most leather items are made from legal species (e.g., cows, pigs, etc.), but some protected species (e.g., sea turtles, arapaima, etc.) are also used making those items illegal. Thus, determining the exact species used is paramount for prosecution. Due to the chemical and mechanical processes associated with processing leather, the DNA of the source species is heavily degraded and in low quantities. As PCR-based methods yield little success, we applied a new highly sensitive molecular method to sequence full mitochondrial genomes from leather items for species identification – hybridization capture coupled with next-generation sequencing. Using a custom panel designed for over 100 species regularly encountered in the leather trade, we initially tested two pilot sample cohorts: 10 sea turtle leather samples, and 16 naturally degraded or chemically fragmented non-leather samples. Each sample was prepared into a DNA sequencing library, subjected to sequencing, and had their mitochondrial genomes assembled. The assembled genomes were evaluated for quality and compared to references to evaluate their species match. 15/16 of the non-leather samples were correctly matched to their species indicating the myBaits panel could identify a variety of taxa, while none of the leather samples were matched correctly, likely due to competition with the higher quality non-leather samples. From here, the wet-laboratory workflow is being refined and applied to process ~200 unknown leather samples provided by our partner-laboratory (U.S. Fish and Wildlife Service).

Funding Source
U.S. Department of Justice (Award No. 15PNIJ-24-GG-01549-MUMU)
NC State University Office of the Associate Dean for Research and Graduate Studies - Advancing Animal Health and Wellbeing Fund

Research Type
Basic

Primary Subject Category
Genetics

PRELIMINARY QUANTIFICATION OF FUNCTIONAL STRENGTH IN NON-FRAIL DOGS

Curtis Greene; Veterinary Student
Zoe Loh, Eleanor McNamee, Colt Nichols, Yifan Wu, Dave Roberts, Natasha Olby, Alper Bozkurt, Margaret Gruen
cmgreen8@ncsu.edu

Affiliations
Comparative Behavioral Research, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
Department of Electrical and Computer Engineering, College of Engineering, North Carolina State University, Raleigh, North Carolina, USA

Mentor
Dr. Margaret Gruen, DVM, PhD, Comparative Behavioral Research
megruen@ncsu.edu

Abstract

Loss of muscle condition is a characteristic of frailty in dogs, however functional assessments of strength are lacking. As muscle weakness is a key characteristic of frailty in humans, assessment of muscle strength in dogs is of great interest. The objective of this study is to quantify functional strength, represented as pull-force, in both non-frail and frail dogs. We hypothesized that non-frail dogs would exhibit significantly greater pull force than age- and weight-matched frail dogs. Frailty was assessed on a 5-point scale and incorporated factors such as mobility, activity level, and weight loss. Pull-force was quantified by having participants pull toward a food reward while wearing a harness and leash equipped with a tension force sensor tethered to the wall. Three one-minute trials were completed by each dog, and calculated pull force metrics included average pull force, maximum pull force, and kilogram-force. To date, pull-force testing has been completed with 38 dogs ranging in age from 1-14 years and weight from 3.2-52.8 kg. Preliminary results have shown that with increasing age and weight, there is a decline in average pull force in non-frail dogs. In addition, dogs with increasing frailty scores demonstrate lower average and max pull-force values than dogs from the non-frail population. Repeatability will also be assessed in a subset of dogs. These findings contribute normative data for functional strength in aging dogs, aiding clinicians in identifying early neuromuscular decline in geriatric patients.

Funding Source

NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Title:
NEUROLOGIC SIGNS IN DOGS FOLLOWING BEDINVETMAB (LIBRELA™) ADMINISTRATION: A SURVEY OF NEUROLOGISTS

Author:
Summer Grubbs- Veterinary Student
Dr. Natasha Olby, VSP PI
sgrubbs@ncsu.edu

Affiliations:
Department of Neurology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Subject Category:
NEUROSCIENCES

Abstract:
Bedinvetmab (Librela™), a monoclonal antibody targeting nerve growth factor, has become a widely adopted therapeutic option for canine osteoarthritis, particularly in geriatric patients unable to tolerate NSAIDs. While pre-approval and pharmacovigilance studies reported a favorable safety profile, growing clinical use—especially in geriatric patients—has led to concerns about potential neurologic side effects. Reports of ataxia, paraparesis, behavioral changes, and seizures have circulated across clinical settings, but there remains little published data that explores these signs in depth.

A survey was administered to veterinary neurologists via the ACVIM and VIN veterinary neurology listserves. Neurologists were invited to submit data on dogs presented to them for neurologic signs following Bedinvetmab treatment. Information collected included patient signalment, dosing history, time to onset, clinical signs and diagnosis.

There were 32 unique responses, representing dogs ranging in age from 3 to 16 years, with the majority over 10 years old. A wide range of breeds was reported, including German Shepherds, Labradors, Pugs, and Golden Retrievers. The number of Librela doses administered prior to the onset of neurologic signs ranged from 1 to 24, with most cases occurring within 1 to 3 injections. Of the 23 dogs that underwent full diagnostic workups, an alternative diagnosis was identified in 20 (87%).

While these findings do not confirm a causal link between Bedinvetmab and neurologic disease, they underscore the need for continued post-market monitoring—particularly in geriatric dogs or those with underlying neurologic vulnerability. This study adds clinical context to broader surveillance data.

Funding sources

NC State University Fluoroscience Endowment

EVALUATION OF MEDICAL INFRARED THERMOGRAPHY AS A SCREENING TOOL IN DOGS WITH OSTEOARTHRITIS

Amber L. Hall & **Nathan P. Hall** (Undergraduate Students)
Co-Authors: Dr. Melissa Lewis, Dr. B. Duncan X. Lascelles, & Dr. Masataka Enomoto
Emails: alhall6@ncsu.edu, nphall@ncsu.edu, mjlewis@ncsu.edu, dxlascel@ncsu.edu, menomot@ncsu.edu
Mobiles: (984) 484-0702, (919) 946-6246
Affiliation: Translational Research in Pain (TRiP) Program, at NCSU CVM

Abstract:
Infrared thermography non-invasively quantifies body surface temperature. Its potential as a biomarker of joint inflammation or pain in dogs with osteoarthritis (OA) has not been clearly established. This study aimed to examine associations between surface temperature and (1) joint pain, (2) radiographic OA (rOA), and (3) combined clinical OA (SxOA = pain + rOA). Thermal images were prospectively collected from 18 client-owned dogs with radiographic evidence of OA in at least one joint, using a standardized imaging protocol. Fourteen regions of interest (ROI) were created per dog centered over left and right limb joints and the spine and mean joint temperature within the ROI were compared between affected and unaffected joints for pain, rOA, and SxOA using bootstrap estimation of mean difference and linear mixed models. Across 250 joints (rOA=83, SxOA=61), pooled comparisons showed no significant mean temperature differences between affected and unaffected joints for pain (0.21°C [95% confidence interval (CI) −0.39 to 0.81]), rOA (0.41°C [95%CI −0.20 to +1.03]), or SxOA (0.28°C [95%CI −0.34 to +0.92]); effect sizes were minimal (d=0.08–0.12). Body region had a significant effect ($p<0.05$): elbow and stifle ROIs were warmer, and the lumbosacral region cooler than carpus. Thermography was not a reliable indicator of OA status in dogs with mild to moderate disease burden. Substantial variability between dogs and joints suggests anatomic and coat effects may mask detection of disease-related thermal changes. Future work will focus on if thermography can complement gait and clinical assessments to refine predictive models of OA.

Ethics Statement:
This study and all procedures were approved by North Carolina State University Institutional Animal Care and Use Committee (IACUC # 25-203, 22-245). The work was conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Funding Statement: The authors declare that this study received funding from NIH [R01AR080733-01-A1].

Research Type: Clinical
Primary Category: Pain
Presentation Preference: Poster presentation

EVALUATION OF NEOPLASIA ACROSS BEAR SPECIES

Helena L. Harrison, BS[1,2,3*]: *veterinary student*
Elizabeth Duke, DVM,[2,3] Brigid Troan, DVM,[2,3] DACVP, Amy Boddy, PhD, [3,4] Lisa Abegellan, PhD,[5] Tara M. Harrison, DVM, MPVM, Dipl ACZM, Dipl ACVPM, Dipl ECZM (ZHM), CVA[2,3]
Email addresses: hlharri4@ncsu.edu, ecgraebe@ncsu.edu, bvtroan@ncsu.edu, boddy@anth.ucsb.edu, lisa.abegglen@hci.utah.edu, scotth@ncat.edu
Mobile #: 517-489-1254
[1]Beloit College, Beloit, Wisconsin 53511 USA
[2]Department of Clinical Sciences, North Carolina State University, Raleigh, NC 27607 USA
[3]Exotic Species Cancer Research Alliance, North Carolina State University, Raleigh, NC 27607 USA
[4]University of California- Santa Barbara, Santa Barbara, CA 93106 USA
[5]Huntsman Cancer Institute, Salt Lake City, UT 84112 USA

Abstract

Numerous species of bears have had neoplasia reported.[1,2] The purpose of this study was to evaluate the prevalence of neoplasia in bears reported in literature and the Exotic Species Cancer Research Alliance (ESCRA). Cases of histologically confirmed neoplasia from published literature and records from ESCRA database were compared across age, sex, species, diagnosis, tumor behavior, treatment, and survival time. Forty-four cases of neoplasia were found in published literature and 57 cases were found in ESCRA database. Fifteen species of bears were reported in the literature and nine cases were reported in the database. The most commonly reported species with neoplasia in the literature were brown bears (*Ursus arctos*) and sloth bears (*Melursus ursinus*). North American black bears and asiatic black bears (*Ursus thibetanus*) were most common in ESCRA database. There were more females reported in the literature 51.1% (n=23) and more males 43*% (n=22*) in the database. Carcinomas were the most common neoplasia found in the literature, while adenocarcinomas were most common in ESCRA. Most bears were diagnosed at necropsy, however surgery was the most common treatment in the literature. The average survival time post diagnosis was eight months in the literature and one month in ESCRA. This data emphasizes the importance of performing routine medical examinations since many bear cancer cases were diagnosed at death. Through continued collaborations, knowledge of bear neoplasia risk factors can help continue to improve their health and medical care.

Funding Source: N/A
Clinical research
Primary subject category for presentation: Clinical Medicine

TRANSCRIPTOMIC ALTERATIONS IN EQUINE COLONIC MONOLAYERS EXPOSED TO C. DIFFICILE TOXIN B

Lilly Haywood: Graduate Student
Keeley Cooper, Casey Theriot, Breanna Sheahan
lmhaywoo@ncsu.edu
(919) 801-6743
Department of Clinical Sciences, North Carolina State University, College of Veterinary Medicine

Abstract

Clostridioides difficile is an important cause of enterocolitis in the horse. Up to 25% of horses with colitis test positive for *C. difficile;* these horses have a higher mortality rate than horses with non-*C. difficile* diarrhea. In this study, we applied a toxin produced by *C. difficile* (TcdB) for 8 (n=8) or 24 (n=10) hours to equine colonic enteroid monolayers. Tissue collection for monolayer production was from a varied population of healthy horses; each horse served as its own control. We demonstrated a significant reduction in barrier function by 8 hours via disruption of tight junction proteins and induction of apoptosis.

Total RNA was extracted from the above monolayers, and rRNA depletion was performed followed by whole transcriptome sequencing. As expected from a diverse donor population, gene expression varied between individuals. However, there were 10 genes that were consistently and significantly (padj<0.05) differentially expressed between control monolayers and TcdB-treated monolayers at either 8 or 24 hours post-exposure. These include genes involved in mucin production (*MUC6, ONECUT3),* bile acid metabolism (*CYP7B1)*, ion transport (*ATP12A),* cell differentiation and proliferation (*GABPR, HOX9B, SOX17),* epithelial barrier repair (*KRT16)*, and small GTPase activity (*ARGHAP33, RAB6B).* These pathways have been implicated in the pathogenesis of *C. difficile* in humans and other species. Further work is needed to determine the role of these genes in the pathogenesis of *C. difficile* infection in equines. Future directions will focus on determining if these genes and their associated pathways can be manipulated for therapeutic purposes.

Funding Sources:

1. Morris Animal Foundation Equine Training Fellowship - Haywood
2. AAEP Foundation for the Horse - Sheahan

Basic Research - Gastroenterology

QUANTIFICATION OF NEUROINFLAMMATION AND DOPAMINE NEURON SURVIVAL IN A SARS-COV-2

Infectious Disease

Madison M. Henderson[1]; Veterinary student
Hannah E. Croy[2], Emma L. Hughes[2], Jeffrey Eells[1,2]
Mmhende2@ncsu.edu
(802) 477-2786

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

Abstract
Following the COVID-19 pandemic, with emergence of variants and long-term disease, little is known about the long-term effects of infection on the body's immune system and physiological states. There has been a recent occurrence of rapid onset of Parkinson's Disease (PD) symptomology in hospitalized cases of COVID. Early research shows that, following infection, dopamine neurons are more susceptible to MPTP. This neurotoxin damages dopamine neurons and results in elevated levels of inflammatory cytokines in the central nervous system. It is currently unknown whether SARS-COV-2 must cross the blood-brain barrier to initiate prolonged inflammation in the brain. We hypothesized that SARS-COV-2 infection needs to enter the brain to sensitize dopamine neurons and enhance neuroinflammation. To test this hypothesis, we infected mice with SARS-CoV-2, using adeno-associated virus (AAV) to express human angiotensin-converting enzyme 2 in the lung and confine infection. Then, following recovery, treated mice with MPTP. Using immunofluorescence in mouse brain tissue, we analyzed the survival of dopamine neurons within the substantia nigra pars compacta (SNpc) and the corresponding microglial presence throughout the SNpc. Preliminary findings illustrate that dopamine neuron loss was significant when comparing mock-vehicle administration to mock-MPTP and SARS-MPTP, suggesting that SARS-COV-2 infection does not contribute to dopaminergic loss. Microglial presence was only increased in the presence of MPTP. These findings support our hypothesis that SARS-COV-2 infiltration of the blood-brain barrier is essential to induce PD-like pathology for dopaminergic neurons. This study provides further evidence of SARS-COV-2 interactions with the CNS and its impact on neuroinflammation and neurodegenerative disease.

Funding sources
Companion Animal Research Endowment (CARE) for Infectious Disease
American Parkinson's Disease Association Research Grant

DEVELOPING A HIGH-THROUGHPUT MULTI-PARAMETER CHEMICAL SCREEN FOR PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) USING ZEBRAFISH AND A MULTI-CAMERA ARRAY MICROSCOPE

Emma Marie Wilber Hepworth[2-5], Graduate Student

Co-authors: John Efromson[7], Mark Harfouche[7], and Jeffrey A. Yoder[1-6]

Emails: EMWH - ewhepwor@ncsu.edu, JE - john@ramonaoptics.com, MH - mark@ramonaoptics.com, JAY - jayoder@ncsu.edu

EMWH Mobile #: (804) 832-7639

Affiliations: [1]Department of Molecular Biomedical Sciences, [2]Department of Biological Sciences, [3]Center for Environmental and Health Effects of PFAS, [4]Toxicology Program, [5]Genetics and Genomics Academy, [6]Center for Human Health and the Environment, North Carolina State University, Raleigh, NC, USA. [7]Ramona Optics, Durham, NC, USA.

Abstract:

Per- and polyfluoroalkyl substances (PFAS) are a class of chemicals that have become widespread and persistent environmental pollutants due to their unique chemistry and extensive use in consumer products. There is substantial evidence that exposure to PFAS is linked with multiple adverse health effects in humans, such as altered immune function, development, and behavior. Zebrafish (*Danio rerio*) are an excellent model for assessing the impact of chemical exposure on these biological outcomes. For example, previous studies demonstrated that exposing developing zebrafish to the PFAS perfluorohexanoic acid (PFHxA) suppressed the neutrophil respiratory burst, a critical component of the immune response, and led to altered behavior. Due to the vast number of PFAS that lack toxicity data, there is significant need for new high-throughput screens that can assess multiple endpoints over a short period of time. The success of such pipelines will increase our knowledge of PFAS-related toxicological outcomes while reducing the numbers of experimental animals. To develop a chemical screen incorporating immunological, developmental, and behavioral outcomes, we explored strategies to perform multiple targeted assays using the same zebrafish larvae. Initial studies reported here utilized wild-type (AB) and transgenic zebrafish lines: *Tg(NFkB:EGFP)*, which expresses a general marker for immune stimulation, and *Tg(lyz:GFP)*, which expresses fluorescently labeled neutrophils. Larvae were evaluated at multiple timepoints for a range of immunological, morphological, and behavioral endpoints using a multi-camera array microscope (MCAM). This rapid workflow allows for assessment of potential immunotoxic, developmentally toxic, and neurotoxic effects of PFAS, while reducing the number of experimental animals.

Funding Sources: NIH P42-ES031009, NIH T32-ES007046, and acquisition of the MCAM was supported by an Innovation Impact Grant from the North Carolina Biotechnology Center.

Research Category: Basic - Immunology

Title:
The *Xenopus* intestine exhibits left-right asymmetry prior to gut looping events

Authors:
Abbie Wright, staff; and **Taylor Hinnant, Ph.D.**, postdoc
Co-Authors:
Meghan Dillon, & Nanette Nascone-Yoder, Ph.D.
tdhinna2@ncsu.edu, anwrigh7@ncsu.edu, nmnascon@ncsu.edu (919)901-9831
Institutional Affiliation:
Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607

Abstract:

Intestinal malrotation occurs when the normal counterclockwise rotation of the embryonic intestine is disturbed during development. If left untreated, malrotations can result in lifelong digestive issues or death, yet the cause of this common (1 in 500) birth defect is largely unknown. Using a vertebrate model (*Xenopus*) that undergoes an intestinal rotation process similar to humans, we discovered that a right-sided concavity initially forms in the embryonic gut tube between the midgut and hindgut. We hypothesized that this previously unrecognized anatomical left-right (LR) asymmetry plays a role in establishing the chirality of intestinal rotation. We first determined the cellular morphology underlying the formation of this asymmetry using immunohistochemistry to observe cell shapes before, during, and after the formation of the concavity in wildtype animals.The cells on the left and right sides of the hindgut were similar in appearance and size across a symmetrical lumen. However, we found that columnar epithelial cells lined the left gut wall were more rounded, disorganized cells comprised the right wall. Since the process of epithelialization drives the lengthening of the gut tube, these results suggest that the rotational curvature of the intestines is driven by differential elongation of the left and right sides of the gut tube. To determine the relationship between this cellular asymmetry and intestinal curvature, embryos were exposed to chemical compounds known to cause intestinal malrotation (propylthiouracil, SB505124, and atrazine). In such embryos, the concavity was found to be reversed, absent, or in a different region of the gut tube, accompanied by cellular level changes. Likewise, loss of function of the master regulator of LR asymmetry, the transcription factor Pitx2, also leads to malrotated digestive tracts and disrupted cell morphology.This study provides new insight into cellular and molecular events that shape normal intestinal rotation and illuminates the potential etiology of a common birth defect.

This work is funded by grants from the NIH (R01HD117958, R21HD111145) to Dr. Nascone-Yoder.

Basic science research in Developmental and Cell Biology.

MORPHOLOGICAL AND MOLECULAR EFFECTS OF JUN-N-TERMINAL KINASE (JNK) ON LEFT-RIGHT ASYMMETRY IN THE *XENOPUS* STOMACH

Giovanna Horta, Undergraduate

Nanette Nascone-Yoder

gaafonso@ncsu.edu, nmnascon@ncsu.edu

Affiliation: NCSU CVM

Funding resources: National Institutes of Health

Basic Research

Primary subject category: Developmental Biology

Heterotaxy is a congenital defect in which the left-right (LR) asymmetry of the body forms incorrectly, leaving internal organs within the chest and abdomen abnormally shaped or in the wrong position.,., The molecular and cellular mechanisms by which individual organs acquire normal or abnormal LR asymmetries are poorly understood. . To address this question, we used the vertebrate model organism *Xenopus laevis*, focusing on the stomach, which forms a leftward curvature similar to mammals. Our lab previously showed that LR differences in cellular epithelial morphogenesis make the left stomach wall convex and the right wall concave. We exposed *Xenopus* embryos to a small LR molecule inhibitor of Jun-N-terminal kinase (JNK), a signaling pathway which our lab previously found to be associated with epithelial morphogenesis during digestive tract development, at. Our preliminary results show that JNK inhibition eliminates stomach curvature, i.e., embryos develop a straightened stomach. is. . Immunohistochemistry was then used to assess cellular epithelial and extracellular proteins on the left and right sides of the JNK-inhibited stomachs. We found that the spatial organization of the stomach epithelium and levels of extracellular matrix proteins, like laminin, are altered by JNK inhibition in a concentration dependent manner. Our work suggests that JNK signaling is required for the formation of LR asymmetry in the developing stomach and possibly other digestive organs. This project reveals potential underlying molecular and cellular causes of heterotaxy, providing etiological explanations to support families affected by severe birth defects.

Title: SARS-CoV-2 AGE-COORELATED EXACERBATION OF MIDBRAIN DOPAMINE DAMAGE

Emma Hughes, undergraduate

Co-Authors: Hannah Croy, Dr. Jeffrey Eells

Elhughe2@ncsu.edu, hecroy@ncsu.edu, jbeells@ncsu.edu

Mobile: (919) 324-2401

College of Sciences, CVM undergraduate research program

Parkinson's Disease (PD) is a progressive neurodegenerative condition marked by the loss of substantia nigra pars compacta (SNpc) dopamine neurons in the midbrain, resulting in characteristic declining motor coordination and control including resting tremors, muscle rigidity, and impaired balance. Our lab has previously established that SNpc dopamine neurons (DN) are susceptible to infiltration SARS-CoV-2 (Severe Acute Respiratory Coronavirus 2) during the acute phase in transgenic K18-hACE2 mice. Given that aging is an integral factor for DN loss, our study sought to assess how age impacts the degree of dopaminergic degeneration during acute SARS-CoV-2 infection. Researchers administered 1 x 10^3 TCID50 of SARS-CoV-2 USA1 strain intranasally to young (90-120 day) and aged (10-14 months) (K18-hACE2 mice and collected tissue five days post-infection, representative of the height of infection. Histological techniques were utilized to visualize DN via tyrosine hydroxylase staining and astrocyte activation using glial fibrillary acidic protein. When assessing the dopamine neuron population in the midbrain, we anticipated that at five days post-infection, elderly mice would exhibit an additive depletion of dopamine neurons and elevated astrocyte activation as compared to younger mice. These findings would suggest that older adults experiencing acute or prolonged SARS-CoV-2 infection may be more susceptible to severe DN loss, and this disruption to the dopaminergic signaling network would place this population at an elevated risk for both the onset and progression of PD.

Funding: American Parkinson Disease Association

Basic Research Format

Neuroscience

A NONINFERIORITY TRIAL EVALUATING THE EFFICACY OF BEDINVETMAB COMPARED TO GRAPIPRANT FOR OSTEOARTHRITIS-PAIN IN DOGS USING FORCE PLATE GAIT ANALYSIS

Danielle Huston: Staff
Masataka Enomoto[1], Lauren Buslinger[1], Connor Thonen-Fleck[1], Rubia Tomacheuski[1], Kim Love[2], John F. Innes[3], B. Duncan X Lascelles[1]
dthuston@ncsu.edu, menomot@ncsu.edu, ldbuslin@ncsu.edu, cjthonen@ncsu.edu, rmtomach@ncsu.edu, kim@krloveqcc.com, J.F.Innes@liverpool.ac.uk, dxlascel@ncsu.edu
703-869-3467
1. NCSU CVM, 2. K. R. Love Quantitative Consulting and Collaboration, 3. Movement Referrals Veterinary Specialists

Bedinvetmab (Librela™) is a fully caninized monoclonal antibody targeting nerve growth factor (NGF), a key mediator of osteoarthritis (OA) pain in dogs. Its efficacy has not previously been evaluated using force plate gait analysis (FPGA), an objective measure of limb function and pain. This randomized, double-blind, non-inferiority trial aimed to compare bedinvetmab with the nonsteroidal anti-inflammatory drug grapiprant in managing naturally occurring OA. Thirty-two client-owned dogs (>20 kg, ≥1 year) with predominantly single-limb lameness due to hip or stifle OA were randomized 1:1 to receive monthly subcutaneous bedinvetmab (0.5–1.0 mg/kg) plus a daily oral placebo, or daily oral grapiprant (2 mg/kg) plus monthly subcutaneous saline. FPGA, client-reported outcome measures (CROMs), and clinical examinations were performed at screening, baseline (day 0), and every 14 days for 8 weeks. Treatment success was defined as a ≥3.5% increase in peak vertical force (PVF) of the affected limb from baseline, with a non-inferiority margin of 21.25% at day 42. Treatment success occurred in 68.8% of bedinvetmab-treated and 56.3% of grapiprant-treated dogs at day 42 (difference = 12.5%, 90% CI = –37.5, 18.8). Given that the upper bound of this interval (18.8%) was below the non-inferiority margin, bedinvetmab was concluded to be non-inferior to grapiprant. Both groups showed significant improvements in FPGA variables ($p \leq 0.05$) and CROMs ($p \leq 0.05$). Adverse events aligned with label expectations. These findings support both bedinvetmab and grapiprant as appropriate first-line treatments for canine OA pain.

Funding Sources: Zoetis Inc. (NC State Grant # 2023-2676)
Research Type: Clinical
Primary Subject Category: Pain
Ethics Statement: This study and all procedures were approved by North Carolina State University Institutional Animal Care and Use Committee (IACUC # 23-193). The work was conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Presentation Type: Poster

INVESTIGATING CANCER-TESTIS ANTIGENS AS IMMUNOTHERAPY TARGETS FOR PERIPHERAL T-CELL LYMPHOMA

Sekwon Im[1]; Veterinary Student
Jennifer C. Holmes[1]
Ching-Yen Lee[1]
Paul R. Hess[1]
sim4@ncsu.edu
984-683-4432

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

Abstract
Peripheral T-cell lymphoma (PTCL) is a chemoresistant blood cancer of dogs and humans with a poor prognosis due to a small population of residual cells that survive chemotherapy and drive relapse. Vaccine-based immunotherapy may help eliminate these cells, but requires discovery of cancer-specific proteins, or antigens. One promising antigen class are cancer-testis antigens (CTAs)—proteins normally restricted to germ cells but aberrantly expressed in some cancers. Their cancer-exclusive expression limits vaccine-induced autoimmunity, though no effective CTA vaccine has been developed. We analyzed the transcriptome of four canine PTCL biopsies using bulk RNA sequencing and identified 14 genes involved in sperm development/growth. These genes may be reactivated in PTCL due to shared demands of rapid proliferation. Of the 14, six were prioritized based on lack of expression in human somatic tissues. Four were previously studied, with one (CCTA4) used in our prototype anti-PTCL vaccine. To move toward a multivalent vaccine, we investigated the remaining two uncharacterized gametogenesis genes (ENTHD1, SPMAP2), and CCTA4. We hypothesized that these genes may be transiently expressed during T-cell development or proliferation. Using endpoint RT-PCR, we screened 1) immune tissues with diverse T-cell populations, and 2) anti-CD3 agonist antibody-stimulated and unstimulated peripheral T cells. Stimulation was confirmed via flow cytometry (CD25+). All candidate CTAs were absent from immune tissue cDNA and circulating T cell under resting and stimulated conditions, refuting our hypothesis, but supporting their potential as tumor-specific vaccine targets for immunotherapy in dogs and humans with PTCL.

Funding sources
Shadow Whatley Research Fund
NC State University Fluoroscience Endowment
Clinical Research
Immunology

AN INVESTIGATION OF WATER MIXING TEMPERATURE AND ITS IMPACT ON IMMUNOGLOBULIN G CONCENTRATION IN BOVINE COLOSTRUM REPLACER PRODUCTS

Hakeem Jenkins: Staff
Dr. Karina Cirone, Dr. Clieah Kretsch-York, Dr. Lisa Gamsjäger

hjenkin@ncsu.edu
kmcirone@ncsu.edu
cmkretsc@ncsu.edu
lgamsja@ncsu.edu

Mobile: 828-489-4148
Affiliation: NCSU CVM PHP
Clinical research
Immunology

Colostrum replacement products are a practical alternative to fresh maternal colostrum, particularly when calves cannot receive high-quality maternal colostrum. Most powdered products specify an optimal mixing temperature of 100°F–120°F to ensure proper reconstitution, yet producers rarely measure water temperature on-farm. Understanding how deviations from these recommendations affect antibody integrity, especially immunoglobulin G (IgG), is therefore important. The objective of this study was to assess the effect of low, recommended, and high water mixing temperatures on IgG concentrations in bovine colostrum replacers. Twelve commercially available replacer products were obtained in duplicate from two separate batch numbers (n = 24). Each batch was reconstituted at low (75°F), recommended (110°F), and high (130°F) water temperatures. Immunoglobulin G concentrations were determined by radial immunodiffusion and the mean IgG concentration of the two batches was used for analysis. The percent difference from labeled IgG content was calculated and compared among temperatures. The median (range) percent differences for low, recommended, and high temperatures were -1.58% (-31.20 to 8.87%), 0.35% (-23.80 to 49.80%), and 0.66% (-28.40 to 26.40%), respectively. IgG concentrations did not differ substantially among the three temperature groups for most products, indicating that moderate variation in mixing temperature has minimal effect on IgG content. However, in several products, measured IgG concentrations deviated markedly from manufacturer-labeled claims across all temperatures. These findings underscore the need for periodic verification of product IgG content to ensure consistency and quality.

ARGININE VASOPRESSIN STIMULATION FOR DIAGNOSING RELATIVE ADRENAL INSUFFICIENCY IN HORSES

Gabriella Kabakchieva[1]; Veterinary Student

Javier Perez-Quesada DVM[2], Jenna Schirmer[2], Amanda Silva DVM[2], Katarzyna Dembek DVM, PhD, DACVIM[2]

ghkabakc@ncsu.edu

414-218-1264

Affiliations

[1]College of Veterinary Medicine - North Carolina State University, Raleigh, NC

[2]Department of Clinical Sciences - North Carolina State University, Raleigh, NC

Abstract

The hypothalamic-pituitary-adrenal gland axis (HPAA) is the key regulator of the stress response to critical illness. Corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) activate the pituitary gland to release adrenocorticotropic hormone (ACTH), which then stimulates the adrenal gland to produce cortisol. Relative adrenal insufficiency (RAI) occurs in both human and equine patients and refers to the impairment of the HPAA, resulting in decreased cortisol production and increased mortality rates. AVP has been implicated as the primary ACTH-releasing hormone in horses, giving it the potential to assess HPAA function more effectively in this population. The objectives of this study were to assess the use of arginine vasopressin (AVP) and desmopressin (DES) as stimulants of the HPAA in healthy adult horses. We hypothesized that AVP stimulation in healthy horses would result in significant increases in ACTH and cortisol, and that DES would not yield significant increases in ACTH or cortisol. A baseline blood sample was collected from horses before 10 IU of AVP (n=7) or 20 ug of DES (n=3) were administered IV. Additional blood samples were taken at 15, 30, 60, and 90 minutes after stimulation. AVP administration resulted in significant increases in ACTH and cortisol concentrations at 15 minutes and 30 minutes, respectively ($P<0.05$). DES administration didn't cause any significant changes in ACTH or cortisol concentrations ($P>0.05$). Additional sampling and analyses must be performed in critically ill horses. The findings from this study further support that AVP stimulation can be utilized to diagnose RAI more effectively in horses.

Funding sources

NC State University Herbert Benjamin Endowment, Morris Animal Foundation, CVM Intramural Funding

Clinical Research

Subject category: Clinical Medicine

Assessment of the prognostic value of the respiratory rate–oxygenation index in predicting early need for mechanical ventilation in dogs treated with high-flow nasal cannula oxygen therapy

Keon Kim, Postdoc

Céline Pouzot-Nevoret, Jiwoong Her

kkim44@ncsu.edu

+1-(919)-227-9506

NCSU CVM

Abstract

Objective: To determine whether the respiratory rate–oxygenation index (ROX), the modified ROX (ROX-HR), and the SpO_2 to FiO_2 (SF) predict escalation to mechanical ventilation (MV) in dogs treated with high-flow nasal cannula (HFNC).

Methods: This retrospective, post hoc observational study analyzed a combined cohort of dogs with hypoxemic respiratory failure (HRF) from previous studies, excluding euthanized cases. Dogs were classified as HFNC successes or failures. ROX, ROX-HR, and SF were calculated hourly for 7 hours after HFNC initiation. Logistic regression analyses were performed for each predictor and log-likelihood values, profile-likelihood confidence intervals, and AUCs were recorded with corresponding cut-offs.

Results: ROX, ROX-HR, and SF demonstrated acceptable predictive performance for identifying dogs requiring escalation from HFNC to MV. A ROX value < 2.5 at 4 and 5 hours showed excellent discrimination (AUC = 0.80 and 0.85, respectively; sensitivity 72–80%, specificity 78–87%). By 7 hours, ROX-HR demonstrated the highest predictive ability (AUC = 0.87), and the SF ratio also showed strong discrimination (AUC = 0.86) with an optimal cutoff of <134 (sensitivity 93%, specificity 75%).

Conclusions: In this post hoc analysis, ROX, ROX-HR, and SF remained useful predictors of MV escalation within the first 7 hours of HFNC. Prospective studies are warranted to determine whether the decision making guided by these indices improve clinical outcome in dogs undergoing HFNC.

Clinical Relevance: Time-specific cut-offs for ROX, ROX-HR, and SF within the first 7 hours can help distinguish dogs that require MV from those that can be managed with continued HFNC.

Funding Sources:

Clinical research

Clinical Medicine

ASSESSMENT OF OCULAR TOXICITY FOLLOWING CORNEAL APPLICATION OF COLD ATMOSPHERIC PLASMA IN RABBITS USING MULTIMODAL IMAGING

Isabel Ku: Veterinary student

Co-author**:** BC Gilger,[1] D Roberts,[1] JH Salmon,[1] J Thomas,[2] K Stapelmann[2]

Email: idku@ncsu.edu

Mobile number: 3523282088

Affiliation: College of Veterinary Medicine, North Carolina State University;[1] Nuclear Engineering, North Carolina State University.[2]

Research: Basic Ophthalmology

(Background) Cold Atmospheric Plasma (CAP) is a partially ionized gas composed of reactive species, ionized particles, and electromagnetic radiation, generated at room temperature and atmospheric pressure. It has shown potential in antimicrobial applications, including treatment of infectious keratitis. **(Purpose)** To evaluate ocular safety and structural changes following corneal CAP exposure using multimodal imaging in a rabbit model, (**Methods)** CAP (22 kV, 5 min) was applied to the central 6 mm corneal region of the left eye in New Zealand White rabbits (n = 9). Slit-lamp examination, intraocular pressure measurement, fluorescein staining, and multimodal imaging—including color photography, optical coherence tomography (OCT), in vivo confocal microscopy and histology (H&E)—were performed at pre-treatment, immediately post-treatment, 24 hours, 72 hours, and 7 days. (**Results)** CAP-treated eyes demonstrated transient ocular surface irritation, with significantly elevated ocular examination scores immediately and 24 hours post-treatment. Positive fluorescein staining observed in all treated eyes at 24 hours. OCT revealed a transient increase in corneal thickness at 24 hours post-CAP treatment, suggesting reversible edema that resolved by day 3. Histology and in vivo confocal microscopy confirmed focal epithelial disruption immediately after CAP and corneal edema at 24 hours, with preserved stromal and endothelial integrity at all time points and complete structural recovery by day 7. Intraocular pressure remained within the normal range throughout the study. **(Conclusion)** The use of the maximum dose of CAP in normal rabbit eyes resulted in transient changes limited to the corneal epithelium, suggesting that while epithelial disruption may occur, CAP is unlikely to cause other corneal toxicity.

Supported by AQHA Foundation. None.

REEVALUATING DOMESTIC CATS AS RESERVOIR HOSTS: PREVALENCE OF CYTAUXZOON FELIS SEROREACTIVITY IN QPCR-NEGATIVE SAMPLES

Meghan Leber; Veterinary student
Courtney Robertson, Sarah Clarke, Mike Karounos, Katherine Stalford, Barbara Quorollo, Adam Birkenheuer
mleber@ncsu.edu
757-544-8972
NCSU CVM
Subject category: Infectious disease

Cytauxzoon felis is a tick-borne pathogen of wild and domestic cats. Bobcats are the reservoir host and typically do not develop disease. Domestic cats were historically viewed as an aberrant host, suffering uniformly fatal disease. Consequently, there has been limited exploration of antibody testing, under the assumption that the disease course may precede the production of antibodies. However, there is increasing evidence that some domestic cats can undergo asymptomatic infection and serve as reservoir hosts. To determine the prevalence of *C. felis* exposure in cats, we used a *C. felis* IgG Enzyme-Linked Immunosorbent Assay (ELISA) to test 450 qPCR-negative serum samples submitted to a university laboratory. Seroreactivity was assessed by comparing each sample's optical density (OD) to the OD of a pooled positive control (n=4 *C. felis*-infected cats). Four cutoff values for seroreactive percent positivity were evaluated, one previously published and three calculated. Using these cutoffs, the prevalence of seroreactivity was 7.1% (32/450), 5.3% (24/450), 3.3% (15/450), and 2.0% (9/450). These seroreactive samples were submitted from 16/39, 13/39, 12/39, and 8/39 states or provinces, respectively. During the same time period, only 0.39% (2/507) of samples were qPCR positive. Our findings may indicate prior exposure and the potential for chronic infection in domestic cats. This ELISA offers a valuable tool for detecting prior *C. felis* exposure and provides insight into the role of domestic cats in the maintenance of this pathogen, reshaping our understanding of the pathogenesis and epidemiology of *Cytauxzoon felis*.

Funding Sources:
Vector Borne Disease Diagnostic Laboratory
Andy Quattlebaum Companion Animal Research Endowment

GEF-H1 DRIVES CANCER CELL CONTRACTILITY AND MIGRATION IN CONFINED ENVIRONMENTS

Lee, Hannah C.[1, #] Graduate Student
Guilluy, Christophe[1]
hclee6@ncsu.edu, cdguillu@ncsu.edu
[#](610) 804-6730
1- Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA.

Cancer cell metastasis occurs in dense microenvironments, where the size and stiffness of the nucleus present a significant obstacle to migration. Nuclear transit is the cellular process of translocating the nucleus through confined spaces. Determining how cancer cells perform nuclear transit is key to understanding the 3D migration strategies utilized during metastasis. Recent work has demonstrated that during confined migration, cancer cells upregulate actomyosin contractility at the cortex, building pressure to “push” the nucleus through small pores. While the small GTPase RhoA appears to be critical to this contractility-mediated nuclear transit, the mechanism by which RhoA is activated following nuclear confinement remains unknown. Using an in vitro compression system to confine cancer cell nuclei, we identified RhoA-specific regulators whose activity is changed in response to nuclear confinement using biochemical assays followed by mass spectrometry analysis. We identified GEF-H1 as being selectively active in compressed vs. non-compressed cells. As measured by atomic force microscopy, GEF-H1 knockdown (KD) cells exhibited significantly lower contractile responses to confinement compared to control HeLa cells. Using transwell assays, we observed significantly lower rates of transmigration in GEF-H1 KD MDA-MB-231 cells as compared to control. Our data suggests that GEF-H1 is a key mediator of the contractile response required for confined migration of cancer cells. Future work will assess GEF-H1 as a regulator of Rho-mediated nuclear transit using microfluidic migration assays, exploring its potential as a therapeutic target against cancer metastasis.

• This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number R01GM147823 (C.G.), the Goodnight Doctoral Fellowship (H.L.), and the College of Veterinary Medicine at North Carolina State University.
• Basic
• Cell Biology

OPTIMIZING EQUINE INTESTINAL VIABILITY AND INJURY ASSESSMENTS UTILIZING AN EX VIVO PERFUSION PLATFORM

Author: Sydney L. Lierz (Veterinary Student)

Co-Authors: Carla Lopez Cruz, John M. Freund, Liara M. Gonzalez, and Caroline A. McKinney-Aguirre

Email: sllierz@ncsu.edu and camckinn@ncsu.edu

Mobile number: (828)-778-6607

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

ABSTRACT:
Colic remains the leading emergency cause of death in horses. These cases can progress rapidly, leading to intestinal ischemia, necrosis, and systemic compromise, necessitating surgical intervention. A critical challenge during colic surgery is assessing intestinal viability to decide if resection is needed. These decisions directly impact survival and long-term outcomes. However, current diagnostic tools are limited in sensitivity and consistency. To address this, we adapted an ex vivo intestinal perfusion platform (exVIP) to maintain viable equine jejunal segments for up to 6 hours and simulate ischemic injury resembling strangulating colic. This system provides direct access to tissue and blood supply, enabling repeated sampling and therapeutic intervention. We hypothesize that an improved clinical tool to determine intestinal viability can be developed using an exVIP equine model to quantify ischemic injury severity and correlate with perfusion parameters. Jejunal segments from 8 horses with no prior gastrointestinal disease were perfused post-mortem; 4 were maintained under normal conditions, while the remaining 4 underwent controlled ischemic injury. Physiological stability was monitored using blood gases, lactate levels, vascular pressure, and gross appearance. Samples were collected every 3 hours starting at baseline, up to 6 in healthy grafts, and every 10 minutes post-induced injury in ischemic grafts. Injury severity was assessed using modified Chiu-Park (0-5), Hemorrhage (0-4), and Freeman (0-4) scoring systems. Preliminary findings suggest that serosal and mucosal injury scores reflect ischemic severity. Improving intestinal viability assessment may enhance intraoperative decision-making and advance equine colic management.

Funding: NIH R01AI182590

Research: Basic

Primary Subject Category: Gastroenterology

Title: CROSS-SPECIES EXOME SEQUENCING REVEALS RECURRENT GENOMIC ALTERATIONS IN CALIFORNIA SEA LION (*ZALOPHUS CALIFORNIANUS)* UROGENITAL CARCINOMA

Author: **Isabella G. Livingston**, Graduate Student

Co-authors: Catherine F. Wise, Allison N. Dickey, Rachael Thomas, Alissa C. Demming, Barbie Halaska, Ailsa Hall, Frances M.D. Gulland, Kathleen Colegrove, Padraig Duignan, and Matthew Breen

Email: igliving@ncsu.edu

Mobile #: (503)318-6836

Affilitation: Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University ; Genetics and Genomics Academy, North Carolina State University

Abstract: Human-driven environmental change can promote cancer development in wildlife, yet the underpinnings of wildlife cancers remain largely unexplored. Urogenital carcinoma (UGC) in the California sea lion (*Zalophus californianus)* (CSL) represents one of the highest incidences of a single cancer type documented in any mammal. Although UGC pathogenesis is known to be multifactorial, with links to environmental contaminant exposure and infection by Otarine Herpesvirus-1 (OtHV-1), its genomic features have not been explored. There is a need to understand pathogenesis in the CSL and potential implications for the health of related species that share the environment and resources. To address this, we leveraged the genome similarity between the domestic dog and the CSL to perform cross-species whole-exome sequencing (WES) on eight tumor and matched normal pairs. Bioinformatic analyses identified somatic variants and copy number aberrations (CNAs) associated with tumor samples, including highly recurrent variants in the exons of CD274/PD-L1 and TNFRSF14, accompanied by copy number gains in CD274/PD-L1, TNFRSF14, CD200, CDK4, and PLCG2. We used an additional 65 individuals for validation and screened for OtHV-1 DNA. OtHV-1 was detected in 70 of 73 individuals (95.9%), and a recurrent variant in exon 4 of CD274/PD-L1 was identified in 63 of 94 tumors (67.0%). Together, these results demonstrate that cross-species exome capture can effectively resolve genomic alterations in a non-model marine mammal and provide preliminary insights into the molecular pathogenesis of UGC in CSLs, offering valuable comparative insights that could inform studies of cancer across taxa.

Funding Source(s): NA

Is your research Clinical or Basic?: Basic

Primary subject category for presentation: Genetics

EVALUATION OF HYPERCOAGULABILITY, AND PLATELET ACTIVATION IN CATS WITH DIFFERNT MANIFESTATIONS OF CONGESTIVE HEART FAILURE

Kylee Long – Veterinary Student

Leo Ragazzo, Lunden Simpson, Teresa DeFrancesco, Alex Lynch, Robert Goggs, Ronald H. L. Li, NCSU CVM

klong7@ncsu.edu, rhli2@ncsu.edu

717-977-0032

Recent evidence suggests that cats with congestive heart failure (CHF) presenting with pleural effusion (PF) have a lower incidence of cardiogenic arterial thromboembolism compared to those with pulmonary edema (PE). Platelet reactivity has been shown to play a prominent role in hypercoagulability in cats affected with heart disease, yet current antithrombotic guidelines for at-risk cats do not account for CHF manifestations. This study aims to assess fibrinolysis, global coagulation, and compare platelet activation in cats with cardiogenic PF and PE. We hypothesized that cats with PF would have increased fibrinolysis and decreased hypercoagulability with modulated platelet activation compared to those with PE. A total of 26 cats (11 with PF and 15 with PE) were enrolled following echocardiogram. Blood was collected for viscoelastic measurement using VCM Vet and for platelet activation analysis by flow cytometry, assessing changes in P-selectin in response to adenosine diphosphate (ADP), convulxin and thrombin. VCM Vet including clot formation time, maximum clot formation and clot lysis did not differ between groups. Flow cytometry performed in 6 PF and 5 PE cats showed increased platelet reactivity to ADP ($p=0.03$) and thrombin ($p=0.03$) in PF cats when compared to PE cats. PF cats also exhibited more severe heart disease, characterized by higher left atrium-to-aortic root ratio (PF = 2.3 ± 0.3 vs PE = 1.9 ± 0.4, $p=0.05$). Contrary to our hypothesis, cardiomyopathic cats with PF were more hypercoagulable than those with PE, driven by augmented platelet responsiveness. Further studies should investigate fibrinolytic capacity and molecular regulation of fibrinolysis in cardiomyopathic cats with distinct CHF presentations.

Research Grant: EveryCat Foundation
Student Support: Morris Animal Foundation

Clinical Medicine

APPLICATION OF A FRAMEWORK TO MITIGATE THE RISK OF SURGICAL SITE INFECTION AFTER EXPLORATORY CELIOTOMY IN HORSES: A RETROSPECTIVE STUDY.

Carla Lopez Cruz[1], Graduate student (clopezc2@ncsu.edu, Mobile #: 4059207003)

Kate L. Hepworth-Warren[1], Holly A. Roessner[1], Megan Burke[1,] Caroline A. McKinney-Aguirre[1], Liara M. Gonzalez[1] & Callie A. Fogle[2]

North Carolina State University, College of Veterinary Medicine[1]
Clemson University[2]

Surgical Site Infection (SSI) is the most common incisional complication following exploratory celiotomy in horses. Despite preventive efforts, no universal validated protocol exists. Historically, NCSU Veterinary Teaching Hospital had a low SSI rate of 7.5%, but in 2019-2020, clinicians noted an increased in frequency and severity, with some cases appearing more rapidly and some progressing to herniation or peritonitis.

This study aimed to describe the methodology used to identify contributors to the perceived increase in SSI and evaluate the effect of the designed intervention.

A retrospective cohort study was conducted on horses that underwent exploratory celiotomy between 2019-2024. An internal surgical audit was conducted to assess adherence to best practices and identify factors contributing to increased SSI rates, leading to the development of an evidence-based intervention to address procedural deficiencies and incorporate preventative perioperative strategies.

A progressive increase in SSI was identified from 7.7% in 2019 to 29% in 2021. Following protocol implementation, the SSI rate decreased to 2.3% by 2024.

The sustained reduction in SSI supports a direct relationship between the intervention, through which multiple changes were implemented simultaneously, and improved outcomes. Some SSI cases may have been underreported post-discharge, though communication with referring veterinarians was generally reliable.

This study demonstrates that surgical audits serve as critical measurements of quality-of-care, allowing hospitals to identify procedural deficiencies. There is currently no literature that describes structured processes to manage this kind of problem in veterinary medicine. Surgical audit implementation may help other hospitals faced with similar challenges.

Type of research: Clinical
Primary subject category for presentation: Gastroenterology, Other

A COMPREHENSIVE STUDY ON BLOOD CHARACTERISTICS AND HEALTH IN WILD-CAUGHT MAHI-MAHI (*CORYPHAENA HIPPURUS)*

Sydney E. MacDonald: Veterinary Student - Interested in Oral Presentation
Gregory Lewbart VMD, MS, Dipl. ACZM & ECZM (Zoo Health Management), galewbar@ncsu.edu , semacdon@ncsu.edu
(252)-722-2160
NCSU CVM

Abstract:
With a variety of fishing tournaments occurring worldwide each year,fish are captured and stored on ice for periods of time. This study investigates the validity of blood and ocular fluid samples obtained from deceased fish in a tournament setting that have been kept on ice, highlighting a possibility for analysis in future tournament settings. Three objectives were evaluated for this project: (1) How do blood characteristics of wild-caught mahi change over time when an animal is kept on ice for up to 10 hours? (2) Is there validity in the use of blood characteristics gathered from wild-caught marine species that have been deceased and kept on ice for up to 10 hours?(3) How does the biochemical analysis of post-mortem aqueous ocular fluid and blood plasma compare? Postmortem blood samples were collected from wild caught mahi-mahi (*Coryphaena hippurus*) (n=78), and postmortem ocular fluid samples were collected from specimens 23-78 (n=55). A hand held iSTAT® portable analyser (Abbot Point of Care Inc., Princeton, NJ, USA) and Chem8+ cartridge were used to obtain blood parameters [Total carbon dioxide (TCO2), ionized calcium (iCa), Sodium (Na), Potassium (K), Chloride (Cl), glucose (Glu), blood urea nitrogen (BUN), creatine (Crea), hemoglobin (Hgb), hematocrit (Hct)] from whole blood. Results are pending analysis and are expected to highlight the validity of the use of samples collected from deceased specimens that have been on ice for up to 10 hours. The health data created from this work will lay a foundation for future research and provide needed clinical baseline measures.

Funding Sources: Robert Koller Aquatic Animal Medicine Endowment at the CVM
Clinical Research - Clinical Medicine

COMPARATIVE ASSESSMENT OF HEMOPERFUSION DEVICES FOR CARPROFEN REMOVAL IN IN VITRO AND EX VIVO MODELS

Carley-Martin MacFarlane[1]; Veterinary Student
Hobbs K[2], Ueda Y[1], Sheats K[1], Papich MG[3], Her J[1]
cmacfar@ncsu.edu, jher@ncsu.edu

Affiliations
[1]Department of Clinical Sciences, NCSU CVM, Raleigh, NC
[2]Texas A&M College of Veterinary Medicine & Biomedical Sciences, College Station, TX
[3]Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, NC

Clinical Research Category: Clinical Medicine

Abstract
Hemoperfusion is a novel extracorporeal technique for treating nonsteroidal anti-inflammatory drugs (NSAID) intoxications in small animals. Two hemoperfusion devices, activated carbon and synthetic polymer beads, are available, yet limited data exists comparing their efficacy or the influence of intralipid emulsion, which is commonly administered before hemoperfusion. This study aimed to 1) evaluate plasma carprofen concentrations during hemoperfusion, and 2) assess the impact of intralipid on removal efficacy. We hypothesized that both hemoperfusion devices would significantly reduce plasma carprofen and that intralipid would not interfere. Initial in vitro plasma agitation model showed 66% (3,276 mcg/ml to 1,098) carprofen reduction with carbon beads and 47% (2,221 to 1,108) with lipid administration. In an ex vivo hemoperfusion circuit using whole blood, four 500 mL units of whole blood received 240mg of carprofen and then filtered through a carbon column and a polymer bead column for two hours. The carbon device reduced carprofen from 274 to 41 mcg/mL (85%) without lipid and from 268 to 58 mcg/mL (78%) with lipid. The polymer device reduced carprofen from 276 to 78 mcg/mL (72%) without lipid and from 222 to 105 mcg/mL (53%) with lipid. Both devices effectively reduced plasma carprofen concentrations, with the carbon device demonstrating slightly higher removal. Intralipid administration slightly decreased the removal in both devices. Further analyses are ongoing to quantify carprofen and expand these models to other NSAIDs. Findings from this study will help elucidate the efficacy of hemoperfusion for NSAID removal and lay the foundation for clinical application.

Funding Sources
NCSU CVM Extramural Funding Incentive Program
NC State University Herbert Benjamin Endowment

DEVELOPMENT OF AN IN VIVO CAPRINE MODEL FOR STAPHYLOCOCCUS AUREUS MASTITIS IN WOMEN

William Marchal; Veterinary Student
Madelyn Schwartz, Zach Norman, Sarah Harvey, Jennifer Halleran
wmmarcha@ncsu.edu, jlhaller@ncsu.edu
Mobile: (919) 259-1233

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

Abstract
Staphylococcus aureus causes around one-third of lactational mastitis cases in women. However, few studies exist guiding drug treatment due to the challenges of conducting drug trials in lactating women, indicating the need for a suitable animal model. Goats have similar mammary structure and milk composition to humans, but it's unclear if they can be infected with *S. aureus* that causes human mastitis. The objective of this study is to evaluate the goat as a model for studying *Staphylococcus aureus* mastitis (SAM). We hypothesized inoculating the mammary gland of a goat with *S. aureus* isolated from a human mastitis patient would result in clinical signs similar to women with SAM. To accomplish this, the right mammary gland of two does, initially negative for SAM, was inoculated with S. *aureus* from a human mastitis case, while the left mammary gland served as a negative control. Physical exams were performed, and milk samples collected every 12hr over 96hr with an infected mammary gland being defined as erythematous, painful, swollen and/or having abnormally thick, flakey or discolored milk. Milk was cultured to confirm SAM. The goats were humanely euthanized at the conclusion, and their mammary glands harvested for future studies to assess the local inflammatory response. The infected mammary glands displayed some of the clinical signs as described above. This result demonstrates goats can be infected with *S. aureus* form a case of human mastitis, indicating their potential as a viable model for future SAM studies.

Funding sources
Halleran Start Up
NC State University Herbert Benjamin Endowment

Research Type
Clinical

Subject category
Infectious Disease

DETECTION OF A NOVEL HEPATOZOON IN THE UNITED STATES DURING LARGE-SCALE FELINE VECTOR-BORNE ORGANISM PCR PREVALENCE STUDY

Margaret E. Martinez[1] (staff)
Dr. Barbara A. Qurollo[1], Sarah B. Clarke[1], James M. Wilson[1], Charley V. Hall[1]

memart25@ncsu.edu, baqurollo@ncsu.edu, sbclarkle@ncsu.edu, jmccamiewils@gmail.com, chall6@ncsu.edu

[1] Vector-borne Disease Diagnostic Laboratory (VBDDL), Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC

Cats in North America are exposed to vector-borne organisms (VBOs) but are less frequently screened than dogs. Although regional feline VBO studies exist, large-scale data across the United States and Canada are lacking. Using PCR results from a veterinary diagnostic laboratory, we determined the prevalence of VBOs in naturally infected cats from January 2019 through December 2024. Blood samples submitted by veterinarians were tested by PCR for *Anaplasma*, *Apicomplexa*, *Babesia*, *Bartonella*, *Cytauxzoon*, *Ehrlichia*, hemotropic *Mycoplasma*, and *Rickettsia* spp. *Hepatozoon* spp. detected by the *Apicomplexa* assay were confirmed using *Hepatozoon*-specific PCRs and sequencing. A subset of archived samples from the northeastern US was retested after a novel *Hepatozoon* species was initially identified. Of 4,950 feline samples, 918 (18.6%) were PCR positive for at least one VBO. *Mycoplasma haemominutum* (8.7%) and *Bartonella henselae* (5.6%) were the most prevalent. *Hepatozoon* DNA was detected in 29 (0.6%) cats. Sequence alignment of the 18S rRNA gene revealed 26 (90%) cats had a novel *Hepatozoon* species based on paralogue sequences, sharing 97–98% identity with *H. silvestris*, 96–98% with *H. sp* voucher, and 93–95% with *H. felis*. Two (7%) cats had sequences identical to *H. felis*, and one (3%) Texas cat carried a *Hepatozoon* sp. most like *H. sp* ex *Lynx rufus* (96–100%). We report a large-scale feline VBO PCR prevalence study in the US and a novel *Hepatozoon* species in cats in the northeastern US, emphasizing the importance of *Hepatozoon* PCR screening in feline VBO surveillance.

Vector-borne Disease Diagnostic Laboratory

Basic Research

Infectious Diseases

NO SAMPLE LEFT BEHIND: QUALITY IMPROVEMENT PROJECT IN LABORATORY SAMPLE COLLECTION AND PROCESSING AT A VETERINARY TEACHING HOSPITAL

Oral Presentation Submission

Sydney McManus, Veterinary Student

Karen Tefft DVM DACVIM, Roy Hudson DNP BS RN NEA-BC FABC, Lydia Love DVM DACVAA
semcman3@ncsu.edu, ktefft@ncsu.edu, rlhudso3@ncsu.edu, lclove@ncsu.edu

Mobile # - (336) 928-0115

Affiliation - NCSU CVM Terry Center Small Animal Teaching Hospital

Abstract

Diagnostic samples are essential to timely and accurate clinical decision-making in veterinary medicine. At the NC State College of Veterinary Medicine, a patient safety reporting system identified recurring issues with lost biologic samples during after-hours small animal emergency service operations, leading to delays in diagnostic reporting and impacts on patient care.

Following Institutional Review Board approval, a quality improvement project was conducted following the Institute for Healthcare Improvement (IHI) Model for Improvement. Clinical observations and semi-structured interviews were conducted with staff from Emergency, Internal Medicine, and Clinical Laboratory services to map the after-hours diagnostic sample workflow. The resulting process map detailed each step from sample collection through labeling, storage, transfer, and diagnostic reporting.

Hospital approved write-offs of diagnostic sample orders were tracked throughout 2025 as an outcome measure. Analysis revealed that while lost sample errors occurred, the most frequent error types included incorrect test requests, sample handling errors, communication breakdowns, and laboratory processing issues. Several opportunities for process standardization and simplification were identified and implemented between May and July 2025.

Using an iterative, exploratory approach, this project demonstrates how systems-based analysis can identify latent vulnerabilities and drive sustainable improvements in efficiency and effectiveness of patient care.

Funding Source – Terry Center Small Animal Teaching Hospital

Basic Research

Subject Category – Other, Quality Improvement

“OPTIMIZATION OF MACROPHAGE REPORTING ASSAY FOR CATEGORIZING MACROPHAGE RESPONSE TO INSPIRE THERAPY”

Jeffrey Meier, Veterinary Student

Saba Zia, Connor Bryan-Haigler, Ariel Tysver, Michael B. Sano

NCSU CVM (Meier, Tysver), NCSU Molecular Biomedical Sciences (Zia, Sano), NCSU/UNC Lampe Joint Department of Biomedical Engineering (Bryan-Haigler)

Contact: jameier@ncsu.edu, mike_sano@ncsu.edu

Pulsed electrical fields (PEFs) are a promising cancer treatment, capable of creating reversible (RE) or irreversible (IRE) defects in cell membranes depending on the field strength. Integrated time nanosecond pulse irreversible electroporation (INSPIRE) is an IRE protocol using ultrashort (250–2000 ns), alternating polarity pulses. Recent studies suggest INSPIRE can stimulate an anti-tumor immune response, prompting research into identifying specific immune mediators it upregulates. This may support future combinatorial therapies integrating INSPIRE and immunotherapy. To assess macrophage activation by INSPIRE, we developed an assay using RAW-Blue cells (Invivogen), derived from murine RAW 264.7 macrophages. Upon activation, these cells express secreted embryonic alkaline phosphatase (SEAP), which quantifies immune response. Panc02 cells—a murine pancreatic ductal adenocarcinoma line—were treated with different ablation methods (freezing, heating, monopolar IRE, and INSPIRE) and co-cultured at varying ratios with RAW-Blue cells. After 24–72 hours of incubation, SEAP levels were measured. Initial assay results were inconsistent, leading to an investigation of potential sources of variability. Hypothesized factors included inaccuracies in cell counting and pipetting, poor cell viability due to suboptimal growth conditions, and competitive inhibition at high Panc02:RAW-Blue ratios. To improve consistency, we employed multiple cell counting methods and assessed post-incubation viability. This viability data was analyzed alongside SEAP output to determine whether high Panc02 concentrations altered RAW-Blue cell function. These steps aimed to refine the assay’s reliability for evaluating immune responses to INSPIRE treatment.

Research reported in this publication was supported by the National Cancer Institute of the National Institutes of Health under award numbers R01CA276232, R01CA272550, and R41CA275587

Category: Immunology

PREVALENCE OF SUBCLINICAL LAMINITIS IN WORKING SPORT HORSES

Cary Miller[1]; Veterinary student
Kimberly Hallowell[1] Andrew W. van Eps[2], Caitlyn R. Horne[1], Sara R. Tufts[1], Raul J. Bras[3], Kim R. Love[4], Alexa Frink[1], Lauren V. Schnabel[1]
csmille3@ncsu.edu | 804-405-4406

Affiliations
[1]NCSU CVM, [2]New Bolton Center, University of Pennsylvania CVM, [3]Rood and Riddle Equine Hospital, [4]K. R. Love Quantitative Consulting and Collaboration

Laminitis is a debilitating condition in horses, and 34% of horses experience recurrence after their first clinical episode. While severe pain and lameness are characteristic signs, it is suspected that a subset of horses have radiographic evidence of laminitis without concurrent clinical signs, which we define here as subclinical laminitis. The prevalence of subclinical laminitis in sport horse populations is unknown. This prospective observational study evaluated radiographs of 180 sport horses presented to an academic teaching hospital's Orthopedic Surgery and Sports Medicine Service for reasons unrelated to laminitis. Lateral radiographs were taken for all four hooves, and measurements previously found to be effective for diagnosing laminitis were obtained. Measurements included the middle lamellar zone to palmar cortical length ratio (middle-LLZ-PCL ratio), angle of rotation between the third phalanx (P3) and the dorsal hoof wall, and observation of bony changes to P3, including osteophytosis and remodeling. Radiographic evidence of laminitis was present in 56.1% of horses, with an increased middle-LLZ-PCL ratio (>11%) in 27.8%, rotational change (>2 degrees) in 26.1%, and bony change in 27.2%. Two or more types of laminitis-related changes were present in 21.1% of horses. The high prevalence of radiographic evidence of laminitis underscores the importance of obtaining baseline hoof radiographs during routine assessment of equine athletes. The 21.1% of horses with two or more laminitis-related changes may represent a true subclinical laminitis population, and earlier identification of these cases may allow prevention of future clinical laminitic episodes.

Clinical research, subject category: Clinical medicine

Funding Sources
Stipend support for Cary Miller provided by NC State University Herbert Benjamin Endowment. Project funding provided by The Foundation for the Horse Innovation and Discovery Grant. Stipend support for Dr. Hallowell provided by an NIH T32 grant (T32OD011130).

CHARACTERIZING ALTERNATIVE SPLICING NETWORKS IN SOMATOSENSORY NEURONS

Quiana Mosley; Graduate Student

Javier Lopez Soto

Email Addresses: qtmosley@ncsu.edu ; ejlopezs@ncsu.edu

828.333.0633

Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University. NCSU Genetics and Genomics Program.

Gene expression programs provide the essential instructions that guide neuronal development, connectivity, and function. Alternative pre-mRNA splicing enhances the coding capacity of the genome by enabling multiple transcript isoforms to be generated from a single gene. Although alternative splicing is highly prevalent in central nervous system neurons, its contribution to the molecular diversity of peripheral sensory neurons remains poorly understood.

Here, we performed a genome-wide analysis of alternative splicing in genetically defined subtypes of mouse dorsal root ganglion (DRG) sensory neurons. Using publicly available deep RNA-sequencing datasets combined with splicing-sensitive computational analyses, we identified and quantified alternative splicing events across peptidergic and non-peptidergic nociceptors, proprioceptors, and mechanoreceptor subtypes. By mapping sequencing reads to exon–exon junctions, we measured exon inclusion levels and uncovered hundreds of differential splicing events distinguishing sensory neuron classes. Clustering based on splicing profiles recapitulated neuronal subtype relationships observed with global gene expression, indicating that splicing signatures parallel transcriptional identity. Differentially spliced exons were enriched in genes involved in ion channels, morphine addiction, synaptic function, and endocannabinoid signaling pathways.

Collectively, these findings reveal that extensive alternative splicing underlies molecular specialization among DRG sensory neurons. By shaping ion channel and synaptic gene isoform composition, alternative splicing likely contributes to the intrinsic excitability and functional diversity of sensory neuron populations. This study highlights transcript diversification as a fundamental mechanism of peripheral neuronal identity and plasticity.

R00NS116123 (NIH-NINDS).

Basic Research

Genetics

CHARACTERIZATION OF A BILE SALT HYDROLASE FROM COMMMENSAL BACTERIUM *CLOSTRIDIUM HIRANONIS*

Damian Nikolov: Undergraduate Researcher
Stephanie Thomas, Noah S. Lyons, Casey M. Theriot
danikolo@ncsu.edu
704-689-2572
Basic Research
NCSU CVM, Department of Population Health and Pathobiology, Raleigh, NC
Gastroenterology and or Infectious Disease

Clostridioides difficile is a Gram-positive, spore-forming bacterium that is responsible for *C. difficile* infection (CDI) and is considered a major threat to public health with 500,000 cases and 29,000 deaths per year. Primary bile acids (BAs), such as cholate (CA) and chenodeoxycholate (CDCA), are synthesized in the host liver, and members of the gut microbiome encode enzymes that can modify and diversify the BA pool. Bile salt hydrolases (BSHs) are enzymes that many bacteria encode to deconjugate conjugated BAs like taurocholate (TCA) and glycocholate (GCA), releasing the free amino acids from the BA core of CA. Many microbially derived BAs, such as deoxycholate (DCA) can inhibit *C. difficile* growth and toxin activity. *Clostridium hiranonis,* a Gram-positive, spore-forming commensal bacterium, has shown promising inhibition of *C. difficile* virulence*. C. hiranonis* encodes a BSH, but there is no data on this enzyme's BA specificity. To determine the role of the *C. hiranonis* BSH in *C. difficile* resistance, we recombinantly made and purified the enzyme in *E. coli*. Using a ninhydrin enzyme assay, we defined the substrate specificity of *C. hiranonis* BSH with the following BAs: TCA, TCDCA, TDCA, GCA, GCDCA, and GDCA. Our results show that *C. hiranonis* BSH is active on all BAs tested but is twice as active with glycine-conjugated BAs than taurine-conjugated BAs. The *C. hiranonis* BSH could be important in the gut as it can release the CA from GCA, and subsequently convert CA to DCA via the *bai* operon, which is important for pathogen resistance.

DESIGNING AND OPTIMIZING AN STR PANEL FOR TURKEY INDIVIDUALIZATION TO SUPPORT THE NC WILDLIFE RESOURCES COMMISSION

Natasha Odegard[1] – Veterinary student

Beckett Goodnight[1], Melissa Scheible[2], Kelly A. Meiklejohn[2]

nmodegar@ncsu.edu, bgoodni@ncsu.edu, mkscheib@ncsu.edu, kameikle@ncsu.edu
435-680-0032

Affiliations:
[1]NCSU CVM
[2] Population Health and Pathobiology, NCSU CVM

Wild turkeys (*Meleagris gallopavo*) are a valuable natural resource in North Carolina, managed by the North Carolina Wildlife Resources Commission (NCWRC). Due to past unregulated hunting and habitat loss, turkey populations once declined dramatically but have since rebounded through extensive restoration efforts. Today, harvests are legally restricted to one bird per day and two per season. Recently, however, NCWRC has reported an increase in suspected overharvesting cases, prompting a need for DNA-based tools to support forensic investigations. These tools would enable (a) individual matching of biological samples (e.g., associating meat or feathers found in a suspect's possession with a harvested bird), and (b) determination of the number of individuals present in mixed biological samples. Short tandem repeats (STRs) – repeating sequences of 2–8 nucleotides within the nuclear genome – are commonly used for both human and wildlife forensic individualization. Currently, there is not an assay that permits genotyping of multiple STRs for turkeys. However, the Tennessee Wildlife Resources Agency utilizes 13 STR markers for turkey identification, where each is genotyped individually, which significantly limits efficiency and throughput. To address this knowledge gap, we hypothesized that existing STR markers from Tennessee could be combined into a reliable multiplex STR panel to streamline genotyping. Preliminary results demonstrate robust single-locus amplification using samples from NC wild turkeys and optimization of a multiplex consisting of 9 markers has commenced. This work supports the development of a forensically validated STR panel for turkey individualization to aid NCWRC enforcement and long-term conservation efforts.

Funding Source: NC State University Herbert Benjamin Endowment, Meiklejohn Laboratory Salary Release.

Primary Subject Category: Genetics

THE IMPACT OF CHOLINE SUPPLEMENTATION ON CELL PROLIFERATION AND BARRIER FUNCTION OF EQUINE COLONIC EPITHELIUM

Mary O'Neill[1]: Veterinary Student
Hedaya S Megeed[1], Breanna J Sheahan[1]
mkoneill@ncsu.edu | 440-591-9791

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

Horses that are hospitalized for inflammation of the colon, or colitis, have a 20-50% mortality rate. Death can occur secondary to bacterial translocation across damaged epithelium. Preliminary data suggests that horses with acute undifferentiated colitis exhibit significant downregulation of *SLC5A7,* which encodes for choline transporter, CHT1, in colonic mucosa and submucosa. Choline is the precursor for acetylcholine and phosphatidylcholine in epithelial cells, which are critical for cellular signaling, proliferation, and membrane function. Although choline's uptake, metabolism, and functions are well-characterized in neurons, its role in colonic epithelium is less understood. We hypothesized that choline availability positively affects cellular proliferation and barrier function in equine colonic epithelium. The first objective was to optimize media conditions with minimal choline availability. It was found that 5% Fetal Bovine Serum was required to produce confluent monolayers when using commercial CMRL media (choline-free) with growth-factor supplementation. The second objective was to test the effect of choline availability on cellular proliferation and barrier function in right dorsal colon (RDC) monolayers from healthy horses (n=4). RDC monolayers were grown in media with varying choline concentrations, as well as with a choline uptake inhibitor, hemicholinium-3. Monolayers were tested for cell viability (resazurin assay) and barrier permeability (FITC-dextran 4 kDa). Immunofluorescence was performed for Ki67 (cell proliferation marker) and propargyl choline (choline uptake visualization). We determined that choline supplementation increases cellular proliferation and barrier formation. Choline may offer a therapy in colitis cases by encouraging epithelial barrier recovery, thereby limiting bacterial translocation.

Funding Sources
Boehringer-Ingelheim Veterinary Scholars Program
Sheahan start up funds

Subject Category
Equine Gastroenterology - Basic Research

EFFECTS OF ANTI-THROMBOTICS ON NET-MEDIATED CLOTTING IN HORSES

Aubrey O'Quinn[2,3], undergraduate
Dr. Megan Palmisano[1,2,3], Halle Cantor[1,2,3,4], Elizabeth Byrnes[2,4], Dr. Ashley Brown[2,4], Dr. Michael Daniele[2,4], and Dr. Rosemary Bayless[1,2,3]
amoquinn@ncsu.edu, mgpalmis@ncsu.edu, rlbayles@ncsu.edu
Phone number: 910-658-2994 (Aubrey O'Quinn), 973-818-4560 (Megan Palmisano)
Affiliations: [1]NCSU CVM, [2]CMI, [3]Molecular Biomedical Science, [4]College of Engineering

Abstract:
Introduction: Dysfunctions in hemostasis can lead to life-threatening complications in veterinary and human patients. In inflammatory conditions, neutrophils promote platelet activation and thrombosis through the release of neutrophil extracellular traps (NETs). Our project investigates the effects of clinically relevant anti-thrombotic agents on *ex vivo* NET-mediated thrombosis in equine blood. We hypothesize that NET-mediated clots are resistant to fibrinolysis and anti-thrombotic agents have increased efficacy in control blood compared to neutrophil-stimulated blood.
Materials/Methods: Blood was collected from 10 healthy horses using sodium citrate anticoagulant. Whole blood was stimulated with phorbol 12-myristate 13-acetate (PMA) to induce NET release or left unstimulated. Following a 4-hour stimulation period, plasma containing platelets and white blood cells was treated with anti-thrombotic agents, including tissue plasminogen activator (tPA), antithrombin III (ATIII), clopidogrel, enoxaparin, aspirin, or the vehicle control (VC). Plate-based clot absorbance assays and confocal microscopy were performed to assess prophylactic anticoagulant use. Analysis was performed using ImageJ and JMP.
Results: Treatment with tPA in unstimulated clots resulted in significant degradation ($P=0.0013$). In stimulated samples, the effects of tPA on clot degradation were blunted. Using confocal microscopy, all treatments led to a decrease in clot fiber density when compared to the VC. When stimulated, clots treated with ATIII, clopidogrel, and aspirin no longer degraded and continued to polymerize after four hours.
Discussion: Treatment with commonly used anti-thrombotics are less efficient in NET-mediated thrombosis. The results from this study show that improved therapeutic options are needed for equine patients with inflammatory conditions and secondary coagulopathies.

Funding source: The American Heart Association
Clinical research
Immunology

ATR INHIBITION COMBINED WITH RADIATION THERAPY FOR THE TREATMENT OF FELINE ORAL SQUAMOUS CELL CARCINOMA

Efstathia Palyvou, Research Fellow

Madison Ravotti[2], Joseph Veliz[2], Thitsana Ingkasri[1], Faihaa Ahmed[1], Eda Koseli[1], Amanda R. Thomas[2], Julianne L. Holleran[2], Jianxia Guo[2], Robert Parise[2], Kyle G. Mathews[1], Jan H. Beumer[3], Yvonne M. Mowery[4] and Michael W. Nolan[1]

epalyvo@ncsu.edu, mwnolan@ncsu.edu

#919-937-8477

NC State University, Raleigh, NC[1]; University of Pittsburgh, Pittsburgh, PA[2]; Johns Hopkins University, Baltimore, MD[3]; and UPMC Hillman Cancer Center, Pittsburgh, PA[4]

Radiotherapy (RT) is used for palliation of feline oral squamous cell carcinoma (FOSCC), but prolonged tumor responses are rare and improved treatment approaches are needed. The objective of the study was to evaluate the radiosensitizing effects of the ataxia telangiectasia and Rad3-related (ATR) inhibitor camonsertib in FOSCC. The CCK8 assay and FOSCC cell lines (SCCF3 and SCCF1) were used to assess the impact of camonsertib on cell proliferation, with and without 4 Gy radiation. The pharmacokinetics and maximum tolerated dose (MTD) of camonsertib were characterized in three healthy cats. Later, a pilot clinical trial enrolled 6 tumor-bearing cats to evaluate the combination of camonsertib and RT. Camonsertib reduced cell viability in both FOSCC lines, with 35-55% greater inhibition upon addition of radiation. The dose-limiting toxicities of camonsertib were neutropenia and elevated rectal temperature. The MTD was 5 mg/kg given subcutaneously, with bioavailability of 167%, maximum concentration at 2-to-4 hours, and a half-life of 2 hours. In clinical trial, cats treated with RT alone (8 Gy × 3, 72-hour interfraction interval) experienced no acute radiotoxicity, whereas cats receiving RT plus camonsertib (SC, 90 min pre-RT) developed grade 2-to-3 acute oral mucositis and dermatitis. Four of six cats had a measurable response to RT, and the addition of camonsertib was associated with prolonged progression-free survival (median of 58 days versus undefined, Log-Rank P= 0.058), but no obvious change in overall survival (median 131 versus 139 days, P= 0.43). This study provides preliminary evidence that camonsertib enhances radiosensitivity in cats with FOSCC.

Founding Source: EveryCat Health Foundation

Primary subject category for presentation: Clinical Research Comparative Oncology

FUNCTIONAL CHARACTERIZATION OF GLYCANS ON HIV-1 ENVELOPE GLYCOPROTEIN TRIMER

Anuja Pimplapure (Graduate Student)

Noah Guertin, Amit Sharma

apimpla@ncsu.edu, njguerti@ncsu.edu, asharma66@ncsu.edu

HIV-1 Envelope (Env) glycoprotein trimer is the only viral protein on the outer surface of the viral membrane that plays an integral part in viral entry. Env is a heavily glycosylated protein with ~25 to 30 N-linked glycosylation sites per trimer. These glycans aid in the proper folding and stability of the env structure, which influences HIV infectivity and shielding of immunogenic epitopes. In Transmitted/Founder (T/F) HIV-1 variants that initiate infection, fewer Env-specific glycans are present, with their functions yet to be characterized. The functional characterization of T/F Env glycans could facilitate the rational design of improved rhesus macaque models to study HIV-1 transmission and pathogenesis. We used the HIV-1 BG505 strain, a subtype A T/F virus, to generate a library with individual glycan site deletions in the Env trimer through substitution of alanine (A) with asparagine (N). The loss of glycan at site 197 and 262 led to 10-fold decrease in virus infectivity. Mechanistically, virus entry in the cells expressing human CD4 and CCR5 receptor was not affected; however, env incorporation in the virion was reduced with glycan loss at 262. In contrast, in cells expressing the rhesus macaque CD4 receptor and the CCR5 coreceptor, the loss of an Env-specific glycan at position 301 resulted in a dramatic increase in viral entry. Overall, our study emphasizes the importance of Env-specific glycans for HIV-1 infectivity and elucidates a potential role of glycan in inhibiting cross-species receptor utilization and transmission.

Primary Subject Category – Infectious disease

FUNCTIONAL CHARACTERIZATION OF GLYCANS ON HIV-1 ENVELOPE GLYCOPROTEIN TRIMER

Anuja Pimplapure (Graduate Student)

Noah Guertin, Amit Sharma

apimpla@ncsu.edu, njguerti@ncsu.edu, asharma66@ncsu.edu

HIV-1 Envelope (Env) glycoprotein trimer is the only viral protein on the outer surface of the viral membrane that plays an integral part in viral entry. Env is heavily glycosylated protein with ~25 to 30 N-linked glycosylation sites per trimer. These glycans aid in the proper folding and stability of the env structure, which influences HIV infectivity and shielding of immunogenic epitopes. In Transmitted/Founder (T/F) HIV-1 variants that initiate infection, fewer Env-specific glycans are present, with their functions yet to be characterized. The functional characterization of T/F Env glycans could facilitate the rational design of improved rhesus macaque models to study HIV-1 transmission and pathogenesis. We used the HIV-1 BG505 strain, a subtype A T/F virus, to generate a library with individual glycan site deletions in the Env trimer through substitution of alanine (A) with asparagine (N). The loss of glycan at site 197 and 262 led to 10-fold decrease in virus infectivity. Mechanistically, virus entry in the cells expressing humans CD4 and CCR5 receptor was not unaffected, however, env incorporation in the virion was reduced with glycan loss at 262. In contrast, in cells expressing the rhesus macaque CD4 receptor and CCR5 coreceptor, the loss of an Env-specific glycan at position 301 led to a dramatic increase in viral entry. Overall, our study emphasizes the importance of Env-specific glycans for HIV-1 infectivity and elucidates a potential role of glycan in inhibiting cross-species receptor utilization and transmission.

Primary Subject Category – Infectious disease

RESOLUTION KINETICS OF MIXED ALLERGENS-INDUCED ALLERGIC AIRWAY INFLAMMATION AND AIRWAY REMODELING

Rekha K C*: Graduate Student
Ishita Choudhary*, Sonika Patial[$], and Yogesh Saini*
rkc2@ncsu.edu, 225-288-4364
*Department of Population Health and Pathobiology, CVM NCSU, Raleigh, NC
[$]Division of Translational Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, Durham, NC

Allergic asthma is characterized by chronic airway inflammation, which when fails to resolve leads to a progressive decline of lung function. Resolution of inflammation is considered an active cellular and biochemical process, and it is crucial to understand the kinetics of resolution of airway inflammation and further investigate the cellular and molecular pathways involved in the resolution process. Accordingly, we intranasally challenged 8-week-old C57BL/6 mice with mixed allergens (MA) or normal saline (NS), three times a week for four weeks and examined the endpoints at 16h, 3d, 7d, 14d, 21d, and 42d post-last challenge. At 16h time-point, MA-challenged mice exhibited all the hallmark features of allergic asthma, including eosinophilic/lymphocytic cell infiltration, production of Th2 cytokines/chemokines, and mucous cell metaplasia (MCM). At 3d time-point and onwards, the inflammatory mediators including IL-4, IL-5, Eotaxin-1, Eotaxin-2, RANTES, MIP-1α, TARC, and MDC decreased in bronchoalveolar lavage fluid (BALF) and gradually returned to the baseline. Eosinophilic and lymphocytic infiltration significantly reduced at post challenge phase. MA-induced MCM subsided gradually during post challenge phase. The levels of specialized pro-resolving mediators (SPMs) such as maresins, protectins, resolvins and their lipid precursors were increased during post-challenge phase. These SPMs decreased at later timepoints during post-challenge phase when inflammatory markers were ameliorated, suggesting the role of SPMs in mediating resolution of MA-induced chronic inflammation. Overall, this study delineates the resolution kinetics of chronic allergic airway inflammation in MA-induced allergic asthma.

Funded by: NIEHS R01 (R01ES035439)
Primary subject category: Immunology and Toxicology

INHIBITION OF GLYCOGEN SYNASE KINASE 3β (GSK3β) SUPPRESSES HSV-1 REPLICATION IN EPITHELIAL CELLS

Jiayi Ren[a] (Graduate Student)

Kasturi Chandra[a], Divya Kinha[a], Amol Suryawanshi[a, *]

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

assuryaw@ncsu.edu, jren9@ncsu.edu, kchandr9@ncsu.edu, fdivya@ncsu.edu

Herpes simplex virus 1 (HSV-1) is a highly successful and ubiquitous human pathogen that primarily infects the epithelial cells of the oral-labial region and establishes life-long latent infection in the trigeminal ganglion. The HSV-1 reactivation and recurrence at the corneal epithelial surface can lead to the development of chronic immuno-inflammatory and vision-impairing disease called herpetic stromal keratitis (HSK). The current HSK treatments targeting inflammation or virus replication are partially effective and promote HSV-1 latency, and long-term use can cause side effects and the emergence of drug-resistant HSV-1 strains. Therefore, there is an urgent and unmet clinical need to develop novel, safe, and effective immunotherapies that selectively induce antiviral responses and suppress inflammation to prevent and treat HSK and permanent blindness caused by multidrug-resistant HSV-1 strains. In this study, we investigated whether targeting glycogen synthase kinase 3 (GSK3) enzyme, a known regulator of the inflammatory process in immune cells, regulates HSV-1 replication and anti-inflammatory responses. Using human corneal epithelial cell lines (HCECs), we demonstrate that a GSK3β inhibitor suppresses HSV-1 replication and reduces the expression of acyclovir-resistant HSV-1 proteins at 16 hours post-infection. Furthermore, GSK3β inhibition increases the expression of type III interferon-dependent interferon-stimulated genes (ISGs), such as ISG-15, MX-1, and OAS, in HSV-1-infected cells. Overall, our data indicate that targeting GSK3β using small molecule inhibitors can suppress HSV-1 replication by enhancing ISG production and represents a promising therapeutic approach to treat recurrent corneal HSV-1 infection and HSK progression.

Funding Source: NCSU CVM Startup fund, National Eye Institute Grant EY034495 (R01)
Research Category: Basic
Subject Category: Immunology

FROM BEHAVIOR TO CIRCUITS: UNCOVERING THE EMERGENCE OF VALUE PERCEPTION IN THE DEVELOPING MOUSE BRAIN

Elizabeth Rexroat[1], Veterinary Student
Mehdi Aliomrani[1], Yasaman Ghaemi[1], Ryan Garry[2], Razi Sayyed[1], Swaranjali Jadhav[1], Jinhyun Ahn[2], Alon Greenbaum[2] and Troy Ghashghaei[1]
erexroa@ncsu.edu

Affiliations

[1]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA
[2]Joint Department of Biomedical Engineering, North Carolina State University, Raleigh, North Carolina, USA and University of North Carolina, Chapel Hill, North Carolina, USA

Abstract
Disruptions in the development of neural networks that encode value (valence) are thought to underlie a range of behavioral deficits in animals and humans, including aggression and anxiety, yet the precise structure and function of aversive circuits remain incompletely mapped. Here, we examined how developmental defects in *Nes:Egfr-null* mice, lacking EGFR in the brain, affect behavioral and neural responses to novel appetitive and aversive stimuli. Mice were exposed to either a cookie (appetitive) or Trimethylthiazoline (TMT, a predator odor), and behavior was monitored using AI-assisted video analysis. *Nes:Egfr-null* mice showed significantly reduced freezing to TMT compared to controls. Brains were collected post-exposure, sectioned, and immunostained for CFOS, an immediate early gene used as a proxy for neuronal activation. Brain-wide CFOS mapping was performed using a high-throughput, AI-assisted pipeline developed in the Ghashghaei lab, which allows for detailed quantification and anatomical registration of CFOS expression across the entire brain. Quantitative comparisons between *Nes:Egfr-null* and control brains revealed significant region-specific differences in CFOS activity in the gene-deleted mice, aligning with their blunted behavioral responses ($P < 0.05$, Kruskal-Wallis with Dunn's post hoc test). These findings support the use of TMT exposure combined with CFOS mapping as a reliable and scalable strategy to understand the neural circuits involved in aversive behavior. This approach may prove useful for identifying circuit-level vulnerabilities in disease models and evaluating the effects of environmental or pharmacological influences on aversive processing.

Funding Sources
NC State University Fluoroscience Endowment
NIH R56NS117019-01A1, R01NS134593-01A1, R21NS129093

Subject Category
Basic Neurosciences

COMPARATIVE EVALUATION OF RADIOGRAPHIC AND ULTRASONOGRAPHIC IMAGING IN PULLETS

Ripper M.[1], Veterinary Student

I. Poudel[2], R. Wysocky[2], A. Frias-De-Diego[1], K. E. Anderson[2] and R. Crespo[1].

maripper@ncsu.edu, ipoudel@ncsu.edu, becca_wysocky@ncsu.edu, afrias@tntech.edu (current), kanderson@ncsu.edu, rcrespo@ncsu.edu,

[1]College of Veterinary Medicine, Department of Population Health and Pathobiology; [2]College of Agricultural Sciences, Prestage Department of Poultry Science, North Carolina State University

This study evaluated bone density and dimensions, and keel fractures by radiography and ultrasound in post-peak production laying hen. Nine hundred and sixty, 50-wk-old hens were randomly divided into two dietary groups supplemented with phytase at 0 or 1000 FYT/g. Each of these groups was further subdivided into 3 groups receiving Azomite® supplementation at 0%, 0.25% and 0.5%. Each experimental unit consisted of 32 hens, with 5 replicates per treatment. At 75 weeks of age, one hen from each replicate was randomly sampled for radiographs and ultrasound images of the keel and the left tibiotarsus. Radiographs were obtained in lateral and ventrodorsal projections. Ultrasound was performed using a handheld portable ultrasound, while the bird was in lateral recumbency. Mean grayscale values were measured for the entire cross section, cortex and medulla of the tibiotarsus. Ultrasound images of the keel were evaluated for their reliability in identifying bone fractures compared to radiographs. Data were analyzed using a linear regression model in JMP® Student Edition 18.2.1, with a significance level of $P<0.05$. Results showed no difference in the diameter of the tibiotarsus measured by radiograph and ultrasound. No differences in the diameter of the tibiotarsus were observed between the phytase or Azomite® treatment groups either. However, phytase supplementation significantly increased total and cortical bone density but not medullary bone density. Azomite® had no significant effect on the bone density. Neither imaging modality nor supplementation had a significant effect in the incidence of keel fractures. In summary, 1) phytase but not Azomite® supplementation enhanced bone density in post peak production laying hens; 2) both radiography and ultrasound are comparable for measuring the diameter of the tibiotarsus; 3) neither imaging technique nor dietary treatments were able to detect significant differences in the number of keel fractures. Radiographs provided clear visualization of long bone density and their cortical margins. In contrast, ultrasound offered real-time, dynamic imaging but was limited in the ability to evaluate bone density of long bones. Overall, radiography provided superior bone structure and density evaluation compared to ultrasound.

Funding: US Poultry & Egg (Project title: “Effects of Phytase and Dacitic Tuff Breccia Supplementation Programs to Support Extended Lay in Laying Hens

Research: Clinical

Category: Other

Mobile Phone: 252-531-3291

DIETARY MODULATION OF GUT MICROBIOTA ALTERS SALMONELLA COLONIZATION AND DIVERSITY IN CHICKENS SUPPLEMENTED WITH PROBIOTICS AND PREBIOTICS.

Natalie Roberts (graduate student)
Matthew Koci, Hosni Hassan, Mary Mendoza, Lauren Anderson
nbhodges@ncsu.edu, mdkoci@ncsu.edu
Mobile #: 919-260-4026
Comparative Biomedical Sciences Graduate Program (CBS), North Carolina State University, Raleigh, NC, USA
Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC, USA

Annually 1.35 million cases of salmonellosis are reported in the US, with 25% of these cases attributed to poultry. Chickens primarily acquire Salmonella through horizontal transmission after hatching, suggesting that manipulating the gut microbiota could be a promising strategy to prevent Salmonella colonization and reduce infection risk. To test this hypothesis, we modulated the chicken microbiome using diets supplemented with probiotics, prebiotics, or both. Day-old chicks were randomly assigned to four dietary treatments: Control Diet, Control Diet + Prebiotic (galacto-oligosaccharide, GOS), Control Diet + Probiotic (three Lactobacillus strains isolated from healthy chicken cecca), and Control Diet + Prebiotic + Probiotic. On day 28, half the birds from each group were orally challenged with a 1:1.5 mixture of *Salmonella typhimurium* ($1x10^8$ CFUs) and *Salmonella enteritidis* ($1.5x10^8$ CFUs). Cecal contents were collected seven days post-infection, and assayed for Salmonella,16S rRNA gene sequencing, and metabolomic analysis. Diet significantly influenced both cecal *Salmonella* colonization and microbial community composition. Interestingly, probiotic-fed birds exhibited higher cecal *Salmonella typhimurium* (CFU/g) than control or control + prebiotic-fed birds. Preliminary 16S analysis revealed reduced alpha diversity in probiotic fed birds. Additionally, there are distinct metabolic signatures associated with each dietary treatment in the cecum and serum metabolite results. These findings indicate that dietary modulation of the gut microbiota can influence *Salmonella* colonization. A better understanding of how differences in the microbial community, and the metabolites they produce, are able to prevent Salmonella from colonizing the chicken intestine will help us engineer poultry diets that prevent foodborne disease.

Funding Source: USDA NIFA: 2012-68003-1962

Research Type: Basic Research
Research Category: Infectious Disease

ALPHA2EQ DOWNREGULATES INFLAMMATORY GENE EXPRESSION IN EQUINE SYNOVIAL FIBROBLASTS IN VITRO

Fabiola K. Ruiz Rosario[1], BS (Veterinary Student)
Brenna R. Pugliese[1,2], DVM, MS, DACVS-LA, Lauren V. Schnabel[1,2], DVM, PhD, DACVS-LA, DACVSMR
fkruizro@ncsu.edu (787-691-9381), brpuglie@ncsu.edu, lvschnab@ncsu.edu
[1]Department of Clinical Sciences, NCSU CVM, Raleigh, NC,
[2]Comparative Medicine Institute, NCSU, Raleigh, NC

Osteoarthritis (OA) is a debilitating inflammatory joint disease leading to cartilage degradation and pain. Alpha2EQ, an autologous intra-articular OA treatment, consists of concentrated alpha-2-macroglobulin (A2M) from equine blood. A2M's anti-inflammatory properties have been studied in other species, however, the equine product remains unexplored. Our objective was to evaluate Alpha2EQ's ability to reduce expression of OA-related genes in equine synovial fibroblasts (SF) that either had endogenously high IL-6 expression or were stimulated with IL-1β in an OA model. To optimize the OA model, cells were stimulated with IL-1β at 1, 2.5, 5, 7.5 and 10 ng/mL, while endogenous IL-6 ^{HIGH} SF remained in serum-free media; after 24hr both groups were treated with 25% Alpha2EQ pooled from 6 healthy horses. Non-treated cells served as baseline control. After 24hr gene expression was analyzed using NanoString nCounter and reported as mean gene expression fold change (log2FC) from baseline. One-tailed t-tests assessed for differences in log2FC between stimulated cells (IL-1β) and stimulated cells treated with Alpha2EQ, and between endogenous IL-6 ^{HIGH} and IL-6 ^{LOW} SF treated with Alpha2EQ ($p < 0.05$). In cells stimulated with 10 ng/mL IL-1β (optimal dose), Alpha2EQ treatment significantly decreased expression of CCL5/RANTES, CXCL6, IL-1β, and PPBP/CXCL7, compared to stimulated cells. Treatment with Alpha2EQ resulted in significantly decreased IL-6, IL-15, CCL2/MCP1, and ADAMTS4 expression in endogeous IL-6 ^{HIGH} compared to IL-6 ^{LOW} SF. Both models decreased expression of CXCL6/GCP-2 and TNF-α. Our results demonstrating Alpha2EQ's downregulation of OA-related genes will inform the use of this orthobiologic, while increasing understanding of disease mechanism.

Funding sources
NIH T35OD011070 Interdisciplinary Biomedical Research Training Program (FKR)
Morris Animal Foundation (FKR)
Triangle Community Foundation (FKR)
Astaria Global, LLC.
F.O.R.G.E.- Fund for Orthopedic Research in honor of Gus and Equine athletes

Primary subject: Regenerative Medicine, Basic Research

EVALUATION OF THE OXYGEN SATURATION INDEX (OSI) AS A PREDICTOR OF OUTCOME AND SURROGATE FOR OXYGENATION INDEX (OI) IN DOGS UNDERGOING MECHANICAL VENTILATION

Luis J. Schmidt[1], House Officer

Jiwoong Her[1], Faculty Mentor

lschmid5@ncsu.edu

(509) 2969984

NCSU CVM[1]

Abstract

Introduction: The Oxygenation Index (OI) is used to evaluate oxygenation in ventilated patients but requires arterial sampling. The Oxygen Saturation Index (OSI), which replaces PaO_2 with SpO_2, has shown strong correlation with OI and has been proposed as a non-invasive alternative for monitoring and prognostication in ventilated patients.

Objective: To evaluate the ability of OSI to predict outcome in mechanically ventilated dogs, assess its correlation and agreement with OI, and establish OSI thresholds corresponding to clinically relevant OI values.

Study Design: Retrospective multicenter observational cohort study including client-owned dogs requiring mechanical ventilation at three teaching hospitals. Only data from the first three days of ventilation were analyzed due to a small sample size beyond that time.

Methods: OSI and OI were calculated using standard formulas, restricting OSI to SpO_2 values between 80–97%. Associations between OSI and survival were assessed with logistic regression, while correlation and agreement with OI were evaluated using Spearman analysis, linear mixed models, and Bland–Altman plots.

Results: Sixty dogs met inclusion criteria, with an overall survival rate of 57% (34/60). Median OSI and OI values did not differ significantly between survivors and non-survivors (all $p > 0.05$), but showed strong correlation ($r = 0.91$, $p < 0.0001$) and high agreement with OI. OSI cutoffs of 4.2, 6.0, and 13.0 predicted OI values >4, >8, and >16, respectively.

Conclusions: OSI did not predict outcome but demonstrated strong correlation and agreement with OI, supporting its potential as a non-invasive surrogate for assessing oxygenation in ventilated dogs.

Funding Sources: N/A

Is the research clinical? Yes

Primary subject category: Clinical Medicine (Veterinary). Preference: Poster presentation

INVESTIGATION OF VECTOR-BORNE AND RELATED DISEASES IN THE HOOFSTOCK SPECIES OF THE SAN DIEGO ZOO SAFARI PARK

Katherine Stalford; Veterinary Student
Sarah Clarke[1], Anthony Cerreta[2], Rob Browning[2], Michael Karounos[1], Meghan Leber, Barbara Quorollo[1], Adam Birkenheuer[1]
klstalfo@ncsu.edu
Subject Category: Infectious Disease

Affiliations
NCSU CVM Department of Population Health and Pathobiology
San Diego Zoo Wildlife Alliance

Abstract
The San Diego Zoo Safari Park (SDZSP) is an 1800 acre facility with eight large, mixed-species field habitats that house dozens of hoofstock species. This layout results in a unique interface between native and nonnative species with the potential for exposure to vector-borne infections (VBI). For example, zoo hoofstock could be exposed to native VBI and native wildlife could be exposed to VBI carried by zoo species. The animals at the SDZSP have never been screened for VBI. The purpose of our study is to screen hoofstock from the SDZSP for Babesia/Theileria, Mycoplasma, Anaplasma/Ehrlichia, Rickettsia, and Bartonella. We hypothesized that the hoofstock species would be infected with native and exotic VBI.
We performed quantitative real-time PCR tests on DNA extracted from whole blood samples from 149 animals representing 51 different species from the SDZSP. 26/149 animals tested positive for a single VBI and 2/149 tested positive for more than one VBI. The number of animals that tested positive for each category of infection is outlined below;

	Babesia/Theileria	Mycoplasma	Anaplasma/Ehrlichia	Rickettsia	Bartonella
positives	12	14	3	0	1

Results demonstrate the presence of an exotic VBI in all 11 Dama Gazelles. We have identified the presence of one or more VBI in 10 additional species and need to perform additional investigation to determine whether or not these infections are native or exotic. These findings at least represent the potential for exposure of native wildlife to exotic infections.

Funding
Vector Borne Disease Diagnostic Laboratory
Andy Quattlebaum Companion Animal Research Endowment

ADVANCING CANINE BEHAVIORAL RESEARCH: DEVELOPMENT AND VALIDATION OF OWNER-COMPLETED QUESTIONNAIRES FOR ASSESSING ANHEDONIA IN DOGS

Michelle Stern[1]; Veterinary Student

Maggie Creamer[1,2], B. Duncan X. Lascelles[2,3,4,5], Margaret E. Gruen[1,3]

mstern@ncsu.edu

310-795-6504

Affiliations

[1]Comparative Behavioral Research and Thinking Pets Program, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[2]Translational Research in Pain, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[3]Comparative Pain Research and Education Centre, Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, United States
[4]Center for Translational Pain Research, Department of Anesthesiology, Duke University, Durham, NC, United States
[5]Thurston Arthritis Center, UNC, Chapel Hill, NC, United States

Abstract

Anhedonia (diminished ability to experience pleasure) is a symptom of depression in humans. Its clinical recognition in dogs remains minimally explored. While the Dimensional Anhedonia Rating Scale (DARS) is validated for human populations, no equivalent tool exists for dogs. This study aimed to develop and evaluate two questionnaires to assess anhedonia in dogs: a version of DARS adapted for dogs (DARS-d) and a new Canine AnhedoNia Scale (CANS). Items in both were related to social interaction, activity, and food motivation. The questionnaires were distributed online, with repeated distribution to assess test–retest reliability. Dog owners (n = 146) completed the DARS-d, CANS, and the validated Canine Owner-Reported Quality of Life Questionnaire (CORQ). Thirty-nine owners completed the questionnaires twice.

Preliminary analyses support convergent validity across both questionnaires and discriminate validity in the DARS-d. DARS-d and CANS scores correlated with CORQ subcomponents conceptually related to anhedonia: vitality (DARS-d: $r = 0.29$, $p < 0.001$, CANS: $r = 0.53$, $p < 0.001$) and companionship (DARS-d: $r = 0.23$, $p = 0.010$, CANS $r = 0.40$, $p<0.001$). The DARS-d was not significantly correlated with the overall CORQ score, which suggests discriminate validity from a measure of quality of life. Test–retest reliability (mean interval 36 days) demonstrated strong temporal stability in the DARS-d scores ($r = 0.75$) and CANS scores ($r = 0.48$).

These findings provide initial evidence for the use of owner-reported tools to assess anhedonia in dogs, which may be used to advance behavioral health assessment in companion animals.

Funding statement: Research reported in this abstract was supported by NIAMS, NIH, under Award Number R01AR085335. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. MS was supported by the Jacobsen Trust Research Fund.

Ethical statement: The study was conducted in accordance with the local legislation and institutional requirements. Behavior tests were approved under NC State University IACUC protocol #24-135. No IRB was required for this work.

Research is Clinical

Primary Subject Category - Pain

ANALYZING THE ROLE OF HISTONE H3 METHYLATION DURING *Hydra vulgaris* REGENERATION

Brooke Stonemetz[1,*] (veterinary student)
Claire H. Parker[1], Abi Fetzer[1], Igor Silva[1], Eric Brooks[1], Christophe Guilluy[1]

[*] bstonem@ncsu.edu, 704-425-9950

Affiliations
[1]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Hydra vulgaris, a freshwater invertebrate known for stem cell-driven regenerative properties, can provide a model for identifying molecules and pathways relative to regeneration. However, the mechanisms orchestrating gene regulation during *Hydra* regeneration are not yet fully understood; specifically, the contribution of epigenetic mechanisms remain unclear. Histone-3 lysine-9 trimethylation (H3K9me3) plays a regulatory role in gene expression by causing chromatin condensation–resulting in transcriptional blockage and gene silencing. Preliminary work, using proteomic screening and immunofluorescence imaging, showed an accumulation of H3K9me3 at the site of regeneration following tissue injury. These results lead to our hypothesis that tissue injury increases H3K9me3, which in turn, may play a role in modifying chromatin accessibility and gene expression important for *Hydra* regeneration. To test this hypothesis, we quantitatively analyzed head regeneration using darkfield microscopic imaging to observe the number of emerging tentacles in *Hydra* treated with histone methyltransferase inhibitors (HMT-2 and Chaetocin) or a vehicle control. In addition, we isolated mRNA from *Hydra* treated or untreated with histone methyltransferase inhibitors during regeneration. mRNA sequencing was then performed to analyze differences in gene expression at varying time points. So far, results from regeneration assays show that HMT-2 prevents head regeneration. Results from gene expression analysis at T24 hpa (treated and untreated with HMT-2) showed significant differences in genes related to the regenerative processes such as transcription factors, cytoskeleton regulators, and prosurvival signaling proteins. This suggests that H3K9me3-mediated regulation of gene expression may be central to the *Hydra's* regenerative processes.

Funding Sources
NC State University Office of the Associate Dean for Research and Graduate Students–Advancing Animal Health and Wellbeing Fund

Subject Categories
Cell Biology; Genetics; Regenerative Medicine

Research Type
Basic

Stride length in relation to age and owner reported cognitive performance in aging dogs

Akiya Stywall; Veterinary Student

Sherry Rafatpanah, Natasha Olby

adstywal@ncsu.edu, srafatp@ncsu.edu, njolby@ncsu.edu

Affiliations
Department of Neurology, College of Veterinary Medicine, North Carolina State University, Raleigh NC, USA

Abstract
Motor and cognitive function are indicators of age-related changes and frequently used in geriatric evaluations of humans. Despite its potential to reflect early functional decline, stride length (SL) remains underused as an objective measure in aging dogs. In this study we investigated age-related changes in SL in senior and geriatric dogs and evaluated the relationship between SL and owner reported cognitive performance using the Canine Dementia Scale (CADES).

We hypothesized that thoracic limb SL would decrease with age and deteriorating cognitive status. We used banked videotapes and cognitive data from 87 dogs participating in a longitudinal cohort study on aging. Stride length (SL) was calculated for each limb by counting the number of steps taken by dogs walking over a 5m distance and dividing distance by steps. SL was divided by dog height to control for differing limb lengths (SL_{adj}) . JMP Pro18 was used to perform cross sectional and longitudinal multivariate analyses with mixed effect models of SL_{adj} in relation to age and CADES score.

Results: In a cross-sectional analysis, age and thoracic limb SL_{adj} were not correlated However, longitudinally both age and CADES score were significantly associated with thoracic limb SL_{ad}. In a multivariate mixed effects model incorporating both age and CADES, the relationship with CADES remained significant. Findings from this study mirror the effects of cognitive function on changes in SL in people and highlight a simple functional measure that could be used to monitor dogs clinically.

Funding Sources
The Dr Kady M. Gjessing and Rhanna M. Davidson Distinguished Chair of Gerontology Daniel Memorial Research Grant

Neurosciences

INVESTIGATING THE GENETIC ORIGIN OF COR TRIATRIATUM SINISTER IN THE DOMESTIC CAT

Primary subject category: Genetics; basic science

Connor J. Thonen-Fleck[1], Veterinary student; cjthonen@ncsu.edu, 336-662-7195
Michael W. Vandewege, Victor N. Rivas, Sandra M. Losa, Josue Gonzalez, Kursten V. Pierce, Joshua A. Stern

Affiliations
[1]The Feline Health Center, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Abstract
Cor triatriatum sinister (CTS) is a rare congenital heart disorder that is estimated to afflict less than 0.004% of human children and 0.002% of the general cat population. The disease is characterized by a fibromuscular membrane partitioning the left atrium into a high-pressure (proximal) and a low-pressure (distal) chamber resulting from failure of the common pulmonary vein to regress. In the absence of medical intervention, affected patients ultimately progress to left-sided congestive heart failure and, in some cases, death. The molecular pathogenesis of CTS is largely unknown in both human and veterinary medicine and no study to date has conclusively identified a genetic etiology. The aim of this study was to elucidate novel CTS-associated genetic variant(s) using previously phenotyped feline CTS case DNA via whole-genome sequencing (WGS). We hypothesized that feline CTS is attributed to variant(s) harbored within genes of cardiac development ontology. A whole-genome association study (WGAS) was employed on four echocardiographically-confirmed CTS-affected and 18 geriatric, cardiovascularly-healthy cats. Expanded genotyping was performed on 404 previously WGSed cats for allele frequency (AF) calculation of candidate variants. A total of 53 variants in 28 genes with an AF of 0 in both the control and expanded genotyped populations were identified in the CTS-affected cats. Continued functional investigation of these candidate variants is currently underway to elucidate the genotype-phenotype relevance. This study reports the results of the first-ever performed genetic study for CTS in any companion animal species and holds promise in contributing to a translational understanding of human disease.

Funding source
NIH T35OD011070-16 Interdisciplinary Biomedical Research Training Program
Stern Translational Cardiac Genetics and Pharmacogenomics Laboratory Fund

THE COMPARISON IN PHARMACOKINETICS AND ADVERSE EFFECTS OF SINGLE SUBCUTANEOUS EXTENDED-RELEASE BUPRENORPHINE (ETHIQA XR®) BETWEEN INTACT FEMALES AND INTACT MALES OF HEALTHY BEAGLES

Kornelia Tiffinger-- ktiffin@ncsu.edu, Hiroko Enomoto- henomot@ncsu.edu, B Duncan X Lascelles-duncan_lascelles@ncsu.edu, Ronald E Baynes-rebaynes@ncsu.edu, Laura M Neumann-lmneuman@ncsu.edu, Amber Hall-alhall6@ncsu.edu, Nathan Hall-nphall@ncsu.edu, Michael Howard-mahowar4@ncsu.edu, Dani Huston-dthuston@ncsu.edu, Masataka Enomoto-menonot@ncsu.edu
Mobile: 541-360-9844
Affiliation: Translational Research in Pain laboratory NCSU CVM

Abstract:

This study aimed to evaluate the pharmacokinetics of an extended-release buprenorphine formulation (Ethiqa XR) in dogs and explore potential sex differences. Twelve healthy intact beagles (6 males and 6 females) received a single subcutaneous injection of Ethiqa XR (0.2mg/kg). Blood samples were collected at multiple timepoints up to 168 hours after administration, and plasma buprenorphine concentrations were measured using liquid chromatography tandem mass spectrometry. Vital signs, sedation score, nauseous score, and adverse events were recorded. Therapeutic plasma concentrations were sustained for approximately 60-90 hours in both sexes, depending on the previously proposed therapeutic threshold (0.6 ng/mL or 1.0 ng/mL). Although no significant differences in buprenorphine pharmacokinetic parameters were detected between sexes, drug exposure and elimination were greater in females than males; median peak plasma buprenorphine concentrations (male:1.6 ng/mL, female:2.9 ng/mL), median terminal half-life (male:36.6-hour, female:24.7-hour), area under the curve (AUC 0-12 hour) (male:12.8 h*ng/mL, female:16.9 h*ng/mL) and AUC (0-96 hour) (male:94.4 h*ng/mL, female:137.7 h*ng/mL). Time to maximum concentrations were 24-hour in both sexes. Higher buprenorphine concentrations were associated with decreased body temperature and lower heart rate in both sexes and were positively correlated with nauseous and sedation scores only in females. Frequently observed adverse events were vomiting, diarrhea, anorexia.

Ethiqa XR provided reliable systemic absorption and maintained therapeutic plasma concentrations up to 96-hours in both sexes. Ethiqa XR may be an alternative treatment option to control post-operative pain, but further study is needed to assess its clinical efficacy.

Funding Statement: This project was funded by Fidelis Animal Health (Grant #PAM-P25-002754) and Extended-release buprenorphine (Ethiqa XR®) was provided by them.

Ethics statement: This study and all procedures were approved by North Carolina State University Institutional Animal Care and Use Committee (IACUC # 25-231). The work was conducted in accordance with the local legislation and institutional requirements.

Research: Clinical

Subject category: Pharmacology

TOLL-LIKE RECEPTOR AGONISTS INDUCE CANINE OSTEOSARCOMA CELL DEATH

Emily Tomblin[1]; Veterinary Student
Ashley Lynn[2], Erika Gruber[2]
egtombli@ncsu.edu, alynn@ncsu.edu, ejgruber@ncsu.edu
(336) 553-7720

Affiliations
[1] NCSU CVM
[2] Department of Population Health and Pathobiology, NCSU CVM

Abstract
Osteosarcoma is a highly aggressive primary bone tumor in dogs, with a median survival time of 10-12 months despite standard-of-care treatment consisting of surgery and chemotherapy. Most patients die from metastatic disease, and few improvements in treatment have been made in decades. Bacteria-based immunotherapies have shown some promise in shrinking other tumor types by initiating an antitumor immune response to prolong patient survival. Bacteria express conserved microbial molecules that are recognized by innate immune receptors such as toll-like receptors (TLRs). TLR agonists may also enhance anti-tumor immunity and tumor regression. We hypothesized that agonists for TLR2 (lipoteichoic acid from *Staphylococcus aureus* (LTA-SA)), TLR3 (polyinosinic-polycytidylic acid (Poly (I:C)) and NexaVant (NVT)), TLR4 (lipopolysaccharide (LPS)), TLR7/8 (imiquimod (IMQ) and resiquimod (R848)), and/or TLR9 (ODN 2395) would kill osteosarcoma cells *in vitro*. Canine osteosarcoma cells were incubated with each agonist and viability was assessed with a biochemical cell viability assay. Select results were confirmed with live/dead microscopy assay. TLR3, TLR4, TLR 7/8 and TLR9 agonists induced cell death, which differed by cell line (Poly (I:C), NVT) and dose (NVT, LPS, IMQ). No effect was observed with TLR2 agonism. These findings suggest a role for TLR signaling in osteosarcoma cell death, uncovering a potential new therapeutic target for canine osteosarcoma.

Funding sources
Boehringer-Ingelheim Veterinary Scholars Program
Gruber Lab Funds
MG Biologics Boehringer Ingelheim Animal Health through the Veterinary Comparative and Clinical Immunology Society

Basic Science
Immunology

INTERACTION OF AVIAN MACROPHAGE WITH *ENTEROCOCCUS CECORUM* AND ANTIBACTERIAL EFFECTS OF PROBIOTIC *LACTOBACILLUS* BACTERIA

Becky Tran[1] ,Veterinary Student

Matthew Browning[1], Hosni Hassan[2], Rocio Crespo[1], Ravi Kulkarni [1]

bytran@ncsu.edu, mhbrowni@ncsu.edu, hmhassan@ncsu.edu, rcrespo@ncsu.edu, ravi_kulkarni@ncsu.edu

[1]College of Veterinary Medicine Department of Population Health and Pathobiology; [2]College of Agricultural Sciences, Prestage Department of Poultry Science, North Carolina State University

Enterococcus cecorum (EC) is an emerging bacterium causing significant mortality due to septicemia and spondylitis in chickens. Despite its economic burden on the poultry industry, there are no effective non-antibiotic control strategies available currently, which is partly due to a poor understanding of EC-specific immune responses. Here, we evaluated the chicken macrophage interactions with EC followed by evaluating the anti-EC effect of two chicken probiotic *Lactobacillus* strains, *L. animalis* (P38) and *L. crispatus* (C29) in-vitro. Macrophages were interacted with two strains of EC, CE1 (non-pathogenic) and SA3 (pathogenic) for 24 hours and nitric oxide (NO) production was quantitated. Results showed that SA3 interaction led to higher ($P<0.05$) NO production, indicating cellular response was targeted against pathogenic but not non-pathogenic strains. Next, we evaluated the effect of P38 and C29 lactobacilli against EC using the Disc-diffusion test to determine the antimicrobial activity of cell-free supernatant (CFS), while assessing the direct EC inhibition using the agar-spot test and coculture assay. The disc-diffusion and agar-spot tests showed inhibition ($P<0.05$) of CE1 growth but not SA3. The coculture of EC strains with lactobacilli showed that both P38 and C29 could reduce ($P<0.05$) the growth of CE1 and SA3, as determined by *SodA* (house-keeping gene) expression. Additionally, P38 coculture showed downregulation ($P<0.05$) of SA3 expression of *cspO* virulence gene. Collectively, our findings suggest that pathogenic EC can induce a robust inflammatory activation of macrophages and that it is possible to prevent EC infection in chickens using certain well-characterized *Lactobacillus* strains such as *L. animalis* (P38).

Research Grant: US Poultry and Egg Association [Kulkarni]

Student Support: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program

Research Type: Basic

Category: Immunology

EXTENDING IFN-λ RELEASE AT THE CORNEAL SURFACE USING A NOVEL HYDROGEL FORMULATION SUPPRESSES CORNEAL IMMUNOPATHOLOGY AFTER OCULAR HSV-1 INFECTION

Author: **McKenna Trull** (Undergraduate student)
Co-authors: Divya Kinha, Jiayi Ren, Amol Suryawanshi
Email: matrull@ncsu.edu, fdivya@ncsu.edu, jren9@ncsu.edu, assuryaw@ncsu.edu
Mobile: (336)-830-6868 (McKenna Trull)
Affiliation: Department of Population Health and Pathobiology, College of Veterinary Medicine (CVM), NC State University, Raleigh, NC

Herpes simplex virus 1 (HSV-1) is a successful and ubiquitous human pathogen that establishes life-long latent infection in the trigeminal ganglion. The HSV-1 reactivation and recurrence at the corneal epithelial surface can lead to the development of chronic immuno-inflammatory and vision-impairing disease called herpetic stromal keratitis (HSK). HSK treatments targeting inflammation or virus replication are partially effective and promote HSV-1 latency, and long-term use can cause side effects and the emergence of drug-resistant HSV-1 strains. Therefore, there is an urgent and unmet clinical need to develop novel, safe, and effective immunotherapies that selectively induce antiviral responses and suppress inflammation to prevent and treat HSK and permanent blindness caused by multidrug-resistant HSV-1 strains. In this study, we investigated whether recombinant IFN-λ (rIFN-λ) can suppress acyclovir-resistant HSV-1 replication in human corneal epithelial cells (HCECs) and control HSK progression. We formulated rIFN-λ in a novel thermo-sensitive poloxamer-407 hydrogel (rIFN-λ-HG) to improve corneal surface retention and minimize frequent applications. Our results show that topical application of rIFN-λ-HG in HSV-1-infected mice significantly diminishes corneal HSV-1 replication, inflammatory immune cell (neutrophils and CD4 + T cells) infiltration, and HSK disease severity compared to rIFN-λ in PBS or vehicle-treated animals. Further, we show that rIFN-λ promotes a strong anti-viral state through the induction of IFN-stimulated genes (ISGs) and can suppress acyclovir-resistant HSV-1 replication in HCECs. Collectively, topical rIFN-λ treatment after corneal HSV-1 infection promotes strong anti-viral and anti-inflammatory responses and represents a promising therapeutic approach to treat HSK, especially caused by acyclovir-resistant HSV-1 strains.

Funding Source: NCSU CVM Startup fund, National Eye Institute Grant EY034495 (R01)
Research Category: Basic
Subject Category: Immunology

DEFINING THE HISTOPATHOLOGIC AND MOLECULAR CHARACTERISTICS OF FELINE EXOCRINE PANCREATIC CARCINOMA

Abby L. Turner : veterinary student
Gabriella Alexander, Yea Ji Jeong
alturne8@ncsu.edu
252-560-5782
NCSU CVM

Feline exocrine pancreatic carcinoma is one of the most lethal cancers in companion cats. It is often detected at an advanced stage with widespread metastases, rendering it incurable. Most patients succumb within a year, even with surgical resection and chemotherapy, highlighting the need for improved diagnostic and therapeutic strategies. Molecular profiling in human oncology has identified genetic mutations linked to pancreatic carcinogenesis, enhancing detection and treatment; however, comparable advances in veterinary medicine remain underdeveloped. This study aims to (1) evaluate histopathologic features of feline pancreatic carcinoma, (2) identify hotspot mutations involved in tumorigenesis and correlate them with histologic subtypes, and (3) trace genomic evolution from primary tumors to distant metastases. Thirteen cases were selected from the NC State Anatomic Pathology archive. Digital pathology was used to analyze histologic subtypes, cellular changes, and metastatic spread. Acinar carcinoma was predominant (n=9), followed by anaplastic carcinoma (n=4). Anisocytosis with anisokaryosis (n=10) and pancreatic necrosis (n=6) were frequent cytologic findings. Metastasis occurred in 9 cases, most commonly in the liver (n=6). Going forward, DNA from neoplastic cell populations will be isolated via laser capture microdissection for Next Generation Sequencing to determine the prevalence of genomic mutations enriched in tumorigenesis and metastatic progression. This data will be pivotal for developing molecular diagnostics and targeted therapeutics. The comprehensive histologic and molecular profiling of feline pancreatic carcinoma will also accelerate the integration of precision oncology into veterinary medicine.

Funding sources: NC State Feline Health Center, NC State Animal Health and Nutrition Consortium

Clinical research
Oncology

INVESTIGATING IMMUNOLOGICAL AND PATHOLOGICAL CHANGES IN LAYER CHICKENS AFFECTED WITH OVARIAN ADENOCARCINOMA

Aakriti Tyagi (Staff)

Pok Man Chan, Jazz Stephens, Michael Rahe, Rocio Crespo, Ravi Kulkarni

atyagi7@ncsu.edu, pchan3@ncsu.edu , jstephe8@ncsu.edu , mrahe@ncsu.edu , rcrespo@ncsu.edu , rrkulkar@ncsu.edu

+1984-357-2257

NCSU CVM

Human epithelial ovarian cancer (EOC) manifests a dysregulated immune phenotype. Older (≥2 years) hens (*Gallus gallus domesticus*) develop spontaneous ovarian cancer that closely resembles EOC in women, including its course of development and pathology; thus, providing a relevant model to study tumor–immune interactions. Here, we aimed at investigating the immunological and pathological changes within the ovaries collected from hens with spontaneous ovarian tumors. Ovarian tumor (OT) tissues from affected chickens, along with healthy control (*n*=6-8), were employed in this investigation. While tissue sections were examined for histopathology, the immunological evaluation included quantifying the expression of interleukin (IL)-1β, IL-10, TGFβ, IFNγ, iNOS, PD-1, PD-L1, CD25, CD40, and FoxP3 genes. Results showed that OT group had histological changes characteristic of endometroid ovarian carcinoma. Immune analysis showed a significant upregulation of IL-10, PD-1, CD25, and FoxP3 gene transcription in OT birds compared to controls, suggesting immunosuppressive changes possibly mediated by an expansion of regulatory and exhausted T-cell subsets. Additionally, gene expression levels of IL-1β, iNOS, and TGFβ were significantly decreased while that of CD40 was elevated, underscoring an immune suppression/tolerance within the tumor microenvironment. Furthermore, immunohistochemistry analysis revealed reduced intra-tumoral $CD3^+$ T-cell infiltration compared to control, indicating an immune-excluded microenvironment of late-stage carcinomas. Collectively, it seems that OT tissues display a transition from inflammatory to immunoregulatory dominance, paralleling the checkpoint- and Treg-enriched landscape of human EOC, and thus, presenting hen as a potential translational model for studying human EOC, including testing future immunotherapeutic interventions or identifying biomarkers of ovarian cancer in women.

Subject Category: Immunology

Funding: PI's college research support funds.

Optimization of Cell Transfection with Electroporation in 3D Culture

Ariel Tysver; Veterinary Student[1]
Robert Williamson[2], Michael B. Sano[1]
amtysver@ncsu.edu, (574) 222-3399

Affiliations
[1] Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

[2] Department of Biomedical Engineering, College of Engineering, North Carolina State University, Raleigh, North Carolina, USA

Abstract
Electroporation utilizes electric fields to induce pores along the cell membrane. This process can be irreversible or reversible. Irreversible electroporation results in permanent damage and cell death, which can be used for cancer treatment and other tissue ablation applications. Reversible electroporation results in transient pore formation without causing permanent cell damage. This can be harnessed to improve the uptake of molecules into the cell that are otherwise difficult to absorb. Reversible electroporation shows clinical promise for local delivery of chemotherapeutics, as well as enhancing transfection of cells with nucleic acids for next generation vaccinations and gene editing therapies including CRISPR-Cas9. Current reversible electroporation protocols utilize monopolar pulses on the order of 100μs and show significant promise in vitro, but present many challenges when translated into the clinic, including intense muscle stimulation, cardiac interference, high rates of cell death, and low rates of transfection. Previous studies in our lab have shown that novel bipolar, 1-2 microsecond pulses alleviate these undesirable side effects significantly, making them preferable in a clinical setting. Our study aims to characterize and optimize these novel pulses for reversible electroporation mediated transfection in a 3D cell culture model for translation into in vivo models. These results will provide a bridge to move this technology from cell suspension to an in vivo model to determine efficacy for nucleic acid delivery to cells in practical therapeutic applications compared to traditional waveforms.

Funding Sources
NIH Interdisciplinary Biomedical Research Training Program (T35OD011070); NIH R01CA276232; NIH R01CA272550; NIH R41CA275587; NCSU Chancellor's Innovation Fund

Subject Category: Biomedical Engineering, basic research

PHARMACOKINETICS OF INTRAVENOUS AMPICILLIN SODIUM IN HOSPITALIZED CALVES

Haley Vaughan[1]; Veterinary Student
Jennifer L. Halleran[1], Derek M. Foster[1], Ronald E. Baynes[1], Danielle A. Mzyk[1]
hccook@ncsu.edu, dalindqu@ncsu.edu
(336) 407-1915

Affiliations
[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

Abstract
The objective of this study was to evaluate the pharmacokinetics (PK) of intravenous (IV) ampicillin sodium in six hospitalized calves presenting for clinical signs of systemic inflammatory response syndrome (SIRS). Calves are commonly administered antibiotics for systemic infections when hospitalized for SIRS; however, minimal research exists investigating the pharmacokinetics of antibiotics administered to septic food producing species. Ampicillin is a beta-lactam antibiotic widely used in the field of veterinary medicine for treatment of bacterial diseases. Calves enrolled in the study were hospitalized, received a clinical sepsis score $\geq$ 5, and had immature neutrophils present on a pre-enrollment hemogram. A dose of 20-22 mg/kg body weight of ampicillin sodium was administered IV every 6-8 hours. Plasma samples were collected at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours following initial IV administration. Ampicillin sodium concentrations were quantified using high-performance liquid chromatography with mass spectrometry. PK parameters were derived using compartmental analyses. The geometric mean ± standard deviation for elimination half-life, total systemic clearance (CL), and area under-the-curve to infinity ($AUC_{0-\infty}$) of ampicillin after IV administration was 0.60 ± 1.6 hr, 392.8 ± 2.0 mL/hr/kg, and 57.0 ± 1.9 hr*ug/mL, respectively. Sepsis scores ranged from 7-11. High variability of plasma CL, maximum plasma concentrations, and drug exposure were noted. The results provide evidence of the need for further support to examine the influence of disease on the pharmacokinetics of critical antibiotics used in calves.

Funding Sources
Food Animal Residue Avoidance Databank (USDA - National Institute of Food and Agriculture)

Stern Translation Cardiac Genetics and Pharmacogenomics Laboratory Fund

Category
Pharmacology

TOLERABILITY, TOXICITY, AND PHARMACOKINETICS OF EPISCLERAL SUSTAINED RELEASE IMPLANTS CONTAINING TACROLIMUS AND RAPAMYCIN IN NEW ZEALAND WHITE RABBITS

Mary Wells[1] veterinary student
Darby Roberts[1], Kaelyn Dausch[1], Isabelle Ku[1], Jacklyn H Salmon[1],
Brian C. Gilger[1]
mmwells2@ncsu.edu
(910) 617-9172

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

Abstract

Sustained-release cyclosporine implants, placed into the ocular episcleral space are beneficial in animals with chronic ocular surface diseases, such as keratoconjunctivitis sicca. However, the size of the implants and limited duration of action have limited their clinical use. To develop smaller and more potent implants, this study investigated the *in vivo* drug release, tolerability, and ocular distribution of potent immunosuppressant drugs, tacrolimus and rapamycin, from silicone episcleral implants in New Zealand white rabbits over 84 days. We hypothesized that these implants would be well tolerated and provide extended high-dose drug release to the ocular surface in rabbits. Twelve New Zealand white rabbits received a blank silicone implant, a tacrolimus implant, or a rapamycin silicone matrix implant surgically implanted into the episcleral space in both eyes. Ocular examinations, consisting of slit lamp biomicroscopy, indirect ophthalmoscopy, and IOP measurements, were done before surgery and on days 1, 3, 5, 7, 10, 14, 21, 28, and monthly through day 84 after implantation. Additionally, for drug tear levels, Schirmir tear test strips were used to collect tears at each examination time. Further, aqueous humor and serum samples will be collected at days 28, 56, and 84 after implantation. Drug levels will be measured using LC-MS/MS on tear, AH, serum, and ocular tissues. To date (through 28 days), ocular examinations have shown only mild conjunctival swelling and redness. Tacrolimus and rapamycin episcleral silicone implants appear well-tolerated in the rabbits, and if release rates are acceptable, may represent a significant advancement in sustained-release ocular therapeutics.

Funding sources

Boehringer-Ingelheim Veterinary Scholars Program

Subject
Clinical Medicine

NORMOTHERMIC MACHINE PERFUSION ENABLES REPERFUSION INJURY ASSESSMENT AND EX VIVO SIMULATION OF INTESTINAL TRANSPLANTATION

Abigail Williams (Undergraduate Student)

Carla Lopez Cruz, Juan Pesantez Valdivieso, Lydia Poisson, John Freund, Caroline Mckinney-Aguirre, Elizabeth Goya-Jorge, Liara M. Gonzalez

aewill28@ncsu.edu, clopezc2@ncsu.edu, jjpesant@ncsu.edu, lpoisso@ncsu.edu, jmfreun2@ncsu.edu, camckinn@ncsu.edu, egoyajo@ncsu.edu, lmgonza4@ncsu.edu

828-729-3525

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

A major cause of intestinal transplantation (ITx) failure is ischemia-reperfusion injury (IRI), which is especially critical in the intestine due to its high metabolic activity and susceptibility to oxygen deprivation. Injury resulting from ischemia during storage and reperfusion upon restoration of blood flow following transplantation, remains difficult to study, as traditional *in vivo* models provide limited insight into these events.

We have shown that Normothermic Machine Perfusion (NMP) is a superior intestinal preservation method compared to traditional clinical practices of static cold storage. In this study, we leveraged this novel platform to study both storage and to simulate *ex vivo* transplantation.

Porcine intestines stored for 12h using either NMP (n=3) or cold storage (n=1) were subjected to 3h of machine reperfusion. Perfusate (blood) and dialysate (waste) were collected hourly from the NMP machine. Lactate, pH, bicarbonate, glucose, and hematocrit were analyzed as real-time biomarkers of intestinal viability, allowing comparison to clinical reference values. Intestinal biopsies were taken every 3h and evaluated histopathologically using Chiu-Park grading to assess injury and repair.

Gross appearance, machine parameters, biochemical biomarkers, and histomorphometric analyses were consistent with viable and functional intestinal allografts. While optimization is ongoing, preliminary findings demonstrate that this novel application of the NMP device effectively replicates key features of ITx.

This newly developed method can be an important breakthrough in the study of IRI and how the intestine responds upon transplantation. Future studies will aim to extend reperfusion duration and refine conditions to investigate molecular mechanisms behind IRI.

Funding Source: NIH 5R01AI182590-02

Basic science, Gastroenterology - Regenerative medicine

BACLOFEN CLEARANCE ACROSS MULTIPLE EXTRACORPOREAL THERAPIES: AN EX VIVO EVALUATION

Zsofia Vigh[1], graduate student (ECC and Extracorporeal Therapies fellow)
Ronald Baynes[1], Jim Yeatts[1], Carley-Martin Macfarlane[1,] Faculty Mentor: Jiwoong Her[1]

zvigh@ncsu.edu, jher@ncsu.edu

(765)775-0880

[1]:NCSU CVM

Introduction: *Baclofen can cause severe adverse effects in dogs and cats when accidentally ingested. The optimal modality of extracorporeal therapies (ECT) for baclofen removal in veterinary patients remains unclear. The aim of this study was to compare the clearance efficacy of baclofen using low- and high-efficiency intermittent hemodialysis (IHD), continuous renal replacement therapy (CRRT), and carbon- and polymer-based hemoperfusion (HP). We hypothesized that IHD would provide the greatest clearance, while CRRT would be the least effective modality.*
Method: *For each modality (F4-IHD, F160-IHD, CRRT, Dublin 100 HP, and VetResQ 50 HP), heparinized equine blood was spiked with baclofen to achieve concentrations of 1800 ng/mL and 900 ng/mL. The blood was divided into five blood bags to simulate the circulating blood volume of a 3 kg dog, with the remaining spiked blood serving as a control. Each modality was run for two hours, targeting a total processed volume equivalent to at least 15 blood volumes. Blood samples were collected from each bag prior to treatment and at 20-minute intervals.*
Results: *Baclofen measurement in equine plasma was validated within a range of 0.01–2.5 µg/mL. Baclofen concentrations at the beginning of the ECT modalities were verified. Baclofen concentrations fell below the lower limit of detection in blood treated with both Dublin 100 and VetResQ 50 HP devices, demonstrating 95-99% elimination.*
Conclusions: *Baclofen assay were successfully validated in horse plasma. Both IHD and HP were effective in drastically eliminating baclofen. Full result is pending and will be presented furthermore in the poster presentation.*

Funding Source: NCSU Extramural Funding Incentive Program

Clinical Research

Clinical Medicine (Veterinary). Preference: Poster presentation