Time	Room	Topic area	Speaker	Title
8:00-8:10	South	Infectious Disease	Bush, Janice (GS - Breitschwerdt)	VIABILITY AND DESICCATION-RESISTANCE OF BARTONELLA HENSELAE IN BIOLOGICAL AND NON-BIOLOGICAL FLUIDS: EVIDENCE FOR PATHOGEN-ENVIRONMENTAL STABILITY
8:12-8:22	South	Infectious Disease	Manvell, Charlotte (GS - Breitschwerdt)	FIRST DETECTION OF ZOONOTIC BARTONELLA ALSATICA IN FLEAS FROM EASTERN COTTONTAIL RABBITS (*SYLVILGUS FLORIDANUS*)
8:24-8:34	South	Infectious Disease	Curtis, Savannah (GS - Lanzas)	MODELING OF HEALTHCARE-ASSOCIATED CLOSTRIDIOIDES DIFFICILE INFECTION AND QUANTIFICATION OF ACQUISITION ROUTES IN ONCOLOGICAL UNITS
8:36-8:46	South	Infectious Disease	Wang, Annie (VS - Lanzas)	QUANTIFYING FITNESS EFFECTS OF RESISTANCE GENES USING PHYLODYNAMIC MULTI-TYPE BIRTH-DEATH MODELS AMONG *CAMPYLOBACTER COLI* FROM CONVENTIONAL AND ANTIBIOTIC-FREE AGRICULTURAL SWINE POPULATIONS
8:48-8:58	South	Infectious Disease; Epidemiology	Frias-De-Diego, Alba (PD - Lanzas)	HORIZONTAL GENE TRANSFER IN HEALTHCARE SETTINGS
9:00-9:10	South	Infectious Disease; Epidemiology	Jara, Manuel (PD - Lanzas)	PANGENOME-GWAS ANALYSIS OF *CLOSTRIDIOIDES DIFFICILE*: IDENTIFYING SELECTION PRESSURES IN HEALTHCARE AND BEYOND
9:12-9:22	South	Infectious Disease	Sykes, Abagael (GS - Machado)	MODELING THE TRANSMISSION AND CONTROL OF AFRICAN SWINE FEVER IN COMMERCIAL SWINE POPULATIONS OF THE UNITED STATES
9:24-9:34	South	Infectious Disease	Sykes, Abagael (GS - Machado)	APPLICATION OF INTERPRETABLE MACHINE LEARNING TO ON-FARM BIOSECURITY PRACTICES AND PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS
9:36-9:46	South	Infectious Disease	Sulaiman, Lanre (PD - Crisci)	PREDICT AND PROTECT AGAINST PRRSV (PREPROPRRSV): COMBINING PRRSV FORECASTING TECHNOLOGY WITH VACCINE EFFICACY PREDICTION TO PREVENT PRRSV OUTBREAK
9:48-9:58	South	Immunology	Byrne, Jake (NS - Crisci)	INTRANASAL AD5 INFLUENZA VACCINE ELICITS HEMAGGLUTININ-SPECIFIC ANTIBODY RESPONSE IN PREGNANT AND LACTATING PIGS
10:00-10:10	South	Infectious Disease	Walker, Grayson (VS/GS - Borst)	A PAN-GENOME-WIDE ASSOCIATION STUDY TO DECIPHER VIRULENCE MECHANISMS OF AVIAN PATHOGENIC *E. COLI*
10:12-10:22	South	Infectious Disease	Gaghan, Carissa (NS - Kulkarni)	EFFECTS OF *LACTOBACILLUS* ISOLATES ON PREVENTION OF NECROTIC ENTERITIS IN BROILER CHICKENS
10:24-10:34	South	Immunology	Criollo Vinueza, Valeria (GS - Kulkarni)	IMMUNE RESPONSES AND IMMUNOPATHOLOGY IN TURKEYS EXPERIMENTALLY INFECTED WITH CLOSTRIDIAL DERMATITIS PRODUCING STRAINS OF *CLOSTRIDIUM SEPTICUM*
8:00-8:10	D239	Gastroenterology	Elane, George (GS - Fogle/Blikslager)	TIME OF COLIC SURGERY IS ASSOCIATED WITH SURVIVAL IN HORSES WITH SURGICAL LARGE INTESTINAL SIMPLE OBSTRUCTION
8:12-8:22	D239	Gastroenterology	McMillan, A.S. (GS - Theriot)	LOSS OF *BACTEROIDES THETAIOTAOMICRON* BILE ACID ALTERING ENZYMES IMPACT BACTERIAL FITNESS AND THE GLOBAL METABOLIC TRANSCRIPTOME
8:24-8:34	D239	Microbiology and Gastrointestinal Biology	Kisthardt, Samantha (GS - Theriot)	STICKLAND AMINO ACID AVAILABILITY ALTERS THE EXPRESSION OF THE BILE ACID INDUCIBLE (*BAI*) OPERON IN COMMENSAL *CLOSTRIDIA*
8:36-8:46	D239	Infectious Disease	Cooper, KC (UG - Theriot)	DETERMINATION OF BILE ACID TOLERANCE OF *CLOSTRIDIUM SPOROGENES* AND *CLOSTRIDIOIDES DIFFICILE*
8:48-8:58	D239	Gastroenterology, Neurosciences.	Mariant, Chloe (GS - Van Landeghem)	CHEMOGENETIC ACTIVATION OF GFAP EXPRESSING ENTERIC GLIAL CELLS PROMOTES DNA INTEGRITY IN CRYPT INTESTINAL EPITHELIAL CELLS AFTER 5-FLUOROURACIL INDUCED INJURY
9:00-9:10	D239	Gastroenterology; Neurosciences	Cook, Caleb (UG - Van Landeghem)	ESTABLISHMENT AND MAINTENANCE OF PRIMARY CULTURES OF COLONIC ENTERIC GLIAL CELLS FROM THE SUBMUCOSAL AND MYENTERIC PLEXI OF NEONATAL AND JUVENILE PIGS
9:12-9:22	D239	Cell Biology	Bacola, Gregory (GS - Van Landeghem)	ENTERIC GLIAL CELL-SECRETED FSTL3 PROMOTES ATM-DEPENDENT CHEMORESISTANCE IN COLON CANCER STEM CELLS
9:24-9:34	D239	Genetics	Connors, Ashley (GS - Yoder)	PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IMPACT MACROPHAGE FUNCTION *IN VITRO*
9:36-9:46	D239	Genetics	Tiedge, Teresa (GS - Meiklejohn)	NEW TOOL IN THE BELT – ENVIRONMENTAL DNA FOR THE FORENSIC ANALYSIS OF SOIL AND DUST
9:48-9:58	D239	Immunology	Diveley, Kayleigh (GS - Peng)	USING HIGH-THROUGHPUT APPROACHES TO IDENTIFY FUNCTIONAL FC GAMMA RECEPTOR SNPS
10:00-10:10	D239	Immunology	Tollison, Tammy (NS - Peng)	DEVELOPMENT OF A SINGLE-CELL-BASED Ig AND TCR FULL REPERTOIRE SEQUENCING ASSAY FOR THE DOMESTIC FERRET
10:12-10:22	D239	Infectious Disease	John, Kristen (GS - Peng)	CHARACTERIZATION OF NOVEL HUMAN LONG NON-CODING RNAS THAT RESPOND TO MAJOR RESPIRATORY VIRAL INFECTIONS AND INTERFERON TREATMENT
10:24-10:34	D239	Immunology	McKinney-Aguirre, Caroline (GS - Gonzalez)	PRE-TRANSPLANTATION STORAGE METHOD IMPACTS IMMUNE CELL POPULATIONS IN INTESTINAL GRAFTS FOLLOWING TRANSPLANTATION
1:00-1:10	South	Anesthesia	Klein, Sarah (HO - Varner)	COMPARSION OF XYLAZINE, LIDOCAINE, AND LIDOCAINE-XYLAZINE FOR DISTAL PARAVERTEBRAL ANESTHESIA IN DAIRY CATTLE
1:12-1:22	South	Behavior	Dunn, Samantha (VS - Gruen)	CAN TAIL WAGGING OF CANDIDATE SERVICE PUPPIES IN RESPONSE TO A NOVEL OBJECT PREDICT SUCCESS OR FAILURE IN THE PROGRAM?
1:24-1:34	South	Clinical Medicine	Chung, Meghan (VS - Lewbart)	USE OF TONOMETRY READINGS AS A DIAGNOSTIC INDICATOR IN ARACHNIDS
1:36-1:46	South	Clinical Medicine	Gareau, Alexandra (HO - Intile)	SERUM SDMA AS A PREDICTOR OF TOXICITY OF CARBOPLATIN IN DOGS
1:48-1:58	South	Neuroscience	Yang, Chin-Chieh (PD - Olby)	VOXELWISE ANALYSIS OF WHITE MATTER IN THE CENTRAL HEARING PATHWAY OF SENIOR DOGS
2:00-2:10	South	Clinical Medicine	Simon, Katherine (GS - Olby)	A RANDOMIZED CONTROLLED CLINICAL TRIAL OF A SENOLYTIC AND NAD+ PRECURSOR SUPPLEMENT COMBINATION IN SENIOR DOGS: OWNER REPORTED COGNITIVE OUTCOMES AT ONE MONTH
2:12-2:22	South	Equine; Neonatology	Coley, Kinnidy (VS - Dembek)	BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME
2:24-2:34	South	Clinical Medicine	Hallowell, Kimberly (HO - Dembek, Horne, Schnabel)	SYSTEMIC ABSORPTION OF TRIAMCINOLONE ACETONIDE FOLLOWING INTRASYNOVIAL AND EXTRASYNOVIAL ARTICULAR INJECTION AND ITS EFFECT ON GLUCOSE, INSULIN, CORTISOL, AND ACTH
2:36-2:46	South	Neurosciences	Nakanishi-Hester, Aoi (GS - Ninomiya-Tsuji)	ABERRANT INFLAMMATORY SIGNALING IMPAIRES NEURONS IN MURINE ALZHEIMER'S DISEASE MODEL
2:48-2:58	South	Neurosciences	Scofield, Kimberly (GS - Marsden)	AN OPTOGENETIC APPROACH TO UNCOVERING MECHANISMS UNDERLYING SENSORY PROCESSING DISORDERS
1:00-1:10	A101	Cell Biology	Huffstetler, Carley (GS - Nascone-Yoder)	*IN VIVO* AND *IN SILICO* LEFT-RIGHT ASYMMETRIES IN EXTRACELLULAR MATRIX SHAPE STOMACH CURVATURE
1:12-1:22	A101	Cell Biology, Gastroenterology	Church, Faith (VS - Stewart)	ISOLATION AND CULTURE OF EQUINE GLANDULAR STOMACH FOR MODEL DEVELOPMENT
1:24-1:34	A101	Cellular Biology	Ludwig, Elsa (PD - Gonzalez)	NORMOTHERMIC MACHINE PERFUSION REDUCES ISCHEMIA REPERFUSION INJURY TO INTESTINAL ALLOGRAFTS
1:36-1:46	A101	Cell Biology; Pain	Velez-Contreras, Raisa (VS - Lascelles/Nolan)	DOES THE INTENSITY OF PERIOPERATIVE ANALGESIA ALTER THE METASTATIC PROPENSITY OF EXTRMITY OSTEOSARCOMA?
1:48-1:58	A101	Cell Biology; Radiation Induced Pain	Ingkasri, Thitsana (GS - Nolan/Mishra/Lascelles)	RADIATION-ASSOCIATED PAIN IN GFRα3 MUTANT MICE
2:00-2:10	A101	Pain	Ahmed, Faihaa (PD - Nolan/Mishra/Lascelles)	THE ROLE OF TRPM8 EXPRESSING NEURONS IN ACUTE OROFACIAL RADIATION ASSOCIATED PAIN
2:12-2:22	A101	Pain	Gupta, Ankita (VS - Mishra/Lascelles)	DIFFERENTIAL CONTRIBUTION OF GFRα3 IN NAÏVE AND OSTEOARTHRITIS-ASSOCIATED PAIN STATES
2:24-2:34	A101	Neurosciences	Tamamoto-Mochizuki, Chie (PD - Mishra)	TRANSCRIPTOMIC PROFILING OF ITCH-SIGNALING PATHWAY IN CUTANEOUS SENSORY GANGLIA IN ATOPIC DOGS
2:36-2:46	A101	Oncology	Horne-Reid, Briana (VS - Nolan)	IN DOGS WITH SINONASAL CARCINOMAS AND SARCOMAS, A LACK OF CYTOLOGICALLY DETECTABLE REGIONAL LYMPH NODE METASTASIS IS PREDICTED BY NORMAL PALPATION AND NORMAL TOMOGRAPHIC APPEARANCE
2:48-2:58	A101	Radiology	Porcel Sanchez, Maria (HO - Lascelles	DETERMINATION OF THE RATE OF PROGRESSION OF DEGENERATIVE JOINT DISEASE IN DOMESTIC CATS

THE ROLE OF TRPM8 EXPRESSING NEURONS IN ACUTE OROFACIAL RADIATION ASSOCIATED PAIN

Faihaa Ahmed: Postdoc

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Radiation Associated Pain (RAP) is a common complication of head and neck irradiation. Biological mechanisms underlying RAP are unknown. Cold-sensing neurons (those which express the TRPM8 ion channel (Transient Receptor Potential Melastatin family member 8) may contribute to RAP. This study sought to determine whether tongue irradiation forces neurons to upregulate TRPM8 in a manner that might cause pain. We use TRPM8-DTR mice; their TRPM8 channels fluoresce (eGFP signal) unless the neuron has been selectively deleted with diphtheria toxin treatment. Their tongues were irradiated (27 Gy single fraction, or sham irradiation). Daily assessments included: glossitis scoring, body weight measurements, behavioral pain phenotyping. Mice were sacrificed on day 11, the time of maximal RAP. Trigeminal ganglia were collected. With severe glossitis, there were indicators of severe pain: weight loss, decreased nest-building and decreased burrowing. Male mice with severe glossitis wiped their eyes more after applying cold saline and had increased TRPM8 expression in trigeminal ganglia neurons (assessed via fluorescent microscopy). We therefore conclude that tongue irradiation caused upregulation of TRPM8 in afferent (trigeminal ganglia) neurons of male mice, and this occurred in conjunction with increased sensitivity to TRPM8 agonists (cold) in the trigeminal pathway (corneal sensitivity). Future experiments are planned to assess whether: (1) this change is restricted to male mice, (2) whether there is true correlation between TRPM8 expression and cold pain, and (3) whether that pain is reduced in mice whose TRPM8 neurons were deleted prior to tongue irradiation.

Funding Source: NIH grant # R37CA248797

Primary subject category for presentation: Pain

ENTERIC GLIAL CELL-SECRETED FSTL3 PROMOTES ATM-DEPENDENT CHEMORESISTANCE IN COLON CANCER STEM CELLS

Gregory Bacola[1] (Postdoc)
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Enteric glial cells (EGC) have recently been shown to impact tumor development. Here, we investigate the impact of EGC on colon cancer stem cell (CSC) chemoresistance. EGC promoted growth of CSC-derived tumors in the presence of 5-FU *in vivo* and *in vitro*. EGC-conditioned medium (CM) reduced 5-FU-induced apoptosis and DNA damage in CSC. Ataxia telangiectasia mutated (ATM) mRNA was significantly enriched in 5-FU-treated CSC grown with EGC *vs.* alone. EGC pro-chemoresistance effects were reduced in CSC derived from cell lines knocked-down for ATM. Inhibition of ATM activity using KU-55933, or upstream MRN activity using mirin abolished EGC-induced CSC resistance to 5-FU. Mass spectrometric analyses identified Follistatin Like 3 (FSTL3) was highly enriched in the CM of 5-FU-treated EGC. Treatment with recombinant FSTL3 protein increased tumor formation, reduced apoptosis, and increased DNA integrity in ATM-expressing CSC in the presence of 5-FU. FSTL3 treatment did not promote tumor formation in CSC knocked-down for ATM, or in CSC treated with KU-55933. EGC knocked-down for FSTL3 expression did not promote CSC tumor formation in the presence of 5-FU. Treatment with FSTL3 neutralizing antibodies inhibited the impact of EGC on CSC chemoresistance.

Altogether our data show that EGC stimulate CSC chemoresistance by promoting activity of the MRN/ATM pathway, driven by release of FSTL3. Future studies will elucidate the signaling cascade by which FSTL3 promotes ATM activity.

Category: Cell Biology

VIABILITY AND DESICCATION-RESISTANCE OF *BARTONELLA HENSELAE* IN BIOLOGICAL AND NON-BIOLOGICAL FLUIDS: EVIDENCE FOR PATHOGEN-ENVIRONMENTAL STABILITY

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Abstract:
Environmental stability is an often-neglected research priority for vector-transmitted pathogens. *Bartonella henselae*, the etiologic agent of Cat Scratch Disease, has become a “pathogen of interest” in several serious human illnesses, which include neoplastic, cardiovascular, neurocognitive, and rheumatologic conditions. Survival in the flea gut and feces as well as the association with a biofilm in culture-negative endocarditis provides insight into this organism’s ability to adjust to environmental extremes. Detection of *B. henselae* DNA in blood and tissues from marine mammals also raises questions about environmental stability and modes of transmission. We investigated the ability of *B. henselae* to survive in fluid matrices chosen to approximate potential environmental sources of infective materials. Feline whole blood, serum and urine, bovine milk and physiologic saline inoculated with *B. henselae* strain San Antonio 2 were subsequently evaluated by culture and qPCR at specified time intervals. Bacterial viability was also assessed following air-desiccation and reconstitution of each inoculated fluid matrix. *B. henselae* SA2 was cultured from feline urine up to 24 hours after inoculation, and from feline blood, serum, cow’s milk, and physiologic saline for up to 7 days after inoculation. Remarkably, bacteria were cultured following air-desiccation of all fluid inoculates. Viability and stability of *Bartonella* within biological and non-biological fluids in the environment may represent a previously unrecognized source of infection for animals and human beings.

Funding Source: This research was supported by the State of North Carolina and donations to the North Carolina State University College of Veterinary Medicine Bartonella and Vector Borne Diseases Research Fund. Janice C. Bush is supported by the GAANN Fellowship for Molecular Biotechnology Training Program (NIH 1T32GM133366).

Primary subject category: Infectious Disease

Presentation preference: Oral

INTRANASAL AD5 INFLUENZA VACCINE ELICITS HEMAGGLUTININ-SPECIFIC ANTIBODY RESPONSE IN PREGNANT AND LACTATING PIGS.

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Influenza A virus can cause severe complications for pregnant women and infants. New vaccines and strategies are being implemented to increase global access to vaccination in these vulnerable populations. Additionally, there are no influenza vaccines approved for infants younger than six months. While inactivated intramuscular (IM) vaccines are currently available for pregnant women, IM immunization may not be an ideal route to boost neutralizing specific antibodies in breastmilk. The aim of the study was to evaluate the capacity of a hemagglutinin (HA) (A/California/2009(H1N1)) Ad5 vector vaccine to induce specific passive immunity in pregnant and lactating pigs using different routes of administration. Pigs were used as a translational model to investigate the protective level of passive maternal antibodies in infants, after mucosal immunization. Influenza naïve pregnant pigs were immunized via oral vaccine tablet or intranasal route three weeks prepartum and boosted four weeks later (one week postpartum). Serum, colostrum, and milk samples, as well as samples from the nasal mucosa and saliva were collected to measure the level of HA-specific antibodies induced by the vaccine over time. Preliminary data showed a significant difference of HA-specific IgG antibody response in serum, colostrum, and milk was induced by vaccination through the intranasal route. However, significance in HA-specific IgA was only found in colostrum and milk. HA-specific IgG and IgA antibodies were only detected in piglets from sows intranasally vaccinated. Future research will evaluate the neutralization capacity of these HA-specific antibodies and determine if mucosal vaccination induces passive protection in a piglet challenge trial.

Funding: Bill & Melinda Gates Foundation

Primary Subject Category: Immunology

USE OF TONOMETRY READINGS AS A DIAGNOSTIC INDICATOR IN ARACHNIDS

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Diagnostics and health evaluation for invertebrates of any species is limited, specifically in the class Arachnida. These limitations are becoming more relevant as more people seek care for their non-traditional pets. Trauma, dysecdysis, hair loss, and prolonged anorexia appear to be the most common medical problems seen in captivity. Recent methods on how to address trauma, amputation of limbs, leaking hemolymph, anesthetization of tarantulas have been published, but healthy diagnostic indicators are relatively new to invertebrate medicine. Dysecdysis is a common reason for owners and keepers to seek out veterinary care, and unless closely monitored in between ecdysis periods, can be difficult to determine where an animal is within their ecdysis cycle. Hydration status is an important variable for an animal to successfully shed. If dysecdysis is caught early, administration of intracardiac fluids has been shown to aid in the process. Keepers and owners have historically assessed hydration status based on body position and ability to move normally, as limb movement is dependent on hemolymph and hydraulics. A possible solution to monitoring an animal throughout their ecdysis cycle is by using tonometry. Rebound tonometry, often used in animals to assess intraocular pressure, was utilized to assess the abdominal pressure of six Curlyhair tarantulas. These pressures were monitored over one year to determine if there is a correlation between pressures and molt cycle. Further research could be done to determine how values compare when a female is gravid, if type of nutrition alters values, and how the pressures differ between species.

Funding: n/a
Category: Clinical Medicine

ISOLATION AND CULTURE OF EQUINE GLANDULAR STOMACH FOR MODEL DEVELOPMENT

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Introduction- Equine Gastric Ulcer Syndrome is classified into Equine Squamous Gastric Disease (EGSD) and Equine Glandular Gastric Disease (EGGD). Diagnosis of EGGD is increasing with a prevalence of up to 65%. Despite this, the exact pathophysiology of EGGD remains unknown and the therapeutics available for ESGD are often less effective. Development of an *in vitro* model of the equine glandular stomach would be invaluable to investigate EGGD mechanisms and test novel therapeutics.
Methods- Glandular stomach was collected from horses without gastrointestinal disease via endoscopic biopsy (n=1) or immediately following euthanasia (n=5). Epithelial cells (glands) were isolated, plated into a 3D matrix, and supplemented with growth factors. Resultant gastric organoids were cultured for a total of 5-10 days. Mature gastric organoids were exposed to control or acidic media (pH ~3.5) for 15 minutes, 3 times daily for 3 days, totaling 9 exposures. Culture media was collected and frozen for analysis of the proinflammatory cytokines TNF-α and IL-8.
Results- Gastric glands were successfully isolated and cultured from both endoscopic biopsies and full thickness tissue samples. Following acid exposures, the gastric organoids subjectively appeared normal, however the surrounding matrigel was visibly disrupted. Preliminary data did not indicate production of TNF-α or IL-8 via ELISA.
Conclusion- Equine glandular stomach can be cultured *in vitro*, however the current model failed to demonstrate production of proinflammatory cytokines following acid exposure. Future investigation is needed to optimize the protocol, including the use of a 2D-monolayer and additional caustic agents (bile salts and/or NSAIDs).

Funding Sources: NC State University Office of the Associate Dean for Research and Graduate Studies, Fund for Discovery
Primary Subjects: Cell Biology, Gastroenterology

BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME

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Neonatal maladjustment syndrome (NMS) is a common disease of neonatal foals resulting in neurological dysfunction and increased mortality. Historically, the central nervous system (CNS) damage was attributed to peripartum hypoxia and ischemia. However, post-mortem evaluation of these foals does not consistently show characteristic histological changes associated with hypoxia. Furthermore, foals with uncomplicated births can show similar behavioral changes which they spontaneously recover. Although several studies have investigated mechanisms involved in neuronal pathology in neonatal foals, the exact pathogenesis of NMS and diagnostic options are not well described. Therefore, the goal of this study is to measure blood biomarkers associated with neuronal damage in foals with NMS, healthy foals, and foals with other disorders to evaluate their association with the clinical signs of NMS and survival. We hypothesize that the blood biomarkers of neuronal damage will be altered in foals with NMS and associated with disease severity and poor outcome. Blood samples were collected from 37 hospitalized foals and 6 healthy foals <7 days old on admission. Additional blood samples were collected on days 1, 2, and 5 of hospitalization. Blood concentration of brain-derived neurotrophic factor (BDNF), glial fibrillary acidic protein (GFAP), neuropeptide Y (NPY), and pigmented epithelium-derived factor (PEDF) were measured with ELISAs. Preliminary analysis revealed that PEDF and GFAP concentrations were lower in hospitalized foals with NMS and sepsis compared to healthy foals. This is clinically relevant since the pathogenesis of NMS is unknown and may aid in its diagnosis and provide novel therapeutic options in the future.

Funding Sources: Boehringer-Ingelheim Veterinary Scholars Program, North Carolina State College of Veterinary Medicine Intramural Seed Grant, and The Morris Animal Foundation

Subject Category: Equine, Neonatology

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IMPACT MACROPHAGE FUNCTION *IN VITRO*

Ashley Connors, Graduate Student

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Presentation Type: Oral talk preferred

Abstract:

Immune function can be impaired by environmental contaminants. One class of chemicals recently shown to interfere with the immune system is per- and polyfluoroalkyl substances (PFAS). Previous studies on the innate immune system indicate that PFAS exposure can influence the numbers of innate immune cells, cellular signaling, and functional endpoints. We are evaluating how macrophages are affected by a 2-day *in vitro* exposure to ten PFASs: perfluorobutanesulfonic acid (PFBS), perfluorohexanesulfonic acid (PFHxS), perfluorooctanesulfonic acid (PFOS), perfluorohexanoic acid (PFHxA), Perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), Nafion Byproduct 2, perfluoro-2-methoxyacetic acid (PFMOAA), and hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). In single-PFAS cytotoxicity studies with human macrophage-like THP-1 cells, exposure to 320 μM PFDA, PFNA, PFOS, and Nafion Byproduct 2 significantly reduced viability. We observed no changes in cell viability at or below exposures to 80 μM PFAS. To test how PFAS modulate phagocytosis, macrophage-like THP-1 cells were exposed to 80 μM PFAS for 48 hours, then challenged with fluorescent heat-killed *E. coli*. Phagocytic index and number are measured with flow cytometry. Thus far, we have observed that PFOS and PFNA increase the average extent of phagocytosis. Additionally, we are measuring cytokine production by both unstimulated and stimulated THP-1 macrophages. Thus far, we have observed that PFOS and PFNA modulate secreted levels of IL-6. Understanding how PFASs affect innate immunity will help us better understand how these chemicals can alter an organism's ability to recognize and destroy pathogens in its environment as well as infected or transformed cells.

Funding source: NIH P42-ES031009
Primary subject category: Immunology

ESTABLISHMENT AND MAINTENANCE OF PRIMARY CULTURES OF COLONIC ENTERIC GLIAL CELLS FROM THE SUBMUCOSAL AND MYENTERIC PLEXI OF NEONATAL AND JUVENILE PIGS

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The enteric nervous system (ENS), comprised of enteric neurons and enteric glial cells (EGC), innervates the gut to regulate essential functions, including motility and mucosal functions. The ENS is organized into two major ganglionated networks: the submucosal plexus (SMP) located in the submucosa and the myenteric plexus situated between the two muscle layers. While neuronal bodies are confined to ganglia, EGC are distributed throughout the entire colonic wall. Disturbances to the EGC network have been linked to various gastrointestinal diseases, emphasizing the importance of studying these cells further. EGC primary cultures represent crucial in vitro tools for deepening our understanding of EGC biology. Here we describe a novel method allowing for the first time robust and reproducible establishment of EGC primary cultures from both inner (referred to as SMP) and outer (aka LMMP) layers of the colonic wall of pigs.
Microdissection was performed on spiral colons from 2-week and 6-week old pigs to isolate SMP and LMMP, followed by enzymatic digestion and mechanical dissociation to obtain single-cell preparations, subsequently grown in conditions favoring EGC growth.
Using $3cm^2$ specimens, an average yield of $1.5\pm0.3x10^6$ million cells for SMP cultures and $0.7\pm0.1x10^6$ million cells for LMMP was obtained, with no significant differences between age groups. Immunofluorescence analyses using EGC markers GFAP and S-100β revealed a purity of 92.6 ± 1.47% in our cultures, with no notable differences between plexi or age groups.
Our new culture system represents a valuable platform to elucidate EGC role in animal and human gut health and diseases.

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Category: Gastroenterology; Neurosciences.

Determination Of Bile Acid Tolerance Of *Clostridium sporogenes* and *Clostridioides difficile*

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Funding Sources –
OUR Summer 2023 Research Award
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Subject Category – Infectious Diseases

Clostridioides difficile infection (CDI) is the leading cause of nosocomial infection in the United States, impacting nearly half a million people each year. Antibiotics are a major risk factor for CDI as they alter the gut microbiota and decrease colonization resistance to this pathogen. It is hypothesized that commensal *Clostridia* are able to prevent *C. difficile* growth due to their ability to make inhibitory secondary bile acids from primary bile acids. The bile acid-inducible (*bai*) operon is necessary to carry out 7α-dehydroxylation of deconjugated primary bile acids – cholic acid (CA) and chenodeoxycholic acid (CDCA) thereby converting them into the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. A recent study was able to knock in the *bai* operon into *C. sporogenes* or MF001. To determine the relationship between *C. sporogenes* wildtype (WT), MF001, and *C. difficile,* we will measure the minimum inhibitory concentrations (MICs) and growth kinetics of these strains in the presence of bile acids CA, CDCA, DCA, LCA at different concentrations. *C. sporogenes* WT and MF001 have similar MICs in CA (>10 mM), CDCA (0.96 mM), and DCA (1.25 mM). Whereas, *C. difficile* has different MICs in CA (~5 mM), CDCA (~0.625 mM), and DCA (~1.25 mM). These MICs will inform future experiments that will determine how *C. difficile* growth is affected by secondary bile acids made by MF001. These findings will help further our understanding of how commensal gut bacteria are able to inhibit *C. difficile*.

IMMUNE RESPONSES AND IMMUNOPATHOLOGY IN TURKEYS EXPERIMENTALLY INFECTED WITH CLOSTRIDIAL DERMATITIS PRODUCING STRAINS OF *CLOSTRIDIUM SEPTICUM*

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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.

Funding source: US POULTRY and EGG ASSOCIATION

Primary subject category for presentation: Immunology

Title: MODELING OF HEALTHCARE-ASSOCIATED *CLOSTRIDIOIDES DIFFICILE* INFECTION AND QUANTIFICATION OF ACQUISITION ROUTES IN ONCOLOGICAL UNITS

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Abstract: *Clostridioides difficile* infection (CDI) is one of the most common healthcare-acquired infections worldwide. Oncological patients have the highest incidence of CDI because they are severely immunocompromised, have near ubiquitous antimicrobial exposures, and are hospitalized for prolonged periods of time. In this population, there are four routes to CDI: importation of- and transmission from- asymptomatic carriers, and importation of- and transmission from- symptomatic patients. However, the extent to which asymptomatic carriers contribute to the overall CDI burden is unknown. To estimate this, we defined a CDI network model that explicitly measures asymptomatic carriers in 48 beds across two oncological wards. Exact admission/discharge dates and patient room assignments were taken directly from data, and healthcare worker (HWC) staffing data was used to incorporate HCW movement. The data we used tested patients regardless of their CDI status which allowed for the identification of asymptomatic shedders. Thus, we were able to simultaneously fit counts of weekly positive tests for both asymptomatic and symptomatic patients to obtain posterior estimates for remaining model parameters. We used the parameterized model to run simulations under various conditions and calculated the average number of new cases arising from each pathway. Our preliminary findings lead us to conclude that at least half, and potentially up to 90%, of all hospital-acquired CDI cases occur due to asymptomatic carriers. This study is a first opportunity to measure and model the asymptomatic population directly, and the developed model will serve as a foundation for more investigations to reduce the burden of this disease.

Funding Source: CDC BAA 200-2018-02926, CDC U01CK000587, NSF DGE-2137100

Primary Subject Area: Infectious Disease

USING HIGH-THROUGHPUT APPROACHES TO IDENTIFY FUNCTIONAL FC GAMMA RECEPTOR SNPS.

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Analysis of the HIV-1 vaccine trials has associated multiple genetic variations in the Fcγ Receptor (FcγR) region with disease susceptibility and vaccine protection. However, the genetic complexity exhibited within the human FcγR region presents a challenge for post-GWAS and translational functional analyses. Therefore, the identity and regulatory role of functional Single Nucleotide Polymorphisms (fSNPs) in the FcγR region have been limited to date.

Using the high-throughput Regulatory Element (REEL)-Seq screen, we have systematically identified 549 candidate functional FcγR SNPs in human monocytes. Candidate FcγR fSNPs with HIV-1 relevance were individually evaluated for allele-specific protein binding, indicative to functionality in the nucleus. The alleles at candidate fSNPs were also observed to cause alterations to transcriptional activity of an in vitro reporter protein. These results lend support to the hypothesis that these non-coding variations act as transcriptional regulators of FcγRs. To evaluate this hypothesis further, we are utilizing the CRISPR and CRISPRi systems to edit the identified SNPs and perturb their interacting proteins to examine the effects upon FcγRs at the cell level. As FcγR receptor abundance may influence monocyte-based antibody-dependent cellular phagocytosis (ADCP) activity, understanding the regulatory impact of these fSNPs will inform the development of antibody-based therapy and prevention.

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Primary Subject Category: Genetics

CAN TAIL WAGGING OF CANDIDATE SERVICE PUPPIES IN RESPONSE TO A NOVEL OBJECT PREDICT SUCCESS OR FAILURE IN THE PROGRAM?

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Funding Source: National Institutes of Health and NC State University Office of the Associate Dean for Research and Graduate Studies

Behavior

Currently, there is a 50% success rate for candidate service dog puppies. As such, there is a loss of time and money when dogs fail as adolescents. One potential refinement in the selection of dogs for service work may be their emotional arousal in response to a novel object. The aim of this study was to evaluate tail wag as a predictive measure of emotional arousal. Every two weeks, puppies were presented with an unfamiliar mechanical, motion-activated toy animal for a 90-second trial. Their response was coded from video for frequency of tail wag, vocalizations, and a subjective score ranging from 0-5 where the two ends of the scale indicate high positive and negative arousal, respectively. A total of 332 videos from 79 puppies ranging from 8-20 weeks of age were analyzed. We found a significant relationship between tail wag frequency and subjective score, with subjective score explaining 29.9% of variability in tail wag even when controlling for puppy age. Puppies with a subjective score of 0, 1, or 5 wagged significantly more than puppies with scores of 2, 3, or 4 ($F = 26.6$; $p = 0.0001$). This indicates that tail wag is significantly associated with high emotional arousal. These findings will be further evaluated for their predictive value in success or failure in training.

TIME OF COLIC SURGERY IS ASSOCIATED WITH SURVIVAL IN HORSES WITH SURGICAL LARGE INTESTINAL SIMPLE OBSTRUCTION

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Abstract:

Surgical outcomes after business hours in people show increased morbidity and mortality. Our objective was to compare morbidity and mortality for emergency colic surgery performed during daytime and after-hours. We hypothesized that horses receiving colic surgery after-hours would be less likely to survive to discharge and predisposed to postoperative complications. Medical records of horses presenting for colic were reviewed. Horses were excluded if they did not receive or had previous colic surgery, received surgery more than six hours after admission, or were euthanized intra-operatively. Lesions were categorized as small intestinal or large intestinal non-strangulating or strangulating obstruction (four categories). Surgical lesions, starting time of surgery (daytime [7am-7pm] or after-hours [7pm-7am]), and complications were recorded. Case-matching was performed with each daytime case paired with a similar lesion operated on after-hours (within 36 months of each other). Association was determined using chi-squared tests. Large intestinal non-strangulating obstructions receiving colic surgery after-hours were found to be at increased risk for non-survival ($p = 0.03$) and postoperative colic ($p = 0.01$). We believe this may be due to a perceived lack of urgency for non-strangulating cases compared to strangulating cases, and an inclination for clinicians allow the lesion to resolve with medical management, resulting in damage to the large colon, which complicates recovery and may result in postoperative colic complications seen here. Limitations include the retrospective nature and small number of complications between lesion categories. Large intestinal non-strangulating obstructions are predisposed toward non-survival and postoperative colic if operated on after-hours.

Funding: No funding sources were required for this project.

Primary subject category: Gastroenterology

HORIZONTAL GENE TRANSFER IN HEALTHCARE SETTINGS

Alba Frias-De-Diego, Postdoctoral Research Scholar

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Antimicrobial resistance (AMR) poses a significant public health challenge, particularly in healthcare settings where patients are highly susceptible to infections caused by drug-resistant bacteria. To effectively combat this issue, it is crucial to understand the molecular mechanisms and epidemiological factors driving the dissemination of AMR genes among bacterial populations. This can be achieved by using tools such as mathematical models, which play a pivotal role in bacterial epidemiology, specifically in healthcare settings by enabling the analysis and prediction of AMR dynamics. Horizontal gene transfer (HGT) plays a critical role in the emergence and spread of multidrug-resistant bacteria, particularly due to the impact of mobile genetic elements (MGE) like plasmids. In this study, we conducted a systematic review spanning the period between 2013 and 2022, focusing on AMR within healthcare. Using predefined search criteria, we identified 23 studies that specifically investigated horizontal plasmid transfer in healthcare facilities. Over time, Illumina sequencing and polymerase chain reaction (PCR) emerged as the dominant platforms and detection methods, respectively, while whole genome sequencing gained prominence for genetic analysis. *Klebsiella spp*. and *E. coli* consistently emerged as primary pathogens in both the literature and the final subset of studies. However, we found that investigations on HGT in hospital settings were predominantly descriptive, highlighting the need for more comprehensive studies. A major challenge in developing accurate models to study AMR is the scarcity of detailed metadata associated with the sequences. This limitation hampers the application of data for mathematical modeling purposes. Nevertheless, mathematical modeling remains a powerful tool in the field of antimicrobial resistance, providing valuable insights into AMR transmission dynamics and the impact of interventions within healthcare settings. Despite the data constraints, it is crucial to promote data comparability and foster collaboration among researchers to collectively address the global threat posed by AMR. Enhancing our understanding of the molecular mechanisms and epidemiological factors driving plasmid-mediated HGT is essential for the development of targeted interventions against AMR.

• Primary subject category for presentation: Infectious Disease, Other (Epidemiology)

EFFECTS OF *LACTOBACILLUS* ISOLATES ON PREVENTION OF NECROTIC ENTERITIS IN BROILER CHICKENS

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Abstract:

Necrotic enteritis (NE) in chickens is an economically important disease caused by virulent *Clostridium perfringens* bacteria. In the current era of 'no-antibiotics-ever' farming, NE incidences are on the rise, and *Lactobacillus*-based probiotics seem to offer a promising non-antibiotic alternative. In the current study, we evaluated the ability of four *Lactobacillus* isolates of chicken origin, namely, *L. crispatus* (Str. C25), *L. animalis* (Str. P38), *L. acidophilus* (Str. P42), and *L. reuteri* (Str. P43), in preventing NE. Broiler chickens were orally administered with each of the lactobacilli on 1, 8, and 15 days of age followed by an experimental *C. perfringens* challenge. The results showed that treatment with P38 and P43 resulted in a significant reduction of NE severity when compared to the untreated control, as determined by intestinal gross pathology. Additionally, P38 and/or P43 treatment showed a significant reduction in the expression of pro-inflammatory cytokine (IL-1β and IFNγ) genes in the duodenum and jejunum tissues compared to the untreated control. Furthermore, the transcription of IL-13 in the duodenum, IL-4 in the bursa of Fabricius, and IL-10 and TGFβ in the cecal tonsils were found significantly elevated in these birds, suggesting anti-inflammatory effects of P38 and P43 strains. Collectively, our findings suggested that certain lactobacilli can prevent NE in chickens, and that the probiotic mechanisms seem to involve immunomodulation of intestinal inflammation. Further studies are currently underway to develop probiotic *Lactobacillus*-based recombinant vaccines against NE in chickens.

Funding Source: US POULTRY & EGG FOUNDATION and PI Start-up funds

Subject Category: Infectious diseases

SERUM SDMA AS A PREDICTOR OF TOXICITY OF CARBOPLATIN IN DOGS

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Glomerular filtration rate (GFR) predicts carboplatin clearance and myelotoxicity in humans and cats. This relationship is unknown in dogs. Elevated serum SDMA correlates with reduced GFR in dogs. This study aimed to determine whether dogs with increased SDMA were more likely to experience adverse hematological and gastrointestinal toxicity from carboplatin chemotherapy.

This was a prospective observational study of 30 dogs with confirmed neoplasia. Dogs weighing >15 kg received carboplatin intravenously at 300 mg/m^2. SDMA was measured on the day of treatment. Plasma clearance pharmacokinetics (PK) of carboplatin was measured using high-performance liquid chromatography. Adverse effects were monitored with weekly CBCs and owner assessment forms.

Five dogs had increased SDMA (>14 µg/dL). Four of those dogs had significantly decreased carboplatin clearance (mL/hr/kg) compared with dogs with normal SDMA (mean 93.9, range 76.1-116.8 vs. mean 185.4, range 114.3-268.3, p = 0.0001). One dog had an elevated serum creatinine (1.7 mg/dL, reference range 0.5-1.4 mg/dL). Three had grade 4 hematological toxicity; one was euthanized due to febrile neutropenia. The remaining two dogs were euthanized at days 5 and 13 post treatment due to adverse gastrointestinal signs. Of the 25 dogs with SDMA ≤14 µg/dL, 10 had grade 3 or 4 hematological toxicity; none were euthanized or died due to treatment.

Increased SDMA is associated with decreased carboplatin clearance in dogs. SDMA may predict the risk of carboplatin-associated toxicity when dosing based on body surface area.

Funding provided by the Veterinary Cancer Society-Dr. Gordon Theilen Resident Research Grant.

DIFFERENTIAL CONTRIBUTION OF GFRα3 IN NAÏVE AND OSTEOARTHRITIS-ASSOCIATED PAIN STATES

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Osteoarthritis (OA) is a leading cause of disability, with ~100 million US adults suffering from chronic pain and widespread sensitization. Clinically efficacious and safe analgesics for OA-pain are limited partly due to a lack of understanding of neural pain mechanisms. We have linked synovial fluid concentrations of a neurotrophic factor, artemin, to naturally occurring joint pain in dogs and identified upregulation of GDNF family receptor alpha 3 (GFRα3, artemin's receptor) expression in dog sensory neurons in association with OA-pain. Sequestering artemin reversed pain and hypersensitivity in an induced mouse model of OA-pain. Using *Gfrα3*$^{-/-}$ (mutant; nonfunctional GFRα3) and wildtype mice, this study explored whether artemin mediates sensitivity via GFRα3 in naïve states and the role of GFRα3 in OA-pain and hypersensitivity in two induced mouse models of stifle OA-pain. In naïve *Gfrα3*$^{-/-}$ (mutant; nonfunctional GFRα3) and wildtype mice, we evaluated artemin-induced thermal and mechanical hypersensitivity up to 24 hours post-injection. In *Gfrα3*$^{-/-}$ mice, we also evaluated changes in hypersensitivity and limb asymmetry in the chemical monoiodoacetate (MIA at week 6) and surgical destabilization of the medial meniscus (DMM at week 16) mouse models of OA-pain. In the naïve state, artemin-induced hypersensitivity is dependent on GFRα3. *Gfrα3*$^{-/-}$ mice are protected from MIA-induced cold hypersensitivity and limb asymmetry and DMM-induced mechanical hypersensitivity and limb asymmetry. GFRα3 differentially mediates hypersensitivity across naïve and pain states but appears to mediate joint pain as measured by limb use. GFRα3 may be a viable target to mitigate OA-pain.

Research Funding and Student Support: NIH R01AR079713

Category: Pain

SYSTEMIC ABSORPTION OF TRIAMCINOLONE ACETONIDE FOLLOWING INTRASYNOVIAL AND EXTRASYNOVIAL ARTICULAR INJECTION AND ITS EFFECT ON GLUCOSE, INSULIN, CORTISOL, AND ACTH

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Laminitis remains a major concern with use of corticosteroids in horses. Individual case factors influencing steroid-induced laminitis risk have not been investigated. This study aimed to determine if systemic absorption of triamcinolone acetonide (TA) varies between intrasynovial (antebrachiocarpal) and extrasynovial (sacroiliac) injection sites, and to determine the metabolic effects of TA absorption from those sites. We hypothesized that there would be increased absorption from the extrasynovial site, and that TA administration would increase insulin and glucose and decrease cortisol and adrenocorticotropic hormone (ACTH) levels.

12 adult horses were randomized into intrasynovial or extrasynovial injection groups. Each horse received bilateral injections with a total dose of 18 mg triamcinolone. Blood was collected prior to and for 72 hours following injection and TA, insulin, glucose, cortisol, and ACTH levels were measured at each time point. Data were analyzed using 2-way repeated measures ANOVA.

Contrary to our hypothesis, plasma TA levels were significantly higher in the intrasynovial group from 8-36 hours post-injection. In both groups, insulin levels significantly increased 10-72 hours post-injection, in many cases with sustained insulin values well above 100 µIU/mL. Glucose values in both groups significantly increased from baseline, while cortisol and ACTH significantly decreased.

This study is the first to demonstrate that extrasynovial injection sites have different drug absorption properties than intrasynovial injection sites. The decrease in cortisol and ACTH suggests suppression of the hypothalamic-pituitary-adrenal axis. The marked hyperinsulinemia present in both groups post-injection warrants further investigation into insulin dysregulation as a major risk factor for steroid-induced laminitis.

Subject category: Clinical medicine

Funding sources: NCSU Department of Clinical Sciences VPP Grant and the AAEP Innovation & Discovery Research Grant

IN DOGS WITH SINONASAL CARCINOMAS AND SARCOMAS, A LACK OF CYTOLOGICALLY DETECTABLE REGIONAL LYMPH NODE METASTASIS IS PREDICTED BY NORMAL PALPATION AND NORMAL TOMOGRAPHIC APPEARANCE

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Abstract: In canine sinonasal cancer, prognosis and treatment approach can be impacted by lymph node (LN) metastasis. Hence, despite uncertain cost effectiveness, staging plans often include aspiration and cytology of the regional LNs. This retrospective study evaluates how well physical examination and computed tomography (CT) perform as surrogates for LN cytology in dogs with sinonasal carcinomas or sarcomas. Dogs were included if they underwent radiotherapy between 2013 and 2023, after staging with physical examination, CT, and cytology of at least one regional LN. Physical exam parameters and tomographic appearance of the LN were compared with cytologic descriptions to enable calculation of sensitivity and specificity, plus positive and negative predictive values (PPV and NPV). A total of 150 dogs were included (108 with carcinoma, and 42 with sarcoma). Fourteen dogs had cytologically confirmed LN metastasis; all 14 had carcinomas. Metastases were detected ipsilaterally in 12 dogs (10 mandibular, 1 medial retropharyngeal [MRP], 1 mandibular and MRP); 1 dog had bilateral mandibular LN metastases, and 1 had bilateral mandibular and MRP LN metastases. The sensitivity, specificity, PPV and NPV of LN palpation alone were 71.4%, 64%, 17%, and 96%, respectively; for CT alone, these were 100%, 46.3%, 16.1%, and 100%; and for the combination of palpation and CT, these were 100%, 59.5%, 23.8%, and 100%. Based on these results, detection of a palpably and tomographically normal LN in a dog with sinonasal carcinoma or sarcoma appears sufficient to confidently rule out cytologically detectable metastasis, thus obviating the need for LN aspiration and cytology.

Funding: N/A

Primary Subject Category: Oncology

IN VIVO AND *IN SILICO* LEFT-RIGHT ASYMMETRIES IN EXTRACELLULAR MATRIX SHAPE STOMACH CURVATURE.

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Proper embryonic left-right (L-R) asymmetry sculpts many organs; thus, understanding the morphogenetic mechanisms behind simple L-R asymmetries, like the leftward curvature of the stomach, can provide etiological insights into laterality-related birth defects. We previously established that conserved L-R patterning cues promote curvature via cell rearrangements in the left stomach endoderm; however, the potential influence of the mesoderm tissue layer and the extracellular matrix (ECM) on stomach curvature morphogenesis is unknown.

To examine the role of ECM in stomach curvature, we conducted immunohistochemical analyses on *Xenopus* stomach sections at stages before, during, and after curvature. In the left (convex) stomach, fibronectin (FN) fibrils become aligned and localized within a compact basement membrane between the endoderm and mesoderm. However, on the right side, FN is broadly distributed and disorganized, and forms a concavity with an irregular endoderm-mesoderm border. Importantly, these FN asymmetries are reversed or bilateral in embryos with experimentally-induced stomach curvature defects, indicating that LR asymmetric ECM distribution patterns locally influence organ topology.

We hypothesized that L-R differences in FN generate distinct L-R tissue topologies by modulating interfacial tension between endoderm and mesoderm on the contralateral sides of the stomach. To directly test this, we developed a unique, multi-layer, multi-scale computational model of stomach curvature. *In silico* simulations of L-R asymmetries in surface tension mimicked *in vivo* morphologies. Overall, our results indicate that the morphogenetic events shaping stomach curvature involve complex, interdependent L-R asymmetries in multiple 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

RADIATION-ASSOCIATED PAIN IN GFRα3 MUTANT MICE

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Abstract: Successful use of radiotherapy for head and neck cancer often causes painful side effects. We hypothesize that acute orofacial radiation-associated pain (RAP) is mediated by neurons that express TRPM8, and that those neurons are activated by interaction between artemin, and its receptor GFRα3. To better define the role of GFRα3, GFRα3 mutant and wild-type (WT; C57BL6/NJ) mice underwent high-dose tongue-irradiation (21 Gy, single fraction) or sham treatment (0 Gy). Body wight, glossitis severity, and grooming were assessed daily; behavioral pain assays were done before and 10-11 days post-irradiation. Mice were then euthanized, and trigeminal ganglia (TG) were collected for downstream analysis. Radiation caused glossitis of similar severity in both strains, but the GFRa3 mutant mice had evidence of more pain: more weight loss (23.29% vs. 12.59%, $p<0.0001$), worse grooming and nesting scores ($p<0.0001$, and $p=0.0080$, respectively) and greater ocular sensitivity to topically applied cold saline solution ($p=0.0020$). As assessed with calcium imaging, afferent (TG) neurons from irradiated GFRα3 mutant mice were more responsive to TRPM8 agonists (icilin and menthol) than neurons from WT mice. Interestingly, neuronal expression of TRPM8 protein was higher in irradiated WT mice (vs. GFRα3 mutant), whereas mRNA was numerically (albeit non-significantly) higher in the mutant mice. These data suggest that for a given level of tissue injury, loss of *GFRα3* leads to more pain, and more *in vivo* and *ex vivo* sensitivity to TRM8 agonists (cold, icilin, menthol). Future experiments are planned to verify these results and clarify the differences between protein and gene expression of TRPM8.

Funding source: National Institutes of Health (NIH)

Subject categories: Cell Biology, Radiation induced pain.

Pangenome-GWAS analysis of *Clostridioides difficile*: Identifying selection pressures in healthcare and beyond

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Clostridioides difficile (*C. difficile*) is an anaerobic, spore-forming gastrointestinal pathogen. It is a leading cause of healthcare-associated infections worldwide. *C. difficile* possesses an extensive repertoire of mechanisms for conferring resistance. As a consequence, it demonstrates a remarkable capacity to acquire antimicrobial resistance (AMR) and rapidly adapt to novel environments. These environments extend beyond healthcare settings and encompass non-healthcare or community settings, where diverse utilization of antimicrobials engenders distinct selection pressures. This study aimed to investigate the presence of potential selection pressures associated with healthcare and non-healthcare settings and its impact on the prevalence of AMR and virulence gene repertoires of *C.difficile* worldwide. By applying pangenome genome-wide association studies (pangenome-GWAS) on 7,785 whole genome sequences (WGS) of *C. difficile,* we identified genes significantly associated with healthcare and non-healthcare settings, indicating the presence of differential selection pressures. In healthcare settings, macrolide resistance genes, including *erm(B)*, were consistently detected across all sequence types (STs). Furthermore, a higher number of virulence genes, such as *tcdR* and cdtR, were strongly associated with healthcare settings, indicating the influence of constant exposure to drugs and other selective pressures. On the other hand, tetracycline resistance gene *tet(M)* and *sat4* gene (aminoglycosides) were consistently prevalent in non-healthcare settings, which are widely used in agriculture and veterinary medicine. This study underscores the significance of considering both healthcare and non-healthcare settings in understanding the transmission and evolution of AMR and virulence in *C. difficile*. The identification of specific resistance and virulence markers associated with each setting provides valuable insights for surveillance and control strategies, resulting in a broader implications for AMR stewardship.

• Primary subject category for presentation: Infectious Disease, Other (Epidemiology)

CHARACTERIZATION OF NOVEL HUMAN LONG NON-CODING RNAS THAT RESPOND TO MAJOR RESPIRATORY VIRAL INFECTIONS AND INTERFERON TREATMENT

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Long non-coding RNAs (lncRNAs) are a newer class of non-coding transcripts identified as key regulators of biological processes, including viral infection. We aimed to identify novel lncRNA targets that may play critical roles in major human respiratory viral infections by systematically mining large scale transcriptomic datasets. Using bulk RNA-seq analysis, we identified multiple lncRNAs that were consistently upregulated after influenza infection across multiple human epithelial cell types and influenza A strains. We experimentally confirmed the response of these lncRNAs to influenza infection and interferon-beta (IFN-β) treatment in human epithelial cells and found their expression was robustly induced by IFN-β treatment in a time and dose-specific manner. In addition, we found that they responded to IFN-β treatment in immune cell types including human T cell and monocyte cell lines. Together, our results suggest that these lncRNAs are novel interferon stimulated genes that may play broad roles in the host immune response to viral infection. We propose to characterize the function of these lncRNAs with the goal of better understanding host-pathogen interactions and identifying potential targets for viral intervention.

Funding Sources:

National Institutes of Health (Grant R21AI147187) and North Carolina State University College of Veterinary Medicine, Raleigh, NC

Primary Subject Category: Infectious Disease

STICKLAND AMINO ACID AVAILABILITY ALTERS THE EXPRESSION OF THE BILE ACID INDUCIBLE (*BAI*) OPERON IN COMMENSAL *CLOSTRIDIA*

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Clostridium scindens is a commensal that metabolizes primary bile acid cholate (CA) into secondary bile acid deoxycholate (DCA), which are inhibitory to *Clostridioides difficile*. Recent work showed that the *C. scindens bai* operon decreased expression in the presence of the proline precursor hydroxyproline. Proline and glycine are important for Stickland fermentation in both commensal *Clostridia* and the pathogen *C. difficile*. The consumption of these amino acids by *C. scindens* provides competition, further contributing to the inhibition of *C. difficile* by an intact gut microbiota. We hypothesize that the availability of amino acids used for Stickland fermentation alters the expression of the *bai* operon in commensal *Clostridia,* ultimately controlling secondary bile acid metabolism. To test this, we grew *C. scindens* in excess proline or glycine, in the presence and absence of CA or DCA. At mid-log growth, supernatant was collected for RNAseq and metabolomic analysis. Supplementation of CA significantly increased expression of the *bai* operon in *C. scindens*. Supplementing proline and CA maintained the same magnitude of expression, while the addition of glycine significantly decreased expression. Proline and CA altered the global transcriptome with 270 genes being differentially expressed, compared to only 50 genes with glycine. Genes important for carbohydrate metabolism, proline usage (*prd*), and cellular energetics in the form of molybdenum transfer and biosynthesis increased, while genes involved in the synthesis of proline and arginine metabolism (*pro* and *arg*) decreased. These findings suggest an avenue to modulate secondary bile acid production in commensal *Clostridia* with amino acid supplementation via diet.

Funding Sources: NIH R35 GM149222, NIH T32 GM133366, NCSU GGA Genetics and Genomics Scholars

Subject Category: Other (Microbiology and Gastrointestinal Biology)

COMPARSION OF XYLAZINE, LIDOCAINE, AND LIDOCAINE-XYLAZINE FOR DISTAL PARAVERTEBRAL ANESTHESIA IN DAIRY CATTLE

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Funded source: USDA NIFA Animal Health Formula
Primary subject category for presentation: Anesthesia
Abstract:
We assessed duration of action of distal paravertebral blocks (DPB) at L1, L2, and L4 in adult Holstein dairy cows using xylazine (X), lidocaine (L), and L+X (LX). Six cows received 3 treatments (X, L, LX) in a blinded random cross-over design. X did not cause anesthesia and data was excluded. Due to treatment failure, four additional cows were enrolled and received L and LX treatments. L (1800 mg; 90 ml) was administered in L and LX treatments. In X and LX treatments, 0.025 mg kg^{-1} of X was given in in 90 mL of 0.9% NaCl. 30 mL of test solution was injected per site for each treatment. Anesthesia was assessed by response to needle prick with a 22 g needle every minute for 15 minutes and then every 15 minutes. Anesthesia onset and duration, and heart rate (HR) were recorded. Data was used only from cows with successful blocks with both L and LX (N = 5) and analyzed with a paired t-test or a Wilcoxon signed-rank test. A Fisher's Exact Test compared success rate between treatments. A p-value < 0.05 was considered significant. Duration of anesthesia was 251.6 +/- 96.94 and 105.8 minutes +/- 80.3 minutes in the LX and L treatments, respectively. Average HR was 55.9 +/- 2.6 and 58.9 +/- 2.9 bpm in the LX, and X groups, respectively. Success rate between L and LX treatments did not differ and was 75% overall. DPB with LX produced longer duration anesthesia and lower HR than L.

NORMOTHERMIC MACHINE PERFUSION REDUCES ISCHEMIA REPERFUSION INJURY TO INTESTINAL ALLOGRAFTS

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Intestinal transplantation (IT) is the final treatment option for intestinal failure. Intestinal allografts are traditionally preserved using cold storage (CS). However, CS and subsequent transplantation induce ischemia-reperfusion injury (IRI). Severe IRI impairs epithelial barrier function including loss of intestinal stem cells (ISC), critical to epithelial regeneration. Normothermic machine perfusion (NMP) preservation has been shown to minimize CS-associated IRI in other organs but has not been described for intestine. We hypothesized that, compared to CS, intestinal NMP will reduce IRI and better protect ISC regenerative potential and viability. Twenty-four porcine intestines were stored for 6hr at 4°C (CS-T6) or perfused with 34°C perfusate (NMP-T6) and transplanted (n=18). Recipient pigs were recovered from anesthesia. Jejunal and ileal segments were collected immediately after procurement (T0), after storage, and 1hr post reperfusion. Histologic injury and cellular apoptosis was assessed. ISC viability and proliferation were quantified using crypt culture as well as gene and protein biomarker expression. Histologically, NMP-T6 tissue had mild villus tip epithelial erosion, although increased apoptotic cells were counted in CS-T6 compared to T0. In NMP-T6 tissues, elevated ISC gene and protein biomarker expression and increased spheroid areas and proliferating cell numbers were measured compared to T0 and CS-T6. Post-reperfusion, increased histologic injury and apoptotic cells were identified in CS grafts compared to uninjured T0 and NMP. Finally, post-transplant survival was markedly better for recipients of NMP intestine. Compared to CS, NMP appears to reduce IRI and improve graft regeneration, resulting in transplantation of healthier bowel and superior recipient survival.

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

Subject category: Cellular Biology

FIRST DETECTION OF ZOONOTIC *BARTONELLA ALSATICA* IN FLEAS FROM EASTERN COTTONTAIL RABBITS (*SYLVILGUS FLORIDANUS)*

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Subject Category: Infectious Disease

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Few studies have investigated the *Bartonella* and *Rickettsia* spp. vectored by fleas infesting wildlife. Case studies have documented *Bartonella alsatica* infection in humans with endocarditis, granulomatous lymphadenitis, and an aortic prosthesis infection. This *Bartonella* species is typically associated with rabbit and rabbit flea species and has been detected in Europe and the Western US. The presence and prevalence of *Bartonella alsatica* in Eastern cottontail rabbits (*Sylvilagus floridanus*) and their fleas (*Cediopsylla simplex)* in the midwestern US is unknown. To investigate the presence of *Bartonella* and *Rickettsia* species in small wildlife fleas, we collected fleas from *S. floridanus* and Virginia opossums (*Didelphis virginiana*) in southern Wisconsin. Fleas were speciated and pooled by host for qPCR to detect *Bartonella* and *Rickettsia.* Analysis compared the flea species, host species, pathogen detection, and geographic location. *Cediopsylla simplex* (n = 114, n = 24 pools) were collected from 20 rabbits. *Ctenocephalides felis* (n = 1, n = 1 pool) and *Nosopsyllus fasciatus* (n = 232, n = 15 pools) were collected from 7 opossums. *Bartonella alsatica* DNA was amplified from 50% (12/24) of *C. simplex* pools. *Rickettsia felis* DNA was amplified from a single *N. fasciatus* pool (1/15, 7%). This study is the first to report *B. alsatica* from *C. simplex* fleas collected from *S. floridanus*, the most common rabbit species in North America. Further research is necessary to assess pathogen and flea epidemiology across the United States, the range of disease manifestations in animals and human patients, and pathogen phylogenetic diversity in North America.

Funding: This research was supported by the State of North Carolina and donations to the North Carolina State University College of Veterinary Medicine Bartonella Vector Borne Diseases Research Fund. Charlotte O. Moore is supported by NIH 1T32GM133366.

CHEMOGENETIC ACTIVATION OF GFAP EXPRESSING ENTERIC GLIAL CELLS PROMOTES DNA INTEGRITY IN CRYPT INTESTINAL EPITHELIAL CELLS AFTER 5-FLUOROURACIL INDUCED INJURY

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Maintaining the integrity of the intestinal epithelium is critical to preserve digestive functions while preventing harmful agents from penetrating the body. Fortunately, the intestinal epithelium possesses one of the highest regenerative capabilities of all tissues in the body both in homeostatic conditions and after injury. Extrinsic signals, including those emanating from surrounding cells such as enteric glial cells (EGC), are potent regulators of intestinal epithelial cell functions. However, whether and how EGC impact epithelial response to a genotoxic stress remains to be defined. To investigate this, we used the chemogenetic GFAP-hM3Dq mouse model, in which GFAP-expressing EGC are activated upon Clozapine-N-oxide (CNO) administration. To study the impact of EGC stimulation on epithelial response to genotoxic stress, mice received CNO 24h prior to and after a single dose of 150mg/kg of the chemotherapeutic drug 5-fluorouracil (5-FU). Small intestines were collected at different time points post-5-FU, and the impact of EGC activation was assessed on epithelial DNA damage, apoptosis and regeneration. In chemotherapy-treated mice, EGC activation expanded the pool of intestinal epithelial cells in S-phase at 24 and 72 hours after chemotherapy administration. Consistent with this, morphometric analyses revealed that EGC activation increased villus and crypt density, as well as crypt depth at 72h post-chemotherapy treatment. Finally, chemoactivation of GFAP+ EGC decreased DNA damage and apoptosis in intestinal epithelial cells 12h after 5-FU administration. Altogether, this preliminary study indicates that activation of GFAP+ EGC promotes DNA integrity and regeneration in the small intestinal epithelium after a genotoxic stress.

Fundings: NIH 1R01CA270462-01, UNC Lineberger Comprehensive Cancer Center Developmental grant; UNC CGIBD NIH NIDDK P30 DK034987 Pilot/Feasibility grant.

Subject category: Gastroenterology, Neurosciences.

PRE-TRANSPLANTATION STORAGE METHOD IMPACTS IMMUNE CELL POPULATIONS IN INTESTINAL GRAFTS FOLLOWING TRANSPLANTATION

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Intestinal transplantation (IT) is the only treatment for intestinal failure patients who cannot tolerate parenteral nutrition. Although cold storage (CS) is the gold standard for intestinal allograft preservation, normothermic machine perfusion (NMP) has improved transplantation success in other organs. Allograft immune modulation is thought to contribute to this success. We hypothesized that NMP would reduce proinflammatory immune cell infiltration thus improving overall IT success.

Porcine intestines were stored for 6hr at 4°C (CS-T6, n=6) or perfused at 34°C (NMP-T6, n=8) and transplanted. Samples were collected following intestinal procurement (T0), storage, 1-hour post-transplant (T1PT), and euthanasia (T48). Flow cytometric profiling and quantification of intraepithelial (IEL) and lamina propria (LP) lymphocytes and NK cells was performed at T0, T6, and T48. Immunofluorescence (IF) identified CD3+ T-cells at T1PT. One-way ANOVA analyzed cell counts; significance set at $p< 0.05$.

Ileal IEL $\lambda\delta$ T-cells were reduced throughout NMP compared to CS (T6 28.7% vs 44.0%; T48 16.65% vs 22.37%; $p< 0.0001$). NK cells were decreased in ileal and jejunal LP at NMP-T6 compared to T0 (3.6% vs 5.6%, $p< 0.01$; 2.4% vs 4.3% $p< 0.05$). CD3+ crypt IELs increased following NMP compared to CS in the jejunum (4.9% vs 2.1%, $p< 0.05$) and ileum (6.9% vs 2.8%, $p< 0.001$).

NMP reduces NK cells associated with rejection. Although T-cell numbers within the crypt increased, the subpopulation of $\lambda\delta$ IELs were reduced in NMP. Further characterization of T-cell subpopulations is needed. Understanding immune cell dynamics will improve IT outcomes for patients requiring this critical surgery.

Funding: U.S. Department of Defense PR181265; NIH K01OD010199 SERCA, NIH 5T32OD011130-15
Subject Category: Immunology

Preferred presentation format: Oral presentation

LOSS OF *BACTEROIDES THETAIOTAOMICRON* BILE ACID ALTERING ENZYMES IMPACT BACTERIAL FITNESS AND THE GLOBAL METABOLIC TRANSCRIPTOME

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Bacteroides thetaiotaomicron (*B. theta*) is a Gram-negative gut bacterium that encodes enzymes that alter the bile acid pool in the gut. Primary bile acids are synthesized by the host liver and are modified by gut bacteria. *B. theta* encodes two bile salt hydrolases (BSHs), as well as a hydroxysteroid dehydrogenase (HSDH). We hypothesize that *B. theta* modifies the bile acid pool in the gut to provide a fitness advantage for itself. To investigate each gene's role, different combinations of genes encoding bile acid altering enzymes (*bshA, bshB*, and *hsdhA*) were knocked out by allelic exchange. Bacterial grow th and membrane integrity assays were done in the presence and absence of bile acids. To explore if *B. theta's* response to nutrient limitation changes due to the presence of bile acid altering enzymes, RNASeq analysis of WT and triple KO strains in the presence and absence of bile acids was done. WT *B. theta* is more sensitive to deconjugated bile acids (CA, CDCA, and DCA) compared to the triple KO, which also decreased membrane integrity. The presence of *bshB* is detrimental to growth in conjugated forms of CDCA and DCA. RNASeq analysis also showed bile acid exposure impacts multiple metabolic pathways in *B. theta*, but DCA significantly increases expression of many genes in carbohydrate metabolism, specifically those in polysaccharide utilization loci or PULs, in nutrient limited conditions. This study suggests that bile acids *B. theta* encounters in the gut may signal the bacteria to increase or decrease its utilization of carbohydrates.

Funding Sources: NIH NCSU MBTP T32 GM133366

Primary Subject Category: Gastroenterology

ABERRANT INFLAMMATORY SIGNALING IMPAIRES NEURONS IN MURINE ALZHEIMER'S DISEASE MODEL

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Neuroinflammation is causally associated with Alzheimer's disease (AD) pathology. When the brain is inflamed, reactive microglial cells secrete various neurotoxic factors that impair neuronal functions, eventually leading to neuronal loss. While microglial activation has been relatively well studied, the mechanisms of inflammatory signaling in neurons and oligodendrocytes promote neuronal dysfunction/loss remain poorly understood. Mitogen-activated protein kinase kinase kinase 7 (MAP3K7), widely known as TAK1, is the central intracellular signaling molecule of inflammation. We previously found that TAK1 is sustainedly activated in neuron lineage cells, which include neurons and oligodendrocytes, in the aged and AD model mouse brain. This encourages us to hypothesize that sustained TAK1 inflammatory signaling in neurons and oligodendrocytes is associated with AD pathology. Given the essential role of oligodendrocytes in memory formation, we further postulate that sustained inflammatory signaling leads to memory impairment by damaging oligodendrocyte function.
To validate these hypotheses, we are conducting a 2-part study determining:

1. Whether and how intraneuronal inflammatory singling promotes neuron death.
2. The role of inflamed oligodendrocytes in neuron loss and memory impairment.

We have thus far found:

1. TAK1 is sustainedly activated in neuron lineage cells, particularly in the hippocampus in the AD mouse model.
2. *Tak1* gene deletion in neuron lineage cells alleviates AD pathology.
3. Inflammatory cytokine TNF and calcium influx together but no single stimulation kills neurons through TAK1.

We are currently investigating how TAK1-mediated inflammation affects memory consolidation using genetically modified mice with oligodendrocyte-specific TAK1 activation and inhibition.

Funding Source: NIH R35GM139601, 2019-AARG-NTF-641347

Subject category for presentation: Neurosciences

Title: DETERMINATION OF THE RATE OF PROGRESSION OF DEGENERATIVE JOINT DISEASE IN DOMESTIC CATS

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ABSTRACT

There are no data on the rate of progression of radiographic signs of DJD in cats, nor factors affecting this progression. To address this, we performed an observational clinical study using cats from previous clinical studies. Cats were identified who had full body radiographs with at least 1 set of follow-up radiographs as part of various clinical trials. An additional 6 cats with only one set of radiographs underwent follow-up radiography. Radiographs were anonymized and each joint and section of the axial skeleton was scored for severity of DJD using a previously described 10-point scale. Change in total DJD scores and individual joint scores over time were calculated for each cat. Age, weight, body condition score, degree of disability, joint pain and radiographic severity at the first timepoint (T1) were evaluated as possible risk factors for progression of DJD in cats using regression models. Many cats had evidence of radiographic progression of DJD in one or more joints; full results are under analysis. Understanding the rate of progression of DJD in cats will allow practitioners to better assess their patients over time and evaluate the effect of therapeutics on radiographic changes to the joints.

Primary subject category: Radiology description

AN OPTOGENETIC APPROACH TO UNCOVERING MECHANISMS UNDERLYING SENSORY PROCESSING DISORDERS

Author**: Kimberly Scofield**[1] (Graduate Student)

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Primary Subject: Neuroscience

Sensory over-responsiveness occurs when an individual is unable to ignore irrelevant external stimuli, and it is often a debilitating component of conditions including autism spectrum disorder and anxiety. To understand the mechanisms that enable sensory filtering, we study the acoustic startle response in larval zebrafish, an evolutionarily conserved defensive response that allows organisms to quickly escape from danger. Through a forward genetic screen, previous work found that mutations in *Cytoplasmic FMRP Interacting Protein 2* (*cyfip2*) cause startle hyper-responsiveness. However, it is not known whether this is due to enhanced detection of auditory stimuli or reduced filtering of sensory input. To address this question, we have developed an optogenetic approach that enables us to bypass hair cells and activate auditory nerve neurons directly. If the hyper-responsiveness observed in *cyfip2* mutants is driven by enhanced detection by hair cells, then directly exciting the auditory nerve will abolish mutants' hyper-responsiveness. Our data show that we can reliably elicit startle responses with light that are distinct from light-driven behavioral responses and kinematically indistinguishable from acoustic startle responses. We are complementing this optogenetic approach by measuring the activity of hair cells using a vital dye and calcium indicator. Additionally, we are developing a transgenic line that will allow us to express Cyfip2 in specific neuronal populations to determine where it is acting in the acoustic startle circuit. In total, these experiments will allow us to better understand the genetic and neural circuit mechanisms that control sensory filtering, thereby providing key insight into sensory processing disorders.

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

A RANDOMIZED CONTROLLED CLINICAL TRIAL OF A SENOLYTIC AND NAD+ PRECURSOR SUPPLEMENT COMBINATION IN SENIOR DOGS: OWNER REPORTED COGNITIVE OUTCOMES AT ONE MONTH

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Abstract: Cellular senescence and decreasing concentrations of the NAD+ metabolite are two hallmarks of aging. These contribute to inflammation, mitochondrial dysfunction, and oxidative stress; all of which exacerbate the aging phenotype. We hypothesized that targeting these hallmarks with a senolytic and NAD+ precursor will delay age-related cognitive and mobility decline. In this randomized controlled trial, seventy senior dogs were assigned to one of three treatment groups (placebo, dose 1 and dose 2) and were evaluated at months 0, 1, 3 and 6. The purpose of this interim analysis is to report the 1 month outcome of owner-reported cognitive function, as measured by the Canine Dementia Scale (CADES). Summary data were generated for fractional lifespan and questionnaire scores at baseline and month 1. Change in score was calculated and groups were compared using pairwise Wilcoxon Rank Sum (Table below). Of the 49 dogs analyzed, fractional lifespan was 0.77-1.24. The CADES scores did not differ between groups at study start. There was no significant difference in score change between groups.

	Group 1 (n=17)	Group 2 (n=15)	Group 3 (n=17)	p value
CADES M0 Median (Range)	30 (14 – 61)	33 (16 – 60)	35 (12 – 53)	0.80
CADES change Median (Range)	0 (-21 – 9)	-9 (-19 – 10)	-2 (-24 – 12)	0.13

We conclude that there is a large placebo effect for aging dogs in owner reported assessments of cognitive status. It is possible placebo effect will wane with time, allowing us to determine whether the supplements improve cognition.

Funding Source: Animal Biosciences

Primary Subject Category: Clinical Medicine

PREDICT AND PROTECT AGAINST PRRSV (PREPROPRRSV): COMBINING PRRSV FORECASTING TECHNOLOGY WITH VACCINE EFFICACY PREDICTION TO PREVENT PRRSV OUTBREAK

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Abstract
The high mutation rate of PRRSV represents a big challenge and raises two important questions for swine producers - which PRRSV strain will hit my farm next and which vaccine can best protect my herd against it? Currently, no technology can adequately answer those questions. To overcome this issue, we have combined two technologies – PRRSV forecasting and heterologous vaccine efficacy prediction. These technologies will create the first proactive PRRSV mitigation system: Predict and Protect against PRRSV (PreProPRRSV).

The establishment of PRRSV Forecasting Technology uses computer-based prediction algorithms based on relevant surveillance data to predict PRRSV spread – both intrinsic (e.g., variation of pathogen strains) and extrinsic (e.g., landscape) variables, pig transporting, and farm locations. This technology is projected to precisely predict the spread of PRRSV strains in North Carolina (NC). The Vaccine Efficacy Prediction System consists of an immune biobank (cells + serum) from pigs which received different PRRSV vaccinations. This biobank is established at the North Carolina State Veterinary College and will be used to screen virus isolates, thereby enabling us to determine which vaccine induces the strongest immune response to an approaching NC PRRSV strain. This interdisciplinary combination of computer-algorithm-based forecasting with translational immunology to enable precision animal management for PRRSV in North Carolina will determine the most effective vaccine before the emerging PRRSV strain arrives at a production site. The PreProPRRSV system is therefore expected to enhance pig health and production by decreasing the impact of PRRS with a proactive outbreak mitigation approach.

Funding Source(s): USDA, NIFA

Primary subject category: Infectious Disease

MODELING THE TRANSMISSION AND CONTROL OF AFRICAN SWINE FEVER IN COMMERCIAL SWINE POPULATIONS OF THE UNITED STATES

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As the risk of African swine fever (ASF) introduction into the U.S. rises, mathematical simulations are imperative to predict the dissemination and impact of the virus within the U.S. swine industry and develop mitigation strategies. We developed a spatially-explicit stochastic farm-level transmission model for ASF, incorporating six transmission routes, including between-farm swine movements, vehicle movements, and local spread. To assess the trajectory of an outbreak and assess the effectiveness of the ASF national response plan.

During the first 60 days of the outbreak, we observed 73 secondary infections, on average, with finisher farms being the worst affected with an average of 50 secondary cases. The predominant transmission was identified as between-farm movements of swine, contributing an average of 71.4% to ASF dissemination, while local spread and vehicle movements contributed less, with 14.5% and 14.2%, respectively. We demonstrated that combining all the control actions (quarantine and depopulation, movement restrictions, contact tracing, and control zones) was the most effective strategy, reducing secondary cases by 74%, on average, within 140 days. Under this strategy, 485,138 animals were depopulated, 1,634,623 diagnostic tests were required, and 53,648 single-movement permits were issued. Despite substantial reductions in cases, ASF was only eliminated in 29% of simulations, indicating that an outbreak would likely last longer than four months. Nonetheless, our results critically evaluate the current national response plan, estimating the resources needed and highlighting the need to further investigate control action implementation to mitigate a potential ASF outbreak.

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Primary Subject Category: Infectious Disease

APPLICATION OF INTERPRETABLE MACHINE LEARNING TO ON-FARM BIOSECURITY PRACTICES AND PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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Due to the highly connected nature of the U.S. swine industry, effective on-farm biosecurity practices are key in preventing the introduction and dissemination of infectious pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV). With the lack of quantitative supporting evidence, on-farm biosecurity practices are often chosen based on potentially biased experiences or beliefs, rather than their impact on bio-containment and bio-exclusion. We developed an interpretable machine learning methodology to quantify and rank biosecurity practices by their efficacy in reducing disease risk to facilitate better-informed implementation of biosecurity practices for PRRSV prevention. We trained an ensemble machine learning algorithm with data on biosecurity practices, farm demographics, and previous PRRSV outbreaks from 139 herds, to classify farms by PRRSV status and produce a predicted outbreak risk. Farms and production systems were then benchmarked by predicted risk, and the impact of each practice on disease risk was quantified at the individual farm level. The results determined a substantial contribution to predicted outbreak risk from biosecurity practices relating to the turnover and number of employees, the surrounding density of swine premises and pigs, the sharing of haul trailers, distance from the public road, and farm production type. At the individual level, we develop detailed biosecurity assessments that can guide biosecurity implementation on a case-by-case basis. This methodology can be applied through an R package, *MrIML-biosecurity*, to reduce the incidence of PRRSV outbreaks within the swine industry, with potential applications to other livestock systems and industry-relevant diseases.

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Primary Subject Category: Infectious Disease

TRANSCRIPTOMIC PROFILING OF ITCH-SIGNALING PATHWAY IN CUTANEOUS SENSORY GANGLIA IN ATOPIC DOGS

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Abstract
In recent years, accumulative studies have proposed the dysregulation of neuroimmune circuits causing pruritus and neurogenic skin inflammation as the new key component in the pathomechanism of atopic dermatitis (AD). Such a neurogenic role in AD remains unexplored in companion animal species. Our objectives were to uncover key differences in gene expression in sensory neurons in atopic dogs. Dorsal root ganglia were collected from two atopic (AD1 and AD2) and four normal dogs (Dog1-4) to compare their transcriptional profiles using RNA sequencing. Then, we restricted the analysis to itch-associated receptors, neurotransmitters/neuropeptides, and signaling molecules to focus on the neuronal pathway. Principal component and heatmap analyses revealed two distinct clusters separating atopic from healthy dogs. Consistent with this observation, we identified 627 (543 up-regulated and 84 downregulated) differentially-expressed genes (DEGs) in atopic compared to normal dogs. Due to a significant individual difference between the two atopic dogs, we further narrowed down our genes of interest to common DEGs in each atopic dog, which revealed 159 (132 up-regulated and 27 down-regulated) DEGs. Among these genes, when we focused on itch-signaling-associated molecules, *P2RY12*, *TLR1,* and *POSTN* were significantly up-regulated, whereas *MRGPRD* and *LPAR3* were significantly down-regulated in both atopic dogs compared to those in healthy dogs. Pathway analysis showed a significant upregulation of CREB signaling in neurons, myelination signaling pathway, and neuroinflammation signaling pathway in atopic dogs. Our study suggested that dysregulation of neuroinflammatory pathways might play a role in the pathomechanism of canine AD as in humans.

Funding Source: NCSU and NIH
Primary subject category: Neurosciences

NEW TOOL IN THE BELT – ENVIRONMENTAL DNA FOR THE FORENSIC ANALYSIS OF SOIL AND DUST

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Soil and dust are often submitted to crime laboratories as trace evidence and can be used to link an individual to a crime or to determine provenance. Forensic geologists that analyze these geologic materials aim to characterize physical properties (*e.g.* color and pH) and inorganic components (*e.g.* mineral content). However, sample size is often a limiting factor in these analyses; supplemental methods requiring a small amount of geologic material as input could provide additional evidentiary information. DNA metabarcoding is commonly used to identify biological taxa present in environmental samples by amplifying and sequencing short, yet informative, regions of the genome and is not restricted by sample amount. The goal of this research is to determine the utility and stability of environmental DNA associated with mock soil and dust evidence for sample-to-sample comparisons and determining sample origin. In this study, five mock geologic evidence items were collected monthly from an agricultural and urban location in North Carolina over a one-year period. DNA metabarcoding was applied to characterize bacteria (16S), fungi (ITS1), arthropods (*COI*), and plants (ITS2, *trnL*) associated with each sample (n, 1026). Libraries were generated using custom indexed primers that target each barcode region and were sequenced using the Illumina MiSeq. Raw sequencing reads were processed through a bioinformatic pipeline that identifies amplicon sequence variants (ASVs) via DADA2 and searches ASVs against GenBank for taxonomic identification. This presentation will include a preliminary assessment of temporal and spatial variables on the recovery of the four biological taxa from mock geologic evidence.

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Primary Subject: Genetics

DEVELOPMENT OF A SINGLE-CELL-BASED Ig AND TCR FULL REPERTOIRE SEQUENCING ASSAY FOR THE DOMESTIC FERRET

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Mustela putorius furo, the domestic ferret, is the preferred model for studying influenza due to the similarities of transmission and pathogenesis that these animals share with humans. Additionally, domestic ferrets are well suited for the *in vivo* study of SARS-CoV-2 infection due to their possession of ACE2 receptors which require engineered strains of mouse models. A major and consistent issue with the use of domestic ferrets for the study of infectious disease has been the general lack of species-specific reagents for immunological studies. In particular, no ferret specific reagents are commercially available for single-cell immune repertoire sequencing (scIRS). scIRS is critical for determining the isotype specificity of individual B and T cells, allowing researchers to explore processes such as somatic hypermutation, isotype switching, maturation, and clonal proliferation. To address this critical need, we developed an assay alongside systematically annotated germline Ig/TCR variable (V), diversity (D), joining (J), and constant (C) genes that allows users to individually capture each known isotype. We then validated this assay using a commercially available single-cell-based workflow and were able to recover all known isotypes of the ferret immune repertoire. This assay should allow future studies to obtain a deeper and more highly resolved picture of immune function through capture of the full diversity of the ferret immune repertoire.

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Primary Subject Category: Immunology

DOES THE INTENSITY OF PERIOPERATIVE ANALGESIA ALTER THE METASTATIC PROPENSITY OF EXTRMITY OSTEOSARCOMA?

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Osteosarcoma (OS) is an aggressive bone cancer in dogs and children. Recent data suggest that the types of pain medications used during and after canine OS surgery (limb amputation) can impact time to metastasis. Here, we aimed to establish methods for modeling this phenomenon in mice. Female SCID-Beige mice underwent intratibial injections of 143B human OS cells. Ten days later, affected limbs were amputated. Mice were randomly assigned to receive low intensity analgesia (one dose of meloxicam and then buprenorphine for three days), or high intensity analgesia (meloxicam and buprenorphine for three days, plus liposomal bupivacaine at the surgical site). Amputated legs were processed for histopathology. To evaluate surgical site sensitivity, the von Frey assay was performed. Lung tumor burden was quantified using bioluminescent imaging and computed tomography. Survival was quantified. One day post-surgery, mice in the high-intensity analgesia group had lower surgical site sensitivity than mice that received low-intensity analgesia. IVIS imaging was performed 10 days postoperatively and showed no difference in lung-tumor burden between the treatment groups. Based on Kaplan-Meier survival curve analysis, there was also no detectable difference in overall survival. Leg histopathology did show variability in the amount of intra-osseous tumor. Despite significant differences in surgical site sensitivity, the magnitude of difference was small. The tumor induction technique also led to inconsistent amounts of tumor within the medullary cavity. Future investigations should: (1) further exaggerate the differences in analgesic intensity, and (2) aim to use more consistent and more biologically relevant tumor modeling techniques.

Subject Category: Cell biology, Pain

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A PAN-GENOME-WIDE ASSOCIATION STUDY TO DECIPHER VIRULENCE MECHANISMS OF AVIAN PATHOGENIC *E. COLI*

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Avian pathogenic *Escherichia coli* (APEC), the agent of colibacillosis, is the leading cause of mortality in poultry. Treatment of APEC infections is confounded by widespread antimicrobial resistance, genetic diversity, and numerous overlapping APEC virulence mechanisms. To develop effective interventions, it is crucial to understand the genetic basis of APEC virulence. In this investigation, a pan-genome-wide association study was used to correlate APEC genotypes with virulence phenotypes to identify APEC genes associated with embryo lethality. The whole-genome sequences of 97 APEC strains isolated from septic chickens and turkeys were annotated. Binary virulence phenotypes (virulent vs avirulent) were assigned to each strain using an established embryo lethality assay with a survival threshold of 50%. Embryos were challenged with APEC strains at 12 days of incubation and cumulative viability over 5 days was plotted as Kaplan-Meier survival curves. After constructing a pan-genome for the 97 APEC strains, the resulting genotype matrix was aligned with the binary virulence phenotype assignments to complete the pan-genome-wide analysis. Predicted proteins required for exopolysaccharide production including lipopolysaccharide (LPS) and colanic acid exhibited 97% sensitivity in the identification of virulent APEC strains (Odds Ratio = 15.5). Additionally, 180 hypothetical proteins were 100% specific to virulent APEC strains suggesting a number of APEC virulence factors remain uncharacterized. This genetic screen provides insight into APEC virulence, which is important for the development and evaluation of interventions for the prevention and treatment of colibacillosis.

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Category: Infectious Diseases

QUANTIFYING FITNESS EFFECTS OF RESISTANCE GENES USING PHYLODYNAMIC MULTI-TYPE BIRTH-DEATH MODELS AMONG *CAMPYLOBACTER COLI* FROM CONVENTIONAL AND ANTIBIOTIC-FREE AGRICULTURAL SWINE POPULATIONS

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Antimicrobial resistance (AMR) is a major public health challenge, threatening the future efficacy of lifesaving antibiotic therapies. Most AMR mitigation strategies prioritize responsible antimicrobial use through stewardship programs, which are most effective when resistance genes carry a fitness cost in absence of antimicrobial exposure. However, such fitness costs are not universal. Previous research on *Campylobacter coli* from conventional and antibiotic-free (ABF) agricultural swine cohorts detected resistance to fluoroquinolone and macrolide antibiotics, which are used to treat human campylobacteriosis. Notably, a high prevalence of over 20% macrolide-resistance was observed among both production systems. This study quantified fitness effects of resistance-conferring genes among these ABF and conventional *C. coli* populations using a likelihood-based phylodynamic approach. We used multi-type birth-death models that allow multiple resistance features and interactions between features to determine lineage-specific growth rates, and thus fitness. We focused on the T86I *gyrA* and A2075G *23S rRNA* point mutations, which confer resistance to fluoroquinolone and macrolide antibiotics respectively. Among *C. coli* lineages from conventional swine farms, the fitness of lineages with the T86I *gyrA* mutation was 1.35 times the fitness of lineages without the mutation. Yet, T86I *gyrA* had a significant fitness cost among *C. coli* from ABF farms. In contrast, the fitness of *C. coli* from ABF farms with the A2075G *23S rRNA* mutation was 1.025 times the fitness of lineages without the mutation, and the opposite trend was observed among conventional isolates. Our results suggest that fluoroquinolone resistance in *C. coli* may respond antimicrobial stewardship practices, but not macrolide resistance.

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Subject Area: Infectious Disease

VOXELWISE ANALYSIS OF WHITE MATTER IN THE CENTRAL HEARING PATHWAY OF SENIOR DOGS

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Presbycusis, or age-related hearing loss, affects both dogs and humans, primarily due to defects in the peripheral auditory system. Emerging evidence in humans suggests changes in the central auditory system also play a role, but research on this in dogs is limited. Diffusion tensor imaging (DTI) can be used to detect white matter abnormalities in the brain. Various DTI scalars, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), can serve as indicators of different pathological changes. This prospective cross-sectional study aimed to enhance our understanding of presbycusis in senior dogs by investigating DTI scalars of the central hearing pathway. Senior dogs were recruited. To select dogs before the development of severe hearing loss, brainstem auditory evoked response (BAER) was performed, and only those with a threshold ≤ 70 dB nHL were included. Fourteen dogs meeting the criteria underwent 3T magnetic resonance scanning. Tract-Based Spatial Statistics (TBSS) was used to examine age-related changes in DTI scalars. The streamlines connecting regions of the central hearing pathways (caudal colliculus, medial geniculate nucleus, and middle ectosylvian cortex) were used as regions of interest in the TBSS. There was a significant negative correlation between FA and fractional lifespan ($p<0.05$) in the region connecting the medial geniculate nucleus and middle ectosylvian cortex, suggesting age-related white matter changes in the hearing pathway. These changes occur before severe hearing loss manifests and may contribute to central presbycusis development. This finding warrants further studies to advance our knowledge of central presbycusis in dogs.

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