Poster #	Author (Classification-Mentor)	Title	Time
1	Alexander, Gabriella / Hobbs, Avery (UG - Meiklejohn)	ASSESSING THE IMPACT OF LAND USE ON THE GUT MICROBIOME OF THE COMMON EASTERN BUMBLEBEE (BOMBUS IMPATIENS)	12:00-1:00 pm
2	Arya, Sankalp (PD - Lanzas)	DEVELOPING A MULTI-SCALE MODEL OF C. DIFFICILE DYNAMICS TO INVESTIGATE THE RELATIONSHIP BETWEEN PATHOGEN LOAD AND INFECTION OUTCOMES	10:30-11:30 am
3	Atwood, Lainey (VS - Lewis)	THERMOGRAPHY IN A HETEROGENEOUS POPULATION OF HEALTHY DOGS	12:00-1:00 pm
4	Bopp, Kaitlyn (VS - Hepworth-Warren)	CHANGES IN THORACIC ULTRASONOGRAPHY IN HORSES BEING EVALUATED FOR EQUINE ASTHMA SYNDROME BEFORE AND AFTER BAL	12:00-1:00 pm
5	Brown, Aniya (VS - Camacho / Foster)	EVALUATION OF CLINICAL BIOMARKERS IN CATTLE EXPERIMENTALLY INFECTED WITH *MANNHEIMIA HAEMOLYTICA*	12:00-1:00 pm
6	Browning, Matthew (GS - Kulkarni)	IMMUNE GENE EXPRESSION IN MUCOSAL AND LYMPHOID TISSUES DURING NECROTIC ENTERITIS DISEASE IN BROILER CHICKENS	10:30-11:30 am
7	Byrne, Jake (S - Crisci)	HIGH THROUGHPUT 96 WELL PLATE BASED PORCINE ANTIBODY ISOLATION PROTOCOL	10:30-11:30 am
8	Caldwell, Madison (VS - Blikslager / Ziegler)	NEONATAL ENTERIC GLIA ENHANCE INTESTINAL EPITHELIAL RESTITUTION IN VITRO FOLLOWING EXPOSURE TO STERILE COLONIC LUMINAL CONTENT OF JUVENILE BUT NOT NEONATAL PIGS	12:00-1:00 pm
9	Castillo, Anna (UG - Fisher / Schnabel)	MESENCHYMAL STEM CELL LICENSING FOR IMPROVED TENDON HEALING	10:30-11:30 am
10	Chan, Pok Man (S - Kulkarni)	PRO-INFLAMMATORY AND IMMUNO-REGULATORY RESPONSES DURING EXPERIMENTAL CLOSTRIDIUM PERFRINGENS-INDUCED NECROTIC ENTERITIS IN CHICKENS	10:30-11:30 am
11	Chang, Ashley (VS - Sano)	HARNESSING ELECTROPORATION FOR DNA VACCINATION	12:00-1:00 pm
12	Charron, Kimberly (GS - Marsden)	DEFINING HOW CYFIP2 REGULATES THE DEVELOPMENT OF VISUAL BEHAVIOR CIRCUITRY	10:30-11:30 am

Poster #	Author (Classification-Mentor)	Title	Time
13	Choudhary, Ishita (PD - Saini)	MYELOID CELL-SPECIFIC IL-4Rα SIGNALING DIFFERENTIALLY REGULATES THE MUCOINFLAMMATORY PROTEIN SIGNATURE IN THE BALF IMMUNE CELLS IN ALLERGIC AIRWAY DISEASE	10:30-11:30 am
14	Cornatzer, Elijah (UG - Ninomiya-Tsuji)	HIPPOCAMPAL INFLAMMATION IN CANINE COGNITIVE DYSFUNCTION SYNDROME	10:30-11:30 am
15	Creamer, Maggie / Loh, Zoe (PD - Lascelles / Gruen)	CHARACTERIZATION OF COGNITION AND AFFECT IN PET DOGS WITH NATURALLY OCCURRING OSTEOARTHRITIS AND ASSOCIATED PAIN	10:30-11:30 am
16	Criollo, Valeria (GS - Kulkarni)	HOST IMMUNE RESPONSES AGAINST CLOSTRIDIUM SEPTICUM STRAINS CAUSING GANGRENOUS CLOSTRIDIAL DERMATITIS IN TURKEYS	10:30-11:30 am
17	Curry, Kourtni (VS - Buchler)	ISOLATION AND IDENTIFICATION OF ANAEROBIC GUT FUNGI FROM THE RUMEN AND FECES OF CATTLE	12:00-1:00 pm
18	Daeschner, Melissa (GS - Lopez Soto)	UNCAPPING NOCICEPTION	10:30-11:30 am
19	Darrow, Sean (VS - Bonin Ferreira)	EVALUATION OF TRANSPORT VEHICLES IN THE TRANSMISSION OF PORCINE EPIDEMIC DIARRHEA VIRUS	12:00-1:00 pm
20	Diaz, Lindsey (UG - Meiklejohn)	OPTIMIZING NON-DESTRUCTIVE SAMPLING OF PARCHMENT FOR GENOMIC SEQUENCING	10:30-11:30 am
21	Diehl, Caroline (VS - Ozawa)	TOXICOLOGY REPORTS IN EXOTIC ANIMALS	12:00-1:00 pm
22	Dion, Hannah (VS - Gamsjäger)	INVESTIGATING GOAT BLOOD TRANSFUSION DELIVERY TECHNIQUE AND ITS IMPACT ON RED BLOOD CELL STABILITY IN VITRO	12:00-1:00 pm
23	Eckert, Lynn (VS - Bennett)	POST-ADOPTION OUTCOMES OF NORTH AMERICAN SHELTER DOGS WITH BITE HISTORIES, 2021-2024	12:00-1:00 pm
24	Edel, Margaret (VS - Lascelles/Enomoto)	COMPARISON OF AX6 AND GT3X ACCELEROMETER DEVICES FOR MEASURING ACTIVITY IN DOGS	12:00-1:00 pm
25	Emanuel, Deanna (S - Gimeno)	USE OF A NOVEL MEDIUM TO GROW CHICKEN EMBRYO FIBROBLASTS THAT INCREASES THE YIELD OF MAREK'S DISEASE VACCINES	10:30-11:30 am
26	Fares, Abdelhamid (GS - Gimeno)	DIFFERENCES IN THE EARLY REPLICATION AND IMMUNE RESPONSES INDUCED BY VARIOUS CVI-988 STRAINS IN EGG-TYPE CHICKENS	10:30-11:30 am

Poster #	Author (Classification-Mentor)	Title	Time
27	Farrow, Isla (VS - Messenger)	EVALUATING THE EFFICACY OF INTRANASAL NONSTEROIDAL ANTI-INFLAMMATORY DRUGS FOR PAIN MITIGATION IN PIGLETS USING A NOVEL DRUG DELIVERY DEVICE	12:00-1:00 pm
28	Fitzgerald, Sara (VS - Halleran)	COMPARISON OF THE PINNA TO THE JUGULAR VEIN AS A SITE FOR MEASURING BLOOD GLUCOSE CONCENTRATIONS IN GOATS	12:00-1:00 pm
29	Flynn, Jason (GS - Buchler)	USING FUNCTIONAL GENOMICS AND TIMELAPSE MICROSCOPY TO ELUCIDATE THE CELL CYCLE TIMING AND ARCHITECTURE OF A HYBRID G1/S REGULATORY NETWORK IN A CHYTRID	10:30-11:30 am
30	Ford, Earl (GS - Baynes)	APPLICATION OF AN UPLC-MS METHOD FOR THE DETERMINATION OF METHYLENE BLUE RESIDUES IN CATTLE TISSUES	10:30-11:30 am
31	Forrest, Kaitlyn (VS - Mzyk)	BIOAVAILABILITY OF FLUNIXIN MEGLUMINE AFTER TRANSDERMAL ADMINISTRATION TO WOOL AND HAIR SHEEP	12:00-1:00 pm
32			
33	Gallagher, Robin (VS - Olby)	EXAMINING DEMENTIA LINKED GENES IN DOGS AND WOLVES	12:00-1:00 pm
34	Gallagher, Robin (VS - Stern)	BLOOD PRESSURE CHANGES IN AGING DOGS	12:00-1:00 pm
35	Glass, Reagan (VS - Li)	INVESTIGATING THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN PROCOAGULANT PLATELET FORMATION USING AN IN VITRO MODEL OF IMMUNE MEDIATED HEMOLYTIC ANEMIA IN DOGS	12:00-1:00 pm
36	Gupta, Shivani (PD - Lascelles)	ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 & 2 (VEGFR) ANTAGONIST IN ATTENUATING THE MIA INDUCED OSTEOARTHRITIS PAIN IN A RAT MODEL OF OSTEOARTHRITIS PAIN	10:30-11:30 am
37	Hagopian, Katy (VS - Bayless)	EFFECTS OF WITHAFERIN A ON CYTOKINE PRODUCTION IN LEUKOCYTES FROM PERIPHERAL BLOOD AND BRONCHOALVEOLAR LAVAGE SAMPLES	12:00-1:00 pm

Poster #	Author (Classification-Mentor)	Title	Time
38	Hamacher, Sam (GS - Theriot)	CHARACTERIZATION OF GENETICALLY MODIFIED CLOSTRIDIUM SPOROGENES AND ITS IMPACT ON GUT BILE ACID BIOTRANSFORMATIONS	10:30-11:30 am
39	Harris, Lindsey (VS - Meiklejohn)	ASSESSING GAPS AND TRAINING OPPORTUNITIES FOR ANIMAL ABUSE AND NEGLECT CASES IN NORTH CAROLINA	12:00-1:00 pm
40	Haupt, Emily (VS - Kendall)	LOW DOSE RADIATION THERAPY: A NOVEL TREATMENT FOR FELINE IDIOPATHIC/INTERSTITIAL CYSTITIS	12:00-1:00 pm
41	Hay, Katie (VS - Hess)	CHEMOKINE RECEPTORS AS TARGETS OF MONOCLONAL ANTIBODIES IN CANINE T-CELL LYMPHOMA	12:00-1:00 pm
42	Hertzig, Isabella (VS - Guilluy)	HYDRA VULGARIS REGENERATIVE CAPACITY UNDER HYPEROSMOTIC STRESS	12:00-1:00 pm
43	Hoot, Renee (VS - Bennett)	FAMILIES WITH CHILDREN: A GOOD FIT FOR ALL SHELTER DOGS?	12:00-1:00 pm
44	Horta, Giovanna (UG - Nascone-Yoder)	PHARMACOLOGICAL DISRUPTION OF LEFT-RIGHT ASYMMETRY CAUSES DISTINCT MORPHOLOGICAL PHENOTYPES IN THE XENOPUS STOMACH	10:30-11:30 am
45	Huffstetler, Carley (GS - Nascone-Yoder)	LEFT-RIGHT ASYMMETRIES IN THE EXTRACELLULAR MATRIX SHAPE STOMACH CURVATURE.	10:30-11:30 am
46	James, Rebekah (VS - Lewbart)	PRELIMINARY ASSESSMENT OF HEALTH BIOMARKERS OF TWO SHARK SPECIES (CARCHARHINUS BREVIPINNA AND CARCHARHINUS OBSCURUS) IN THE NEW YORK BIGHT	12:00-1:00 pm
47	Jameson, Peyton (VS - Panchan Sitthicharoenchai)	EQUINE GASTROINTESTINAL INFLAMMATION AT NC STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE PATHOLOGY SERVICE FROM 2019-2023	12:00-1:00 pm
48	John, Feba Ann (S - Kulkarni)	SCREENING AND SELECTION OF EUBIOTIC COMPOUNDS WITH IMMUNOMODULATORY AND ANTI-CLOSTRIDIUM PERFINGENS PROPERTIES	10:30-11:30 am
49	Johnson, Jake (HO - Peterson [Tufts])	DIFFUSE LARGE B-CELL LYMPHOMA PRESENTING AS CONGESTIVE HEART FAILURE IN A CAT	10:30-11:30 am

Poster #	Author (Classification-Mentor)	Title	Time
50	Jones, Anna (VS - Messenger)	POPULATION PHARMACOKINETICS OF FLUNIXIN MEGLUMINE IN PIGLETS	12:00-1:00 pm
51	Jones, Molly (VS - Almond)	REGULATION OF PERIWEANING BODY TEMPERATURE IS IMPROVED IN PIGLETS GIVEN HIGER DOSES OF INJECTABLE IRON DEXTRAN DURING THE EARLY SUCKLING PERIOD	12:00-1:00 pm
52	K C, Rekha (GS - Saini)	TIME-OF-DAY DIFFERENCE IN INFLAMMATORY RESPONSES AND CIRCADIAN CLOCK GENE EXPRESSION IN ACUTELY OZONE-EXPOSED MICE	10:30-11:30 am
53	Kamra, Arushee (UG - Blikslager / Ziegler)	DETERMINING THE EFFICACY OF IPEC-J2 DERIVED EXOSOMES ON INTESTINAL EPITHELIAL WOUND HEALING	10:30-11:30 am
54	Kaur, Gurjinder (GS - Suryawanshi)	IL-27 MODULATES MACROPHAGE IMMUNOMETABOLISM TO PROMOTE DUAL ANTI-VIRAL AND ANTI-INFLAMMATORY EFFECTOR FUNCTIONS DURING OCULAR HSV-1 INFECTION	10:30-11:30 am
55	Khaled, Nagwa (GS - Gimeno)	EARLY CHANGES IN THE THYMUS OF MEAT TYPE CHICKENS AFTER VACCINATION WITH VARIOUS MDV-1 VACCINES	10:30-11:30 am
56	Knight, Paige (VS - Martin)	EQUINE EXTRACELLULAR STEM CELLS EXERT DIRECT BACTERICIDAL EFFECTS	12:00-1:00 pm
57	Koehler, Kelly (VS - Ben-Horin)	PREVALENCE OF HEMATODINIUM SP. INFECTION IN JUVENILE NORTH CAROLINA BLUE CRABS (CALLINECTES SAPIDUS)	12:00-1:00 pm
58	Kohls, Madeline (VS - Shelton)	COLORECTAL CANCER METASTASIS THROUGH THE ENDOTHELIUM	12:00-1:00 pm
59	Korla, Praveen Kumar (PD - Birkenheuer)	THE UPDATED CYTAUXZOON FELIS GENOME	10:30-11:30 am
60	Krogman, Nicole (VS - Ben-Horin)	PATHOLOGY ASSOCIATED WITH SUDDEN UNUSUAL MORTALITY SYNDROME (SUMS) IN DIPLOID AND TRIPLOID OYSTERS	12:00-1:00 pm
61	Ku, Isabel (VS - Gilger)	CORNEAL SAFETY PROFILE OF COLD ATMOSPHERIC PLASMA (CAP) AS A THERAPEUTIC FOR FUNGAL KERATITIS: EFFECTS ON EX VIVO PORCINE EYES	12:00-1:00 pm

Poster #	Author (Classification-Mentor)	Title	Time
62	Kumar, Rahul (GS - Saini)	COMBINED LIVER-SPECIFIC DELETION OF RNA-BINDING PROTEINS, ZFP36L1 AND ZFP36L2, INITIATE CHOLESTATIC LIVER INJURY AND FIBROSIS	10:30-11:30 am
63	Lamichhane, Richa (GS - Saini)	TRISTETRAPROLIN (TTP) PROTECTS AGAINST OZONE-INDUCED ACUTE LUNG INJURY AND INFLAMMATION IN MICE	10:30-11:30 am
64	Leber, Meghan (VS - Rhea)	WILD NORTH CAROLINA FRESHWATER TURTLES: POTENTIAL BIOINDICATORS OF ENVIRONMENTAL ANTIMICROBIAL RESISTANCE?	12:00-1:00 pm
65	Lierz, Sydney (VS - Gonzalez)	OPTIMIZING PERMEABILITY ASSAYS TO EVALUATE THE PROTECTIVE EFFECTS OF NORMOTHERMIC MACHINE PERFUSION PRESERVATION	12:00-1:00 pm
66	Livingston, Isabella (GS - Breen)	MOLECULAR DISCOVERY OF DNA FROM FILARIAL NEMATODES IN AN ENDANGERED GALAPAGOS PINNIPED (ZALOPHUS WOLLEBAEKI)	10:30-11:30 am
67	López Rivera, Karen (VS - Gruber)	EFFECTS OF PRO-INFLAMMATORY CYTOKINES ON TNFα SECRETION BY CANINE OSTEOSARCOMA CELLS	12:00-1:00 pm
68	Lorenz, Lizette (Faculty)	THE ROLE OF TEXTILE DYES IN THE IMMUNE SYSTEM DYSREGULATION	10:30-11:30 am
69	Luzzi, Angelica (VS - Traverson)	INFLUENCE OF MATERIAL ON ACCURACY OF NOVEL CUSTOM 3D-PRINTED CUTTING GUIDE IN CANINE SEGMENTAL MANDIBULECTOMY: A CADAVERIC STUDY	12:00-1:00 pm
70	McDermott, Lexa (UG - Sheahan)	OPTIMIZING PROTOCOL FOR IN VITRO EQUINE RECTAL MONOLAYER GROWTH	10:30-11:30 am
71	Mitlyng, Natalie (VS - Hobbs)	EX VIVO EVALUATION OF AN ACTIVATED CARBON HEMOPERFUSION COLUMN FOR REMOVAL OF CYTOKINES AND IMPACT ON LABORATORY PARAMETERS IN HORSES	12:00-1:00 pm
72	Mooring, Margaret (VS - Varner)	PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS OF INTRAVENOUS MORPHINE AND BUTORPHANOL IN CATTLE	12:00-1:00 pm
73	Mosley, Quiana (GS - Lopez Soto)	CHARACTERIZING ALTERNATIVE SPLICING CONTRIBUTIONS TO MOUSE SENSORY NEURONS	10:30-11:30 am

Poster #	Author (Classification-Mentor)	Title	Time
74	Murray, Gail (VS - Gruen)	EVALUATING THE EFFECT OF THE CALM & COZY WRAP ON THE BEHAVIOR OF CATS DURING VETERINARY EXAMS	12:00 - 1:00 pm
75	Ortiz, Eric (VS - Sheats)	THE SAFETY OF TRAP-DEXAMETHASONE ITS IMPACTON THE HPA-AXIS	12:00-1:00 pm
76	Owens, Anya (VS - Moore / Fowlkes)	CLINICOPATHOLOGIC EVALUATION OF RADATION-INDUCED SKIN TOXICITY IN A PORCINE MODEL	12:00-1:00 pm
77	Pan, Isabella (UG - Dembek / Gilger)	EFFECT OF THE REDUCTION OF BLUE LIGHT ON SLEEP AND STRESS LEVELS IN EQUINES	10:30-11:30 am
78	Paudel, Kshitiz (GS - Saini)	THE HISTORY OF ACUTE OZONE EXPOSURE AFFECTS LUNG'S RESPONSE TO REPETITIVE OZONE EXPOSURE	10:30-11:30 am
79	Perkins, Cypress (S - Theriot)	NEW ANTIMICROBIAL EVG7 PREVENTS RECURRENT CLOSTRIDIOIDES DIFFICILE INFECTION IN A MOUSE MODEL BY SPARING MEMBERS OF THE LACHNOSPIRACEAE	10:30-11:30 am
80	Perry, Erin (VS - Traverson)	INFLUENCE OF MATERIAL ON ACCURACY OF NOVEL CUSTOM 3D-PRINTED CUTTING GUIDE IN CANINE SEGMENTAL MANDIBULECTOMY: A CADAVERIC STUDY	12:00-1:00 pm
81	Petry, Jessica (VS - Lewis)	VARIABILITY OF MEDICAL INFRARED THERMOGRAPHY IN HEALTHY SHELTER DOGS	12:00-1:00 pm
82	Phillips, Lanie (VS - Gamsjäger)	AN INVESTIGATION OF ERYTHROCYTE HEALTH AND BIOCHEMICAL CHANGES IN GOAT BLOOD STORED AS WHOLE BLOOD AND PACKED RED BLOOD CELLS	12:00-1:00 pm
83	Pugliese, Brenna (GS - Schnabel)	DOES ALPHA-2-MACROGLOBULIN CONTROL INFLAMMATION IN AN IN VITRO MODEL OF EQUINE OSTEOARTHRITIS?	10:30-11:30 am
84	Ren, Jiayi (GS - Suryawanshi)	THE PROTECTIVE ROLE OF IFN-λ AT THE OCULAR MUCOSAL SURFACE DURING CORNEAL HSV-1 INFECTION	10:30-11:30 am
85	Rogers, Erica (UG - Yoder)	DEVELOPMENTAL TOXICITY ASSESSMENT OF PER- AND POLYFLUOROALKYL SUBSTANCES-BASED FIREFIGHTING FOAMS & ALTERNATIVE FLUORINE-FREE FOAMS USING THE ZEBRAFISH MODEL	10:30-11:30 am

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86	Ruiz Rosario, Fabiola (VS - Schnabel)	GENE EXPRESSION IN EQUINE JOINT CELLS TREATED WITH ALPHA-2 MACROGLOBULIN IN AN IN VITRO OSTEOARTHRITIS MODEL	12:00-1:00 pm
87	Ruterbories Culbreth, Laura (S - Lynch)	COMPARISON OF THE PHARMACOKINETICS AND PHARMACODYNAMICS OF APIXABAN AND RIVAROXABN IN DOGS	10:30-11:30 am
88	Santiago Rivera, Fabiola (VS - Pairis-Garcia)	DEVELOPING AND VALIDATING A PAIN SCALE FOR MEAT-PRODUCING GUINEA PIGS UNDERGOING CASTRATION	12:00-1:00 pm
89	Scheible, Melissa (S - Meiklejohn)	BRIDGING THE GAP BETWEEN GENOMICS AND THE HUMANITIES: MITOCHONDRIAL GENOME SEQUENCING FOR DETERMINING SPECIES OF ORIGIN IN PARCHMENT SPECIMENS	10:30-11:30 am
90	Scott, Samantha (VS - Olby)	DEVELOPMENT OF A FUNCTIONAL VISION TEST FOR COMPANION DOGS	12:00-1:00 pm
91	Shah, Diya (UG - Nascone-Yoder)	JUN N-TERMINAL KINASE (JNK) AND RHO KINASE (ROCK) ARE REQUIRED FOR STOMACH CURVATURE	10:30-11:30 am
92	Shankar, Aditi (GS - Lascelles/Mishra)	LOCALIZATION AND EXPRESSION OF THE GENE FOR ARTEMIN IN MOUSE AND CANINE OSTEOARTHRITIC JOINT TISSUES	10:30-11:30 am
93	Shankar, Aditi (GS - Lascelles/Mishra)	ARTEMIN-INDUCED NEURONAL SPROUTING IN OSTEOARTHRITIS: IMPLICATIONS FOR PAIN MECHANISMS AND THERAPEUTIC STRATEGIES	10:30-11:30 am
94	Silva, Igor (GS - Bayless)	DEVELOPMENT AND VALIDATION OF NOVEL WITHAFERIN A ANALOGS AS CHEMOPROTEOMIC PROBES	10:30-11:30 am
95	Singamsetty, Dhruthi (GS - Saini)	IDENTIFYING THE AIRWAY EPITHELIAL CELL-SPECIFIC ROLE OF IL-4Rα IN ALLERGEN-INDUCED MUCOUS CELL METAPLASIA	10:30-11:30 am
96	Soto Montes, Dileydis (VS - Halleran)	PHARMACOKINETICS OF PHENAZOPYRIDINE IN HEALTHY GOATS AT TWO DIFFERENT DOSAGES	12:00-1:00 pm
97	Stoop, Seline (VS - Sheahan)	INVESTIGATING THE ROLE OF SGLT1 AND INCRETINS IN EQUINE INSULIN DYSREGULATION USING DUODENAL SAMPLES	12:00-1:00 pm

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98	Talic, Emily (PD - Cruse)	CHRONIC EFFECTS OF ALLERGIC HYPERSENSITIVITY-RELATED CYTOKINES IL-4 AND IL-33 ON MAST CELL FUNCTION AND RESPONSIVENESS	10:30-11:30 am
99	Tamamoto-Mochizuki, Chie (PD - Mishra)	INFILTRATION OF NEUTROPHILS IN AN EXPERIMENTAL MODEL OF CANINE ACUTE ATOPIC DERMATITIS SKIN LESIONS	10:30-11:30 am
100	Tang, Aaron (VS - DeFrancesco)	ASSESSMENT OF HYPERCOAGULABILITY, FIBRINOLYSIS, AND INFLAMMATION IN CATS WITH DIFFERENT MANIFESTATIONS OF CONGESTIVE HEART FAILURE	12:00-1:00 pm
101	Thomas, Stephanie (S - Theriot)	CLOSTRIDIOIDES DIFFICILE TOXINS ALTER HOST BILE ACID SYNTHESIS PATHWAY GENE EXPRESSION	10:30-11:30 am
102	Tomblin, Emily (VS - Sano)	INDUCTION OF AN IMMUNOLOGICAL ANTI-TUMOR RESPONSE USING PULSED ELECTRIC FIELDS	12:00-1:00 pm
103	Tran, Becky (VS - Kulkarni)	EVALUATION OF IMMUNE RESPONSES IN CHICKENS IMMUNIZED WITH CLOSTRIDIAL DERMATITIS VACCINE	12:00-1:00 pm
104	Vair, Elizabeth (VS - Meiklejohn)	EPIGENETIC DIFFERENCES BETWEEN CAPTIVE AND WILD AMERICAN BLACK BEARS (URSUS AMERICANUS) IN NORTH CAROLINA	12:00-1:00 pm
105	Wade-LaHart, Telea (PD - Westermeyer/Gruen/Olby)	OPHTHALMIC FINDINGS IN AGING DOGS	10:30-11:30 am
106	Wills, Maya (VS - Frey)	CHARACTERIZING SUBCLINICAL BACTERIURIA IN AGING FEMALE CATS	12:00-1:00 pm
107	Wood, Elyse (GS - Van Landeghem)	IDENTIFICATION OF A NOVEL CHEMOPROTECTIVE FACTOR IN THE COLONIC TUMOR MICROENVIRONMENT: FOLLISTATIN-LIKE 3	10:30-11:30 am
108	Wright, Abigail (UG - Nascone-Yoder)	INVESTIGATING THE INFLUENCE OF LEFT-RIGHT ASYMMETRY ON XENOPUS HINDGUT DEVELOPMENT	10:30-11:30 am
109	Yasa, Krithika (UG - Ziegler)	DETERMINING THE EFFECTS OF MICROBIOTA-PRODUCED FACTORS ON INTESTINAL EPITHELIAL CELL CYCLE DYNAMICS AND PROLIFERATION	10:30-11:30 am

Poster #	Author (Classification-Mentor)	Title	Time
		WITH DIFFERENT MANIFESTATIONS OF CONGESTIVE HEART FAILURE	
100	Thomas, Stephanie (S - Theriot)	CLOSTRIDIOIDES DIFFICILE TOXINS ALTER HOST BILE ACID SYNTHESIS PATHWAY GENE EXPRESSION	10:30-11:30 am
101	Tomblin, Emily (VS - Sano)	INDUCTION OF AN IMMUNOLOGICAL ANTI-TUMOR RESPONSE USING PULSED ELECTRIC FIELDS	12:00-1:00 pm
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108	Yasa, Krithika (UG - Ziegler)	DETERMINING THE EFFECTS OF MICROBIOTA-PRODUCED FACTORS ON INTESTINAL EPITHELIAL CELL CYCLE DYNAMICS AND PROLIFERATION	10:30-11:30 am

ASSESSING THE IMPACT OF LAND USE ON THE GUT MICROBIOME OF THE COMMON EASTERN BUMBLEBEE (*BOMBUS IMPATIENS*)

Authors: **Gabriella Alexander (undergraduate), Avery Hobbs (graduate student)**
Co-authors: Teresa M. Tiedge, Kelly A Meiklejohn
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Affiliations: NCSU CVM

Abstract:
The common eastern bumblebee, *Bombus impatiens*, is an integral player in the stability of the Eastern North American environment. As a generalist pollinator, *B. impatiens* visits wildflowers and tree flowers in natural areas, as well as a broad range of crops. Human influences such as urbanization and pesticide use have been shown to decline the conditions of wildlife including insects. Urbanization and management strategies are likely to be challenging for bumble bees, disrupting population numbers and the pollination services they provide. Previous studies examining the impact of urbanization in insects have reported that gene expression, pathogen load, and the gut microbiome can be altered as a response to stress based on changing land use. This research aims to identify the potential impacts of anthropogenic change on the gut microbiome of *B. impatiens*. To achieve this, over 2,400 bumblebees were collected across four land use types (industrial, urban-garden, agricultural and natural) in both Alabama and Washington D.C. during foraging seasons in 2018 and 2019. A cohort of 434 bees spanning various land use and collection times were selected for gut microbiome analysis. The gut from each bumblebee was carefully dissected and used for DNA extraction using the *Quick*-DNA Fecal/Soil Microbiome kit (Zymo Research). The *Quick*-16S Plus NGS Library Prep Kit was used to simultaneously amplify the V3-V4 region of 16S and index samples for Illumina sequencing. The results of this research will assist in highlighting the environmental conditions suitable for the health of *B. impatiens* bumblebees.

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Subject category: Genetics

DEVELOPING A MULTI-SCALE MODEL OF *C. DIFFICILE* DYNAMICS TO INVESTIGATE THE RELATIONSHIP BETWEEN PATHOGEN LOAD AND INFECTION OUTCOMES

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In healthcare settings *Clostridiodes difficile* infections (CDI) are a significant source of morbidity and mortality, particularly for high-risk individuals such as immunocompromised patients. Colonization with *C. difficile* is a key precursor of infection and asymptomatically colonized patients are an important source of transmission of the organism to other patients. Thus, preventing colonization and reducing pathogen load in those colonized could significantly reduce CDI. Despite these benefits, no approved agents target *C. difficile* colonization. A primary reason for this is the lack of understanding between pathogen load and CDI progression. Mathematical models can help in quantifying this relationship, especially multi-scale models, which explicitly link pathogen load to CDI progression and patient status. We present here an individual-based model that couples the between-host transmission of *C. difficile* in a hospital with within-host *C. difficile* dynamics. The model incorporates the different concentrations of *C. difficile* in patients to classify them as asymptomatically colonized or symptomatically infected. We analyze the model to determine the number of CDI cases, colonized patients and transmission events in the hospital. We utilize the model to compare the impact of utilizing antimicrobials as decolonizing agents. From the model output, we see that reducing colonization leads to reduction in CDI cases, however frequency of transmission remains the same. We will further expand this model to understand the effects of colonization in different synthetic patient cohorts as well as the impact of other decolonization strategies. This model can thus help in designing better trials for novel biotherapeutics.

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Infectious Disease

IDENTIFICATION OF SMALL MOLECULE INHIBITORS FOR BLOCKING ENTRY OF SARS-COV-2 AND RELATED BAT CORONAVIRUSES

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel and highly pathogenic coronavirus and the causative agent of COVID-19, an ongoing pandemic. Delays in vaccine deployment at a global scale, vaccine hesitancy, and ongoing evolution of the virus is leading to emergence of SARS-CoV-2 variants that are potentially more transmissible and pathogenic. SARS-CoV-2 virions display the characteristic club-shaped projections formed by trimers of viral Spike glycoprotein on their surface. To invade the host cell, the receptor-binding domain (RBD) of Spike protein binds to the host cell's ACE2 receptor, followed by cleavage events that allow the Spike protein to fuse with the host cell membrane. Thus, the Spike protein is a prime target for therapeutic interventions.

Our study focused on identifying small molecule inhibitors that block the Spike protein-ACE2 interaction. We identified "SAI4", a candidate small molecule, which inhibits SARS-CoV-2 pseudovirus entry with an IC50 of ~18 μM and binding to the Spike protein's RBD with a Kd of ~20 μM. SAI4 effectively inhibited pseudovirus entry in cells expressing both engineered and physiological levels of ACE2. We validated the antiviral efficacy of SAI4 against genuine SARS-CoV-2 using a murine model of infection, observing a significant reduction in viral titers and proinflammatory cytokines compared to the control group. Furthermore, we demonstrated antiviral potential of SAI4 against four SARS-CoV-2 variants of concern (α, β, γ, and δ). Finally, we explored the antiviral potential of SAI4 against two circulating bat coronaviruses, BANAL-20-53 and BANAL-20-103, which utilize ACE2 as their entry receptor. Remarkably, the S-enantiomer of SAI4 exhibited an IC50 of 1.4 μM against both variants. These findings suggest that SAI4 is a promising candidate for further development as a broad-spectrum antiviral agent targeting SARS-CoV-2 and related coronaviruses.

CHANGES IN THORACIC ULTRASONOGRAPHY IN HORSES BEING EVALUATED FOR EQUINE ASTHMA SYNDROME BEFORE AND AFTER BAL

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Equine asthma syndrome is a chronic non-infectious respiratory disease affecting adult horses. Diagnosis is based on cytology of bronchoalveolar lavage (BAL) fluid and clinical signs. Thoracic ultrasonography (TUS) is used to rule out other disease processes. There's an effect of BAL on radiographic, TUS, and CT appearance of the lungs for up to 24 hours in humans, macaques, and dogs. However, the effect of BAL on the ultrasonographic appearance of the lungs has not been investigated in horses. The objective of this study was to compare TUS changes in the thorax of horses evaluated for equine asthma before and after BAL using a validated scoring system. We hypothesized that scores would be higher following BAL and there would be a correlation between magnitude of change in TUS score, percent fluid recovery, and asthma group classification based on BAL cytology. 28 adult horses, with a median age of 15 (range 8-25), suspected to have equine asthma were enrolled. TUS was performed before BAL and repeated within 2 hours after BAL. Horses were classified as normal, mild-moderate, or severe equine asthma based on BAL cytology. There was a statistically significant difference between pre-BAL (median= 16) and post-BAL (median= 18) scores (p= 0.04). There was no statistically significant difference in magnitude of change between pre and post BAL between groups (normal, mild-moderate, severe). There was no significant correlation between percent recovery of BAL fluid and magnitude of change (p=0.26). Clinicians should be aware that BAL may change the ultrasonographic appearance of the lungs.

Category: Clinical Medicine
Funding: Boehringer Ingelheim

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EVALUATION OF CLINICAL BIOMARKERS IN CATTLE EXPERIMENTALLY INFECTED WITH *MANNHEIMIA HAEMOLYTICA*

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Bovine respiratory disease (BRD) impacts cattle welfare and is the largest cause of economic loss in the beef industry. *Mannheimia haemolytica* is the most commonly isolated pathogen of BRD worldwide. This study aimed to evaluate the changes of twenty selected biomarkers throughout the course of BRD caused by *M. haemolytica* to identify the trends that exist in the disease as part of a larger study evaluating flunixin meglumine residues in diseased calves. Twenty-four calves were exposed to transport stress and then inoculated intratracheally with *M. haemolytica* to mimic stress and infection. Calves were treated with flunixin meglumine intravenously upon meeting enrollment criteria. Samples were taken before transport, on arrival but before inoculation, and daily until 144h post inoculation depending on group assignment or until euthanasia. Clinical assessments, respiratory and depression scores, and thoracic ultrasonography were performed daily. We hypothesized that fibrinogen would be the best indicator of disease severity as it is easily measured and analyzed in ruminants as part of the complete blood count (CBC), and it is the most commonly evaluated acute phase protein. Fibrinogen increased significantly from the pre-transport to 120h time points. Data from the other biomarkers measured within the study are pending. This study demonstrated that BRD is associated with significant alterations in specific biomarker levels. Our data indicate that measuring biomarkers in conjunction with physical examination and thoracic ultrasonography could aid in determining the disease severity of cattle infected with *M. haemolytica*.

NC State University Office of the Associate Dean of Research and Graduate Studies

Infectious Disease

IMMUNE GENE EXPRESSION IN MUCOSAL AND LYMPHOID TISSUES DURING NECROTIC ENTERITIS DISEASE IN BROILER CHICKENS

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Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an enteric disease of chickens negatively affecting the economy of the broiler industry worldwide. Despite its huge economic burden, there are no non-antibiotic control measures, such as vaccines, currently available, which is largely because the host response during NE is not well understood. In the present study we used two NE predisposing models, namely the 'dietary' and 'dietary + Coccidia', to evaluate the mucosal (duodenum and jejunum) and lymphoid (cecal tonsil 'CT' and bursa of Fabricius 'Bursa') immune gene expression in broiler chickens during NE. We also used two virulent strains of *C. perfringens*, Str. CP44 and Str. CP64, to reproduce NE. Results showed that Coccidia-predisposition followed by CP44 or CP64 infection induced increased (P<0.05) expression of IL-1β, IL-6, IL-13 or FoxP3 genes in the cecal tonsils compared to the Coccidia-alone group. Additionally, the Coccidia+CP44 infected group had higher (P<0.05) IFNγ transcription in the duodenum and jejunum tissues. Furthermore, birds receiving dietary predisposition+CP44 (but no coccidia) also had elevated (P<0.05) expression of the IL-6 gene when compared to the negative (uninfected) control group. In summary, our findings suggested that coccidia-predisposition increases the severity of NE and results in an increased gene expression of pro-inflammatory cytokines (IL-1β/IL-6/IFNγ) in both mucosal and lymphoid tissues, as well as increased immunoregulatory transcription factors such as FoxP3. Further studies are currently underway to investigate the mechanisms of immunity against NE in broiler chickens.

Subject Category: Immunology

HIGH THROUGHPUT 96 WELL PLATE BASED PORCINE ANTIBODY ISOLATION PROTOCOL

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Abstract

For decades, scientists have been using column chromatography to purify an array of analytes. This same system has also been deployed for the isolation and purification of antibodies to increase sensitivity and specificity of assays such as Western Blot, ELISA, Immunohistochemistry, etc. Here we describe an antibody isolation method that was specifically developed for use in pregnant/lactating pigs when measuring influenza specific antibodies. In this context, antibody isolation was required because of the inherent nature of the samples, in particular serum, milk and colostrum, that generate high background in the assay due to the presence of hyper sialylation that prevents the analysis of finer differences in the system. We developed a swine specific high throughput 96 well plate-based method that allowed for both a rapid and consistent specific antibody isotype yield from individual animals, from various tissues and across multiple timepoints. The following protocol describes a modified column chromatography technique utilizing multiple 96-well membrane-bottom plates in conjunction with isotype specific resins to isolate both IgG and IgA to be used in a variety of antibody-based immunoassays to control the high background noise from pregnant/lactating pigs. The results show that after sample processing and antibody isolation the protein yield was similar between single column chromatography and 96 well based system but less time consuming. In comparison to the standard single column chromatography, taking between 2-3 hours per 12 samples for isolation and quantification, we have been able to increase the antibody isolation to 192 samples isolated and quantified in 8-9 hours.

Funding Source: partially funded by Bill & Melinda Gates Foundation

Subject Category: Immunology

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

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Neonates exhibit significantly poorer outcomes from intestinal epithelial damage following ischemia for unknown reasons due to a defect in epithelial restitution. However, ischemia-injured neonatal pig intestine can be rescued by application of homogenized juvenile mucosa, but the mechanism remains unclear. Enteric glial cells (EGC) promote epithelial restitution following injury via paracrine signaling and are abundant in the intestinal mucosa of juvenile but not neonatal pigs. In mice, postnatal maturation and maintenance of this EGC network is driven by colonization of gut microbiota. Therefore, we believe that changes to luminal microbiota during weaning plays a key role in EGC maturation required for proper epithelial restitution. We hypothesized that treatment with juvenile (>6 weeks of age), but not neonatal (<3 weeks of age), luminal content (LC) would improve restitution of neonatal epithelial monolayers co-cultured with neonatal EGC. We compared the effect of sterile-filtered neonatal or juvenile LC addition on scratch wound restitution in neonatal porcine IPEC-J2 monolayers grown in monoculture or co-culture with primary porcine neonatal submucosal EGC using a transwell system. Our results support a significant effect of LC treatment in the presence of EGC (P=0.0081). Specifically, juvenile LC treatment enhanced IPEC-J2 wound closure in co-culture with EGC but not in monoculture (P=0.0005). Mass spectrometry of EGC supernatants identified the damage-associated molecular pattern HMGB1 as significantly decreased in neonatal EGC treated with juvenile LC compared to those treated with neonatal LC. Future work will further investigate the role of EGC-secreted HMGB1 on intestinal epithelial wound healing and its regulation by LC.

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Subject category: Gastroenterology

MESENCHYMAL STEM CELL LICENSING FOR IMPROVED TENDON HEALING

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Abstract: (250 word limit)
Tendon injuries cause significant pain and loss of function in both veterinary and human patients, with Achilles tendon injuries being particularly common. Horses serve as an excellent model for studying these injuries, as the superficial digital flexor tendon (SDFT) in horses is equivalent to the Achilles tendon in humans. Mesenchymal stem cells (MSCs) have shown promise in treating equine tendon injuries, but healed tissue often lacks the composition and strength of native tendon, leading to high reinjury rates. Recent studies suggest that "licensing" MSCs with IL-1ß and TGF-ß2 can enhance their therapeutic efficacy. In vitro research has shown that dual licensing improves tenocyte migration, metabolism, and gene expression, prompting our in vivo investigation. The aim of this study is to compare dual licensed and naïve MSCs on tendon healing in vivo. A total of 20 horses will be included in the study. Bone marrow is collected to obtain MSCs and surgically induced core SDFT lesions are created in one forelimb under general anesthesia. At 14 days postoperatively, either licensed or naïve MSCs are injected intralesionally using ultrasound guidance. Horses follow a 90-day exercise protocol with regular lameness and ultrasound evaluations, followed by postmortem analysis. Preliminary ultrasound images from 9 horses show subjective improvement with licensed MSCs, with biomechanical data yet to reveal definitive insights. Completion of lameness, ultrasound, biochemical, biomechanical, histological, and gene expression analyses will determine if dual licensed MSCs improve tendon healing in vivo and their potential to enhance clinical management of these injuries.

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Hong Kong Jockey Club Equine Welfare Research Foundation (LVS)
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Subject Category: Regenerative medicine

PRO-INFLAMMATORY AND IMMUNO-REGULATORY RESPONSES DURING EXPERIMENTAL *CLOSTRIDIUM PERFRINGENS*-INDUCED NECROTIC ENTERITIS IN CHICKENS

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Host immune responses against virulent *Clostridium perfringens* during NE in chickens are poorly understood. The present work investigated the CD4+, TCRγδ+ T cells and IgM+ B cell responses along with evaluating the immune gene expression in the mucosal lymphoid organ, the cecal tonsil (CT), and the tissues (duodenum and jejunum), of broiler chickens infected with virulent *C. perfringens* (Str. CP64). Results showed that the infected chickens had reduced (P=0.0524) body weight gain and increased (P<0.05) NE severity as indicated by the gross pathology lesions in the small intestine. Immunophenotyping analysis of CT cells revealed a reduction (P<0.05) in the frequencies of CD4+CD25+ and TCRγδ+CD25+ T cells, while IgM+ B cell frequencies were higher (P<0.05) in comparison to uninfected control. Gene expression analysis showed an increased (P<0.05) transcription of IL-1β inall tissues from the infected group compared to the uninfected control. In the CT and jejunum, infected birds also hadelevated (P<0.05) IL-6 and/or IFNγ, and reduced (P<0.05) CD25 transcription. These results suggested that virulent *C. perfringens* seem to induce an inflammatory mucosal andlymphoid responses via augmenting the expression of pro-inflammatory cytokine genes, while suppressing the regulatory CD4+ and γδ T cell responses, as well as the CD25 and FOXP3 molecular transcription. Further investigation is currently underway to determine whether these findings have any implications for NE pathogenesis in chickens.

Funding: US Poultry and Egg Association

Subject Category: Immunology

HARNESSING ELECTROPORATION FOR DNA VACCINATION

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Abstract: Electroporation is the application of an electric field to a cell which causes the permeabilization of the membrane. Higher doses of pulsed electric fields lead to cell death, while lower doses form pores that the cell can repair. Such reversible permeabilization could be exploited for the uptake of large molecules. Clinically, reversible electroporation could be harnessed for the purposes of DNA vaccination, gene editing or chemotherapy, however, it faces numerous challenges in becoming the standard of care. Current protocols for reversible electroporation cause significant muscle stimulation, high rates of cell death, and low rates of transfection. The purpose of this study was to improve transfection efficacy *in vivo*. In previous studies in our laboratory, bipolar microsecond pulses have been found to lead to superior gene transfection with greater reversible thresholds than longer duration monopolar pulses in 3D cell cultures. In this study, we sought to replicate this in a murine model. Mice were injected intramuscularly with DNA plasmid encoding for a bioluminescent reporter enzyme, then treated using microsecond pulsed electric fields. Transfection efficacy was evaluated using whole-animal optical imaging. Higher doses and faster rates of treatment delivert led to greater levels of gene expression, though the standard protocol performed better than our proprietary protocols. In spite of its lagging performance in these data, the clinical viability of bipolar microsecond pulses is significantly greater than current standards. Further modeling and experimentation is needed to determine optimal protocols for the implementation of bipolar microsecond pulses for the purposes of DNA delivery.

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Biomedical Engineering

Title: DEFINING HOW CYFIP2 REGULATES THE DEVELOPMENT OF VISUAL BEHAVIOR CIRCUITRY

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Development of the neural circuits that enable detection, processing, and response to visual stimuli requires a complex genetic program that controls neuronal migration, axonal growth and guidance, and synapse formation and function. *cytoplasmic FMRP interacting protein 2 (cyfip2)* has roles in these processes, and *cyfip2* mutant zebrafish have subtle defects in retinal lamination and patterning of retinotectal axon projections. Here we show that *cyfip2* mutants display severe behavioral defects that suggest *cyfip2* has important roles in visual system development beyond retinotectal axon guidance. Our current work is focused on defining when, how, and where *cyfip2* acts in the developing visual system to enable normal behavioral responses. To define the critical window for when *cyfip2* expression is needed, we established a heatshock-inducible transgenic line and found that transient expression of Cyfip2 from can partially restore visually-mediated responses in *cyfip2* mutants. To determine if Cyfip2 regulates visual behavior circuitry through its role in modulating actin dynamics and/or through its role in translation regulation, we will use heatshock-inducible transgenics with point mutations in Cyfip2's protein binding domains that disrupt actin remodeling or translational repression and measure the ability of these transgenes to restore visually-driven behavior. Finally, we will identify the precise location(s) of the functional deficit(s) in the visual system of *cyfip2* mutants using electroretinograms, calcium imaging, and cell-specific rescues. Together, these experiments will both spatially and temporally define Cyfip2's roles in the neural circuits required for each step in translating visual input into proper behavioral responses.

Funding Source: National Institute of Neurological Disorders and Stroke (NINDS), R01-NS116354-01A1
Primary Subject: Neuroscience

MYELOID CELL-SPECIFIC IL-4Rα SIGNALING DIFFERENTIALLY REGULATES THE MUCOINFLAMMATORY PROTEIN SIGNATURE IN THE BALF IMMUNE CELLS IN ALLERGIC AIRWAY DISEASE

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Cell-specific IL-4Rα signaling differentially contributes towards the development of allergic airway disease pathology. However, the role of myeloid cell-specific IL-4Rα signaling in regulating protein signatures in the BALF immune cells from allergens-challenged lung airspaces remains unknown. We hypothesized that myeloid cell-specific IL-4Rα signaling is required for the mucoinflammatory protein signatures in BALF immune cells in allergic asthma. To test our hypothesis, we challenged IL-4Rα sufficient (Het), IL-4Rα KO (KO), and myeloid cell-specific IL-4Rα-deficient (Mye KO) mice with either mixed allergens (MA) or normal saline and performed the proteomic analyses on BALF immune cells. While the principal component analyses of the BALF immune cell-bound proteome did not reveal clear distinction between KO-MA and Mye KO-MA mice, they both showed clear separation from Het-MA mice. Compared to Het-MA mice, 570 and 549 proteins were upregulated and 506 and 492 proteins were downregulated in BALF immune cells from KO-MA and Mye KO-MA mice, respectively. Compared to KO-MA mice, BALF immune cells from Mye KO-MA mice showed 12 upregulated and 7 downregulated proteins. Mucoinflammatory proteins, including ARG1, CHIL3, MMP-12, EAR1, EAR2, EPX, and FETUB that were enriched in BALF immune cells from Het-MA mice, were reduced in KO-MA and Mye KO-MA mice. Other mucoinflammatory proteins enriched in BALF immune cells from Het-MA mice, including MUC5AC, CHIA, RETNLA, MUC5B, and MUC5AC, were reduced in KO-MA but not in Mye KO-MA mice. Collectively, this study identifies BALF immune cell-specific proteins whose expressions can be either dependent or independent of myeloid cell-specific IL-4Rα signaling in allergic asthma.

Primary subject category for presentation: Immunology

HIPPOCAMPAL INFLAMMATION IN CANINE COGNITIVE DYSFUNCTION SYNDROME

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This research aims to introduce a novel approach to Alzheimer's Disease (AD) by investigating the pathology of Canine Cognitive Dysfunction Syndrome (CCDS). AD is a neurodegenerative disease that causes memory loss and cognitive decline in elderly individuals. Although it is one of the leading causes of death, our understanding of its pathology is limited. One of the difficulties in AD research is the lack of effective model systems. While mouse models with human AD-associated mutations are widely used, they lack two critical features of AD: a link between amyloid-β and tau pathologies and their natural occurrence. Thus, I present a novel, unique, and complementary approach using canines as a naturally occurring disease model. Some aged dogs develop a dementia-like condition called CCDS. It is known that both AD and CCDS are caused by neuron death induced by neuroinflammation in the brain; however, the specific mechanisms are unknown.
In our lab, we have previously discovered that a protein kinase, mitogen-activated protein kinase kinase kinase 7 (MAP3K7), also known as TAK1, induces inflammatory signaling. This was causally associated with neuron loss in AD and aged mouse groups. We hypothesize that the TAK1 inflammatory signaling pathway is also associated with the pathology of CCDS. We examine our hypothesis by observing inflammation in the hippocampus of several dogs using qPCR, immunofluorescent staining, and western blotting. This research will provide a potential therapeutic target for both AD and CCDS.

Category: Neurosciences

CHARACTERIZATION OF COGNITION AND AFFECT IN PET DOGS WITH NATURALLY OCCURRING OSTEOARTHRITIS AND ASSOCIATED PAIN

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The chronic pain experience in humans encompasses adverse effects on mobility, sleep, social interactions, quality of life, cognitive, and affective dimensions. Painful osteoarthritis (OA) is a leading cause of chronic pain in humans yet translational research is not producing new therapeutics. This is partly because existing animal models fail to fully recapitulate and measure the complexity of the chronic pain experience, particularly the cognitive and affective dimensions. We hypothesize that pet dogs with naturally occurring OA pain display alterations in cognition and affect, similar to humans experiencing OA pain. In exploratory work we evaluated cognitive function and affective state in dogs with painful OA (N = 20) and healthy controls (N = 29). These groups were confirmed using owner evaluations and veterinarian examinations. The holeboard and cylinder tasks assessed cognitive domains, including memory, attention, processing speed, and executive function while the Positive and Negative Activation Scale (PANAS) owner questionnaire was used to measure affect. We found that OA dogs performed worse overall on cognitive tasks in both training and test phases (cylinder task, $p<0.05$; holeboard $p<0.01$). Additionally, dogs with OA pain had significantly diminished positive affect ($p<0.05$) across four domains of the PANAS. These early data suggest that pet dogs, like humans, also exhibit cognitive and affective impairments with OA pain, establishing a compelling case for their use as a model for developing innovative pain management therapies. These findings and future work could also improve the lives of pet dogs suffering with this common affliction.

Primary Subject Category: Pain
Funding: Salary release (Lascelles; Gruen)

HOST IMMUNE RESPONSES AGAINST *CLOSTRIDIUM SEPTICUM* STRAINS CAUSING GANGRENOUS CLOSTRIDIAL DERMATITIS IN TURKEYS

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Clostridium septicum causes clostridial dermatitis (CD), an emerging disease of turkeys, characterized by sudden deaths and necrotic dermatitis. Pathogen-specific immune responses during CD are poorly characterized. Here, we infected turkeys with three field strains of *C. septicum*, namely Str. A1, Str. B1 and Str. C1, to evaluate local (skin and muscle) and systemic (spleen) pathological and immunological responses. Results showed that strains A1 and B1 caused significantly higher mortality when compared to Str. C1. Gross and histopathology showed that birds infected with A1 and B1 had severe inflammatory/edematous, granulomatous and necrotic lesions in the skin, muscle and spleen, while these lesions in C1-infected birds were less severe and confined to skin and/or muscle. Immune gene expression showed that B1-infected birds had higher expression of Interleukin (IL)-1β, IL-6 and Interferon (IFN)γ genes compared to uninfected control, suggesting robust inflammatory response locally and systemically. The transcription of IL-1β and IFNγ in the muscle/spleen of A1-infected birds and IL-1β in the skin of Str. C1-infected group was also significantly higher than control. Additionally, A1 or B1-infected groups also had higher IL-4 transcription in these tissues, while birds infected with all three strains developed *C. septicum*-specific serum antibodies. Furthermore, splenic cellular immunophenotyping showed a marked reduction in CD4+ cells. Collectively, it can be inferred that host defense against *C. septicum* involve a marked inflammatory response coupled with antibody production and that the disease severity, as assessed by the mortality and pathological parameters, and the associated immune responses seem to be strain dependent.

ISOLATION AND IDENTIFICATION OF ANAEROBIC GUT FUNGI FROM THE RUMEN AND FECES OF CATTLE

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Anaerobic gut fungi (AGF, Neocallimastigomycota) are essential microorganisms that degrade plant fibers and contribute to the alimentary tract microbiome of herbivorous animals, including larger mammals and some reptilian, avian, and marsupial species. Current research is focused on improving ruminant nutrition by feeding AGF direct-fed microbials to improve fiber degradation, as well as inoculating silage with AGF enzymes to aid in cleaving lignocellulosic structures for higher energy exploitation from forages. Methods like these require that we discover, isolate, maintain, and manipulate AGF species in the lab. However, the complete taxonomy and diversity of AGF species remains unknown. The aim of this project was to identify AGF species from the rumen and feces of cattle at NC State University to further contribute to improving the poorly characterized genomes of AGF. We isolated genomic DNA from cattle rumen fluid and feces, amplified and cloned the AGF DNA sequence of the large subunit ribosomal RNA (LSU rRNA), and sequenced the plasmid insert. Bioinformatic analysis using the LSU rRNA Database of AGF revealed that 39 of our sequences clustered into four distinct clades: *Neocallimastix* genus, *Pecoramyces* genus, NY08 genus, and a novel clade that couldn't be confidently placed within the existing LSU rRNA Database of AGF. Comparison of our DNA sequence results from rumen fluid and fecal samples indicated that *Neocallimastix* and *Pecoramyces* AGF were exclusively present in rumen fluid samples. The novel clade, which was present in both rumen fluid and feces, opens the door for future classification of a new genus or species.

Funding Sources: NIH/T35 Interdisciplinary Biomedical Research Training Program

Subject: Microbiology

Presentation Type: Oral

UNCAPPING NOCICEPTION

Melissa Daeschner: graduate student

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The calcium-dependent secretion activator protein (CAPS1) regulates large dense core vesicle exocytosis from neurons. In mammals, release of neuropeptides such as CGRP and substance P from vesicles contributes to post injury neuronal sensitization or inflammation in skin. We have found that CAPS1 is highly expressed in all mouse sensory neurons, including pain-sensing neurons called nociceptors, but CAPS1 role in pain is unknown. Here, we focus on describing CAPS1 expression in sensory neurons and determining CAPS1 contributions to pain-related behavior in mice. Using western blotting and splicing-sensitive bioinformatics, we show that CAPS1 is expressed in sensory neurons innervating skin and that *Cadps*, CAPS1 gene, transcripts undergo extensive alternative splicing depending on neuronal type. We use the Hargreaves apparatus to evaluate heat sensitivity in wild-type mice and in mice lacking CAPS1 in TRPV1 nociceptors. Intraplantar injection of capsaicin, a TRPV1 channels agonist, is used as a neurogenic inflammation model as it induces transient hypersensitivity to sensory stimuli at 15 minutes post-injection and a return to baseline responses within 30 minutes. We found that capsaicin-induced heat hypersensitivity was 61% reduced in mice lacking CAPS1 in nociceptors as compared to wild-type mice. Moreover, capsaicin-induced CGRP release in skin was also decreased by about 20% compared to wild-type mice. Our data suggest that CAPS1 supports CGRP exocytosis following exposure to capsaicin and CAPS1 is required for normal heat hypersensitivity.

Funding sources: R00NS116123 (JLS), 2024 CVM Intramural Research Grant (JLS)

Primary category: Pain

EVALUATION OF TRANSPORT VEHICLES IN THE TRANSMISSION OF PORCINE EPIDEMIC DIARRHEA VIRUS

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Porcine epidemic diarrhea virus (PEDV), a highly contagious enteric pathogen, causes severe acute vomiting, diarrhea, and dehydration with up to 100% mortality in neonatal piglets. Biosecurity and disinfection practices are the most effective means of prevention, as variants of PEDV have led to vaccine failure. PEDV is primarily spread through the fecal-oral route, however, pigs can also be exposed indirectly via contaminated fomites. Previous studies identified transportation vehicles as a major concern in dissemination of swine diseases. Vehicle cleaning and disinfection (C&D) is primarily used to control the spread of disease, however, the frequency of vehicle contamination is unknown. In this study, samples were collected bi-weekly at three eastern NC C&D sites from four different truck types that move between swine farms: crew, feed, pigs-to-farm, and pigs-to-market. Swabs were collected from truck cabins, trailers, and tires before and after C&D with two commercial disinfectants. Total RNA from samples was extracted in the laboratory and the presence of PEDV confirmed via RT-qPCR, which has been used previously to show the presence of PEDV in trailers after C&D. Positive RT-qPCR samples indicate only the presence of viral RNA, not necessarily viable, infectious virus. This study will use a swine-model bioassay to determine the infectivity of positive samples collected. Results from this study will provide data that can be used by the swine industry to standardize C&D protocols to eliminate PEDV, as well as other foreign animal diseases with shared sensitivities to disinfectants, such as African swine fever virus.

Funding Source: USDA NADPRP

Primary Subject: Infectious Disease

OPTIMIZING NON-DESTRUCTIVE SAMPLING OF PARCHMENT FOR GENOMIC SEQUENCING

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Parchment is a writing surface derived from animal skins, which contains valuable genetic information from the source animal, surface contaminants, and more. DNA sequencing has been successfully used to identify the animal source used in parchment documents and current research has examined many destructive sampling methods of parchments. However, a limited number of non-destructive methods have been tested, which are pivotal to ensure preservation of cultural artifacts. This study examines various non-destructive methods to develop an optimal procedure for isolating cellular material from parchment. Genetic material was collected from four different parchments, ranging from the 15th century to the modern-day. During collections, researchers utilized many non-destructive sampling methods, which were categorized into two phases. Phase 1 of sampling consisted of collecting cellular material from parchment documents through cytology brushes both with and without a 30-second pre-eraser cleaning. In Phase 2, fiber lift tape and gecko tape were used to collect cellular material; samples were collected with both tapes using two and five lifts both with and without a 30-second pre-eraser cleaning. Generated sequencing reads will be processed through an existing bioinformatics pipeline in CLC Genomics Workbench (Qiagen).
Non-destructive methods have shown to be promising for future studies and need to be explored more thoroughly. Non-destructive sampling will preserve documents with historical significance while still allowing for investigations that will reveal important information.

Funding Sources: Ronald E. McNair Scholars Program; 2024 GGA Summer Team Research Mini-Grant

Subject: Genetics

TOXICOLOGY REPORTS IN EXOTIC ANIMALS

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NCSU CVM

The American Society for the Prevention of Cruelty to Animals, Animal Poison Control Center (ASPCA APCC) fields thousands of calls each year regarding animal toxicosis cases. Few reports are published regarding the prevalence of exotic animals exposed to toxins in domestic and nondomestic settings. The objective of this study was to report the most prevalent toxins reported in these species to the ASPCA APCC. All exotic animal records were retrieved from January 1 through December 31, 2022 and species, age, outcome, substance, route, and exposure amount were extracted from the records. In total, 2,008 cases were reported. Cases were categorized by taxa including amphibians, avians, fish, large and small exotic mammals, and reptiles. Small mammals accounted for the largest taxa reported with 1,351 cases (67.3%) with rabbits being the most commonly represented species (912 cases, 45.6%). The most common substance was food with 507 cases reported (25.2%). Of those generalized as food, 272 cases were called in with a chocolate specific exposure (53.6%). Toxin exposures in these species occurred primarily via dermal, inhalation, intramuscular or oral routes with the most common being of the oral route with 1,792 (89%). These results can provide insight for veterinarians treating these species as to the variety of toxicosis cases that should be considered. Additionally, the results of this study can aid to educate pet owners on the risks associated with keeping exotic pets in domestic environments.

Funding Source: Boehringer-Ingelheim Veterinary Scholars Program

Subject: Toxicology

INVESTIGATING GOAT BLOOD TRANSFUSION DELIVERY TECHNIQUE AND ITS IMPACT ON RED BLOOD CELL STABILITY *IN VITRO*

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Funding: NCSU Fluoroscience Endowment, Dr. Gamsjäger Start-up funds
Primary Subject Category for Presentation: Clinical Medicine, Cell Biology

The administration of blood, either as whole blood or packed red blood cells (pRBCs), is critical for treatment of anemia in goats. The method with which blood products are delivered to caprine patients varies greatly, and currently there is no consensus on best practices.

Thus, the objective of this study is to identify the impact of transfusion technique on the stability of RBCs *in vitro*. Based on literature spanning multiple species, we hypothesize that linear peristaltic volumetric infusion pumps will lead to increased hemolysis and erythrocyte fragility compared to gravity flow.

6 healthy does from the NCSU Small Ruminant Educational Unit were enrolled, and 900 mL of blood was collected from each subject and processed into pRBCs and whole blood. All blood bags were stored in a designated refrigerator for the duration of the study. On day 0, 14 and 28, blood was administered through gravity flow and then a linear peristaltic pump sequentially. A sample was collected and lactate concentration, hematocrit, and erythrocyte osmotic fragility was measured.

Statistical analysis of these results was performed using a paired t-test for normally distributed data and a Wilcoxon matched-pairs signed rank test for non-normally distributed data in GraphPad Prism 10.

Preliminary results indicate that there are no biologically relevant, statistically significant differences in lactate concentration, hematocrit, and erythrocyte osmotic fragility measurements between the two transfusion techniques.

Future studies should include a direct measurement of hemolysis and an *in vivo* component.

POST-ADOPTION OUTCOMES OF NORTH AMERICAN SHELTER DOGS WITH BITE HISTORIES, 2021-2024

Lynn Eckert[a], BS, MA (DVM student)

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Behavioral concerns, particularly aggression, are a common reason for dog relinquishment and shelter dog adoption returns, yet little is known about the post-adoption outcomes for dogs that have bitten while in a shelter. Similarly, there is scant research about the long-term impacts on owners after adopting dogs with bite histories.

Formal behavioral assessments in shelter contexts do not accurately predict future behavior, nor do they foretell owners' attitudes toward their pets. The context of a shelter intake and stay may either inhibit or elicit undesirable behaviors, including aggression, making shelter behavior an unreliable predictor of future behavior. Shelters strive to safely place animals in homes while not putting people or animals at avoidable physical or emotional risk, and the Post-Adoption Outcomes Study aims to contribute important additional information for shelters, veterinarians, and owners for dogs with bite histories.

This is a large outcomes study drawing on roughly 150,000 records from 11 shelter organizations across the United States and Canada over a three-year period. Adopters of all dogs from these shelters will receive invitations to participate in an online survey assembled from two validated instruments that cover dog temperament and behavior, the quality of life of dog owners, and the relationship between a dog owner and their dog. The analysis of responses from owners of dogs with and without bite histories will focus on characterizing and quantifying aggression after adoption, dogs' welfare in their homes, owners' experiences with their dogs, and any effect that body weight may have on these parameters.

Funding: NC State University Herbert Benjamin Endowment

Primary subject categories: Behavior, Animal Welfare, Shelter Medicine

Keywords: aggression, animal welfare, behavior, dogs, human-animal bond, pet adoption, shelter medicine, temperament

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Funding: NC State University Herbert Benjamin Endowment

Primary subject categories: Behavior, Animal Welfare, Shelter Medicine

Keywords: aggression, animal welfare, behavior, dogs, human-animal bond, pet adoption, shelter medicine, temperament

VALIDATION OF AX6 ACCELEROMETER DEVICE FOR MEASURING ACTIVITY IN DOGS - A PILOT STUDY

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Subject Category: Pain
Funding: Salary release (Lascelles)

Osteoarthritis (OA) and associated persistent pain results in disability and decreased quality of life, affecting approximately 40% of dogs. Assessing the impact of persistent pain is crucial in clinical and research sciences, yet hindered by a lack of validated objective measures. Physical activity monitors (PAMs), such as accelerometers, offer a promising objective measure of the impact of pain on physical activity in dogs with OA. However, few accelerometers have been validated for canine patients. One approach to assessing validity is to assess performance of a new device against a validated device. This pilot study aimed to validate a novel device, the Axivity (AX6), comparing it with the previously validated Actigraph. The AX6 offers advantages such as a longer battery life and the ability to record at higher acquisition rates which could provide more insight into specific activities performed. Under IACUC approval and informed owner consent, six healthy dogs (age: 3.8 y/o ± 2.6 y/o; weight 37.5 lbs ± 13.7 lbs) wore both the AX6 as well as the previously validated Actigraph (GT3X) on their collar for one week, with devices collecting data at one-minute intervals. Output was downloaded onto computers using proprietery software. Correlation between device output was assessed by reporting the R^2 correlation. There was a high correlation between the GT3X and AX6 activity counts (R^2=0.776; $p<0.0001$). Future studies should should confirm these preliminary findings in a larger number of animals and over a wider range of activity levels.

USE OF A NOVEL MEDIUM TO GROW CHICKEN EMBRYO FIBROBLASTS THAT INCREASES THE YIELD OF MAREK'S DISEASE VACCINES

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Abstract

The cornerstone in the control of Marek's disease (MD) is vaccination. MD vaccines are cell-associated and grow in chicken embryo fibroblasts (CEF). One of the major limitations when growing MD vaccines is that infected cells tend to die and it is critical to trypsinize cells when the largest number of infected cells can be retrieved. Infectivity rate, however, is usually low and that is one of the factors why there is great variability in the dose administered to chickens even when using the same vaccine vial. Also, the low infectivity rate limits the amount of vaccine doses that could be added into a vial and increases vaccine production costs. In the present study we have evaluated the growth of HVT, one of the MD vaccines most used, in a novel medium (Diploid Growth Serum Reduced Medium). This medium allows CEF to grow with limited amount of calf serum or even without serum. In our study, the best results to grow HVT were achieved when supplementing the medium with 1% calf serum. With this medium, CEF tend to become multilayer and HVT grows extensively in the plates for a longer period of time compared to using conventional media (modified Leibovitz-McCoy). Yield of HVT in the Diploid Growth Serum Reduced Medium was at least twice than when using conventional media and infectivity rate increased as well. The impact of these results on the production of MD vaccines will be discussed.

Funding: NA

Subject category: Other

Litwack Abstract for Undergraduate Presentation
Subject category: Infectious Disease

INFLUENCE OF *CLOSTRIDIOIDES DIFFICILE* ENDOTOXINS, TCDA AND TCDB, in CACO-2 CELLS ON HOST BILE ACID SYNTHESIS GENE EXPRESSION

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Gastroenterology and or Infectious Diseases (for presentations)

Clostridioides difficile infection (CDI) is a major threat to public health and non-antibiotic therapies are urgently needed. The pathogen is exquisitely sensitive to alterations in the gut bile acid (BA) pool. BA production is primarily regulated by the host nuclear receptor Farnesoid X receptor (FXR). Bile acids bind to and activate FXR, where it binds to target promoters to regulate BA genes that create a negative feedback signal to downregulate BA synthesis in the liver. Preliminary data shows FXR expression and its target genes increase when Caco-2 cells are exposed to *C. difficile* toxins. However, the relationship between CDI and other host nuclear receptors is not well understood. Differentiated Caco-2 cells were exposed to *C. difficile* toxins, TcdA, TcdB or both at concentrations ranging from 10-100 pM for 24 hr. RNA was extracted from cells for qRT-PCR to assess gene expression of nuclear receptors *PPAR-gamma* and *CAR*, as well as target genes *CYP27a1*, *SCDI,* and *CPT1*. *ASBT*, a target gene of FXR, was also assessed. When exposed to TcdB, expression of *ASBT* and *CPT1* increased in a dose-dependent response while *PPAR-gamma* expression decreased. When exposed to 100 pM of TcdB, expression of *CAR* and *CYP27A1* was altered. TcdA did not alter the expression of any of the genes. This data suggest *C. difficile* toxin TcdB is potentially able to regulate host BA synthesis and other nuclear receptor regulated metabolic pathways. Further exploration between CDI, nuclear receptors, and the host response may help identify novel therapeutics for treating CDI.

DIFFERENCES IN THE EARLY REPLICATION AND IMMUNE RESPONSES INDUCED BY VARIOUS CVI-988 STRAINS IN EGG-TYPE CHICKENS

Abdelhamid M. Fares[1]: Graduate Student

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Control of Marek's disease (MD) has been successfully achieved by vaccination since 1970. CVI-988 strain is the gold standard vaccine against the very virulent plus MD virus (vv+MDV). However, efficacy of CVI-988 strains from various commercial sources differs greatly. The objective of this study was to evaluate the biological properties of three commercial CVI-988 strains and a novel vaccine CVI-LTR (CVI-988 strain with the insertion of the promotor of reticuloendotheliosis virus). Vaccine replication in the lymphoid organs (2-5 days post vaccination or dpv) and feather pulp (7 and 21 dpv), lymphoid organ relative weight (7 dpv), immune responses in the spleen at 5 dpv, and ability of the vaccine to decrease vv+MDV DNA load in the feather pulp at 21 dpv were evaluated. Vaccines differ significantly in their ability to replicate in lymphoid organs and feather pulp. While none of the evaluated vaccines affected the relative weight of bursa or thymus, all CVI988 strains but not CVI-LTR, increased the spleen relative weight at 7 dpv when compared to unvaccinated control. The most protective CVI-988 strains increased the frequency of activated macrophages and CD4+ T cells in the spleen at 5 dpv. The CVI-LTR strain also increased the frequency of activated macrophages but it reduced the frequency of cytotoxic T cells in the spleen at 5 dpv. Our results demonstrated that there are significant differences in the replication and in the early immune responses elicited by various CVI-988 and CVI-LTR that might contribute to the differences in their protective efficacy.

Primary subject category: Infectious Diseases

EVALUATING THE EFFICACY OF INTRANASAL NONSTEROIDAL ANTI-INFLAMMATORY DRUGS FOR PAIN MITIGATION IN PIGLETS USING A NOVEL DRUG DELIVERY DEVICE

Isla Farrow. Category: Veterinary student
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There are currently no FDA-approved medications for the mitigation of pain in pigs, and large-scale commercial farms are in need of a means of efficacious, inexpensive, easy, and safe administration of pain medications to these animals. The primary aim of this study is to evaluate the efficacy of intranasally-administered nonsteroidal anti-inflammatory drugs (NSAIDs; INN) for the mitigation of pain and inflammation in piglets using a needle-free drug delivery device. We hypothesize that processed piglets receiving each INN will exhibit fewer pain behaviors and demonstrate minimal to no physiological response to the painful events, thus improving overall animal welfare on the farm. A total of 120 piglets from 30 litters were enrolled in a prospective, randomized trial to receive one of three INN or saline control treatments; half the animals underwent routine processing, while half underwent sham processing. Blood samples were collected via direct venipuncture from the retrobulbar space at baseline, 1, 3, and 24 hours post-INN administration. Plasma cortisol concentrations were analyzed by radioimmunoassay; prostaglandin E2 (PGE2) was analyzed by liquid chromatography/tandem mass spectrometry. Data were analyzed using a mixed model with p-values ≤ 0.05 as significant. Cortisol was increased in meloxicam castration pigs ($p<0.001$) but no other differences were found. In conclusion, INN may have decreased biomarkers of stress and inflammation in processed piglets, although further studies should be performed to confirm the dose and efficacy of INN. These drugs and route may be used to improve welfare in swine production.

Funding Source: NC State University Fluoroscience Endowment, Animal Health and Nutrition Consortium
Primary Subject Category: Pharmacology

COMPARISON OF THE PINNA TO THE JUGULAR VEIN AS A SITE FOR MEASURING BLOOD GLUCOSE CONCENTRATIONS IN GOATS

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Abstract:
Portable blood glucose monitors are valuable tools in veterinary medicine as a quick, inexpensive way to measure blood glucose. In ruminants, blood glucose measures for disease processes including pregnancy toxemia, ketosis and sepsis. While the jugular vein is the most readily accessible venous access, it is difficult to sample alone or inaccurate if intravenous dextrose has been administered. There is scant research assessing the pinna as a sample site for measuring blood glucose in goats. In this study, we aim to compare blood glucose at the pinna and jugular vein during different metabolic states. Based on previous small animal studies, we hypothesize that the pinna will provide an accurate measurement of systemic blood glucose levels. In this study, 5 intact male Boer goats had blood glucose measured during resting, induced hyperglycemic and induced hypoglycemic states. For the hyperglycemic phase, goats were administered 0.2 mL/kg 50% dextrose bolus intravenously. For the hypoglycemic phase, goats were administered 0.1 Units/kg of short acting insulin intramuscularly. Blood samples were then collected via jugular catheters and 25-gauge needles were used to prick the pinna and tested using a POC glucometer at multiple time points. All goats were monitored for lethargy and lateral recumbency. A linear mixed model was conducted and demonstrated location had no statistically significant impact on blood glucose values. The pinna is a viable sample site for measuring blood glucose during normal, induced hyperglycemic and hypoglycemic states.

Funding acquired from Boehringer-Ingelheim Veterinary Scholars Program; Halleran Start Up
Primary subject category: Clinical medicine

USING FUNCTIONAL GENOMICS AND TIMELAPSE MICROSCOPY TO ELUCIDATE THE CELL CYCLE TIMING AND ARCHITECTURE OF A HYBRID G1/S REGULATORY NETWORK IN A CHYTRID

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Our lab recently discovered that a viral KilA-N domain (SBF) restructured the G1/S regulatory network in the fungal ancestor. This led to the ancestral E2F-Rb pathway being replaced by the novel SBF-Whi5 pathway in higher fungi. Chytrids, an early-diverging fungal lineage, have retained both the E2F-Rb and SBF-Whi5 pathways, representing an intermediate step in this evolutionary process. To better understand cell cycle evolution in fungi and animals, our lab is developing the chytrid *Spizellomyces punctatus* as a model organism. The specifics of the chytrid cell cycle, such as the duration of DNA synthesis and mitosis, as well as the structure and potential cross-regulation of the E2F-Rb and SBF-Whi5 pathways, remain unknown. To address this, I developed a two-color fluorescent reporter (PCNA-mScarlet and H2B-mCitrine) to measure the duration of DNA synthesis (S phase) and mitosis (M phase). Time-lapse fluorescence movies of strains with this reporter reveal the presence of G1 and G2 gap phases between DNA replication and mitosis. To investigate the G1/S regulatory pathways, I am using yeast two-hybrid assays to systematically measure protein-protein interactions among all putative chytrid E2F-Rb (3) and SBF-Whi5 (6) regulators. Over the next year, I plan to measure the localization and timing of these nine G1/S regulators relative to the G1 and S phases. Characterizing the cell cycle in chytrids will enhance our understanding of cell cycle evolution and highlight differences between the cell cycles of animals and fungi.

Funding: Comparative Molecular Medicine Training Program (CMI T32)
Subject: Cell Biology

APPLICATION OF AN UPLC-MS METHOD FOR THE DETERMINATION OF METHYLENE BLUE RESIDUES IN CATTLE TISSUES

Earl Ford IV BS, MS[1,2]: Graduate Student
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Abstract: Nitrite toxicity is an important concern for ruminant producers and veterinarians due to its ability to cause significant loss to a herd in a short time frame, and induces methemoglobinemia which if not treated is fatal within forty-eight hours and is treated using intravenous (IV) injection of low doses (2-4 mg/kg) of methylene blue (MB). Currently few pharmacokinetic (PK) studies for methylene blue use in ruminants exist, posing issues to the use of MB in food animals. Due to this, the purpose of the current study was to assess the pharmacokinetics of MB in cattle to provide an estimation of the withdrawal interval (WDI) in tissues and milk. Four Holstein-Jersey cross steers (6-7 months old), two Holstein-Jersey cross heifers (6-7 months old), and two Holstein-Jersey cross cows (4-5 years old) were injected with 2.5% MB solution (10 mg/kg dose) compounded in physiologic saline. Following injection of MB, plasma, urine, and milk samples were collected at a specified interval over 3 and 6 days. The cattle were euthanized and tissues were collected. Tissues and plasma were analyzed for methylene blue residues by ultraperformance liquid chromatography tandem mass spectroscopy following the three- and six-day treatment period. PK analysis of the plasma show that in healthy cattle MB is rapidly eliminated from the body, and tissue analysis shows that residues do not persist in any tissue longer than three days. This greatly improves the knowledge of MB used in cattle allowing for its potential use as an antidote in food animals.

Funding: NIEHS – T32ES007046-41, FARAD – USDA NIFA 2021-41480-35270

Category: Pharmacology

POSTER PRESENTATION ONLY

BIOAVAILABILITY OF FLUNIXIN MEGLUMINE AFTER TRANSDERMAL ADMINISTRATION TO WOOL AND HAIR SHEEP

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The absence of approved drugs for pain control in sheep significantly limits the treatment options available to producers, raising concerns about animal welfare and potentially leading to poor production outcomes. The use of pharmacokinetic (PK) evaluation for assessing the efficacy of a drug assumes that the plasma concentration is related to the concentration at the site of action. Therefore, knowledge of the PK characteristics following different routes of administration of flunixin meglumine is crucial to determine effective control of pain in sheep.

Physiological variables (species, breed, age) may affect absorption, distribution and elimination of drugs. Therefore, the objective of the study was to evaluate pharmacokinetics of flunixin meglumine after transdermal (TD) administration to coat types in sheep (Dorset (wool) and Katahdin (hair)). Flunixin was also administered intravenously (IV) to the same sheep to establish the elimination kinetics and bioavailability. We hypothesize that hair sheep will exhibit plasma concentrations similar to values reported in goats, while wool sheep may demonstrate increased bioavailability, thereby demonstrating a need for tailored pain mitigation strategies across diverse sheep breeds.

A single dose of flunixin meglumine (2.2 mg/kg) was administered IV to each sheep. After a washout period of 10 days, each sheep was administered a dose of a commercial transdermal formulation flunixin (3.3 mg/kg) by TD application. Plasma samples were obtained for 96 hours following both IV and TD administration, respectively. Flunixin concentrations were quantified by use of high-performance liquid chromatography with mass spectrometry and PK parameters derived using non-compartmental analysis.

Funding Source: American Veterinary Medical Foundation (AVMF) and the Veterinary Pharmacology Research Foundation (VPRF)

Field of Research: Pharmacology

INFLUENCE OF SEQUENCING TECHNOLOGY ON PANGENOME-LEVEL ANALYSIS AND DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN ESKAPE PATHOGENS.

Alba Frias-De-Diego, Postdoctoral Research Scholar

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As sequencing costs decrease, short-read and long-read technologies are indispensable tools for uncovering the genetic drivers behind bacterial pathogen resistance. This study explores the differences between the use of short-read (Illumina) and long-read (Oxford Nanopore Technologies, ONT) sequencing in detecting antimicrobial resistance (AMR) genes in ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter cloacae). Utilizing a dataset of 1,385 whole genome sequences and applying commonly used bioinformatic methods in bacterial genomics, we assessed the differences in genomic completeness, pangenome structure, and AMR gene identification. Genome completeness demonstrated higher completeness for Illumina sequences, while ONT identified a broader pangenome. Despite these variations, both long and short-read technologies performed similarly in identifying key AMR genetic determinants. Hybrid assembly successfully combined the strengths of both approaches, getting closer to Illumina's completeness and revealing ONT-like pangenomic content. Notably, Illumina consistently detected more β-lactam resistance genes than ONT, while ONT found higher variation in AMR-related point mutations. This highlights the importance of method selection based on research goals. Differences were also observed for specific gene classes and bacterial species, underscoring the need for a nuanced understanding of technology limitations. Overall, this study reveals the strengths and limitations of each approach, advocating for the use of Illumina for common AMR analysis; ONT for studying complex genomes and novel species, and hybrid assembly for a more comprehensive characterization, leveraging the benefits of both technologies.

Funding source: This material is based upon work supported by the US National Institutes of Health (NIH) (R35GM134934).

• Primary subject category for presentation: Genetics & Infectious Disease.

BLOOD PRESSURE CHANGES IN AGING DOGS

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Abstract -

In humans, systolic blood pressure (SBP) increases with age, while diastolic BP (DBP) decreases. A cross-sectional study of healthy young and geriatric dogs found that SBP did not differ between groups; there are no data surrounding longitudinal changes of BP with age in senior dogs and little is known about factors influencing BP. The study goal was to describe longitudinal changes in BP in aging dogs and to examine BP associated factors. We hypothesized that SBP would increase with age and correlate with body weight (BW), body condition score (BCS), pain and cognition. Data were available from a senior dog cohort studied q6 months since 2019. Data obtained included mean BP, DBP and SBP, age, fractional lifespan (FLS), BW, BCS, sex, composite pain scores, and owner derived pain and cognition scores. Univariate relationships between BP parameters and other variables were examined using mixed models, with individuals as a random effect. Our longitudinal data confirmed previous findings that SBP does not increase with age or FLS. However, DBP increased significantly with age and FLS. There was no correlation between BP parameters and BW, BCS, sex, pain level or cognition score. Mean BP was associated with age when pain score and BW were incorporated into the model. We conclude that canine and human BP parameters do not change with age in the same manner. The increase in DBP was unexpected. The lack of influence of BCS or BW on BP highlights the differences between the canine and human cardiovascular systems with aging.

Funding Source(s) - NIH T35OD011070 Interdisciplinary Biomedical Research Training Program
Category - Neurosciences

EXAMINING DEMENTIA LINKED GENES IN DOGS AND WOLVES

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Abstract -

Dementia, one of the most feared consequences of aging, develops in many neurodegenerative diseases including Alzheimer's Disease, Frontotemporal Dementia and Parkinson's Disease. While environmental and lifestyle factors influence disease development, there are clear genetic risk factors. Many genes involved in the human condition are now known, and we understand that multiple mutations in some genes can cause the same pathogenic phenotypes, but other genes exhibit vulnerabilities at a minor set of nucleotide sites. However, canine dementia and relevance of these genes in canines is less understood. In this exploratory study we aimed to investigate the prevalence of variants and evolutionary pressures among key dementia associated genes. We explored selective pressure variance among 19 human dementia linked genes and found genes with weaker selective pressures were more likely linked to human dementia. To understand these gene properties in canine species, we explored whole genome sequences from 60 wolves and 2707 domestic dogs. We explored variant types (frameshift, missense), their allele frequencies, and identified variants that differ between dogs and wolves. Expectedly, genetic diversity was lower in dogs than wolves, but fixation indices (F_{ST}) revealed only a small number of sites segregating by dog and wolf lineages. Neurodegeneration risk increases with age in dogs, but almost nothing is known about wolf neurodegeneration. However, since few genetic differences were found between dogs and wolves aside from decreased genetic diversity, we can initially hypothesize that wolves are as susceptible to neurodegeneration as domestic dogs, but that remains to be properly examined and tested.

Funding Source(s) - NIH T35OD011070 Interdisciplinary Biomedical Research Training Program
Category - Genetics

INVESTIGATING THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN PROCOAGULANT PLATELET FORMATION USING AN IN VITRO MODEL OF IMMUNE MEDIATED HEMOLYTIC ANEMIA IN DOGS

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Immune-Mediated Hemolytic Anemia (IMHA) is an autoimmune disease in dogs that targets the host's red blood cells and can lead to life-threatening thromboembolism due to systemic hypercoagulability. Limited understanding surrounding the interactions between the innate immune system and coagulation system has impeded the development of effective thromboprophylactic therapies in IMHA dogs. Platelets in different disease states have been shown to undergo persistent activation forming a platelet subpopulation known as procoagulant platelets, rendering them unresponsive to conventional antiplatelet drugs. Our hypothesis was that neutrophil extracellular traps (NETs) production in response to exogenous hemin will prime platelets to adopt procoagulant phenotypes upon exposure to platelet agonists. Our primary objective was to compare platelet procoagulant markers in response to hemin to those treated with NETs from heme-activated neutrophils. Washed platelets were isolated from 4 healthy dogs by centrifugation and filtration. Isolated neutrophils were treated with 10uM hemin or 100nM phorbol myristate acetate in the presence or absence of DNase1. Negative control consists of buffer or DMSO. Following incubation for 120 to 180 minutes, supernatant was sonicated before incubation with autologous platelets ($1x10^7$/mL) in the presence or absence of thrombin/collagen and thrombin/convulxin. Positive control consists of platelets treated with calcium and 10uM A23187. Flow cytometry analysis of procoagulant markers consisted of surface P-selectin, fibrinogen and annexin V. Procoagulant platelets were identified based on simultaneous detection of P-selectin and fibrinogen. Preliminary data showed a trend that supernatants from hemin-activated neutrophils primed platelets to produce more procoagulant platelets upon exposure to thrombin, collagen and convulxin.

ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 1 & 2 (VEGFR) ANTAGONIST IN ATTENUATING THE MIA INDUCED OSTEOARTHRITIS PAIN IN A RAT MODEL OF OSTEOARTHRITIS PAIN.

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Abstract
Osteoarthritis (OA) is a prevalent joint disease characterized by tissue degeneration and pain. Vascular endothelial growth factor (VEGF) has been implicated in its pathogenesis through its roles in neurogenesis, angiogenesis and pro-nociception via VEGF receptors1 & 2. This study evaluated the therapeutic potential of a long-acting nanoparticle form of pazopanib (nanoPAZII-t), a VEGF receptor (VEGFR) 1 and 2 antagonist in attenuating OA associated sensitivity. Using the monosodium iodoacetate (MIA)-induced model of OA pain, 6–8-week-old Sprague Dawley rats (wt.= 180-350g, 50/50 male/female) were randomly assigned to two groups: intra-articular saline (Group 1, N=18) and intra-articular nanoPAZ II-t (Group 2, N=20), both administered 3 days following model induction (MIA injection). OA-associated sensitivity was measured using mechanical (von Frey) and thermal (hot-plate) sensitivity testing at baseline & 7, 14, 21, 28, 35, and 42 days post-MIA injections. Group data were compared using a 2-way ANOVA. Intra-articular treatment with nanoPAZ II-t at the inflammatory stage of OA in the MIA model overall decreased OA-induced mechanical hypersensitivity, significantly so at 14,21,28 & 42 days following model induction, compared to saline controls. Thermal sensitivity was not seen in this experiment. These early findings suggest that targeting VEGFR1 and 2 may offer a novel therapeutic strategy for managing OA pain. Future work will assess more complex pain behaviors to fully determine the effects on OA pain, as well as evaluate effects on disease progression. Additional work will explore joint tissue, sensory nerve and spinal cord gene expression changes effected by nanoPAZ II-t treatment.

Funding source: National Institute of Health (NIH) R01AR077890
Primary Subject category for Presentation: Pain

EFFECTS OF WITHAFERIN A ON CYTOKINE PRODUCTION IN LEUKOCYTES FROM PERIPHERAL BLOOD AND BRONCHOALVEOLAR LAVAGE SAMPLES

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Funding: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program, Bayless Laboratory Startup Funds
Primary Category: Immunology

Equine asthma is a chronic, progressive disease with limited therapeutic options. In some patients, environmental management is not feasible and current therapies have poor efficacy or adverse effects. Withaferin A (WFA) is a plant-derived compound with anti-inflammatory properties that shows promise as a therapeutic for equine asthma.

The goal of this project was to determine the effect of WFA on the production of pro- and anti-inflammatory cytokines by blood and airway leukocytes stimulated with lipopolysaccharide (LPS). Populations of leukocytes from blood and bronchoalveolar lavage (BAL) samples were incubated with WFA and LPS for 2, 6, or 18 hours. Dexamethasone was used as a positive control for inhibition of cytokine secretion. ELISAs were used to measure supernatant concentrations of TNF-alpha, IL-6, and IL-10.

LPS induced a substantial increase in TNF-alpha secretion by peripheral blood mononuclear cells (PBMCs) and mixed blood leukocytes. Median TNF-alpha concentrations were significantly lower in PBMCs and mixed blood leukocytes treated with WFA. No effect from LPS stimulation was observed in IL-6 and IL-10 secretion in untreated cells, preventing interpretation of WFA's effects on cytokine secretion. WFA also suppressed TNF-alpha secretion in LPS-stimulated airway leukocytes from the BAL samples.

In summary, WFA inhibits TNF-alpha secretion in PBMCs, mixed leukocytes, and airway leukocytes. This supports the potential for using WFA as a therapeutic for equine asthma and other inflammatory diseases.

Ongoing research in our lab seeks to determine the effect of WFA on cytokine gene expression and identify anti-inflammatory WFA mechanism(s) of action in leukocytes.

CHARACTERIZATION OF GENETICALLY MODIFIED *CLOSTRIDIUM SPOROGENES* AND ITS IMPACT ON GUT BILE ACID BIOTRANSFORMATIONS

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Gut bacteria provide colonization resistance through nutrient competition and making inhibitory secondary bile acids. Few strains of commensal *Clostridia* encode a bile acid inducible (*bai*) operon, that converts primary bile acid cholate (CA) to secondary bile acid deoxycholate (DCA) through 7-α dehydroxylation, however many are genetically intractable. Recent work has cloned the *bai* operon into *C. sporogenes* (MF001), creating a strain to study the contribution of both nutrient competition and bile acid modifications with genetic control, however they are not well characterized. We sought to characterize WT and MF001 strains, validating the maintenance of the *bai* gene plasmids over 72 hr. We measured the growth kinetics of both strains with and without CA, DCA, and determined the minimum inhibitory concentrations (MICs). Using *bai* specific primers, PCR and gel electrophoresis showed that MF001 maintained *bai* plasmids for up to 72 hr. MF001 supplemented with CA had a rapid decline in growth in CFUs compared to WT after 48 hr. MICs of CA for WT and MF001 were 10 mM. The MIC of DCA differed between the WT and MF001, and was 1.25 mM and 0.625, respectively. The inhibition of growth with MF001 and CA suggests that the *bai* operon is active, and potentially creating an inhibitory amount of DCA. Bile acid metabolomics will be used to measure the conversion of CA to DCA over 72 hr. Fully characterizing this strain will prove to be a valuable tool in mechanistically dissecting the interplay between *C. difficile,* nutrient competition, and bile acid biotransformation.

Funding sources: NIH R35 GM149222, USAR AMEDD LTHET scholarship
Category: Other

ASSESSING GAPS AND TRAINING OPPORTUNITIES FOR ANIMAL ABUSE AND NEGLECT CASES IN NORTH CAROLINA

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Abstract: Cruelty to animals manifests in numerous ways, from the brutal spectacles of dog and cock fighting to the tragic circumstances of animal hoarding and neglect. It is estimated that 10 million animals die from abuse every year in the United States. However, these figures are likely underestimated due to the stigma associated with reporting and the complexities involved in prosecuting such cases. This study aims to evaluate the existing gaps and identify training opportunities in the handling of animal abuse and neglect cases in North Carolina. Despite the strict animal welfare statutes listed under North Carolina General Statutes, Chapter 19A: *Protection of Animals*, inconsistencies in enforcement persist, undermining the effectiveness of the legal protections for animals. This study will use a mixed-methods approach, incorporating qualitative surveys encompassing a) general shelter information, b) employee information, c) cruelty incidence occurrences and case details over the last three years, and d) assessing resources and proper training amongst law enforcement, animal control officers, and animal shelter employees. The survey will be dispersed to animal shelters in counties grouped into one of three tiers based on population size and shelter intake numbers. This research will highlight the specific areas where training is insufficient and propose comprehensive training programs to enhance the skills and knowledge of those directly involved with these particular cases. By addressing these gaps, the study strives to improve the overall handling of animal abuse and neglect cases, ensuring better enforcement of animal welfare laws and promoting justice for affected animals.

Funding Source: Boehringer-Ingelheim Veterinary Scholars Program

Subject: Clinical Medicine

LOW DOSE RADIATION THERAPY: A NOVEL TREATMENT FOR FELINE IDIOPATHIC/INTERSTITIAL CYSTITIS

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Feline Idiopathic/Interstitial Cystitis (FIC) is a poorly understood, yet common, disease that causes pain and inflammation of the lower urinary tract (LUT). 55-69% of LUT disease is attributed to FIC and 5-21% of cats with recurrent clinical signs from FIC are euthanized due to disease severity. Clinical signs include hematuria, pollakiuria, and stranguria, which significantly impact quality of life and burden pet owners. Male cats with FIC can also suffer from urethral obstructions (UO), which is a life threatening condition requiring costly interventions. Current medical management options and environmental modification have not been successful at preventing recurrences of clinical signs, thus the need for alternative therapies. Low dose radiation therapy (RT) has been shown to reduce pain and inflammation in human medicine and veterinary medicine, specifically with palliative care in cancer patients. RT is relatively accessible, cost effective, and has low risk of side effects, making it a good candidate as a novel treatment for FIC. A pilot study was performed at NC State with client owned male cats severely affected by FIC and/or UO to determine safety and efficacy. It was concluded that RT was a safe and viable option for FIC treatment in this population, and with environmental modification, can reduce the frequency and severity of clinical signs. This summer, we began replicating this study with female cats to determine if there is similar efficacy. This work is revolutionizing the treatment of FIC in veterinary medicine and provides hope for many frustrated pet owners.

Research funded by the NC State Feline Fund and personal startup. Student supported by the NC State Feline Health Center.
Category: Clinical Medicine

CHEMOKINE RECEPTORS AS TARGETS OF MONOCLONAL ANTIBODIES IN CANINE T-CELL LYMPHOMA

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Subject Category: Immunology
NC State University Office of the Associate Dean for Research and Graduate Studies

Abstract

Specific monoclonal antibodies (mAbs) bind normal surface proteins (thus, "antigens") that can differentiate lymphocyte types. Engineered mAbs that lead to lymphocyte death upon binding can serve as an anti-lymphoma agent for that type. Rituximab, which recognizes CD20 (antigen exclusively expressed on B cells), helps cure humans with B-cell lymphoma. Normal B cells are killed, too, disabling humoral immunity, but such toxicity is manageable. Analogous mAb-targeting of pan-T-cell antigens in T-cell lymphoma (TCL; our lab's focus) is not tolerable. Global T-cell loss creates AIDS-like susceptibility to infections. Finding a surface antigen that differentiates only a T-cell fraction that includes the TCL is a potential work-around. T-cell expression of chemokine receptors (CCRs) depends on the T-cell subtype, so CCRs are candidates. From preliminary canine TCL RNAseq, CCR9 appears promising. We hypothesize that high CCR9 expression on T-cell subsets, & low expression on somatic cells, is favorable for targeting TCLs with minimal autoimmunity. We predict low CCR mRNA expression in most tissues; hence, our 1st goal – develop CCR9 qPCR assay for surveying. We also predict CCR9 surface expression on canine TCLs; hence, our 2nd goal – validate useful anti-mouse/-human CCR mAbs for flow cytometry. A canine cell line served as a surrogate: GL-1 (CCR7-CCR9+). An efficient qPCR reaction was developed. Results supported our hypothesis with low CCR9 expression in normal tissues and detection of CCR9 in all leukemia and lymphoma tissues tested. Anti-CCR9 antibodies were flow-profiled, demonstrating intracellular expression of CCR9 in GL-1. Tools for assessing CCR9 as mAb targets in canine TCL were successfully developed.

Sponsor: American Kennel Club Canine Health Foundation (AKC CHF)

HYDRA VULGARIS REGENERATIVE CAPACITY UNDER HYPEROSMOTIC STRESS

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CoAuthors: Igor Silva, Christophe Guilluy, DVM, PhD

Hydra vulgaris, a small freshwater cnidarian, exhibits remarkable regenerative abilities that have captivated research for over two centuries. When transected, the 10-30 mm freshwater polyps can fully regenerate a head and foot forming two identical individuals within a few days. This capacity contributes to *Hydra*'s virtually limitless lifespan, as they are considered nearly immortal in a sterile laboratory setting. However, rising salt concentrations in freshwater environments due to human activities are occurring globally, causing osmotic stress, which can impact cellular growth and senescence in many aquatic organisms. To explore the consequences of freshwater salinization on *Hydra* regeneration, we analyzed head regeneration following transection in *Hydra* incubated in either 0, 12.5 or 25 mM NaCl. We performed 3 independent experiments with 30 individuals per group. We found that increasing levels of NaCl significantly inhibited *Hydra* regeneration, with more detrimental effects observed in 25 mM NaCl. Our results suggest that freshwater salinization has deleterious effects on *Hydra vulgaris* physiology. We are now further investigating this effect by quantitatively analyzing the impact of osmotic stress on stem cell proliferation, apoptosis, and differentiation.

Funding: NC State University Fluoroscience Endowment and the NIH

FAMILIES WITH CHILDREN: A GOOD FIT FOR ALL SHELTER DOGS?

L. Renee Hoot, BS (DVM Student)

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Caring for and living with dogs who have a bite history is a complex issue of significant concern for shelters, communities, owners, and, in particular, for homes with children. However, there is a crucial gap in knowledge of how these problems overlap between groups. Our study aims to help fill this gap by examining dog intakes and outcomes over a three-year period in a diverse group of shelters across the US and Canada, focusing on dogs with a bite quarantine during their stay in the shelter. In conjunction with this historical data, a survey comprised of previously validated tools will be sent to adopters to collect information about their dog's behavior once adopted, the quality of life for both the dog and their family, and overall, the impact of placing dogs with bite histories into adoptive homes with and without children. Our goal in analyzing this data is to provide the community with valuable insight into the quality of life for these families and their dogs, thereby providing shelters with data that will offer guidance in shelter outcome decision-making processes, especially for dogs with bite histories. This research has the potential to help support the missions and responsibilities of shelters to not only improve the lives of these dogs but also enhance the safety and success of these types of adoptions within the local communities they serve.

Funding: Veterinary Practice Plan

Primary subject category: Other

PHARMACOLOGICAL DISRUPTION OF LEFT-RIGHT ASYMMETRY CAUSES DISTINCT MORPHOLOGICAL PHENOTYPES IN THE XENOPUS STOMACH

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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. In normal embryos, cilia at the embryonic LR organizer create a leftward flow of signaling molecules to establish differential gene expression on the left vs right sides of the embryo, ultimately resulting in the formation of asymmetric anatomy. However, the downstream cellular events that result in normal or abnormal asymmetries within each organ are unknown. To address this question, we used the vertebrate model organism *Xenopus laevis*, focusing on the stomach. The *Xenopus* stomach forms a leftward curvature, similar to mammals, with the left side convex and the right side concave. We exposed *Xenopus* embryos to 0.8mM propylthiouracil (PTU), a chemical that induces heterotaxy by inhibiting ciliary flow, and then assessed cellular and extracellular proteins on the left and right sides of the developing stomach using immunohistochemistry. We found that 80% (n=20) of PTU-treated embryos develop heterotaxy, with two distinct stomach phenotypes: 1) an organ with an inverted (left side) concavity or 2) a straightened stomach in which both sides are convex. Each case correlates with abnormal protein distribution patterns implicating specific morphogenetic mechanisms. Our work suggests heterotaxy causes specific categories of abnormal asymmetries in embryonic organs. This project reveals underlying causes of abnormal LR asymmetry, providing etiological explanations to support families affected by severe birth defects.

Primary subject category: Developmental Biology

LEFT-RIGHT ASYMMETRIES IN THE EXTRACELLULAR MATRIX SHAPE STOMACH CURVATURE.

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Embryonic left-right (L-R) asymmetry sculpts many organs; thus, understanding the morphogenetic mechanisms behind L-R asymmetries, like the leftward curvature of the stomach, can provide insights into laterality-related birth defects. Our lab previously found that conserved L-R patterning cues promote curvature via cell rearrangements in the left stomach endoderm; however, the potential influence of the mesoderm and the extracellular matrix (ECM) on stomach curvature is unknown. To examine the role of ECM in stomach curvature, I conducted immunohistochemical analyses on *Xenopus* stomach sections at stages before, during, and after curvature. In the left (convex) stomach, fibronectin fibrils become aligned within a compact basement membrane between the endoderm and mesoderm. However, on the right, fibronectin is broadly distributed and disorganized, and forms a concavity with an irregular endoderm-mesoderm border. These fibronectin asymmetries are reversed or bilateral in embryos with experimentally-induced stomach curvature defects, indicating that LR asymmetric ECM distribution patterns locally influence organ topology. To test whether fibronectin is required for stomach curvature, I targeted translation-blocking fibronectin morpholinos to each side of the embryonic stomach. Morpholino injections on either side elicited distorted stomachs, indicating that fibronectin is required on both sides for curvature. These results indicate that stomach curvature involves L-R asymmetries in multiple sides and tissue layers.

Funding: NCSU Genetics & Genomics Academy; NCSU Genetics and Genomics Scholars; Kenan Institute of Engineering, Technology & Science NCSU; NIH

Primary subject category for presentation: Cell Biology (Other: Developmental Biology; Extracellular Matrix; Organogenesis)

PRELIMINARY ASSESSMENT OF HEALTH BIOMARKERS OF TWO SHARK SPECIES (*CARCHARHINUS BREVIPINNA AND CARCHARHINUS OBSCURUS*) IN THE NEW YORK BIGHT

Rebekah James, veterinary student

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NCSU CVM; ZooQuatic Laboratory, LLC; Mississippi Aquarium; Disney's Animals, Science and Environment; Stony Brook University - School of Marine and Atmospheric Sciences; South Fork Natural History Museum, Shark Research Program

Globally, elasmobranch abundance has declined 71% over the last 50 years, with a steady 18% decrease each decade despite regulations. Overfishing, through recreational angling, targeted commercial activities, and accidental bycatch presents the primary threat to shark and ray species. Anthropogenic stressors and climate change are emerging issues for elasmobranch populations. Health assessments of *in situ* populations are critical to establishing baselines in the face of future disturbance, and to understanding individual, population and ecosystem health. Samples are being collected in shark and ray species (target n=255) under typical capture circumstances in advance of and during construction of an offshore wind energy station near Long Island, New York over a 3 year time period. The study goals are to: 1) assess hematologic, biochemistry, protein electrophoresis, acute phase protein and nutritional biomarker levels; 2) measure primary and secondary stress profiles associated with capture; 3) document animal diet and foraging strategies; 4) create species-specific reference ranges for the health, stress, and nutritional biomarkers; 5) determine post-release survivorship and habitat through acoustic telemetry. Here we present a preliminary assessment of health biomarkers in 2 species (*Carcharhinus brevipinna and Carcharhinus obscurus*) prior to activation of an offshore wind cable. This information will contribute significantly to the ability of organizations to evaluate changes in animal health in the Mid-Atlantic and will also inform critical health and dietary baselines needed in managed care.

The American Association of Zoo Veterinarians Wild Animal Health Fund, NC State University Office of the Associate Dean for Research and Graduate Studies.

Clinical Medicine

EQUINE GASTROINTESTINAL INFLAMMATION AT NC STATE UNIVERSITY COLLEGE OF VETERINARY MEDICINE PATHOLOGY SERVICE FROM 2019-2023

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Coauthors: Nora Gardner, Elizabeth Rose, Panchan Sitthicharoenchai
Affiliations: NC State University CVM

Gastrointestinal inflammation is a prevalent condition among equids globally, exhibiting a spectrum of severity from subclinical findings to fatal outcomes. Clinicians and pathologists often face difficulties in pinpointing a definitive cause for the inflammation through routine antemortem and postmortem examinations. This retrospective study evaluates biopsy and necropsy cases of equids diagnosed with gastrointestinal inflammation at the North Carolina State University College of Veterinary Medicine over a four-year period (2019-2023). Out of 233 cases that met selection criteria, 56.7% had an undetermined etiology, 24.7% had an infectious cause, and 18.6% had a non-infectious cause. Infectious cases were split evenly between parasitic and bacterial causes, with roughly 0.2% due to other infections. Non-infectious causes included impactions (25.4%), displacements (19.7%), neoplasia (12.7%), gastric ulcers (8.5%), and other conditions such as entrapment and volvulus (33%).

Several factors may contribute to the high rate of indeterminate diagnoses in equine gastrointestinal inflammation, including limited ancillary testing. Among 57 cases without ancillary testing, 82.5% had undetermined etiologies. In contrast, 69% of 176 cases with ancillary testing remained undetermined. These findings emphasize the need for standardized microscopic evaluation protocols to enhance diagnostic accuracy in equine gastrointestinal diseases. The study highlights the complexity of diagnosing equine gastrointestinal inflammation and the necessity of comprehensive diagnostic approaches, including standardized evaluation and ancillary testing, to improve diagnostic and therapeutic outcomes in equine veterinary practice.

Research Grant: NC State University Fluoroscience Endowment
Primary Subject Category: Equine Gastroenterology

SCREENING AND SELECTION OF EUBIOTIC COMPOUNDS WITH IMMUNOMODULATORY AND ANTI-*CLOSTRIDIUM PERFRINGENS* PROPERTIES

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Eubiotic compounds (EC) are water/feed additives used in poultry to enhance gut health and control pathogens, including *Clostridium perfringens*. Although several EC are being introduced commercially, an in-vitro approach to screen EC for their immunomodulatory and antimicrobial properties prior to their in-vivo testing is needed. Here, we developed a chicken macrophage cell-line-based model to screen properties of 10 EC; Monobutyrin, Monolaurin, Calcium butyrate, Tributyrin, Carvacrol, Curcumin, Green tea extract, Rosemary extract, Monomyristate, and Tartaric acid. First, an optimal concentration for each EC was selected by measuring the effect on cell viability. Next, cells were treated with EC for 6, 12, and 24 hours, to measure expression of immune response genes (IFNγ, IL-1β, IL-6, IL-10, TGFβ) and MHC-II protein. At 6 hours post-stimulation, Monobutyrin, Calcium butyrate, and Green tea extract significantly downregulated IFNγ, IL-6, or IL-1β gene transcription and MHC-II expression, while the IL-10 or TGFβ gene expression in these treatments as well as those receiving Rosemary extract and Tartaric acid was significantly upregulated, when compared to control, suggesting immunomodulatory properties of these ECs. Finally, pre-treatment of macrophages with the selected five ECs for 24 hours before *C. perfringens* infection showed that Monobutyrin, Green tea extract, Rosemary extract, and Calcium butyrate significantly inhibited bacterial growth at 12 and/or 24 hours post-infection compared to control. These results indicated that an avian macrophage cell-based in-vitro model can be used to screen ECs possessing both immunomodulatory and antimicrobial properties so that they can further be tested in vivo for their disease prevention efficacy.

Funding: This work was funded by Eastman Chemical Company through the Eastman Chemical Center of Excellence research partnership with NC State University.

Subject Category for Presentation: Immunology

Title: DIFFUSE LARGE B-CELL LYMPHOMA PRESENTING AS CONGESTIVE HEART FAILURE IN A CAT

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Abstract: Feline congestive heart failure (CHF) is among the most common clinical presentation in small animal emergency and referral practice, with primary cardiomyopathy or left ventricular remodeling secondary to systemic disease routinely identified as antecedent causes. Cardiac lymphoma is uncommon in cats and is rarely considered as a differential diagnosis for CHF. This study examines a 10-year-old neutered male domestic short-haired cat with clinical histories of feline immunodeficiency virus, diabetes mellitus, and congestive heart failure that was humanely euthanized. Post-mortem evaluation demonstrated a transmurally-infiltrative round cell neoplasm of the heart, resulting in pleural and pericardial effusion and pulmonary edema. Immunohistochemistry of neoplastic tissue was consistent with diffuse large B-cell lymphoma (DLBCL). This case demonstrates a peculiar presentation of cardiac DLBCL, with chronic feline lentiviral infection possibly contributing to disease initiation and progression. It remains unresolved whether this presentation represented primary or metastatic disease, as similar foci of neoplastic round cells were observed histologically in in the lung, jejunum, kidney, brain, lymph nodes (mesenteric and mesocolonic), and bone marrow (femur). In cases with echocardiographic myocardial hypertrophy and a history of FIV, appropriate molecular tests for lymphoma are recommended.

Funding: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Category: Clinical Medicine

POPULATION PHARMACOKINETICS OF FLUNIXIN MEGLUMINE IN PIGLETS

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Funding Source: NC State University Fluoroscience Endowment, Animal Health and Nutrition Consortium
Primary Subject Category: Pharmacology

There are currently no FDA-approved medications for the mitigation of pain in pigs, and large-scale commercial farms are in dire need of a means of efficacious, inexpensive, easy, and safe administration of pain medications to these animals. We hypothesized that piglets who received intranasally administered flunixin (INF) would have no differences in plasma concentrations or pharmacokinetic variables between animals undergoing processing or between sexes. The objective of this study was to determine the population pharmacokinetics of INF following administration to piglets for acute pain in a commercial setting. Sixty piglets from 30 litters (30 male and 30 female) were enrolled in a prospective, randomized trial to receive 2.2 mg/kg of flunixin meglumine administered intranasally. The male piglets underwent routine processing. Blood samples were collected via direct venipuncture from the retrobulbar space at baseline, and at 1, 3, and 24 hours post-flunixin administration. Plasma flunixin concentrations were analyzed by ultra high pressure liquid chromatography/tandem mass spectrometry. Pharmacokinetic modeling was performed using non-linear mixed effects modeling. The population estimates and CV% for clearance per fraction absorbed was 376.8 (84.5%) mL/hr/kg, volume of distribution at steady state per fraction absorbed was 2.8 (177%) L/kg, and elimination half-life was 11.3 hr. Our study provides evidence that intranasal flunixin is absorbed in piglets with high variability. Further studies are needed to determine efficacious plasma levels of flunixin.

REGULATION OF PERIWEANING BODY TEMPERATURE IS IMPROVED IN PIGLETS GIVEN HIGER DOSES OF INJECTABLE IRON DEXTRAN DURING THE EARLY SUCKLING PERIOD

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Abstract:
Increased doses of iron dextran given early in a piglets life have been documented to support improved birth-market growth. Recently, a study demonstrated that injectable iron dose also altered the expression of numerous genes of interest in pigs at weaning. One gene (TRPV1), which is known to be involved in the regulation of core body temperature, had a > 4.0-fold increase in expression in piglets receiving higher iron doses. The objective of this study was therefore to evaluate the relationship between injectable iron dose and periweaning body temperature regulation.

Sixteen gilts were allotted to control or treatment groups and at three days of age, all piglets received 200mg of Uniferon® iron dextran, while treatment pigs received a second 200mg three days later. To monitor temperature, Thermochron® data loggers were affixed to each pig the day before weaning for continuous temperature recording. Piglets were anesthetized with IM TKX and the axillary skin was shaved and cleaned with surgical scrub. Loggers were adhered with an adhesive patch then sutured around the circumference using 2-0 vicryl. Loggers were programmed to record every five minutes beginning 12 hours pre-weaning and continuing five days post-weaning.

The mean temperature in the treatment group (101.2°F±1.6) was closer to normal body temperature compared to controls (100.6°F±2.3). Peak temp occurred approximately 4hrs post-placement in the nursery for all pigs but was higher in controls (105.8°F) vs treatment (105.1°F) while minimum temp was higher in treatment pigs (93.3°F) vs controls (91.5°F) indicating a tighter regulation of body temperature.

Funding: Pharmacosmos Inc.
Primary Subject Category: Other, Swine Clinical Medicine

TIME-OF-DAY DIFFERENCE IN INFLAMMATORY RESPONSES AND CIRCADIAN CLOCK GENE EXPRESSION IN ACUTELY OZONE-EXPOSED MICE

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Exposure to ground-level ozone induces inflammatory lung injury and alters lung function. The immune cells and key inflammatory mediators, i.e., cytokines and chemokines, exhibit circadian rhythm in a 24-hour cycle. However, time-of-day dependent inflammatory responses in ozone-exposed mice are unclear. Therefore, Eight-week-old C57BL/6J mice were exposed to 1.5ppm ozone or filtered air (FA) for 4 hours at two different time points, i.e., 6-10 am [Zeitgeber time (ZT=0)] and 6-10 pm (ZT=12). As expected, in comparison with the FA-exposed mice, the ozone-exposed mice exhibited an exaggerated inflammatory response and lung injury. Immune cell recruitment and epithelial injury indicated by increase in total cell counts and total protein levels, respectively, in the bronchoalveolar lavage fluid (BALF), and protein expression of found in inflammatory zone protein 1 (FIZZ1) in airway epithelium were significantly higher in ozone-exposed mice in the night (ZT=12) compared to day timepoint (ZT=0). Inflammatory mediators including KC, MIP-1α, Eotaxin-1, and IL-5 also trended higher in the BALF of ozone-exposed mice in the night timepoint (ZT=12) versus those exposed in the day timepoint (ZT=0). As compared to the day timepoint, ozone exposure in the night timepoint (ZT=12) differentially affects the expression of circadian clock genes, i.e., *Bmal1, Nr1d2, Per2, Per3, Cry1, Dbp, Cry2,* in a sex-specific manner. These findings suggest that ozone exposure in the nighttime (ZT=12) induces exaggerated inflammatory responses and dysregulates circadian clock gene expression. However, the cause-effect relationships between the dysregulated clock gene expression and the altered ozone-induced lung inflammatory responses remain unclear.

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Primary subject category: Immunology and Toxicology

DETERMINING THE EFFICACY OF IPEC-J2 DERIVED EXOSOMES ON INTESTINAL EPITHELIAL WOUND HEALING

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Primary Category: Regenerative Medicine

Intestinal ischemia/reperfusion (I/R) injury occurs when blood flow to a segment of the intestine is obstructed and then restored, damaging the epithelium responsible for nutrient absorption and acting as a barrier to microbes. Intestinal I/R-induced barrier dysfunction is associated with devastating mortality rates of 60-80%, driving the need for innovative therapies. Exosomes, or extracellular vesicles, have emerged as promising regenerative agents. This study explored the efficacy of exosomes derived from neonatal pig intestinal epithelial cells in promoting intestinal epithelial wound healing. We hypothesized that IPEC-J2-derived exosomes will enhance cell migration *in vitro* and improve barrier integrity *ex vivo*. Isolation and characterization of the exosomes revealed a mean size of 205.3 +/- 60.3 nm, and a concentration of 1.31e+10 exos/mL. We conducted scratch assays *in vitro* and quantified percent closure over 8 hours, which revealed that dosages of 5000 exos/cell and 10,000 exos/cell show significantly lower percent closure at T=4 and T=6. We used an *ex vivo* surgically-induced neonatal pig I/R injury model to measure the trans-epithelial electrical resistance (TEER) and mannitol flux using Ussing chambers. The mannitol flux showed that treatment prevented a significant increase in flux between control and ischemic tissues that was present in the vehicle condition, and TEER demonstrated no significant differences between each dosage. This study demonstrated that IPEC-J2 derived exosomes do not significantly affect neonate wound healing *in vitro* or *ex vivo*. However, the lack of toxicity leaves room for their use as drug delivery vehicles in future studies.

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IL-27 MODULATES MACROPHAGE IMMUNOMETABOLISM TO PROMOTE DUAL ANTI-VIRAL AND ANTI-INFLAMMATORY EFFECTOR FUNCTIONS DURING OCULAR HSV-1 INFECTION

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Abstract

Herpetic stromal keratitis (HSK) is a painful and vision-impairing disease caused by recurrent HSV-1 infection of the cornea. The macrophages play a central role in HSV-1 clearance in the cornea through phagocytosis of infected epithelial cells and apoptotic neutrophils. However, whether HSV-1 modulates macrophage immunometabolism to evade anti-viral immunity is unknown. Our study shows that HSV-1 infection dysregulates mitochondrial metabolisms in macrophages with increased immune-responsive gene 1 (Irg1) expression. Our results indicate that the HSV-1-induced Irg1/itaconate axis suppresses IFN-β production by macrophages. Conversely, our data suggest that HSV-1 infection stimulates IL-27 production by macrophages. Using a primary corneal HSV-1 infection mouse model and IL-27 receptor knockout mice, we show that IL-27 plays a critical role in controlling HSV-1 shedding from the cornea, the optimum induction of effector $CD4^+$ T cell responses, and limiting HSK progression. Using in vitro bone marrow-derived macrophages, we show that IL-27 suppresses HSV-1-induced Irg1 expression and plays an anti-viral role by regulating macrophage-mediated HSV-1 killing, IFN-β production, and IFN-stimulated genes expression after HSV-1 infection. Our results indicate that IL-27 promotes endogenous anti-viral and anti-inflammatory responses, and modulating IL-27-mediated metabolic programming in macrophages may represent a promising therapeutic approach to control HSK progression.

Funding Sources: National Eye Institute Grant EY035057 (R15), National Eye Institute Grant EY034495 (R01)

Primary subject category for presentation: Immunology

EARLY CHANGES IN THE THYMUS OF MEAT TYPE CHICKENS AFTER VACCINATION WITH VARIOUS MDV-1 VACCINES

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The most effective vaccines against very virulent plus Marek's disease virus (vv+MDV) are attenuated Gallid alphaherpesvirus 2 (MDV-1) strains. MDV-1 vaccines are protective against vv+MDV-induced tumors but not all of them can protect against vv+MDV-induced immunosuppression (MDV-IS). In this study, we evaluated early thymus changes following vaccination with three MDV-1 strains: CVI-LTR and rMd5ΔMeq-BAC, which protect against both tumors and MDV-IS, and CVI-988, which protects against tumors but not against MDV-IS. The results showed that, the three vaccines replicated efficiently at 3 and 5 days of age. By day 26, only CVI-988 was detected in all chickens, while few had detectable levels of CVI-LTR and rMd5ΔMeq-BAC. Cellular changes were apparent at 5 days, but not at 26 days, compared to the sham-inoculated age-matched group. The three vaccines reduced the percentages of CD4+MHC-II+ (CVI-988 and rMd5ΔMeq-BAC numerically; CVI-LTR significantly), and TCRγδ+ T cells (CVI-988 and rMd5ΔMeq-BAC significantly and CVI-LTR numerically). CVI-988 (significant) and rMd5ΔMeq-BAC (numerical) decreased the percentage of CD3+ T cells, whereas CVI-LTR and rMd5ΔMeq-BAC significantly decreased the percentage of CD8α+ T cells. Dead cell analysis at 5 days showed several common features between CVI-LTR and rMd5ΔMeq-BAC: increased CD4-CD8- T cells and decreased CD3+, CD4+CD8α+, CD8β+, and TCRγδ+ T cell percentages. Our results confirm that the thymus is an initial target of MDV-1 vaccines replication, with immunophenotype changes occurring as early as 5 days and differing between vaccines. A better understanding of early thymus changes is crucial for elucidating the mechanisms of MDV-1 vaccines elicited-protection against MDV-IS.

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Infectious Diseases

EQUINE EXTRACELLULAR STEM CELLS EXERT DIRECT BACTERICIDAL EFFECTS

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Equine septic arthritis is a performance limiting and even life-threatening condition for horses. The gold standard treatment is surgical arthroscopy in conjunction with antibiotics, however treatment failure is not uncommon and new treatment approaches are needed. Bone marrow derived mesenchymal stem cells (BM-MSCs) have shown promise as a novel therapeutic for equine septic arthritis, but have limitations such as cost, production time, and patient rejection. These limitations could be overcome by use of a secreted product of MSCs called MSC-derived extracellular vesicles (BM-MSC EVs), which have also demonstrated antibacterial properties. Unfortunately, little is known about BM-MSC EVs in the horse. Therefore, the objective for this study was to investigate the antibacterial potential of equine BM-MSC EVs. We hypothesized that equine BM-MSC EVs would directly inhibit *S. Aureus* growth *in vitro*. We used previously isolated BM-MSC EVs from 3 healthy horses. BM-MSC EVs or saline (negative control) were incubated at a ratio of 1:10 and 1:1 with specified dilutions of *S.aureus* in LB broth. 96 well plates were incubated overnight and bacteria were quantified using optical density (600 nm fluorescence) and a standard curve. Bacteria were then spotted onto agar plates using a 3-spot plate method, incubated overnight and counted. Results thus far indicate that BM-MSC EVs significantly inhibit the growth of S. Aureus compared to saline and EV bacterial inhibition is concentration dependent. Future studies will evaluate BM-MSC EVs inhibition on growth of other common bacteria found in infected joints, with and without the addition of antibiotics.

Funding Source(s): Boehringer Ingelheim

Subject Category: Clinical medicine, cell biology

PREVALENCE OF *HEMATODINIUM SP.* INFECTION IN JUVENILE NORTH CAROLINA BLUE CRABS (*CALLINECTES SAPIDUS*)

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Blue crab (*Callinectes sapidus*) is North Carolina's largest and most valuable fishery, with approximately 20 million pounds landed annually since 1978. The estuaries of North Carolina provide critical habitat for juvenile blue crabs, where they settle in seagrass beds found in the salt marsh. Habitat loss, disease, and overfishing have all contributed to the decline in North Carolina's blue crab abundance. *Hematodinium sp.* is a parasitic dinoflagellate that infects the hemolymph and hemopoietic tissue of blue crabs along the Atlantic and Gulf coasts of the United States. It is known to cause high mortality in juveniles (< 30 mm carapace width [CW]) within their first year, particularly in higher salinity waters such as coastal bays. This project aimed to assess the prevalence and spatial distribution of *Hematodinium sp.* infection in the juvenile North Carolina blue crab population to better understand the impact of the disease on this important commercial species. The study area included patches of seagrass sampled throughout North Carolina's coastal sounds and bays, including Back Sound and South Core Sound in Carteret County, NC. Juvenile crabs were collected at each site using either a seine net (twice for 25 m) or a dip net (for 20 minutes). The CW of each crab was recorded, and hepatopancreas tissue was collected for DNA extraction using a QIAGEN DNeasy Blood & Tissue Kit. A quantitative PCR assay targeting the parasitic DNA was used to quantify the *Hematodinium sp.* infection in the samples and to compare the prevalence of infection across different sampling sites.

Funding source: NC Sea Grant & NIH T35 IBRTP

Primary subject category: Infectious Disease

COLORECTAL CANCER METASTASIS THROUGH THE ENDOTHELIUM

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Funding Source: NIH Interdisciplinary Biomedical Research Training Program
NC State College of Veterinary Medicine, UNC-Chapel Hill and NC State Joint Department of Biomedical Engineering

Background: Metastasis is the process of tumor cells breaking apart from the initial site of invasion and forming additional tumors in other areas of the body. One hypothesis is that tumor cells may utilize Von Willebrand Factor (vWF) to extravasate from blood vessels into tissue. vWF is essential in coagulation and is expressed by endothelial cells and platelets. Tumor cells can express vWF or trigger endothelial cell activation resulting in increased vWF in vasculature.

Objective: We have generated a microphysiological model of vasculature and tumor perfusion to examine the role of vWF in extravasation and metastasis. We use this *in vitro* model to investigate the role of VWF in six colorectal cancer (CRC) cell lines.

Methods: *In-vitro* models for vasculature were created by combining human umbilical vein endothelial cells (HUVEC) and normal human lung fibroblasts (NHLF) inside a microfluidic device. These cells were able to self-assemble a perfusable network.

Six different CRC cells were perfused through the microfluidic device and imaged on a confocal microscope over three days to observe adhesion and extravasation. The cell lines used were: SW 480, SW 620, DLD1, RKO, HT-29, HCT-116.

Results: We have not observed extravasation from any of the six cell lines. We will use image cytometry to determine how vWF expression on the cell membrane varies across these cell lines and perform ELISA to quantify secreted vWF levels. At the completion of this project, we will be able to determine the role of vWF in CRC metastasis.

TITLE: THE UPDATED CYTAUXZOON FELIS GENOME

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

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Abstract

Background

Cytauxzoon felis, a tick-transmitted protozoal pathogen that causes cytauxzoonosis in felines. This disease has high mortality rates despite the best available treatments. The inability to culture the parasite continuously in vitro complicates traditional vaccine development. There is an urgent need for a protective vaccine and highlights the necessity for detailed genomic data to better understand the pathogen biology and elucidate vaccine targets.

Materials and methods

Whole blood was collected from a naturally infected cat immediately after death. Leukocytes were removed using a high-efficiency filter, and merozoite DNA was extracted from the infected erythrocytes. The entire genome was sequenced using Illumina and PacBio systems. Feline genome sequences were filtered from the raw data, followed by de novo assembly and annotation of the Cytauxzoon felis genome.

Results

We successfully assembled the Cytauxzoon felis genome into 10 contigs using hybrid and long-read techniques, achieving a contig N50 of 2.6 Mb and a 97.5% completeness score according to BUSCO, with no duplicated BUSCOs. The 9.2 Mb genome is distributed across five chromosomes, a 33 Kb apicoplast, and 7.3 Kb mitochondria. We annotated over 4,000 protein-coding genes, demonstrating gene conservation within the Piroplasmida.

Conclusion:

A complete *C. felis* genome sequence and assembly will facilitate vaccine and drug targets. It will help us better understand the pathogen biology which may facilitate our ability to culture the pathogen *in vitro*.

Primary subject category for presentation: Infectious diseases

PATHOLOGY ASSOCIATED WITH SUDDEN UNUSUAL MORTALITY SYNDROME (SUMS) IN DIPLOID AND TRIPLOID OYSTERS

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Recurring mortality events have been a major hurdle to North Carolina's expanding oyster aquaculture industry. This research project has complemented ongoing work evaluating interactions between hatchery selection for fast-growing oysters for aquaculture selection and observed mortality. This summer, weekly field sampling was carried out at Nelson Bay, Owens Bay, Pasture Point, and Williston Creek. Various genetic lines of oysters were deployed at these sites. Each week, the bags were checked for the count of alive and dead oysters, measurements were taken from 15 oysters, and one oyster was taken for histological processing back in the lab. Previous work has identified inflammation and necrosis in oyster digestive tubule epithelial cells prior to observed mortality. In this project, histology slides from 2022 depicting oysters from various genetic lines and different sites were examined and evaluated using a 0-2 grading scale. Various stages of sloughing of the digestive diverticula, and necrosis of the small intestine were observed. Terminal deoxynucleotidyl transferase dUTP nick end label (TUNEL) staining was used to characterize apoptosis in histological sections collected in oyster prior to and through observed mortality events. The goal of this project is to develop a more complete understanding of cellular processes associated with observed mortality and how these processes may be associated with hatchery-selected oyster genetic lines.

North Carolina Commercial Fishing Resource Fund & North Carolina Collaboratory

Aquaculture

Title: CORNEAL SAFETY PROFILE OF COLD ATMOSPHERIC PLASMA (CAP) AS A THERAPEUTIC FOR FUNGAL KERATITIS: EFFECTS ON EX VIVO PORCINE EYES

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Abstract: Cold Atmospheric Plasma (CAP), the fourth state of matter generated at room temperature and atmospheric pressure, composed of ionized particles, reactive species, and electromagnetic radiation, has shown promising applications in biomedicine such as wound healing, cancer cell apoptosis, and microbial sterilization. Prior research indicates that brief CAP exposures can eradicate keratitis pathogens while preserving corneal integrity and enhancing conventional treatments such as antibiotics. This study evaluates the safety of the use of CAP in the cornea for treating fungal keratitis, by evaluating its effect on porcine corneal tissue. Dose escalating CAP treatment intensities (15, 20 kV) and durations (1-5 minutes) were applied to porcine cadaver cornea, and the corneal thickness, epithelial disruption, and endothelial cell morphology were measured using fluorescein stain, optical coherence tomography (OCT), corneal confocal microscopy, and histology. Results demonstrated that CAP treatments of 15 kV for up to 5 minutes and 20 kV for up to 3 minutes maintained corneal thickness and cellular structure without significant damage. However, extending the exposure to 20 kV beyond 4 minutes leads to notable endothelial abnormalities and epithelial injuries. These findings suggest CAP's potential as a safe therapeutic option under controlled conditions, paving the way for future studies using handheld CAP devices, ex vivo and in vivo corneal infection models, its synergy with antifungal drugs, and ultimately its use as an innovative therapeutic for fungal keratitis.

Funding Source: NIH T35OD011070 Interdisciplinary Biomedical Research Training Program, Boehringer-Ingelheim Equine Award, American Quarter Horse Foundation.

Subject Category: Infectious Diseases, Ophthalmology

COMBINED LIVER-SPECIFIC DELETION OF RNA-BINDING PROTEINS, ZFP36L1 AND ZFP36L2, INITIATE CHOLESTATIC LIVER INJURY AND FIBROSIS

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Zinc Finger Protein 36 Like 1 (ZFP36L1) and Zinc Finger Protein 36 Like 2 (ZFP36L2) posttranscriptionally mediate mRNA decay via binding to 3' untranslated regions (3'UTRs) of target mRNAs. Some previous studies have suggested their redundant functions in multiple organs; however, their combined role in liver physiology remains poorly understood. Here, we achieved combined deletion of ZFP36L1 and ZFP36L2 in the liver using the Cre-loxP system. Liver-specific ZFP36L1 and ZFP36L2 double knockout (L1/L2^{dKO}; AlbCre^{+}/*Zfp36l1*$^{flox/flox}$/*Zfp36l2*$^{flox/flox}$) and flox-only control (L1/L2^{FLX}; AlbCre^{-}/*Zfp36l1*$^{flox/flox}$/*Zfp36l2*$^{flox/flox}$) mice were sacrificed at postnatal day (PND) 0, 7, 14, 21, and 56 to assess liver to body weight ratio, liver histology, liver function, and gene expression. The liver to body weight ratio was significantly higher in L1/L2^{dKO} compared to L1/L2^{FLX} mice on PND 14, 21, and 56. Furthermore, L1/L2^{dKO} liver showed bile infarcts, periportal inflammation, biliary hyperplasia, and fibrosis consistent with significantly elevated serum levels of liver function enzymes, ALT and AST, at PND 21 and 56. Moreover, L1/L2^{dKO} mice had increased total bile acid levels in serum and liver compared to L1/L2^{FLX} mice both at PND 21 and 56. Lastly, RNA-seq, 3'UTR analysis, and pathway enrichment analysis revealed enrichment of target mRNAs among differentially expressed genes (~30% of 3085 DEGs) and activation of multiple canonical pathways including hepatic cholestasis and fibrosis in L1/L2^{dKO} liver. Overall, the combined deletion of ZFP36L1 and ZFP36L2 in the liver upregulates target mRNAs that potentially cause increased bile acid synthesis and/or defective bile acid transport and ultimately, cholestatic liver injury and fibrosis.

Subject category: Immunology

TRISTETRAPROLIN (TTP) PROTECTS AGAINST OZONE-INDUCED ACUTE LUNG INJURY AND INFLAMMATION IN MICE

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Tristetraprolin (TTP), a translational product of the *Zfp36* gene, is an anti-inflammatory protein that mediates mRNA decay, especially of transcripts encoding pro-inflammatory cytokines. TTP modulates various pathological outcomes in diverse inflammatory diseases; however, its role in ozone-induced acute lung injury (ALI) has never been tested. Here, we hypothesized that the loss of TTP would exacerbate ozone-induced ALI and that the systemic overexpression of TTP levels would protect against ozone-induced ALI. Accordingly, whole-body TTP-deficient (TTP^{KO}), airway epithelial cell-specific TTP-deficient (TTP^{EpiKO}), myeloid cell-specific TTP-deficient (TTP^{MyeKO}), and TTP-overexpressed ($TTP^{\Delta ARE}$) adult male and female mice, along with their respective littermate control mice, were exposed to either ozone (O_3) or filtered air (FA) for 3-hours. The endpoints, including bronchoalveolar lavage fluid (BALF) cellularity, cytokine levels, and histopathological changes were assessed 21-24 hours after exposure to either O_3 or FA. As compared to the O_3-exposed TTP-sufficient mice, the O_3-exposed TTP-deficient mice exhibited a significant worsening of ALI outcomes, i.e., neutrophil infiltration, cytokine/chemokine production, and lung pathology. The severity of these outcomes was relatively milder in O_3-exposed TTP^{EpiKO} and O_3-exposed TTP^{MyeKO} mice versus O_3-exposed TTP^{KO} mice. Conversely, the O_3-exposed $TTP^{\Delta ARE}$ mice were protected against O_3-induced ALI as indicated by relatively reduced levels of inflammatory cytokines/chemokines, reduced neutrophil infiltration, and mitigated lung pathology. Overall, our data suggest that TTP is a critical regulator of inflammation in O_3-induced ALI. These findings indicate that enhancing TTP expression could be a potential therapeutic strategy for simultaneously targeting multiple inflammatory cytokines in O_3-induced ALI and possibly other inflammatory diseases.

Funding source: This work was supported by LSU COBRE (NIGMS Grant # P20GM130555) and NIH R01 (NIEHS Grant # R01ES030125).

Primary subject category for presentation: Immunology

WILD NORTH CAROLINA FRESHWATER TURTLES: POTENTIAL BIOINDICATORS OF ENVIRONMENTAL ANTIMICROBIAL RESISTANCE?

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Subject category: Population Health

STUDY RATIONALE: Wildlife can harbor and facilitate spread of antimicrobial resistance (AR) genes and antimicrobial resistant bacteria, including those with public health relevance (e.g., carbapenem-resistant *Enterobacteriales*), and could serve as environmental AR bioindicators. However, occurrence of and factors influencing AR in wildlife are not well-understood. We characterized enteric coliform bacteria and assessed AR in select isolates from wild North Carolina (NC) freshwater turtles.
METHODOLOGY: During May−June 2024, we collected cloacal swabs and morphologic data from trapped turtles at eight NC public parks. Swabs were streaked for isolation on MacConkey agar. Following overnight incubation, three lactose-fermenting colonies per turtle were replated on tryptic soy agar with 5% sheep blood for purity. Isolates were speciated. Phenotypic antimicrobial susceptibility testing (AST) was performed on select *Enterobacter* spp., *Klebsiella* spp., and *Escherichia* spp. isolates.
RESULTS: We collected swabs and data from 100 turtles, mainly *Chrysemys picta* (39%) and *Trachemys scripta scripta* (24%). Of 300 isolates obtained, 260 (87%) were identified to the genus-level. Of 102 (*Enterobacter* spp. (N=56), *Klebsiella* spp. (N=34), *Escherichia* spp. (N=12)) isolates that underwent AST, four (4%) (all *Enterobacter* spp.) phenotypically demonstrated AR; one was resistant to multiple agents, including ceftiofur, ceftriaxone, ciprofloxacin, and gentamicin.
CONCLUSIONS: We identified *Enterobacteriales* of public health relevance, including genera *Enterobacter, Escherichia,* and *Klebsiella*, from wild NC freshwater turtles. Phenotypic AR was present, but uncommon, among examined isolates. Investigation of potential associations between isolate characteristics and turtle attributes is ongoing. Routine sampling of NC freshwater turtles could further inform their potential use as environmental AR bioindicators.

OPTIMIZING PERMEABILITY ASSAYS TO EVALUATE THE PROTECTIVE EFFECTS OF NORMOTHERMIC MACHINE PERFUSION PRESERVATION

Sydney L. Lierz (Veterinary Student)

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Though intestinal transplantation is a possible life-saving intervention for patients with intestinal failure, currently it is not the preferred treatment option due to the high risk of allograft loss. The standard procurement and cold storage (CS) technique for transplantation procedures has been associated with ischemia reperfusion injury and damaged mucosal barrier, which can result in graft failure and rejection. An alternative organ preservation method known as normothermic machine perfusion (NMP) has been applied to intestinal allograft storage to maintain the allograft in an environment complete with oxygen and nutrition. Recent findings show that NMP-storage of small intestines can mitigate preservation injury and subsequent graft failure. To better understand the factors that play a role in NMP-graft viability, we aim to assess intestinal architecture, permeability, and absorptive function via histological and mucosal barrier function analyses over time. A modified lactulose-mannitol (LM) flux test was used to measure intestinal permeability. A mixture of mannitol (30 mg/mL) 50 mg/kg body weight and lactulose (300 mg/mL) 500 mg/kg body weight was administered in proximal jejunal lumen (N=2). Plasma perfusate samples were collected hourly spanning NMP-storage time (18 hours). Samples have been submitted for liquid chromatography-mass spectrometry analysis to correlate a LM absorption ratio with barrier function. Optimizing this assay could help to establish a method for real-time measurement of barrier function and intestinal health to validate the status of intestinal allografts being maintained on a NMP device. This will enable the transplant of a healthier bowel and to improve post-transplant outcomes.

NIH Public Health Service 1R01AI182590-01

Gastroenterology

Title - MOLECULAR DISCOVERY OF DNA FROM FILARIAL NEMATODES IN AN ENDANGERED GALAPAGOS PINNIPED (ZALOPHUS WOLLEBAEKI)

Author Name – Isabella Livingston, Ph.D. Candidate / Graduate Student

Co-Authors - Taylor M. Gregory, Eleanor C. Hawkins, Ashley Cave, Andrea Loyola, Shelly L. Vaden, Diane Deresienski, Marjorie Riofrío-Lazo, Gregory A. Lewbart, Diego Páez-Rosas, Matthew Breen

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Primary Subject Category – Genetics / Infectious Diseases

Presentation Preference – Poster presentation

Abstract - Rapidly changing environments contribute to the spread of invasive species and their associated pathogens into new and vulnerable ecosystems, such as the Galapagos archipelago. These pathogens represent a significant wildlife health concern for native Galapagos species. One such species is the Galapagos sea lion (*Zalophus wollebaeki*) (GSL), an endangered and endemic pinniped increasingly at risk of acquiring infectious diseases due to interactions with introduced companion animals. Previously, we reported the first documented case of antigens from Dirofilaria immitis, the parasite that causes canine heartworm disease, in the GSL. To further investigate, we developed a multifilarial PCR assay and detected DNA from D. immitis and the closely related D. repens in 10.7% of our sample cohort. Based on a conserved region in the filarial 28S gene, this assay can be used in conjunction with restriction endonuclease digestion or Sanger sequencing to recover the genus or species of the causative nematode. Our method proved effective without nonspecific amplification in a wide range of host species, detecting as little as one parasite, and can be used in cases of immature, low-worm burden, or all male infections. This molecular approach offers a sensitive and specific method for detecting filarial parasites in wildlife. Further investigations are necessary to confirm the pathology of filarial nematodes in the GSL and their prevalence in the population. Our findings underscore the urgent need for measures to manage the risk of pathogen transmission from introduced species to native wildlife**.**

EFFECTS OF PRO-INFLAMMATORY CYTOKINES ON TNFα SECRETION BY CANINE OSTEOSARCOMA CELLS

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Abstract:
Osteosarcoma (OSCA) is the most common primary bone tumor in dogs. OSCA is an aggressive neoplasm that causes death despite standard treatments, including surgery, radiation, and chemotherapy. There is evidence that OSCA may be sensitive to immunologic intervention; however, considerable gaps exist in our understanding of canine immunology and oncoimmunology. We recently discovered that canine OSCA cells induce a pro-inflammatory phenotype in canine macrophage-like DH82 cells, and this effect increases when we pretreat the OSCA cells with interferon-gamma (IFNγ). In this project, we hypothesized that stimulation of OSCA cells with the pro-inflammatory cytokines IFNγ, tumor necrosis factor-alpha (TNFα), or CXCL-10 would increase secretion of the pro-inflammatory cytokine, TNFα. OSCA-71 cells in culture were stimulated with recombinant canine IFNγ, TNFα, or CXCL-10 for 24 hours, and the TNFα levels in the cell supernatants were measured by ELISA. We found that CXCL-10 induces TNFα secretion by OSCA-71 cells, whereas stimulation with IFNγ and TNFα does not significantly affect TNFα secretion. Understanding whether and how pro-inflammatory cytokines, directly and indirectly, influence tumor cell biology could offer crucial insights for developing treatments and enhancing our comprehension of osteosarcoma in both canines and people.

Funding sources: American Kennel Club Canine Health Foundation Acorn grant, PHP Faculty Startup Funds, NC State University Herbert Benjamin Endowment

Subject category: Clinical Pathology

THE ROLE OF TEXTILE DYES IN THE IMMUNE SYSTEM DYSREGULATION

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Supported by NCSU-Data Science Academy
Category: Immunology
Poster presentation

There has been an increase in the incidence of atopic diseases and cancer in the last decades. There is an urgent need to identify the environmental triggers that fuel these conditions. In recent years, the use of textile dyes has increased. The effects of these chemicals or metabolites on our skin and our immune system have not been well studied. There have been reports on the effects of irritants on the skin and their ability to break the dermal barrier to start a chain of immune reactions that dysregulate the immune system. This study aimed to investigate the effects of the most common textile dyes in the viability, and mitochondrial function of epidermal skin cells, and intestinal epithelial cells using swine as an animal model. Cultures of MPEK-BL6 line and IPEC cells were cultured with disperse textile dyes Red 11, Orange 37, Blue 1, Blue 124, Brown 1, and PPD at low and high concentrations for 3 and 6 hours and 1, 2 and 3 days. Viability was measured with LUNA cell counter and by flow cytometry. Oxygen consumption rate (OCR) was measured by Seahorse Agilent Technology kits. Disperse Blue 124 and Brown 1 impaired cell viability and mitochondrial function as early as 3 hours after exposure with IPEC and MPEK cells. These data suggest that common disperse textile dyes can influence cell viability and possibly inflammation as a starting point to dysregulate the immune system.

INFLUENCE OF MATERIAL ON ACCURACY OF NOVEL CUSTOM 3D-PRINTED CUTTING GUIDE IN CANINE SEGMENTAL MANDIBULECTOMY: A CADAVERIC STUDY

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Segmental mandibulectomy is performed in dogs to remove a portion of the mandible affected by oral tumors. Complete surgical margins are essential to reduce risk of recurrence and increase median survival time. Previous research on canine maxillectomy cutting guides has shown a statistically significant increase in accuracy when using a 3D-printed cutting guide compared to freehand procedure. However, linear deviation from the planned to actual cut was observed, as a result of errors occurring during manufacturing, positioning and cutting. Traditionally, 3D-printed guides are manufactured using polymer materials. We propose that utilizing metal will limit the deviation and improve overall accuracy. For this study, we acquired 20 canine cadaver heads. Preoperative computed tomodensitometry (CT) scans were performed and 3D models of the mandible were extracted to design 40 custom 3D-printed cutting guides personalized to each half mandible. Cutting guides were randomized into two study groups, manufactured in metal (20 guides, test group) vs. polymer (20 guides, control group), and equally subdivided into experienced vs. novice surgeon user subgroups. Design and material investigation phase resulted in manufacturing with titanium and Tough 1500 polymer supplemented with 5% barium sulfate. Segmental mandibulectomy was performed on each head side in a randomized order by either the experienced or novice surgeon. CT scan images were acquired after guide positioning and cutting to measure the linear deviation between the planned, positioned and actual cuts within each group using a point cloud analysis. Analysis of linear deviation will help compare material performance and direct future surgical guide manufacturing. (250 words)

Funding Source: Principal Investigator Start-up funds, DoCS VPP Grant
Category: Biomedical Engineering

OPTIMIZING PROTOCOL FOR IN VITRO EQUINE RECTAL MONOLAYER GROWTH

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Abstract:

Monolayers are 2D cell culture models that can be created from intestinal organoids. They give access to the apical surface of the epithelium for assessment of ion transport and application of treatments. However, this system has not been optimized for experimental use. This project aimed to determine if the frequency of media changes, the concentration of Matrigel, or using differentiation media affect the epithelial barrier function and growth of monolayers as determined by visual confluency and Trans-Epithelial Electrical Resistance (TEER).

The effects of the frequency of media change were determined by plating equine rectal monolayers and either changing the media when discolored (indicator of acid buildup in media) vs changing the media every other day. The effects of the concentration of Matrigel were determined by plating equine rectal monolayers with different Matrigel:media concentrations. The effects of differentiation media application were determined by plating rectal monolayers and changing to differentiation media at different points within their growth (early vs late time points).

Our preliminary results show that there is no difference when media is changed when the media is discolored as opposed to every other day ($p=0.64$). With Matrigel concentrations, our results were influenced by individual variability or plating but did not appear to have a major effect on outcome. There is no difference in confluency or TEER when differentiation media is applied at an early or late stage ($p=0.98$.)

This preliminary information will help us grow monolayers more efficiently and consistently in future experiments.

Funding: North Carolina State University Park Scholarships Park Enrichment Grant
Category: Gastroenterology

EX VIVO EVALUATION OF AN ACTIVATED CARBON HEMOPERFUSION COLUMN FOR REMOVAL OF CYTOKINES AND IMPACT ON LABORATORY PARAMETERS IN HORSES

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Sepsis is a common condition in horses and accounts for one third of foal deaths. Current treatment relies on antimicrobials and supportive care, but targeted modulation of the immune response, including cytokines, is a potential therapeutic strategy. In humans, hemoperfusion has been shown to decrease systemic cytokine concentrations and complications in patients with sepsis. While early studies have shown that hemoperfusion is feasible in horses, it remains underexplored in equine medicine. Therefore, the goal of this study was to investigate a carbon column for equine cytokine removal using an *ex vivo* hemoperfusion model. Our objectives for this proof-of-concept study were to determine the impact of carbon column hemoperfusion on: 1. inflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, IFN-γ) in LPS-stimulated whole blood, and 2. essential blood components such as calcium potassium, albumin, and globulins. We hypothesized that hemoperfusion with a carbon-based column would significantly reduce cytokines commonly associated with sepsis and would have minimal impact on blood parameters. Two liters of heparinized whole blood were collected from two healthy adult horses and stimulated with LPS. Blood was filtered for 180-minutes and samples were obtained at regular intervals, spun, and frozen at -80 for later analysis. Cytokines were measured via multiplex. Biochemical analyses were conducted pre- and post-filtration. Carbon column filtration for 180 min resulted in significant removal of IL-10 ($p=0.003$), IL-1β ($p=0.01$) with TNFαapproaching significance ($p=0.09$). IL-8 removal was not significant. Essential blood components, including globulin ($p=0.003$), potassium ($p=0.005$), calcium ($p=0.003$), and creatinine ($p=0.03$), were significantly decreased, but remained with clinically accepted reference ranges. Other chemistry values remained within reference ranges. Future research will investigate the efficacy of hemoperfusion *in vivo* as an adjunctive sepsis therapy in horses.

Category: Veterinary Student, Clinical Medicine
Funding: NC State CVM Boehringer Ingelheim Veterinary Scholars Program

PHARMACOKINETIC-PHARMACODYNAMIC ANALYSIS OF INTRAVENOUS MORPHINE AND BUTORPHANOL IN CATTLE

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The use of analgesics in cattle has increased in recent years, driven by heightened societal concern for animal welfare. The lack of pharmacokinetic and pharmacodynamic (PK-PD) data on opioids in cattle has hindered the establishment of known dosing strategies and withdrawal intervals (WDI), thereby limiting their use.

Our research aims to investigate PK-PD profiles of morphine and butorphanol in cattle. We hypothesize that morphine will produce greater sedation with a longer duration of action than butorphanol. Our secondary hypothesis is that morphine will reach plasma concentrations consistent with those producing analgesic effects in other species.

Using a blinded, randomized Latin square design, eight Holstein-cross steers will be used in all four treatments (0.1 & 0.2 mg/kg morphine & 0.02 & 0.1 mg/kg butorphanol) with a minimum 72-hour washout period between treatments. Pharmacodynamic parameters collected before and after treatment include heart and respiratory rate, temperature, and sedation scores. PK and PD samples will be obtained at baseline and at predetermined time points for 72 hours following administration. WDI will be established based on tissue sampling and residue analysis following euthanasia.
A generalized linear mixed effects model with a Poisson distribution including treatment effects, repeated measure time points, and their interactions as fixed effects will be utilized. P-value <0.05 will be considered significant.

Data obtained from this project will be used to determine accurate WDIs to avoid tissue residues. This study will likely provide valuable insights, potentially leading to more effective and safe pain management strategies in cattle.

Funding Source: We thank FARAD and NC State University Office of the Associate Dean for Research and Graduate Studies for their support of this research
Subject category: Clinical Medicine, Pain, Pharmacology

CHARACTERIZING ALTERNATIVE SPLICING CONTRIBUTIONS TO MOUSE SENSORY NEURONS

Quiana Mosley, Graduate Student

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GGA Travel Award Grant, GGA Summer Teamwork Mini-Grant, MBL SPINES Award Grant, MBL SPINES SfN Travel Award Grant, NINDS grant R00 NS116123 (EJLS) and the Warren Alpert Foundation (EJLS).

Gene expression programs are essential instructions for neurons to develop, connect, and mature. As neurons differentiate, alternative pre-mRNA splicing further enriches their genetic programs by generating multiple isoforms from single genes. This molecular mechanism is most prominent in the nervous system, and it is key for virtually all neuronal functions. We study how alternative splicing contributes to cell-specific neuronal functions and work to refine gene networks for pain signaling. Using publicly available deep RNA-sequencing datasets, we perform splicing-sensitive analysis to detect and quantify alternative exons in genetically identified Dorsal Root Ganglia (DRG) sensory neurons. We included datasets of nociceptors, proprioceptors, and different subsets of mechanoreceptors. We find that expression of alternative exons clusters neuronal samples based on cell-type and with similar trajectories than global gene expression. Differentially spliced alternative exons between neuronal subtypes are enriched in genes involved in regulation of ion transport, morphine addiction, synapse, and endocannabinoid signaling pathways. Our weighted gene co-expression network analysis, using alternative exons rather than genes, allowed us to detect early developmental relationships across the sensory neurons. Our results demonstrate that extensive alternative splicing occurs in major subsets of sensory neurons affecting intrinsic neuronal and synaptic properties. The splicing of alternative exons in DRG neurons may arise from mechanistically different forms of transcript diversification likely initiated early during development.

Subject Category: Pain

Poster Presentation Abstract

EVALUATING THE EFFECT OF THE CALM & COZY WRAP ON THE BEHAVIOR OF CATS DURING VETERINARY EXAMS

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Annually, only ~44% of cat owners bring their pet to the veterinarian for routine visits. Overall stress experienced by the cat is one of the primary reasons behind the lack of feline visits. Reducing the stress of the veterinary visits is a priority to facilitate veterinary visits and encourage higher owner-compliance with veterinary care. The objective of this prospective crossover clinical trial is to investigate the effectiveness of the Cozy Cat Wrap (CCW) in decreasing anxiety during a physical exam. This wrap was designed to swaddle the torso of the cat, providing deep-touch stimulation and facilitating examination. We aim to determine if the swaddling effect of the CCW produces a meaningful and measurable reduction in the anxiety of cats during physical exams using behavioral scoring, heart rate, blood pressure, and serum glucose concentration. Behavioral scoring is performed live by the veterinarian and the owner, and from video in order to quantify clinically relevant behaviors such as vocalization, position of the ears, and lip-licking using the Fear Anxiety Score and General Anxiety Score. Subjects were seen on two separate clinic days for a standardized veterinary exam, and were randomized to wearing the wrap on the first or second visit. We will compare the physiologic and behavioral outcomes during the wrap and non-wrapped visits, with each cat as their own control. If these measures are improved during the wrap visit, this would indicate that the CCW would be a useful tool in improving the veterinary experience for cats.

Research Grant: Animal Behavior Service Foundation and Veterinary Scholars Program
Primary Subject Source: Behavior

THE SAFETY OF TRAP-DEXAMETHASONE ITS IMPACTON THE HPA-AXIS

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Severe Equine Asthma (SEA) is a debilitating and chronic pulmonary disease. Oral dexamethasone is an effective treatment for SEA, but it has negative long term side effects including HPA-axis suppression. Direct airway administration of some medications can avoid systemic side effects, but this does not hold true for dexamethasone in horses. TRAP (Tissue Reactive Anchoring Pharmaceuticals) is a technology that allows local deposition of chemically linked pharmaceuticals to release slowly over time. Our long term goal is to determine whether nebulized TRAP-dexamethasone is an effective treatment for SEA that avoids HPA-axis suppression. The short-term objectives for this proof-of-concept study for TRAP-dexamethasone in adult horses were to 1. Investigate safety of nebulized TRAP-dexamethasone and 2. Determine its effects on systemic levels of dexamethasone and cortisol. We hypothesized that TRAP-dexamethasone would not cause airway irritation and that nebulized TRAP-dexamethasone would not cause increased plasma dexamethasone or 24 hour cortisol suppression. Nine horses were divided into three groups (n=3 per group) that received the following treatment once daily for 4 days: 5 mg oral dexamethasone (positive control), 5 mg nebulized TRAP-dexamethasone (treatment), 5 mL saline (vehicle control). Measured parameters included physical exam, bronchoalveolar lavage cytology, glucose, insulin, plasma dexamethasone and cortisol. Physical examination showed no signs of airway irritation in any group. BAL cytology showed no evidence for increased airway inflammation in horses receiving TRAP-dexamethasone. Blood glucose levels remained within normal limits. Cortisol levels showed no decrease following TRAP-dexamethasone treatment or saline control group,insulin and whole blood glucose remained within normal limits.

Funding Source: CMI Ideation Grant

CLINICOPATHOLOGIC EVALUATION OF RADATION-INDUCED SKIN TOXICITY IN A PORCINE MODEL

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Abstract (Preference: Poster Presentation)

Background: Radiotherapy (RT) is a common cancer treatment, and many patients experience radiation-induced skin reactions (RISRs), having a significant negative impact on quality of life. Therefore, new strategies to reduce RISRs are critical. Studies in mice have shown FLASH-RT can deliver high doses eliminating cancer cells, while sparing normal tissues. Pigs being a highly relevant model, we evaluated clinicopathological skin changes after FLASH-RT and conventional radiation (CONV-RT). We hypothesized FLASH-RT would result in decreased skin toxicity and improved healing.

Materials & Methods: Four pigs received either CONV-RT or FLASH-RT on both the right and left flank using an IntraOp Mobetron electron linear accelerator. Skin punch biopsies were collected, and pigs were monitored for RISRs. Tissues were processed and microarrays created. Multiplex immunofluorescence (mIF) staining was performed to characterize immune cells and fibrosis. Slides were scanned and biomarkers quantified using HALO v.3.6. Data was analyzed via 2way ANOVA using GraphPad Prism v.10.

Results: FLASH-RT showed reduced acute clinical dermatitis at doses of 20 and 25 Gy characterized by reduced erythema, ulceration, and dry desquamation. Macrophages predominated in acute RISRs at all doses with a significant dose-dependent increase in M2 macrophages. Reduced chronic inflammation and fibrosis were observed clinically in FLASH-RT in comparison to CONV-RT.

Conclusion: FLASH-RT resulted in reduced acute and chronic skin toxicity clinically and improved wound healing. Macrophage infiltration is a critical component of the immune response in acute RISRs and dose dependent M2 polarization may be an early determinant of chronic inflammation, fibrosis, and delayed healing after RT.

Funding Sources: Stipend Funding provided by CATALYST (DISCOVER program, UT MD Anderson Cancer Center)

Subject Category: Other

EFFECT OF THE REDUCTION OF BLUE LIGHT ON SLEEP AND STRESS LEVELS IN EQUINES

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Introduction: Effective sleep cycles and reduced stress levels are essential for improving recovery rates in hospitalized horses and horse performances. Artificial lighting emits blue light, hypothesized to disrupt sleep cycles and increase stress levels. Human studies established that blue light exposure alters circadian cycles, slows melatonin production, and increases stress hormones[1,2]. This study sought to determine whether the reduction of blue light exposure would alter clinical behavior and cortisol production in stabled horses through the use of a sleep mask (SM) with Amber lenses.
Methods: Horses (n=2) were acclimated to SM twice the week before the study. For the first study week, one horse was assigned to the no SM group (noSM) and the other to wearing SM(wSM) for a 24-hour period with blood collected for melatonin and cortisol, vital signs, input/output(I/O's), facial pain, and behavior metrics all obtained every 2 hours. In the second study week, the SM assignment of horses was reversed, and the same data/blood work was repeated.
Results/Conclusion: All horses tolerated SM well without adverse effects. No statistically significant difference was found between the noSM and wSM groups in clinical behavior (calmness, facial pain, activity level), TPR, gut sounds, vital signs, and I/O's. Results of melatonin and cortisol levels are pending. However, wearing SM for longer than 24 hours may be required to alter clinical behavior and vital signs. Further studies are warranted to examine longer periods of SM wearing with larger sample sizes.

References: 1. J Athl Train. 2012 Nov-Dec; 47(6): 673–678; 2. Neurobiology of Stress 28 (2024) 100600

Funding: Equifit Company

Category: Clinical Medicine

THE HISTORY OF ACUTE OZONE EXPOSURE AFFECTS LUNG'S RESPONSE TO REPETITIVE OZONE EXPOSURE

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Ground-level ozone is recognized as a criteria air pollutant with significant adverse effects on pulmonary health, including airway inflammation, reduced lung function, and increased susceptibility to infections. This study investigates the impact of prior acute ozone exposure on the lung response to subsequent repetitive ozone exposures. We exposed 8-10-week-old female C57BL6/J mice to 2 ppm ozone for three hours, followed by a three-week recovery period, and then subjected the mice to nine doses of 1 ppm ozone. Control groups were exposed to filtered air instead of ozone. We evaluated mucus production, mucous cell metaplasia, immune cell recruitment, epithelial injury, and inflammatory mediators in cell-free bronchoalveolar lavage fluid. Mice previously exposed to acute ozone demonstrated a significantly attenuated response to repetitive ozone exposure compared to controls. Specifically, these mice exhibited less body weight loss after 2-5 doses and showed reduced eosinophil and lymphocyte counts but increased neutrophil counts. Notably, mucous cell metaplasia was also attenuated, as evidenced by fewer AB-PAS and MUC5B positive airway epithelial cells. Inflammatory response modulation was evident through altered cytokine profiles, with lower levels of eotaxin, IL-1α, IL-1β, and IL-4, but higher levels of G-CSF, KC, IL-6, IL-10, and IL-12. Furthermore, the immunolocalization of FIZZ1, a marker of inflammation, was significantly reduced. Our findings suggest that prior acute ozone exposure may offer protection against the adverse effects of repeated exposures, indicating an adaptive lung response to environmental stressors. This knowledge could guide the development of strategies to mitigate ozone pollution's health impacts.

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Subject category for presentation: Immunology

Title: NEW ANTIMICROBIAL EVG7 PREVENTS RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION IN A MOUSE MODEL BY SPARING MEMBERS OF THE LACHNOSPIRACEAE

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Abstract: *Clostridioides difficile* is a Gram-positive bacterium that is responsible for the most common hospital acquired infections in the United States. Standard of care antibiotics to treat primary CDI include vancomycin or fidaxomicin, however in up to 30% of patients this results in recurrent CDI (rCDI). There is an urgent need for the development of therapeutics that can target *C. difficile* without affecting other protective members of the gut microbiota. The objective of this study was to assess whether a novel glycopeptide termed EVG7 (developed by the Martin research group, Leiden), could prevent rCDI when compared to standard of care vancomycin, in a mouse model of rCDI. On day 4 post challenge with *C. difficile* 630 spores, mice were given either a high (0.4 mg/ml) or low (0.04 mg/ml) dose of either vancomycin or EVG7 in their drinking water for 5 days to determine if they were able to clear primary CDI, and prevent rCDI, which occurs around day 14 post challenge. We report that the low dose EVG7 treated mice cleared primary CDI at day 9 and did not show any clinical signs of disease by day 14. 16S rRNA sequencing analysis showed an increase in members from the Lachnospiraceae Family in cecal content of mice treated with the low dose EVG7. This data suggests that EVG7 could be a promising anti-*C. difficile* agent that does not target other members of the gut microbiota thus allowing for quicker recovery after treatment of primary CDI and preventing recurrence.

Subject Category: Infectious Disease

INFLUENCE OF MATERIAL ON ACCURACY OF NOVEL CUSTOM 3D-PRINTED CUTTING GUIDE IN CANINE SEGMENTAL MANDIBULECTOMY: A CADAVERIC STUDY

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Canine segmental mandibulectomy procedures are performed to remove a portion of the mandible affected by oral tumors. Complete surgical margins are essential to improve tumor control and limit local recurrence. A 2023 study evaluating canine maxillectomy cutting guides showed increased accuracy when using a 3D-printed surgical guide compared to freehand. However, linear deviation from the planned to actual cut was observed due to errors that occurred during the manufacturing, positioning and cutting process. Custom 3D-printed surgical guides are frequently manufactured using polymer materials; we propose that utilizing metal will limit deviation and improve surgical accuracy. Twenty canine cadaver heads were acquired for this study. Preoperative computed tomography (CT) scans were performed, and the images were used to design 40 customized 3D-printed cutting guides utilizing Materialise software. Guides were randomized into two study groups: titanium metal (20 guides, test) vs. Tough 1500 polymer with 5% barium sulfate (20 guides, control). Segmental mandibulectomy procedures were performed on each head side. CT scan images were acquired after guide positioning and after cutting to measure the linear deviation between the planned, positioned and actual cuts by performing a point cloud analysis with CloudCompare software. Preliminary findings indicate longer duration of placement for the metal (39.35s) compared to polymer guides (14.31s), and an increased incidence of cracking of the cut slot during sawing in the polymer (7/20) compared to metal guides (0/20). Analysis of linear deviation will help compare the performance of each material and guide future manufacturing directions.

Funding Source: Principal Investigator Start-up funds, DoCS VPP Grant
Category: Biomedical Engineering

VARIABILITY OF MEDICAL INFRARED THERMOGRAPHY IN HEALTHY SHELTER DOGS

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Abstract:

Infrared thermal imaging (IRT) has been used as a screening tool in herd and wildlife populations but there is little shelter setting data. Our objective was to utilize IRT to describe temperature variability in a group of apparently healthy shelter dogs.

Images of the left and right lateral flank and facial views were taken of dogs >6 months from two shelters with a medical-grade thermal camera. Regions of interest (ROI) were drawn over the trunk and eyes using lateral and facial views. Mean temperatures for each ROI were calculated and compared based on reproductive status and shelter location.

45 dogs were enrolled (26 shelter A, 19 shelter B) including 14 spayed females (FS), 12 neutered males (MN), 10 intact females (FI), and 9 intact males (MI). Shelter A mean flank temperature (25.6℃, 23.1-28.8℃) was lower than shelter B (27.8℃, 26.1-30.4℃). Mean flank temperature of intact dogs (MI: 28.2℃, 25.6-30.4℃; FI: 27℃, 23.1-30.2℃) was higher than altered dogs (MN: 25.4℃, 23.8-27.3℃; FS: 26.1℃, 24.0-28.8℃). Shelter A mean eye temperature (30.5℃, 26.7-32.5℃) was lower than shelter B (31.5℃, 30.4-33.5℃). Mean eye temperature of MI dogs (32℃, 30.1-33.5℃) was higher than FI, (31.2℃, 29.3-32.5℃), MN (29.9℃, 26.7-31.2℃) or FS dogs (31℃, 29.7-32.5℃).

Findings suggest there is variability in body surface temperatures in shelter dogs based on reproductive status and housing.

Funding Sources: Funded by PI
Primary Subject Category: Clinical Medicine

AN INVESTIGATION OF ERYTHROCYTE HEALTH AND BIOCHEMICAL CHANGES IN GOAT BLOOD STORED AS WHOLE BLOOD AND PACKED RED BLOOD CELLS

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Blood transfusions are life-saving interventions for goats suffering from severe anemia. Little is known about biochemical and hematological changes that occur in goat blood stored as either whole blood or packed red blood cells (pRBC) over time. The objective of this study is to determine how the storage duration of goat whole blood or pRBCs impacts erythrocyte fragility and blood composition at transfusion. We hypothesized that storage lesions would occur after 14 days in refrigerated goat blood products (4-7C), which could negatively impact goat health following transfusion. Blood was collected from 6 healthy Boer x Kiko goats and placed into one bag with CDPA-1 (citrate, dextrose, phosphate, adenine anticoagulant) for whole blood storage and another bag with CPD (citrate, phosphate, dextrose) and Optisol for pRBC storage. On the day of blood collection and weekly for five weeks, osmotic fragility, hematocrit, hemolysis, blood glucose, lactate, and potassium concentrations were measured. Preliminary results show that lactate concentrations increase after one week ($P<0.03$) and potassium concentrations and hemolysis increase after 4 weeks ($P=0.001$ and $P<0.003$, respectively). Glucose concentrations decrease at 2 (pRBC) and 3 (WB) weeks ($P<0.004$). Osmotic fragility in pRBC decreases at 4 weeks ($P<0.04$). There were no significant changes in hemoglobin, hematocrit, and whole blood osmotic fragility until day 28. Storing goat whole blood and pRBC at refrigerated conditions can cause substantial hemolysis and biochemical changes, and the use of fresh blood may be preferable in critically ill patients.

Funding Sources: NC State University Fluoroscience Endowment

Primary Subject Category: Farm Animal Internal Medicine and Clinical Pathology

DOES ALPHA-2-MACROGLOBULIN CONTROL INFLAMMATION IN AN IN VITRO MODEL OF EQUINE OSTEOARTHRITIS?

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Alpha-2-macroglobulin (A2m) is a tetrameric blood glycoprotein capable of inhibiting inflammatory factors such as cytokines and proteases. The macromolecule indiscriminately captures proteases in its bait region which induces a conformational change and prevents the protease from binding its substrate. Within the joint, this inhibition of inflammatory factors attenuates cartilage degeneration contributing to osteoarthritis (OA). Thus, intra-articular injection of A2m is used clinically as a promising orthobiologic for the treatment of OA. However, the ability of the currently available commercial product to control inflammation and protect equine synovial fibroblasts and chondrocytes in OA is unknown. Therefore, the broad objective for this study is to evaluate a commercially available A2m biologic (Alpha2EQ) as a treatment for equine OA. In an in vitro model of OA, we will treat equine synovial fibroblasts and chondrocytes with Alpha2EQ and measure gene expression using NanoString analysis. A2m treatment will be compared to a steroid-treated control. We hypothesize that treatment of cartilage and synovial cells with A2m will alter expression of genes associated with in the pathogenesis of OA. Results of these experiments are forthcoming and we hope to have preliminary findings to share at the CVM Research Forum.

Funding sources:
Astaria Global, LLC
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Primary subject: Regenerative Medicine

THE PROTECTIVE ROLE OF IFN-λ AT THE OCULAR MUCOSAL SURFACE DURING CORNEAL HSV-1 INFECTION

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Herpes simplex virus-1 (HSV-1) is a highly prevalent human pathogen and successful neurotropic virus that undergoes life-long latency in the trigeminal ganglion (TG). The recurrent HSV-1 infection of the cornea causes herpetic stromal keratitis (HSK), the most common cause of infectious blindness in the Western world. The emergence of multi-drug (anti-viral/acyclovir) resistant HSV-1 strains is a major concern for treating latent and recurrent HSV-1 infections. Using a primary mouse model of ocular HSV-1 infection, we show that HSV-1 infection induces a dominant IFN-λ response compared with type I IFNs in the cornea. The topical recombinant IFN-λ (rIFN-λ) treatment during the early viral replication phase reduced viral burden at the primary site of HSV-1 replication, neuronal spread, and HSV-1 levels in the TG, significantly suppressing HSK progression. Early rIFN-λ treatment reduced IL-6, IL-1β, and CXCL-1 production in the cornea and promoted neutrophil-mediated anti-viral effector responses. Further, we demonstrate that rIFN-λ significantly suppresses acyclovir-resistant HSV-1 replication in the epithelial cells compared to acyclovir alone treatment groups. Our data indicate that local delivery of IFN-λ-therapy at the corneal mucosal surface can promote endogenous anti-viral responses and represents a promising alternative therapeutic approach against recurrent corneal HSV-1 infection caused by multi-drug-resistant HSV-1 strains.

Funding Sources: National Eye Institute Grant EY035057 (R15), National Eye Institute Grant EY034495 (R01)

Primary subject category: Immunology

DEVELOPMENTAL TOXICITY ASSESSMENT OF PER- AND POLYFLUOROALKYL SUBSTANCES-BASED FIREFIGHTING FOAMS & ALTERNATIVE FLUORINE-FREE FOAMS USING THE ZEBRAFISH MODEL

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Presentation Type: Oral or Poster Presentation

ABSTRACT:
Aqueous film-forming foams (AFFF) are Per- and Polyfluoroalkyl Substances (PFAS)-based firefighting foams used to fight fires. Numerous studies have indicated that these PFAS-based foams have many harmful human health effects, including developmental abnormalities and immune dysfunction. Because of this, fluorine-free firefighting foams (F3) have been developed as supposedly safer alternatives to PFAS-based firefighting foams. However, minimal research exists on the toxicity of these alternatives. Our research utilizes the zebrafish model to conduct a developmental toxicity assessment of fluorine-free and PFAS-based firefighting foams. Larval zebrafish were exposed to a range of concentrations of each foam from 6-72 hours post fertilization (hpf). At 72 hpf, developmental endpoints such as survival rate and morphology were observed. The potential of fluorine-free and PFAS-based foams to induce oxidative stress was investigated by measuring intracellular reactive oxygen species (ROS), with planned follow up studies assessing glutathione production. Morphology was imaged using a Ramona Optics Multi-Camera Array Microscope: abnormalities observed included scoliosis, cardiac edemas, delayed or halted development, and reduced pigmentation in concentrations of AFFF and F3 greater than or equal to 0.0131%. Adverse impacts on the survival rate were observed in AFFF concentrations greater than 0.0131% and in F3 concentrations greater than 0.00420%. Higher ROS production was seen in higher F3 concentrations. These concentrations are considerably lower than the practical application concentration of 3%. Our findings so far suggest that F3 foams may be similarly or more toxic to PFAS-containing AFFF foams.

Primary Subject Category: Other

This work is supported by the NCSU Comparative Medicine Institute and the NCSU Center for Environmental & Health Effects of PFAS, NIH P42-ES031009.

GENE EXPRESSION IN EQUINE JOINT CELLS TREATED WITH ALPHA-2 MACROGLOBULIN IN AN *IN VITRO* OSTEOARTHRITIS MODEL

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Osteoarthritis (OA) is a prevalent degenerative joint disease which leads to significant morbidity and economic impact in the equine industry. Currently available biologic OA therapies aim to slow disease progression and manage symptoms. Alpha-2 macroglobulin (A2M), a naturally occurring plasma protein, is a promising candidate as an intra-articular regenerative therapy due to its protease inhibitory properties and potential to modulate inflammation and cartilage degeneration. However, the ability of the currently available commercial product (Alpha2EQ) to control inflammation and protect cells within the equine joint has not been investigated. We aim to investigate the gene expression of equine chondrocytes and synovial fibroblasts after treatment with A2M in an *in vitro* OA model. Equine chondrocytes and synovial fibroblasts will be cultured in monolayer in 12-well plates. At 36 hours, cells will be stimulated with IL-1β to create an OA model. Non-stimulated cells will serve as controls. After 24 hours of stimulation, inserts will be placed into the plates and A2M treatment will be added to the inserts at doses of 10% and 25% media volume. Media-treated cells will serve as treatment controls. After 24 hours, cells will be collected and gene expression measured using NanoString analysis to evaluate the alteration of OA-related genes with and without A2M treatment. We hypothesize that treatment of cartilage and synovial cells with Alpha2EQ will alter expression of genes associated with OA pathogenesis. Our study could provide a foundational understanding of A2M's anti-inflammatory properties and therapeutic potential, bolstering evidence for its use as an equine OA treatment.

Funding sources:
Astaria Global, LLC.
F.O.R.G.E.- Fund for Orthopedic Research in honor of Gus and Equine athletes
Primary subject: Regenerative Medicine

COMPARISON OF THE PHARMACOKINETICS AND PHARMACODYNAMICS OF APIXABAN AND RIVAROXABN IN DOGS

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Background: Comparative pharmacokinetics and pharmacodynamics (PK/PD) of apixaban and rivaroxaban have not been studied in dogs and the propensity of these drugs to cause hypercoagulability after discontinuation is unknown.
Hypothesis/Objectives: To compare the PK/PD of clinical dosing regimens of oral apixaban, and rivaroxaban administered repeatedly to healthy dogs and assess the effect of abrupt drug discontinuation on coagulation.
Animals: 6 University-owned, purpose-bred, middle-aged, mixed-breed dogs (4 male, 2 female).
Methods: Dogs were administered oral apixaban or rivaroxaban at 0.5 mg/kg q12h for 7 days with a 14-day washout period between drugs. Plasma drug concentrations were quantitated, and anticoagulant effects measured using clotting times, calibrated anti-Xa bioactivity assays, and measurements of thrombin generation. Potential for rebound hypercoagulability was assessed by measuring D-dimers, thrombin-antithrombin complexes and antithrombin activity after drug discontinuation.
Results: Plasma drug concentrations and anti-Xa bioactivities were closely correlated for both drugs, however drug levels varied considerably among dogs, despite consistent dose regimens. Thrombin generation variables were significantly correlated with the anti-Xa bioactivity of both drugs and no significant differences in the effects of apixaban and rivaroxaban on thrombin generation were observed. Drug discontinuation had no effect on D-dimer concentrations. The concentration of thrombin-antithrombin complexes decreased after apixaban discontinuation and did not change after rivaroxaban discontinuation.
Conclusions and Clinical Importance: Repeated oral administration of apixaban or rivaroxaban to healthy dogs produced comparable anticoagulant effects measured by inhibition of thrombin formation. Rebound hypercoagulability after drug discontinuation was not observed and weaning of these drugs is unnecessary.

Funding source: 2022-23 Honorary Lloyd E. Davis Pharmacology Grant from the Veterinary Pharmacology Research Foundation (VPRF) and the American Veterinary Medical Foundation (AVMF).
Subject category: Clinical medicine

DEVELOPING AND VALIDATING A PAIN SCALE FOR MEAT-PRODUCING GUINEA PIGS UNDERGOING CASTRATION

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In South American countries, guinea pigs are raised and produced as a primary food source. Ensuring the welfare of food animals is crucial because it directly affects their performance and production. To our laboratory's knowledge, there is currently limited peer-reviewed research addressing meat producing guinea pig welfare. This study is aimed to examine the clinical validity and reliability of an acute pain scale modified for guinea pigs undergoing castration. The initial phase of the study involves analyzing videos of guinea pigs post-castration to identify and describe pain-related behaviors to aid observers in recognizing these behaviors. The second phase is a trial in which seventeen male guinea pigs were enrolled in the study and underwent castration in conjunction with an injectable analgesic administered 10 minutes post-castration (1 mg/kg meloxicam subcutaneous). An additional 10, non-painful guinea pigs were included to account for the effect of natural behavioral variation by day on pain scale results. Behavior of each guinea pig was video recorded continuously at four recording periods (15 min pre-castration, immediately post procedure and anesthesia recovery, 1 hour post-castration and 24 h post-castration). These videos will be used to assess pain-related behaviors and create a scale for trained observers to score and validate. In conjunction with this study, a survey was administered to nine Ecuadorian family-type producers to determine their knowledge of animal welfare. This survey will be used to determine areas of improvement, help an underserved part of society, and directly impact the welfare of meat producing guinea pigs.

Funding Sources: NC State Global One Health Academy (GOHA) - Graduate Travel Award
NC State University Office of the Associate Dean for Research and Graduate Studies

Primary Category: Animal Welfare

BRIDGING THE GAP BETWEEN GENOMICS AND THE HUMANITIES: MITOCHONDRIAL GENOME SEQUENCING FOR DETERMINING SPECIES OF ORIGIN IN PARCHMENT SPECIMENS

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Parchment, a specialized writing surface prepared from skins of animals, can be a valuable source of genetic information. As many parchment documents are associated with a defined temporal and geographical region, developing a genetic map of historical domesticated animals may be feasible. While the opportunity is expansive, due to the thousands of manuscripts being meticulously stored in libraries and archives, parchment represents a challenging sample type given: 1) non-destructive sampling techniques are essential to preserve the artifact; 2) the age of the parchment and possibly the production process lead to severe degradation of endogenous DNA; and 3) nucleic acid extracts from parchment often include touch DNA from human handling and other animal-based surface contaminants complicating sequence data analysis.

Here we present interdisciplinary research from a diverse team with combined expertise in genetics, forensic science, bioinformatics, and medieval studies, aiming to glean genetic data from skins collected from well-characterized documents. A total of 351 parchment samples were subjected to an established workflow to sequence full mitochondrial genomes for species identification. Briefly, 1) DNA was isolated from samples and converted to sequence libraries; 2) hybridization capture enriched the source animal mitochondrial DNA; 3) enriched samples were sequenced with Illumina chemistry; and 4) reads were mapped to reference mitochondrial genomes. Using this method, 204 (58%) samples were authenticated with complete mitochondrial genomes. We will share the successes of the method, challenges of the sample type, and provide guidance for future sampling and testing.

Funding source: Research and Innovation Seed Funding Program (RISF)

Category: Genetics

DEVELOPMENT OF A FUNCTIONAL VISION TEST FOR COMPANION DOGS

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Sensory impairments such as vision loss are common in the elderly human population and have adverse effects including cognitive decline. One study in dogs found owner-reported vision loss correlated with worsened cognition based on the canine cognitive dysfunction rating scale (CCDR). However, a method to objectively quantify vision loss has not been developed for companion dogs. While the ophthalmic exam can distinguish whether a dog is sighted or blind using an electroretinogram (ERG), menace response, and cotton ball test, these tests cannot quantify the severity of visual impairment in dogs. Additionally, methods of visual assessment developed for use in laboratory dogs have failed in companion dogs. We hypothesized that we could develop a functional vision test for companion dogs. Our aim was to develop an obstacle course that reliably quantifies vision loss by measuring total number of collisions made. An obstacle course was optimized by testing different obstacles until sighted dogs could negotiate the course with minimal errors. Visually-impaired dogs were then tested. We developed a 5-meter-long obstacle course with 8 obstacles, including 3 clear plexiglass boards, 3 black foam boards and 2 white foam boards. Nine sighted dogs have completed the course, 6 with no collisions and 3 with a single collision into one plexiglass panel. At the time of writing, one visually-impaired dog has been tested. This dog collided with 4 of the 8 obstacles: 3 plexiglass panels and one white foam board. Extensive testing of visually-impaired dogs is underway, but appears promising.

Funding Source: Dr. Kady M. Gjessing and Rahna M. Davidson Distinguished Chair in Gerontology
Category: Gerontology & Neurosciences

JUN N-TERMINAL KINASE (JNK) AND RHO KINASE (ROCK) ARE REQUIRED FOR STOMACH CURVATURE

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The Jun N-terminal kinase (JNK) and Rho kinase (ROCK) signaling pathways play critical roles in the cell rearrangements, i.e., radial intercalation, that drive the elongation of the embryonic gastrointestinal tract. Interestingly, left-right asymmetry in cell rearrangement is thought to drive the leftward curvature of the stomach, as radial intercalation initially occurs only on the left side of the embryonic stomach; we therefore hypothesized that JNK and ROCK signaling would be required for normal stomach curvature. To test this hypothesis, we exposed *Xenopus* embryos to varying concentrations of ROCK or JNK inhibitors during stomach development. We found that both inhibitors resulted in shorter stomachs with more shallow curvatures. Immunohistochemical staining shows that stomach cells in embryos treated with the ROCK inhibitor ("Rockout") display a disruption in the organization of the cell membrane, with enhanced adhesion and diminished presence of cytoskeleton proteins. Rockout exposed cells were also more rounded with less distinct tissue stratification, indicating they were unable to execute proper radial intercalation events, and also showed reduced assembly of basement membrane. Conversely, embryos treated with the JNK inhibitor showed disruptions in cell-cell adhesion proteins which led to cell dissociation, also preventing proper radial intercalation. Thus, while ROCK inhibition primarily disrupted cell shapes and prevented cell rearrangement, JNK inhibition affected cell-cell adhesion, both of which are crucial for proper radial intercalation events. Our findings highlight the importance of both the JNK and Rho/ROCK signaling pathways in maintaining cell and tissue integrity to drive stomach curvature morphogenesis.

Funding Source: NIH R01HD089243

Subject category: Cell Biology

LOCALIZATION AND EXPRESSION OF THE GENE FOR ARTEMIN IN MOUSE AND CANINE OSTEOARTHRITIC JOINT TISSUES

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Painful osteoarthritis (OA) is a major public health problem. In previous work we identified a signaling system (artemin/GFRa3) that was upregulated in painful OA and we found sequestering artemin using systemic anti-artemin monoclonal antibody mitigated pain in rodent OA models. However, the source of artemin is currently unknown. The objective of this study was to elucidate the source of artemin in painful OA-affected joints. Using RT-qPCR, we examined *Artn* gene expression in the chemical monoiodoacetate (MIA) (n = 6-8), and surgical destabilization of the medial meniscus (DMM) (n = 6-11) murine models of OA, evaluating gene expression in subchondral bone, synovium, and dorsal root ganglia (DRG) tissues. Additionally, we measured *ARTN* expression in naturally occurring canine hip OA (n = 6) (subchondral bone, synovium). Results demonstrated increased *Artn* in the tibial subchondral bone in both MIA and DMM mice as compared to their respective saline or sham controls (Fold change: ~5). Similarly, *ARTN* was increased in the femoral head subchondral bone of canine hip OA compared to healthy controls (Fold change: ~17). *Artn* was increased in the synovium of MIA mice as compared to saline (Fold change: ~ 3). *Artn* was also increased in the DRG of both MIA and DMM OA mice as compared to their saline/sham controls respectively (Fold change: ~1.3 - 2). Taken together artemin gene expression appears increased in subchondral bone and DRG of painful OA tissues. In future work, we will evaluate protein expression of artemin and determine its exact tissue/cell source.

Funding Source: NIH NIAMS *R01AR079713*
Category: Pain

ARTEMIN-INDUCED NEURONAL SPROUTING IN OSTEOARTHRITIS: IMPLICATIONS FOR PAIN MECHANISMS AND THERAPEUTIC STRATEGIES

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Pain associated with osteoarthritis (OA) is a major public health problem globally. There is increasing evidence that in painful OA, sensory neurons demonstrate pathologically increased axonal sprouting within the joint, contributing to pain and sensitivity. Previous data from our lab has identified artemin (ARTN), a neurotrophic factor, as playing a significant role in OA-associated pain. We hypothesize that artemin's pro-nociceptive effects may be partly driven by ARTN-induced axonal sprouting. We used dissociated mouse sensory neurons from dorsal root ganglia (age ~3-6 weeks, n = 3) and cultured them in one of four media conditions: ARTN protein (200 ng/mL, 500 ng/mL); nerve growth factor (NGF) protein (positive control; 200 ng/mL, 500 ng/mL); NGF (500 ng/mL) plus ARTN (200 ng/mL); or formulation buffer (FB) (negative control) for 48 hours. Neurite length from ~150 neurons/group was measured using Neurology ImageJ plugin, expressed as mean total neurite per neuron per condition. Data were expressed as percentage increase in neurite length over negative control group. NGF resulted in a ~70% greater neurite length at both concentrations ($p < 0.0001$) and ARTN resulted in a ~35% greater neurite length at 200 ng/mL ($p < 0.001$). Additionally, there was a 93% greater neurite length ($p < 0.0001$) when ARTN and NGF were used in combination. Our results indicate that ARTN does promote axonal sprouting in sensory neurons. In future research, we will focus on identifying the specific neurons that respond to ARTN and extend to *in vivo* studies to better understand ARTN's role in a physiological context.

Funding Source: NIH NIAMS R01AR079713
Category: Pain

DEVELOPMENT AND VALIDATION OF NOVEL WITHAFERIN A ANALOGS AS CHEMOPROTEOMIC PROBES

Presenter: **Igor Silva,[1]** postbaccalaureate researcher

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Withaferin A (WFA), derived from the *Withania somnifera* plant, inhibits inflammatory neutrophil functions and promotes timely apoptosis of neutrophils under inflammatory conditions. These properties likely contribute to published benefits of WFA for inflammatory conditions and are relevant to future use of WFA as a promising cancer therapy. However, the mechanism(s) of action of WFA in neutrophils is unknown, which poses a barrier for the drug development process. Chemoproteomics uses affinity enrichment of proteins bound to a probe (analog of compound of interest) to interrogate targets of drug candidates. We hypothesized that we could synthesize WFA analogs that retain phenotypic effects of WFA in neutrophils and could serve as probes to facilitate high-throughput identification of WFA-binding proteins in neutrophils. We synthesized two WFA analogs with substitutions at the C27 hydroxyl group: biotinylation and alkynylation. We demonstrated that both novel WFA analogs inhibit adhesion and respiratory burst of equine neutrophils, mirroring the effects of WFA on these neutrophil functions. Neither WFA analog compromised neutrophil viability at concentrations and exposure times tested in functional assays. These data support the suitability of our WFA analogs as chemoproteomic probes to identify WFA targets in neutrophils. We are completing the probe validation process by verifying that WFA analogs have similar effects as WFA on neutrophil apoptosis. Next, we will perform proteomic analyses on WFA probe-bound proteins isolated from neutrophil lysate. This project represents an important step towards elucidating the mechanism(s) of WFA in neutrophils, complementing our concurrent *in vitro* and *in vivo* work.

Funding source: 2023-2024 NCSU Research and Innovation Seed Funding Program (RISF)

Primary subject category: Cell Biology

IDENTIFYING THE AIRWAY EPITHELIAL CELL-SPECIFIC ROLE OF IL-4Rα IN ALLERGEN-INDUCED MUCOUS CELL METAPLASIA

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Primary Subject Category: Immunology

Mucous cell metaplasia (MCM), a hallmark feature of allergic asthma, is characterized by an abnormal increase of mucous cells in the airways. IL-4 and IL-13 signaling via IL-4Rα on the airway epithelial cells is known to drive MCM in allergic airway disease. The contribution of IL-4Rα signaling in primary airway epithelial cells i.e., basal cells, club cells, and ciliated cells, towards MCM in allergic asthma is unclear. To study this, we generated basal cell-, and ciliated cell-specific tamoxifen-induced, conditional IL-4Rα knockout mice and challenged them with a single intranasal dose of mixed allergens (MA) followed by the assessment of immune cell recruitment and MCM. First, we performed a time-based kinetics study in wild-type (WT) mice to assess the effectiveness of a single MA dose to induce MCM across different timepoints. The immune cell recruitment and MCM steadily increased from 12h to 96h post-MA, with both peaking at 96h post-MA. As compared to MA-challenged IL-4Rα sufficient mice, the total immune cell recruitment mainly attributable to an increase in macrophages, neutrophils, eosinophils, was unaltered in MA-challenged tamoxifen-inducible KRT5+ basal cell-specific IL-4Rα-deficient mice at 96h post-MA. The extent of MCM, as indicated by the proportion of AB-PAS+ airway epithelial cells was also comparable between two groups. Similar trends in the immune cell recruitment and MCM were seen in MA-challenged IL-4Rα sufficient and MA-challenged tamoxifen-inducible FOXJ1+ ciliated cell-specific IL-4Rα-deficient mice at 96h post-MA. These findings indicate that IL-4Rα signaling in basal and ciliated cells may be dispensable in inducing MCM in allergic airway inflammation.

Title: PHARMACOKINETICS OF PHENAZOPYRIDINE IN HEALTHY GOATS AT TWO DIFFERENT DOSAGES

Dileydis D. Soto Montes, veterinary student
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Abstract:
Phenazopyridine, an over-the-counter genitourinary analgesic for humans, is also used to manage pain in male goats with obstructive urolithiasis. A previous study reported that the analgesic effect of phenazopyridine is dose-dependent in rats. A study conducted in goats undergoing treatment for obstructive urolithiasis found that phenazopyridine is rapidly eliminated from plasma and does not reach detectable levels in urine after oral administration. Further research is needed to determine a safe and effective dosing regimen for goats following surgery to correct obstructive urolithiasis. This study aims to investigate the pharmacokinetic parameters of orally administered phenazopyridine in six healthy Boer cross male goats at two different dosing regimens: 4 mg/kg every 8 hours and 8 mg/kg every 12 hours both administered orally for 24 hours. We hypothesize that higher concentrations of phenazopyridine will be found in the blood and urine at the 8 mg/kg every 12 hours dose, making it more clinically relevant than the 4 mg/kg every 8 hours dose. Following administration of each dosing regimen, plasma and urine samples were collected from each goat at various time points over three days, with a 7-day washout period between study phases. Phenazopyridine concentrations will be determined using HPLC with UV detection. Although results are pending, this research promises to address the lack of characterization of an adequate dosing regimen of phenazopyridine in goats.

Funding Source: Start Up

Subject Category: Pharmacology

Preference: Poster Presentation

INVESTIGATING THE ROLE OF SGLT1 AND INCRETINS IN EQUINE INSULIN DYSREGULATION USING DUODENAL SAMPLES

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Background: Equine metabolic syndrome (EMS) is a major concern for equine welfare due to increased risk of laminitis development. Insulin dysregulation (ID) plays an important role in EMS pathophysiology and is influenced by gastrointestinal hormones (incretins, GIP, GLP-1) secreted by small intestinal epithelium after absorbing glucose via SGLT1. Novel therapies to manage ID are needed.
Hypothesis: Insulin dysregulated horses will express higher levels of *SGLT1* and *GIP* in duodenal biopsies. Expression of *SGLT1* by duodenal organoids can be manipulated by nutrient availability in vitro.
Methods: An oral sugar test (OST) was used to characterize non-insulin dysregulated vs mildly/moderately insulin dysregulated horses. A cohort of 21 healthy horses (8/21 with ID, ages 3-22 years) with similar diets was used. Duodenal biopsies were obtained via gastroscopy for RNA isolation, histopathology, and crypt isolation for organoid culture. After passaging, duodenal organoids were incubated in control, differentiation, and low glucose media. Response to media variation was assessed by gene expression.
Results: Mildly to moderately ID horses were not significantly different for plasma glucose, GIP, active GLP-1, or GLP-2. Gene expression of *SGLT1* and *GIP* in duodenal biopsies was not different between groups. Restricted glucose availability in duodenal organoid culture significantly increased *SGLT1* expression.
Conclusions: Mildly to moderately ID horses did not consistently demonstrate upregulation of *SGLT1* or *GIP* in duodenal epithelium. Nutrient conditions in vitro can influence *SGLT1* expression in duodenal epithelium. Therefore, dietary manipulation of *SGLT1* expression and subsequent glucose absorption could provide avenues for future treatments against ID and EMS.

Funding: NC State University Office of the Associate Dean for Research and Graduate Studies (VSP Stoop), NC State Faculty Research and Professional Development Award (Dr. Sheahan)

Primary Subject Category: Gastroenterology

Title: CHRONIC EFFECTS OF ALLERGIC HYPERSENSITIVITY-RELATED CYTOKINES IL-4 AND IL-33 ON MAST CELL FUNCTION AND RESPONSIVENESS

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Subject Category: Immunology

Abstract:

Chronic allergic diseases, such as asthma and atopic dermatitis, are the result of prolonged activation of immune cells, which results in the sustained release of proinflammatory agents throughout the body. Mast cells are specialized immune cells that function as first-responders against tissue foreign invaders. They are equipped with prestored granules containing inflammatory mediators, cytokines, and chemokines that are released in response to allergen detection. In response to allergens, these granules are released into the tissue through activation of the high affinity immunoglobulin E (IgE) receptor, FceRI, to clear the allergen and damaged cells, increase vascular permeability, and recruit other immune cells. While the acute effects of these inflammatory mediators and cytokines on immune cells are well known, the long-term effects, as would be the case in chronic allergic diseases, need further investigation. By culturing mouse mast cells in IL-4 and IL-33 (2 key allergic response cytokines), this study demonstrates the molecular pathology during the shift from acute to chronic exposure. Over the course of cytokine exposure, mast cell response to IgE was determined using degranulation and signal transduction assays. Further effects on mast cells were investigated through proliferation assays and fundamental protein expression changes. These results illustrate the skewed mast cell response in chronic allergic inflammation, with which we have gained a better understanding of the implications of chronic allergic diseases, as well as identified new potential avenues/targets for therapeutic intervention.

Funding source: NIAID R01AI143985

Preference Format: Poster Presentation

INFILTRATION OF NEUTROPHILS IN AN EXPERIMENTAL MODEL OF CANINE ACUTE ATOPIC DERMATITIS SKIN LESIONS

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Abstract
Inflammatory cells play a critical role in the pathogenesis of atopic dermatitis (AD) with Langerhans' cells, dermal dendritic cells, B- and T-lymphocytes, and mast cells identified as key contributors. Traditionally, neutrophils were considered non-specific to atopic skin lesions; however, recent research redefined their role, demonstrating that skin-infiltrating neutrophils are critical initiators of itch and recurrence of AD skin inflammation in a mouse AD model. This study aimed to investigate the chronological infiltration of neutrophils in an experimental model of canine acute AD skin lesions. Skin samples were collected from five healthy dogs and four dogs with house dust mite-induced canine AD at 0, 24, 48, and 96 hours post-allergen provocation. Neutrophils were immunolabeled using an anti-canine neutrophil monoclonal antibody (CADO48A), and their numbers were quantified. No neutrophils were detected in the skin of either healthy or AD dogs at the baseline (0 hours). Peak neutrophil counts were observed at 24 or 48 hours post-challenge in three out of four AD dogs, with subsequent decline at 96 hours. Notably, there was a significant increase in neutrophil numbers in AD dogs at 96 hours compared to baseline (0 hours) and to healthy dogs. Our study demonstrated early and transient neutrophil infiltration in the acute canine AD skin, paralleling findings in a mouse AD model. Further studies are necessary to elucidate the role of neutrophils in the pathogenesis of canine AD.

Funding Source: NCSU and NIH
Primary subject category: Immunology

ASSESSMENT OF HYPERCOAGULABILITY, FIBRINOLYSIS, AND INFLAMMATION IN CATS WITH DIFFERENT MANIFESTATIONS OF CONGESTIVE HEART FAILURE

Aaron Tang, Veterinary Student

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Objectives: This study aims to determine if cats with congestive heart failure manifesting as large-volume pleural effusion will exhibit different coagulation, fibrinolytic, and inflammatory profiles than those with cardiogenic pulmonary edema.
Methods: Cats with either cardiogenic pleural effusion or pulmonary edema at North Carolina State University (NCSU) Veterinary Hospital will be recruited. Blood samples will be collected for diagnostic assays at NCSU and Cornell's Comparative Coagulation Diagnostic Laboratory. Fibrinolysis will be assessed through Overall Hemostatic Potential (OHP), D-dimer, Plasminogen Activator-Inhibitor-1 (PAI-1), plasminogen, and antiplasmin. Coagulation will be evaluated via fibrinogen, Factor VIII, thrombin generation, von Willebrand antigen, antithrombin, thrombin-antithrombin, and point-of-care viscoelastic monitoring (VCM) system. Inflammation will be examined using complete blood counts, neutrophil extracellular traps, serum amyloid A, and hyaluronan. Ultrasonographic and echocardiographic data will also be collected.
Results: We anticipate differences in fibrinolytic, coagulation, and inflammatory profiles between the two feline congestive heart failure populations. Specifically, we hypothesize that cats with large-volume pleural effusion will exhibit lower risk of feline arterial thromboembolism (FATE) due to its fibrinolytic properties. Additionally, the inflammatory profiles of cardiogenic pulmonary edema may be a risk factor for thrombosis.
Conclusions and relevance: Understanding the hemostatic pathogenesis and mechanisms of FATE will help identify at-risk feline cardiomyopathy populations and guide future prevention and treatment strategies.

Funding Sources: NC State Feline Health Center; NC State University Herbert Benjamin Endowment

Primary Subject Category: Clinical Medicine

Litwack 2024 Poster abstract submission for Stephanie Thomas:
Infectious disease category

CLOSTRIDIOIDES DIFFICILE TOXINS ALTER HOST BILE ACID SYNTHESIS PATHWAY GENE EXPRESSION

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Clostridioides difficile infection (CDI) remains an urgent health threat with an incidence of approximately 500,000 cases and 29,000 deaths per year in the US. *C. difficile* secretes toxins (TcdA and TcdB) that are internalized into host colonic epithelial cells where they disrupt gut barrier function and induce hyper inflammation resulting in severe diarrhea. Bile acids (BA) have a major influence on *C. difficile* lifecycle and pathogenesis. Farnisoid X Receptor (FXR) is a BA responsive nuclear receptor of the host. When activated by intestinal BA, FXR initiates transcription of target genes creating a negative feedback signal to reduce BA synthesis in the liver. FXR activation also reduces inflammation and is therefore an enticing target for interference with CDI. However, little is known about the relationship between *C. difficile* and its toxins regarding FXR signaling in the host. To investigate this, differentiated Caco-2 cells and primary colonocytes from a human donor were exposed to *C. difficile* toxins TcdA, TcdB or both at differing concentrations for 24 hr. Exposure to TcdA and TcdB in primary colonocytes significantly increases expression levels of FXR target genes, namely FGF19 and SHP, but not FABP6. Similar results were seen with Caco-2 cells exposed to TcdB, but not TcdA. These data suggest that exposure of colonic epithelial cells to *C. difficile* toxins alters expression of FXR target genes involved in the BA synthesis pathway, and therefore possibly modify BA synthesis in the host liver.

INDUCTION OF AN IMMUNOLOGICAL ANTI-TUMOR RESPONSE USING PULSED ELECTRIC FIELDS

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In benign or malignant tumor cases in which resection surgery is not possible or desirable, there is a need for a controlled method to selectively destroy target tissues. Irreversible electroporation has emerged as a minimally invasive alternative to resection surgery that is capable of high-volume tissue ablation without harmful thermal affects or the need of adjuvant drugs. A third-generation irreversible electroporation approach, integrated nanosecond pulse irreversible electroporation (INSPIRE), continuously delivers ultrashort duration electrical pulses to create irreversible defects in the cell membrane and thus induce rapid cell death in target tissues. Notably, INSPIRE uses higher voltages and temperature regulation to create large treatment volumes, enhance immunological responses and improved safety. Prior studies on murine tumor models indicate that INSPIRE induces a unique form of cell death that elicits a degree of protective immunity, which is enhanced by adjunctive immunotherapies. This is distinct from traditional therapies which use burning or freezing to focally kill tumors. The goal of this study is to evaluate the degree to which INSPIRE therapy induces an anti-tumor immune response in a murine model and the nature of this response. Murine tumors were treated 6 days post implantation, after which liver and spleen will be harvested to evaluate immunological responses. An understanding of the nature of this anti-tumor immune response induced by INSPIRE therapy and its mechanisms could introduce a new method of immunogenic tumor treatment capable of treating large targets in a minimally invasive, focused, non-surgical manner.

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Biomedical Engineering

EVALUATION OF IMMUNE RESPONSES IN CHICKENS IMMUNIZED WITH CLOSTRIDIAL DERMATITIS VACCINE

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Clostridial dermatitis (CD) caused by Clostridium septicum is an economically important emerging disease affecting poultry. In recent years, a lot of research has been conducted to find an effective non-antibiotic disease control measure, such as vaccine, for CD. We have previously identified a vaccine candidate, the non-toxic domain 2 of C. septicum alpha toxin (ntATX-D2) and showed that turkeys immunized with ntATX-D2 can be protected against CD. More recently, we investigated the efficacy ntATX-D2 vaccine in chickens and found that ntATX-D2 immunization can protect against experimental CD challenge. In this study, we evaluated the vaccine-induced immune responses in chickens by measuring the cytokine gene expression in local (skin and muscle) and systemic (spleen) tissues as well as quantitating serum antibody responses. The treatment groups were; 1) Negative control ('NCx'), 2) Unimmunized but C. septicum-challenged positive control ('PCx'), and 3) ntATX-D2-immunized and C. septicum-challenged ('D2-immunized'). Results showed while the PCx birds had higher ($P< 0.05$) expression of pro-inflammatory cytokine (IL-1β, IL-6 or IFNγ) genes in all three tissues compared to NCx, no significant differences were observed between the D2-immunized and NCx groups. Additionally, in the muscle tissue of D2-immunized chickens, there was a higher ($P< 0.05$) expression of anti-inflammatory cytokines, IL-10 and TGF-β, compared to PCx. Furthermore, the D2-immunized birds had higher ($P< 0.05$) serum IgY antibodies against ntATX-D2/C. septicum secretory antigens compared to NCx / PCx. These results suggests that ntATX-D2-immunization in chickens can result in the modulation of local and systemic inflammatory responses and induce antigen-specific IgY responses.

Funding Source: United States Poultry and Egg Association
Category: Immunology

EPIGENETIC DIFFERENCES BETWEEN CAPTIVE AND WILD AMERICAN BLACK BEARS (*URSUS AMERICANUS*) IN NORTH CAROLINA

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The illegal wildlife trade is estimated to be worth up to $20 billion a year, with a proportion of this trade consisting of wild caught individuals being sold as captive bred individuals for pets or byproducts. To prosecute these cases, methods to differentiate between wild and captive individuals of a given species are needed. Epigenetic changes to DNA, such as genomic methylation, can quantitatively be studied as an indicator of differences amongst individuals of the same species. Research comparing captive and wild populations of species has identified factors such as stress in captivity, social environment, and resource availability as contributing to epigenetic changes between populations. In this pilot project, we used American black bears (*Ursus americanus*) as the model species for assessing if differences in methylation could be observed between captive and wild individuals. We analyzed 3 captive and 3 wild females collected from North Carolina. DNA was extracted from whole blood, prepared into libraries and sequenced for genomic methylation using Nanopore chemistry. EPI2ME was used to a) align generated sequence reads to the black bear reference genome, b) determine the percentage of methylation at each site, and c) compare methylation between captive and wild individuals. Results will be discussed as to whether this is a promising approach to differentiate between captive and wild black bear individuals. The outcome of this pilot study will be valuable in determining whether epigenetics could be applied to other species, providing important information relevant to cross-breeding populations or reintroducing captive-reared animals to wild environments.

Funding Sources: Veterinary Scholars Program, NCSU Biotechnology Program, Meiklejohn salary release

Primary Subject: Genetics

OPHTHALMIC FINDINGS IN AGING DOGS

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Subject Category: Ophthalmology/ Gerontology

Age-related visual impairment in people is well-documented as a risk factor for dementia, impaired independence, and depression. Research of age-related ophthalmic abnormalities and their impact on dogs is sparse. This study aimed to evaluate ophthalmic lesion prevalence in aging dogs and how they relate to cognition, mobility, and quality of life. Forty-eight pet dogs (10-17 years old) participating in a longitudinal study at NC State College of Veterinary Medicine were included in this cross-sectional, descriptive study. Ocular findings were assessed using standard measures and categorized according to severity alongside cognitive testing, mobility assessments, and questionnaires. Univariate analyses were performed using t-tests and Wilcoxon/Kruskal-Wallis Tests followed by multivariate analyses to include covariates. Significant differences were found in executive function testing ($p=0.047$), owner-assessed cognition ($p=0.031$), and quality of life ($p=0.014$) for dogs with and without dense nuclear sclerosis. Similarly, significant differences were noted in owner-assessed cognition ($p=0.0093$) and quality of life ($p=0.049$) between dogs with and without corneal pathology. Multivariate analyses (Least Squares) of owner-assessed cognition and quality of life were conducted with significant covariates from univariate analysis. Owner-assessed cognition was significantly associated with corneal pathology ($p=0.0153$). Aspects of quality of life were significantly associated with corneal pathology and dense nuclear sclerosis **($p <0.0052$).** These findings suggest that common ocular abnormalities in aging dogs may be associated with cognitive decline and contribute to reduced quality of life.

CHARACTERIZING SUBCLINICAL BACTERIURIA IN AGING FEMALE CATS

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Subclinical bacteriuria (SB), the presence of urinary bacteria without associated lower urinary tract signs (LUTS), occurs in cats, particularly older females; however, its clinical outcomes remain understudied. *Escherichia coli* is the most commonly isolated urinary bacteria of cats, and the adhesin fimH is an important mediator of urinary tract colonization. We enrolled female feline patients, 10 years and older, from the NCSU primary care and internal medicine services. Eligible cats had not received systemic antibiotics in the previous 30 days. At enrollment, owners completed a LUTS survey, and a CBC, blood chemistry panel, and urinalysis from cystocentesis were performed. Cats with bacteriuria received a urine culture with antimicrobial susceptibility testing and additional testing at 90 days (urinalysis and culture) and 180 days (urinalysis, culture, and chemistry). Owners repeated a LUTS survey every 30 days during this period. 14.0% (7/50) of cats had SB on day 0 - *E. coli* (2), *Enterococcus faecalis* (3), *Staphylococcus felis* (1), or *Enterobacter cloacae* (1). SB cats had lower USG (1.021 vs 1.035, p=0.026) and were more likely to have a history of LUTS (3/7 vs 3/43, p=0.029). 25% (3/12) of isolates had antimicrobial resistance, but none were multidrug resistant (CLSI VET01Sed7). 75% (3/4) of cats with repeat sampling showed persistent bacteriuria (*E. coli* (1) and *E. faecalis* (2)). No SB cats have been diagnosed with chronic kidney disease since enrolling but 2 developed LUTS. Moving forward, we will enroll 100 total cats and further characterize all *E. coli* isolates through fimH PCR and MLST.

Funding Sources: EveryCat Health Foundation, NC State Feline Health Center

Primary Subject Category: Clinical Medicine

IDENTIFICATION OF A NOVEL CHEMOPROTECTIVE FACTOR IN THE COLONIC TUMOR MICROENVIRONMENT: FOLLISTATIN-LIKE 3

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Colon cancer is one of the leading causes of deaths worldwide, notably due frequent drug resistance. There is increasing interest in developing new therapies that target mechanisms of chemoresistance, many of which are promoted by cells in the tumor microenvironment (TME). Unpublished work from the lab has identified Follistatin-like 3 (FSTL3) expression as increased in the TME following chemotherapy. Work by others suggests that FSTL3 expression levels are positively correlated with metastatic abilities in tumor cells. This work aimed to determine whether FSTL3 impacts colon cancer chemoresistance. To test this, we used human colon cancer cells from cell lines and primary colon adenocarcinomas from patients 3D-cultured as organoids. Supplementation with recombinant hFSTL3 (rhFSTL3) induced an increase in the number of organoids formed in the presence of 5-FU and lower levels of apoptosis, suggesting that FSTL3 is chemoprotective. Comet assays showed that FSTL3 promotes DNA integrity in 5-FU-treated tumor cells. We next investigated whether FSTL3 chemoprotection was dependent on ataxia telangiectasia mutated (ATM), master regulator of DNA double strand break response. ATM knock down or kinase activity inhibition abolished FSTL3 chemoprotection. Immunostainings on human tissue adjacent to colon adenocarcinomas showed that FSTL3 mostly colocalizes with enteric glial cells (EGCs). Cocultures of tumor cells and EGCs showed that EGCs had potent ATM-dependent protective effects against 5-FU. Finally, inhibition of FSTL3 expression or neutralization of secreted FSTL3 abolished EGC-mediated chemoprotection. Overall, this work demonstrates that FSTL3 represents a promising target to promote colon cancer chemosensitivity. Future investigations will identify the mechanism(s) involved.

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INVESTIGATING THE INFLUENCE OF LEFT-RIGHT ASYMMETRY ON *XENOPUS* HINDGUT DEVELOPMENT

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Cell Biology

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 hindgut tube. We hypothesized that this previously unrecognized anatomical left-right asymmetry plays a role in establishing the chirality of intestinal rotation.

To determine the cellular morphology underlying the formation of this asymmetry, immunohistochemistry was performed. Frontal sections were analyzed before, during, and after the formation of the hindgut concavity. While columnar epithelial cells lined the hindgut's left wall, more rounded, disorganized cells were found in the right wall, and the lumen of the hindgut tube was skewed leftward. Since the process of epithelialization drives the lengthening of the gut tube, these results suggest that the rotational curvature of the intestines may be driven by differential elongation of the left and right sides of the gut tube. To determine the relationship between hindgut asymmetry and intestinal development, embryos were exposed to chemical compounds known to cause intestinal malrotation (propylthiouracil and SB505124). In such embryos, the initial hindgut concavity was found to be reversed. Ongoing immunohistochemical staining of these embryos will confirm cellular changes.

This study provides new insight into embryonic events that shape normal intestinal rotation and illuminates the potential etiology of intestinal malrotation.

DETERMINING THE EFFECTS OF MICROBIOTA-PRODUCED FACTORS ON INTESTINAL EPITHELIAL CELL CYCLE DYNAMICS AND PROLIFERATION

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Neonatal necrotizing enterocolitis (NEC) is a devastating gastrointestinal disease in newborns, causing severe inflammation and damage to the intestinal lining. NEC often results in death as this damaged tissue is unable to heal properly, leaving it vulnerable to fatal bacterial infection. Our research hypothesizes that a secretion present in the microbial secretions in lumen contents of the intestinal epithelium is negatively impacting the proliferative response in NEC patients, causing inadequate healing of the damaged areas and subsequent death of the tissue. To test this, we seek to understand the impact of these lumen contents on cell cycle progression and gene expression using neonatal porcine intestinal epithelial cells (IPEC-J2) as a model. We are transfecting plasmids containing fluorescence genes allowing us to detect changes to cell cycle dynamics caused by treatment of healthy and injured IPEC-J2 cells treated with lumen contents. Furthermore, comparative RT-qPCR will provide information about significant changes in cell cycle related gene expression caused by treatment with lumen contents. If successful, this research will shed light on the underlying mechanisms of proliferation in NEC and potentially help to determine clinical targets for NEC and other gastrointestinal diseases.

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