Time	Room	Topic area	Speaker	Title
8:00-8:10	South	Clinical Medicine	Goss, Sarah (HO - Varner)	SURVEY OF U.S. BOVINE PRACTITIONERS' UTILIZATION, TECHNIQUES, AND SUCCESS RATES OF PARALUMBAR FOSSA ANESTHESIA IN CATTLE
8:12-8:22	South	Clinical Medicine	Jones, Katrina (HO - de Linde Henriksen)	PARTICULATE MATTER (PM2.5) FROM WILDFIRE SMOKE IN NORTHERN COLORADO APPEARS TO BE ASSOCIATED WITH CONJUNCTIVITIS IN DOGS
8:24-8:34	South	Clinical Medicine	Collins, Elisabeth (HO - de Linde Henriksen)	PRO-INFLAMMATORY CYTOKINES IN AQUEOUS HUMOR FROM ADAMTS10-MUTANT BEAGLES AT AN EARLY STAGE OF OPEN-ANGLE GLAUCOMA (OAG).
8:36-8:46	South	Clinical Medicine	Young, Kimberly (GS - Gilger)	OCULAR TOXICITY, DISTRIBUTION, AND SHEDDING OF INTRAVITREAL AAV-EQIL-10 IN HORSES
8:48-8:58	South	Genetics	Dillon, Megan (GS - Breen)	IS INCREASED MUTATION DRIVING GENETIC DIVERSITY IN DOGS WITHIN THE CHERNOBYL EXCLUSION ZONE?
9:00-9:10	South	Genetics	Rivas, Victor (GS - Stern)	A NOVEL CARDIAC TROPONIN-I MISSENSE VARIANT (C.593C>T) IS ASSOCIATED WITH FAMILIAL HYPERTROPHIC CARDIOMYOPATHY IN GOLDEN RETRIEVERS
9:12-9:22	South	Genetics	Gonzalez, Aida (VS - Stern)	HERITABILITY AND GENOME WIDE ASSOCIATION STUDY OF ORTHODROMIC ATRIOVENTRICULAR RECIPROCATING TACHYCARDIA IN LABRADOR RETRIEVERS
9:24-9:34	South	Clinical Medicine	Chandra, Anusha (VS - Dembek)	BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME
9:36-9:46	South	Clinical Medicine	Hasapis, Stephanie, (VS - Lewbart)	THE OCCURRENCE AND IMPACT OF INGESTED PLASTICS ON RECREATIONAL MARINE FISHES IN NORTH CAROLINA
9:48-9:58	South	regenerative medicine	Froneberger, Anna (VS - Schnabel)	INITIAL INVESTIGATION INTO THE EFFECTS OF CLINICALLY USED ANTIADHESIVES ON INTRASYNOVIAL TENOCYTES IN VITRO
10:00-10:10	South	Clinical Medicine	Perez Quesada, Javier (HO - Dembek)	BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME
10:12-10:22	South	Clinical Medicine	Zumstein, Jalise (HO - Briley)	DESCRIPTION OF AN ULTRASOUND-GUIDED PUDENDAL NERVE INJECTION IN DOGS: A CADAVERIC STUDY

10:24-10:34	South	Clinical Medicine	LeGrand, Jessica (HO - Silverstein Metzler)	COMPARISON OF INTRARENAL (IR) AND INTRAVENOUS (IV) INJECTIONS OF SODIUM PENTOBARBTAL FOR EUTHANASIA IN RABBITS
8:00-8:10	A101	Genetics & Infectious Disease.	Jara, Manuel (PD - Lanzas)	INVESTIGATING THE ASSOCIATION BETWEEN HEAT TOLERANCE ADAPTATIONS AND ANTIFUNGAL GENES IN *CANDIDA AURIS* IN THE CONTEXT OF CLIMATE CHANGE
8:12-8:22	A101	Pharmacology	Miller, Ranee (GS - Baynes)	INVESTIGATING THE PHARMACOKINETICS AND EFFICACY OF INTRAMAMMARY CEFTIOFUR HYDROCHLORIDE IN NON-LACTATING DAIRY CATTLE
8:24-8:34	A101	Pharmacology	Sheela, Farha (GS - Baynes)	MULTIVARIATE LINEAR REGRESSION IN THE ESTIMATION OF WITHDRAWAL INTERVAL IN GOAT EDIBLE TISSUES FOLLOWING THE EXTRALABEL ADMINISTRATION OF FLUNIXIN MEGLUMINE AT A SINGLE DOSE OF 2.2 MG/KG
8:36-8:46	A101	Genetics & Infectious Disease	Frias, Alba (PD - Lanzas)	INFLUENCE OF SEQUENCING TECHNOLOGY ON PANGENOME-LEVEL ANALYSIS AND DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN ESKAPE PATHOGENS
8:48-8:58	A101	Pain	Ahmed, Faihaa (PD - Nolan)	EVALUATING THE IMPACT OF ARTEMIN DEPLETION IN ACUTE OROFACIAL RADIATION ASSOCIATED PAIN
9:00-9:10	A101	Pain	Caddiell, Rachel (PD - Gruen)	BREED VARIABILITY IN PAIN RECOGNITION AND TREATMENT: INSIGHTS FROM A VETERINARY EMERGENCY ROOM
9:12-9:22	A101	Pain	McNamee, Eleanor (S - Gruen)	INVESTIGATING DOG AND HUMAN FACTORS INFLUENCING CANINE BEHAVIOR AT THE VETERINARY CLINIC
9:24-9:34	A101	Pain	Copeland, Carson (VS - Lascelles / Gruen)	RADIOGRAPHIC PROGRESSION OF DEGENERATIVE JOINT DISEASE IN CATS
9:36-9:46	A101	Immunology	Farkas, Marissa (VS - Cruse)	BETA SUBUNIT OF FcεRI RECEPTOR MAY FACILITATE ANAPHYLAXIS AND, SECONDARILY, IMPACT WEIGHT
9:48-9:58	A101	Infectious Disease	Harden, Sarah (VS - Lanzas)	ASSIGNING PURPOSE OF USE FOR ANTIMICROBIAL DRUG PRESCRIPTIONS USING NATURAL LANGUAGE PROCESSING METHODS IN COMPANION ANIMAL MEDICINE

10:00-10:10	A101	Infectious Disease	Curtis, Savannah (GS - Lanzas)	MODELING OF HEALTHCARE-ASSOCIATED CLOSTRIDIOIDES DIFFICILE INFECTION AND IDENTIFICATION OF TRANSMISSION PAIRS
10:12-10:22	A101	Infectious Disease	Joshi, Umang (GS - Lanzas)	RACIAL AND SOCIODEMOGRAPHIC DISPARITY IN HEALTHCARE ASSOCIATED INFECTIONS
10:24-10:34	A101	Pharmacology	Jolley, Ashlan (VS - Lanzas)	EFFECTS OF REGIONAL DIVERSITY ON ANTIMICROBIAL PRESCRIBING IN COMPANION ANIMALS IN NORTH CAROLINA FROM 2019-2020
1:00-1:10	South	Cell Biology	Cooper, Bethanie (GS - Sheats)	INVESTIGATION OF NETS AS TARGETS IN EQUINE ASTHMA
1:12-1:22	South	Clinical Medicine or Other	Martinelli, Laura (HO - Harrison)	USE OF COMPUTED TOMOGRAPHY TO DETERMINE BODY SURFACE AREA AND K-CONSTANT IN ATLANTIC STINGRAYS (DASYATIS SABINA)
1:24-1:34	South	Neurosciences	Simon, Katherine (VS - Olby)	MAGNITUDE AND DURATION OF PLACEBO EFFECT IN SENIOR DOGS WITH MILD-MODERATE COGNITIVE IMPAIRMENT
1:36-1:46	South	Gastroenterology, Neurosciences.	Mariant, Chloe (GS - Van Landeghem)	GFAP-EXPRESSING ENTERIC GLIAL CELLS PROMOTE DNA INTEGRITY IN INTESTINAL EPITHELIAL PROGENITOR CELLS TO PROMOTE REGENERATION AFTER CHEMOTHERAPY INJURY
1:48-1:58	South	Infectious disease, gastroenterology	Haywood, Lillian (GS - Sheahan)	A NOVEL KIRKOVIRUS MAY BE ASSOCIATED WITH EQUINE GASTROINTESTINAL DISEASE
2:00-2:10	South	Comparative medicine	Hill, Amanda (PD - Piedrahita)	LGR5 STEM CELL RESEARCH IN PIGS: GENERATION OF A NOVEL TRANSGENIC MODEL FOR CELL TRACKING AND ABLATION THROUGH CRISPR- CAS9
2:12-2:22	South	Immunology	Gaghan, Carissa (GS - Kulkarni)	IMMUNIZATION OF BROILER CHICKENS WITH RECOMBINANT CLOSTRIDIUM SEPTICUM NON-TOXIC ALPHA TOXIN DOMAIN-2 CONFERS PROTECTIVE IMMUNITY AGAINST CLOSTRIDIUM DERMATITIS
2:24-2:34	South	Immunology	Hepworth, Emma (GS - Yoder)	INVESTIGATING HOW PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AFFECT NEUTROPHIL METABOLISM

2:36-2:46	South	Immunology	Vo, Thao (GS - Saini)	OPTIMIZATION OF THE X-RAY IRRADIATION DOSE TO ACHIEVE NEAR-COMPLETE RECONSTITUTION OF ALVEOLAR MACROPHAGES WITH DONOR CELLS WHILE MINIMIZING ANIMAL DISTRESS
2:48-2:58	South	Infectious Disease	Ratchford, Andrew (GS - Pierce / Schnabel)	LIPOXAZOLIDINONE NATURAL PRODUCT ANALOG DISPLAYS POTENT IN VITRO ACTIVITY AGAINST STAPHYLOCOCCUS AUREUS BIOFILMS
1:00-1:10	A101	Infectious Disease	Atwood, Emma (GS - Sharma)	IDENTIFICATION OF SMALL MOLECULE INHIBITORS FOR BLOCKING ENTRY OF SARS-COV-2 AND RELATED BAT CORONAVIRUSES
1:12-1:22	A101	Infectious Disease	Kappala, Deepthi (PD - Sharma)	FUNCTIONAL REPERTOIRE OF MACAQUE INTERFERON-INDUCED TRANSMEMBRANE (IFITM) PROTEINS AGAINST DIVERSE PATHOGENIC VIRUSES
1:24-1:34	A101	Infectious Disease	Bamrung, Veeraya (GS - Sitthicharoenchai)	ANTIMICROBIAL RESISTANCE GENOTYPIC AND PHENOTYPIC PROFILE OF STREPTOCOCCUS GALLOLYTICUS ISOLATES FROM DOMESTIC ANIMAL SPECIES: IMPLICATIONS FOR ONE HEALTH
1:36-1:46	A101	Infectious Disease	Bush, Janice (GS - Breitschwerdt)	EFFECT OF BARTONELLA HENSELAE INFECTION ON PHAGOCYTOSIS IN TWO CELL LINES
1:48-1:58	A101	Infectious Disease	Moore, Charlotte (GS - Robveille)	DETECTION OF DIROFILARIA REPENS AND MANSONELLA SPECIES IN THE UNITED STATES BY WOLBACHIA SURVEILLANCE
2:00-2:10	A101	Infectious Disease	Fleming, Christian (GS - Machado)	ENHANCING U.S. SWINE FARM PREPAREDNESS FOR INFECTIOUS FOREIGN ANIMAL DISEASES WITH RAPID ACCESS TO BIOSECURITY INFORMATION
2:12-2:22	A101	Infectious Disease	Sanchez, Felipe (GS - Machado)	PREDICTING COMMERCIAL SWINE FARM LOCATIONS AND DEMOGRAPHIC DATA IN THE U.S. USING DEEP LEARNING AND AERIAL IMAGERY
2:24-2:34	A101	Infectious Disease	Keen, KellyGrace (GS - Rahe)	EFFECT OF DIETARY VITAMIN D3 ON PRRSV DISEASE SEVERITY AND IMMUNE RESPONSE IN NURSERY PIGS

2:36-2:46	A101	Infectious Disease	McMillan, Arthur (PD - Theriot)	METAGENOMIC, METABOLOMIC, AND LIPIDOMIC SHIFTS ASSOCIATED WITH FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT CLOSTRIDIOIDES DIFFICILE INFECTION
2:48-2:58	A101	Infectious Disease	Bourne, Christina (S - Crisci)	IN VITRO ANALYSIS OF SELENIUM-TESTED PORCINE ALVEOLAR MACROPHAGES UPON PRRSV INFECTION

EVALUATING THE IMPACT OF ARTEMIN DEPLETION IN ACUTE OROFACIAL RADIATION ASSOCIATED PAIN

Faihaa Ahmed: Postdoc

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Many patients with head and neck cancer undergoing radiotherapy report severe pain that negatively impacts quality of life and significantly increases morbidity. Biological mechanisms underlying acute Radiation Associated Pain (RAP) are unknown. Activation of cold-sensing TRPM8 (Transient Receptor Potential Melastatin family member 8) channels has been shown to be mediated by ARTN/GFRα3 interactions in several pathological conditions and may also contribute to RAP. To test this, tongues of mice treated with anti-ARTN antibody or control (IgG) were irradiated (19 Gy single fraction, or sham irradiation [0 Gy]). As expected, ARTN expression increased after tongue irradiation, and this was mitigated by anti-ARTN treatment. Daily assessments of glossitis scoring, body weight measurements, and behavioral pain phenotyping indicate that irradiation caused severe pain, but ARTN depletion didn't mitigate this pain. After topical ophthalmic application of cold saline solution, irradiated mice injected with anti-ARTN wiped their eyes less than their IgG-treated counterparts, and as assessed by ex vivo calcium imaging, the afferent sensory nerves were less responsive to cold buffer. We therefore conclude that at the molecular level, ARTN depletion reduces radiation-induced cold sensitivity, but treatment with this anti-ARTN antibody was insufficient to reduce RAP. Ongoing work in tissues from these mice is exploring whether changes in cold sensitivity correlated with TRPM8 expression. Future experiments are planned to assess whether: (1) complete deletion of ARTN (using ARTN-knockout mice) will lower the glossitis severity and the resultant discomfort, (2) whether there is a sex difference in response to ARTN deletion.

Funding Source: NIH grant # R37CA248797

Primary subject category for presentation: Pain

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

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

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

ANTIMICROBIAL RESISTANCE GENOTYPIC AND PHENOTYPIC PROFILE OF STREPTOCOCCUS GALLOLYTICUS ISOLATES FROM DOMESTIC ANIMAL SPECIES: IMPLICATIONS FOR ONE HEALTH

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gGraduate student
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Streptococcus gallolyticus, formerly *S. bovis*, is a significant contributor to infective endocarditis (IE) and is highly associated with colorectal cancer in humans. Although the organism is often found in the gastrointestinal tract of healthy animals and humans, it can lead to opportunistic infections of clinical significance. In animals, it has been known to cause systemic infections, including mastitis and meningitis in cows, septicemia in birds, and endocarditis in pigs. Despite its prevalence in healthy and diseased animals, there are insufficient studies on antimicrobial resistance (AMR) in animals. This study aimed to examine the phenotypical and genotypical antimicrobial susceptibility and resistance in 51 clinical and non-clinical isolates from domestic animal species, including pigs (n=39), cats (n=2), dogs (n=3), ruminants (n=2), turkeys (n=2), chickens (n=1), and horses (n=2), collected from 2020-2023 at Iowa State University Veterinary Diagnostic Laboratory. Whole-genome sequencing (WGS) and antimicrobial susceptibility testing were performed to determine antimicrobial susceptibility and resistance characteristics. WGS analysis divided the isolates into 2 subspecies: *S. gallolyticus* subsp. *gallolyticus* and *S. gallolyticus* subsp. *pasteuranius*. Penicillin, commonly used for treating human IE, showed high susceptibility against both subspecies with 100% (n=51) exhibiting an MIC of 0.25 µg/ml. [98% (50/51)]. However, a significant proportion of the isolates exhibited high resistance to tetracycline (0.5-8 µg/ml) and enrofloxacin, with resistance rates of 76% (39/51)) and 96% (49/51), respectively. RResistance traits were linked to the presence of the tet(M), tet(L), and/or tet(O) + erm(B) genes. Overall, these findings are consistent with previous studies and highlight the need for ongoing surveillance of AMR development in this important One Health pathogen.

Category: Infectious Disease, One Health

IN VITRO ANALYSIS OF SELENIUM-TESTED PORCINE ALVEOLAR MACROPHAGES UPON PRRSV INFECTION

Christina Bourne: staff
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Selenium is a trace mineral with strong antioxidant and anti-inflammatory properties, and its deficiency can lead to immunosuppression. In swine, selenium has been used as a dietary supplement to improve immune function and enhance growth performance. Several studies demonstrated that selenium protects immune cells against oxidative stress during viral infections. Porcine reproductive and respiratory syndrome virus (PRRSV) causes respiratory and reproductive failure in swine, with high mortality rates that lead to up to $1.2 billion per year of economic losses in US industry. The efficacy of available vaccines is greatly hampered by the high mutation rate of the virus, therefore there is a need to evaluate alternative treatments. This study aims to examine the effects of both organic (L-selenomethionine, DL-selenomethionine, yeast-selenium) and inorganic (sodium selenite) forms of selenium on PRRSV infection in vitro. Lung is the primary sites of infection for PRRSV, and the virus has tropism for porcine alveolar macrophages (PAM). In this study PAM were isolated from healthy lungs, infected with PRRSV strains, and treated with different Selenium compounds at different concentrations. After 24h infection, cell supernatant and cell lysate were used for titration and RT-qPCR tests, respectively. No significant differences in viral load between Se-treated and untreated conditions were observed by RT-qPCR, or by titration of infectious viral particles. When we evaluated the mitochondrial function in PRRSV-infected, Se-treated PAM using Seahorse technologies, no significant differences were observed between the Se-treated and untreated infected PAM. Further testing will analyze Selenium immunomodulation capacity upon infection using NanoString technologies.

Funding Source: Christina Bourne salary is supported by USDA NIFA IDEAS Award # 2022-68014-37266

Subject category: Infectious Disease

GUIDELINES

Title – In all CAPITAL letters (line #1)
• **Author Name (Bold)** with Category: undergraduate, veterinary student, house officer, graduate student, postdoc, staff (line #2)
• Co-Author(s), Faculty Mentor (underline) (line #3)
• Email address(es)
• Affiliation(s): Use NCSU CVM when appropriate
• Abstract: Limited to 250 words.
• Funding Source(s) if applicable
• Please provide primary subject category for presentation: Biomedical Engineering, Cell Biology, Clinical Medicine, Gastroenterology, Genetics, Immunology, Infectious Disease, Neurosciences, Pain, Pharmacology, Regenerative Medicine or Other

EFFECT OF *BARTONELLA HENSELAE* INFECTION ON PHAGOCYTOSIS IN TWO CELL LINES.

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Abstract
Intracellular pathogens have evolved ingenious methods to escape the host immune response, often by manipulating host-cell machinery to their benefit. However, these mechanisms can affect normal cellular functioning with the potential for negative impacts on the host. *Bartonella henselae*, a gram-negative, facultatively intracellular bacterium, is capable of infecting several cell types, including monocytes, macrophages and microglia, *in vitro*. Previous research has shown that *B. henselae* uses a Type IV secretion system to inject Bartonella effector proteins, or beps, into the cellular cytoplasm. One of these proteins, bepD, when phosphorylated by a cellular kinase, interacts with the transcription factor, STAT3, which then drives transcription of anti-inflammatory IL-10. This serves to both protect the intracellular bacteria from degradation, but also to suppress the immune response through cellular crosstalk. However, persistent elevation of IL-10 can negatively impact phagocytic ability and impair cellular signaling. Seen in a variety of autoimmune conditions, IL-10 elevation is of particular interest in Alzheimer's Disease, where it may negatively impact microglial clearance of amyloid beta plaques. We hypothesized that human microglia infected with *B. henselae* would produce additional IL-10 and display diminished phagocytosis. *B. henselae* strain San Antonio 2 was utilized to infect the HMC3 line of human microglia, and IL-10 production and phagocytic ability were compared to that of a known phagocytic cell line, the canine monocyte DH82, over different times and multiplicities of infection. We surmise that persistent infection may drive shifts in phagocytic ability.

Funding Sources: Donations to the Vector-Borne Disease and Bartonella fund, NCSU. The Steven and Alexandra Cohen Foundation. The GAANN Fellowship in Molecular Biotechnology.

Primary subject category: Infectious Disease

BREED VARIABILITY IN PAIN RECOGNITION AND TREATMENT: INSIGHTS FROM A VETERINARY EMERGENCY ROOM

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Several studies have demonstrated that veterinarians hold breed-specific beliefs about canine pain sensitivity, but the impact of these beliefs on pain treatment is unknown. We retrospectively analyzed veterinary emergency room (ER) records to evaluate breed effects on pain assessment and treatment for canine patients admitted to an academic ER over a two-year period. Data extracted included signalment and information contained in medical evaluations completed by ER clinicians. The final sample included records from 3,744 patients across 69 breeds/breed types. Regression analyses revealed an effect of breed [$\chi^2(42)=147.840$, $p=1.048\times10^{-13}$] and service the case was transferred to [$\chi^2(7)=280.224$, $p<2.2\times10^{-16}$] on pain scores. Breed [$\chi^2(42)=70.69$, $p=3.664\times10^{-3}$] and transfer service [$\chi^2(7)=118.09$, $p<2.2\times10^{-16}$] were significantly explanatory for whether a pain management plan was prescribed. These findings remained robust when covariates were considered. Overall, pain scores were relatively low in this sample ($\bar{x}=0.68$; [0-4]). Breeds more frequently assigned higher pain scores included Greyhound, Beagle, Dachshund, Corgi, and Siberian Husky, while breeds more frequently assigned lower pain scores include Cavalier King Charles Spaniel, Pug, Golden Retriever, Boston Terrier and Pitbull. Approximately 43% of patients received a pain management plan in the ER but the proportion varied by breed. For example, a Siberian Husky was 1.66 times more likely to have a pain management plan assigned compared to a Golden Retriever. Breed effects do not appear to be driven by presenting conditions suggesting that disparities in pain recognition and treatment exist. Future studies are needed to determine if these findings can be generalized beyond a single veterinary hospital.

Funding Source: American Kennel Club Canine Health Foundation (AKC-CHF Grant Number 67627)
Primary Subject Category for Presentation: Pain

BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME

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Neonatal maladjustment syndrome (NMS) is a common condition in foals in which noninfectious neurologic signs and behavior disturbances develop during the postpartum period. Clinical signs may include absence of suckling, ataxia, seizures, and altered consciousness. Proposed pathophysiology of NMS is not well understood in foals but likely has correlation to abnormal neurosteroid concentration (e.g progesterone) and impaired neuronal function. Progesterone is a hormone crucial for fetal brain development and myelin formation. 17α-hydroxyprogesterone is a derivative of progesterone and is the building block for generating cortisol. Our purpose is to measure the blood concentration of certain biomarkers of brain injury, specifically the neurosteroid progesterone and 17α-hydroxyprogesterone in healthy, sick, and NMS foals. We aim to determine the association of specific biomarkers with foal survival and disease severity to find an objective diagnosis for NMS apart from clinical signs.
We hypothesize that NMS foals will have higher concentrations of progesterone and 17α-hydroxyprogesterone over time in comparison to healthy foals, and that an abnormal concentration of both neurosteroids will be associated with non-survival. Whole blood samples were collected on admission (time 0), and 24h, and 48h. Immunoassays were used to measure steroid concentrations. Results found higher progesterone concentrations trending in sick foals vs. healthy foals over the three time points studied. 17α-hydroxyprogesterone concentrations also follow these trends, and no differences were found between survivor and non-survivor foals. Our research is ongoing for a hopeful future field trial leading to a real-time diagnosis of NMS.

Category: Clinical Medicine

Funding: NC State CVM Boehringer Ingelheim Veterinary Scholars Program, Morris Animal Foundation

PRO-INFLAMMATORY CYTOKINES IN AQUEOUS HUMOR FROM *ADAMTS10*-MUTANT BEAGLES AT AN EARLY STAGE OF OPEN-ANGLE GLAUCOMA (OAG).

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Objective. To investigate the level of pro-inflammatory cytokines in aqueous humor from *ADAMTS10*-mutant research Beagles at an early stage of open-angle glaucoma (OAG).
Animals Studied. Twenty-four research Beagles were enrolled into this case-controlled study. Three groups of *ADAMTS10*-mutant research Beagles were enrolled in this study: (1) Intraocular pressure (IOP) 15-25 mmHg (normotensive IOP), (2) IOP 25-30 mmHg, and (3) IOP 30-36 mmHg. An unaffected control group of Beagles that were either *ADAMTS10*-wildtype (WT) or heterozygote-carrier with normotensive IOPs were also enrolled.
Procedures. Aqueous humor samples were collected and analyzed for 16 pro-inflammatory cytokines using the Canine Cytokine SpikeMix™ and target mass spectrometry via multiple reaction monitoring (MRM-MS). Statistical differences between the four groups' pro-inflammatory cytokines, as well as correlations between pro-inflammatory cytokines and IOPs, were analyzed using Kruskal-Wallis tests and Spearman's rho (ρ) correlations, respectively.
Results. IOP 15-25 mmHg (n=8), mean IOP 19.6 mmHg; IOP 25-30 mmHg (n=6), mean IOP 28.4 mmHg; IOP 30-36 mmHg (n=5), mean IOP 33.1 mmHg; and Control (WT n=3, carrier n=2, total n=5), mean IOP 15.3 mmHg. There were no differences between pro-inflammatory cytokines across the four groups (all p-values > 0.05). IL-13 had a moderate positive correlation with IOP, but was non-significant (ρ=0.373, p=0.073); IL-1β had a moderate negative correlation with IOP but was also non-significant (ρ=-0.344, p = 0.100). All other pro-inflammatory cytokines had only mild correlation with IOPs (|ρ| < 0.229, p > 0.05).
Conclusions. There were no significant changes in the investigated pro-inflammatory cytokines with elevated IOP in canine *ADAMTS10*-OAG.

Funding: AM Komáromy received research funding from PolyActiva Pty. Ltd., CRISPR Therapeutics, Advanced Ophthalmics LLC, and AbbVie Inc.

Category: Clinical Medicine

INVESTIGATION OF NETS AS TARGETS IN EQUINE ASTHMA

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Severe equine asthma (sEA) is a chronic, inflammatory lower-airway disease characterized by cough, increased respiratory rate and effort at rest, and elevated lower airway neutrophils. During disease exacerbation, these neutrophils contribute to ongoing inflammation and host tissue damage through the release of proteolytic enzymes, reactive oxygen species and neutrophil extracellular traps (NETs.) NETs consist of a web of decondensed chromatin decorated with granule and cytosolic proteins. They represent a promising diagnostic biomarker and therapeutic target for sEA. In this study, we hypothesized that NETs would be increased in BAL supernatant from horses with neutrophilic asthma compared to horses with non-neutrophilic asthma and healthy horses. Cell free DNA (cfDNA) concentration was measured in bronchoalveolar lavage supernatant (BAL) using the Qubit 4 fluorometer and 1X dsDNA HS assay kit. ELISAs (BAL supernatant) and immunocytochemistry (BAL cytospins) were used for quantitative and qualitative analysis of two additional components of NETs: myeloperoxidase, and citrullinated-histone 3. cfDNA was significantly higher in BAL supernatant from horses with severe equine asthma compared to healthy horses and horses with non-neutrophilic asthma. Confocal microscopy revealed NETs in BAL cytospins from horses with severe equine asthma, but not in cytospins from healthy horses. CitH3 appears to be increased in supernatant from horses with severe asthma compared to healthy horses and horses with non-neutrophilic asthma. These findings support further investigation of NETs as a biomarker and potential therapeutic target for severe equine asthma. In future studies we will investigate the Qubit fluorometer as a stall-side test diagnostic for asthma in clinical patients.

Funding source:

Foundation for the Horse Young Investigator Grant

HKJC Research Foundation

Graduate Assistance in Areas of National Need Fellowship in Molecular Biotechnology (GAANN)

Subject category: cell biology

RADIOGRAPHIC PROGRESSION OF DEGENERATIVE JOINT DISEASE IN CATS

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Degenerative joint disease (DJD) has a high prevalence in cats, and associated chronic pain negatively affects quality of life in many cases. It is important to understand normal rates of radiographic disease progression to identify abnormal (faster) progression or response to disease modifying therapies (slower progression). Currently, there is a complete lack of information regarding rates of progression of radiographic features of DJD in cats. This longitudinal study was designed to address this knowledge gap. This study will analyze retrospective data from cats with sequential full-body radiographs performed over time (from 2 months to 10 years between scans) as part of clinical studies of DJD-associated pain. Each appendicular joint and spinal segment was scored on a radiographic severity scale of 0-10 and pain severity scale of 0-4. DJD scores for each joint, summed total DJD scores (0-200), and time between radiographic studies will be used to determine the rate of radiographic progression over time for individual joint types and overall. Cat characteristics that may influence rate of progression, including initial body weight and BCS, will be explored. Results are pending for this study. Radiographic scores are expected to worsen over time across all joints, and we hypothesize higher initial pain scores will be associated with faster progression, as supported by evidence that nociceptor activity contributes to disease progression due to neurogenic inflammation. These results will provide reference information relevant to clinical decision-making for cats with DJD.

Funding Sources: This project was supported by NIH grant 1 R01 AR077890-01, an unrestricted gift from Zoetis, and the Animal Behavior Service Foundation.

Primary Subject Category: Pain

Title: MODELING OF HEALTHCARE-ASSOCIATED *CLOSTRIDIOIDES DIFFICILE* INFECTION AND IDENTIFICATION OF TRANSMISSION PAIRS

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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, CDI occurs through the importation of colonized patients who progress towards infection, or it can be healthcare-acquired. However, sampling limitations make it difficult to identify all healthcare-acquired cases, and even more difficult to identify transmission pairs and determine who-infected-whom. To overcome this, we defined and parameterized a CDI network model that explicitly tracks 421 unique patients who visited at least one of two oncological wards over a 6-month period. In this model, we track each time a patient contributes to room contamination and, ultimately, which contamination is responsible for each colonization event. We use exact admission/discharge dates and patient room assignments. We also use healthcare worker (HCW) staffing data to track HCW-mediated room contamination via their movement between rooms. We then compare model transmission pairs to results obtained using whole genome sequencing data from the same study population. Our preliminary results examine the observation strength obtained from a sampling design consisting of testing weekly and upon admission. Our findings indicate that weekly sampling is insufficient to identify all potential pairs of transmissions. This study will inform necessary sampling efforts for future studies on whole genome sequencing to track transmission in healthcare settings.

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

Primary Subject Area: Infectious Disease

IS INCREASED MUTATION DRIVING GENETIC DIVERSITY IN DOGS WITHIN THE CHERNOBYL EXCLUSION ZONE?

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Abstract:
Environmental contamination can have lasting impacts on wildlife communities, though the long-term impacts can be difficult to ascertain. The 1986 disaster at the Chernobyl Nuclear Power Plant and subsequent remediation efforts introduced radioactive material, heavy metals, and additional environmental toxicants into the surrounding environment. Many of these contaminants are mutagenic, and the full effect of these exposures on local flora and fauna has yet to be understood. Several hundred free-breeding dogs occupy the contaminated area surrounding the Chernobyl Nuclear Power Plant, and previous studies have highlighted a striking level of genetic differentiation between two geographically close populations of these dogs. Prior work addressed breed differences and inbreeding as possible causes but did not find evidence that these were tied to the populations' distinctness. With this work, we investigate whether accumulated mutation is the cause of this genetic differentiation. We first considered large-scale mutation by assessing karyotypic architecture. We then search for evidence of mutation through microsatellite diversity analyses, and then by calculating the proportion of recently derived alleles in both populations. Through these analyses, we do not find evidence of differential mutation accumulation for these populations. Thus, we find no evidence that an increased mutation rate is driving the genetic differentiation between these two Chernobyl populations. The dog populations at Chernobyl present a unique opportunity for studying the genetic effects of the long-term exposures they have encountered, and this study will allow researchers to better understand how multi-generational exposure to environmental contamination can influence mutation rate in wild populations.

Funding Sources: Triangle Center for Evolutionary Medicine Graduate Student Award (MND); Cancer Genomics Fund (MB); Clean Futures Fund, SPCA International

Primary subject category for presentation: Genetics

BETA SUBUNIT OF FcεRI RECEPTOR MAY FACILITATE ANAPHYLAXIS AND, SECONDARILY, IMPACT WEIGHT

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Primary subject category: Immunology

Funding: T35

The FcεRI receptor on mast cells binds IgE leading to degranulation and allergic responses. Its beta chain, encoded by full length *MS4A2*, enables trafficking of the complex to the membrane and facilitates signaling. However, forms of FcεRI receptor which lack the beta subunit are also present. In murine mast cell culture, oligonucleotides inducing alternative splicing of *MS4A2* generating a truncated variant, eliminate IgE-mediated degranulation facilitated by FcεRI. However, in human mast cell culture, *MS4A6*, which encodes a similar protein, can rescue FcεRI's cell membrane localization and function with forced alternative splicing of the MS4A2 suggesting a redundant function. It is not clear whether MS4A6 performs a comparable function in mice.

This study expands investigation of *MS4A2* and *MS4A6*'s role through an in vivo murine model. Surprisingly, mice show a sustained decrease in weight over 3 weeks with administration of the oligonucleotide targeting *MS4A2*. While difficulties with the anaphylaxis protocol did not allow for quantitative determination of the impact on anaphylaxis, it was observed that mice receiving the *MS4A2* oligo appeared to undergo more severe anaphylaxis than control. Mice receiving the *MS4A6* oligo appeared to exhibit less severe anaphylaxis. The systemic effect demonstrated by a decrease in weight coupled with the observation of more severe anaphylaxis generated by the *MS4A2-interfering* oligonucleotide suggest that either MS4A6 and MS4A2 competition, or altered splicing of MS4A2 promotes a hyper-responsive mast cell phenotype in vivo. Further studies are needed to confirm the effects on anaphylaxis and elucidate mechanisms of MS4A2 and MS4A6 functions in mice.

ENHANCING U.S. SWINE FARM PREPAREDNESS FOR INFECTIOUS FOREIGN ANIMAL DISEASES WITH RAPID ACCESS TO BIOSECURITY INFORMATION

Christian Fleming graduate student

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This research analyzes data from the Rapid Access Biosecurity (RAB) app™ consortium, comprising swine industry members, government officials, and academics, aimed at facilitating the creation and accessibility of on-farm biosecurity plans.

We overview the RAB app™, describe participating farms, and analyze their Secure Pork Supply (SPS) biosecurity plans, comparing production types, pig capacities, and geographic regions to explore the relationship between biosecurity measures and endemic disease occurrences.

Our study covers farms in 31 U.S. states, with a total reported capacity of 32.7 million head, representing 44.27% of the 2022 U.S. commercial hog population. Biosecurity deserts, where RAB app™ farms' capacity is less than half of the area's hog population, encompass 76.58% of hog-producing Agricultural Statistics Districts.

The average distance between RAB app™ farms is 3.14 km, with 6.98 farms on average within a 5 km radius. North Carolina has the smallest average distance at 1.61 km.

Analysis of SPS biosecurity plans provided insights into practices like manure storage, carcass disposal, and pest control. Rendering was the most common disposal method (67.16% of plans), and lagoon storage was the most common for manure (69.32% of plans).

Analysis of SPS biosecurity maps described spatial data relevant to on-farm biosecurity. The Line of Separation (LOS), a physical boundary to the animal area, averaged 4,381.29 m².

The RAB app™ enhances U.S. swine farm preparedness by providing rapid access to biosecurity information, aiding effective disease response and recovery strategies.

Funding source: NCSU CVM and CGA

Subject category: Infectious disease

PHARMACOKINETIC AND PHARMACODYNAMIC DETERMINATION OF IV BUTORPHANOL AND MORPHINE IN CATTLE

Earl Ford IV, BS, MS[1,2]: Graduate Student
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Abstract: The use of analgesics in cattle has increased in recent years, driven by heightened societal concern for animal welfare. The lack of pharmacokinetic and pharmacodynamic (PK-PD) data on analgesics in cattle has hindered the establishment of known dosing strategies and withdrawal intervals (WDI) limiting their use. Currently there is little known about two commonly used opioid drugs, morphine and butorphanol. We aim to investigate the PK-PD profiles of morphine and butorphanol in healthy steers. We hypothesize that morphine will produce greater sedation with a longer duration of action than butorphanol and that morphine will reach plasma concentrations consistent with those producing analgesic effects in other species. Eight Holstein-cross steers will be used in a blinded, randomized Latin square design consisting of four treatments (0.1 & 0.2 mg/kg morphine & 0.02 & 0.1 mg/kg butorphanol) with a minimum 72-hour washout period between treatments. PD parameters, including heart and respiratory rate, temperature, and sedation scores will be collected before, during and after treatment. PK samples will be obtained at baseline and predetermined time points for 72 hours following administration. A WDI will be established based on tissue sampling and residue analysis following euthanasia based on liver, kidney, muscle, and fat analysis. Preliminary data show that both butorphanol and morphine are rapidly excreted and tolerated well at all doses in healthy steers. This study, in conjunction with future analgesic efficacy testing, will provide valuable insights, potentially leading to more effective and safe pain management strategies in cattle.

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

Category: Pain, Pharmacology

ORAL PRESENTATION ONLY

INITIAL INVESTIGATION INTO THE EFFECTS OF CLINICALLY USED ANTIADHESIVES ON INTRASYNOVIAL TENOCYTES *IN VITRO*

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Abstract
In horses, tenosynovitis most commonly affects the digital flexor tendon sheath (DFTS). Injury to the deep and superficial digital flexor tendons (DDFT, SDFT) within the DFTS can result in the formation of intrasynovial adhesions, which result in persistent lameness and carry a poor prognosis. Tissue plasminogen activator (tPA) and enalapril are clinically used antiadhesive agents; however, their impact on tendon healing has yet to be investigated. The objectives of this study were to examine the effects of tPA and enalapril on equine DDFT- and SDFT-derived tenocyte 1) viability and proliferation, 2) gene and protein expression, and 3) migration *in vitro.* We hypothesized that neither tPA nor enalapril would have negative impacts on tenocytes. Viability and proliferation were performed with tenocytes cultured in control media or media with clinically relevant doses of tPA or enalapril. Gene expression was evaluated using the NanoString nCounter Analysis System and a multiplex immunoassay will be used to quantify supernatant protein concentrations. Migration will be evaluated using a 3D collagen gel assay. Preliminary findings revealed that tPA had no effects, whereas enalapril significantly decreased viability and proliferation in a dose-dependent manner. tPA significantly decreased the expression of some inflammatory genes, but also significantly decreased collagen type I and extracellular matrix gene expression in a dose-dependent manner. Gene expression analyses for enalapril are underway as are protein and migration assays. Findings from this study will elucidate the effects of tPA and enalapril on tenocytes *in vitro* and lay the foundation for future *in vivo* studies.

Funding sources
American College of Veterinary Surgeons Foundation
F.O.R.G.E.- Fund for Orthopedic Research in honor of Gus and Equine athletes
5T35OD011070-15 (AF stipend support 2024), T32OD011130 (SC stipend support 2022-2025), T35OD011070 (CC stipend support 2023)
Primary subject category-regenerative medicine

Title: IMMUNIZATION OF BROILER CHICKENS WITH RECOMBINANT CLOSTRIDIUM SEPTICUM NON-TOXIC ALPHA TOXIN DOMAIN-2 CONFERS PROTECTIVE IMMUNITY AGAINST CLOSTRIDIUM DERMATITIS

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Abstract:

Clostridium Dermatitis (CD) in poultry is an economically important disease caused by *Clostridium septicum* bacteria. In the current era of 'no-antibiotics-ever' farming, CD incidences are on the rise, and unfortunately, there are no effective vaccines currently available to prevent CD. Previously, we identified a non-toxic domain#2 of *C. septicum* alpha-toxin (ntATX-D2) as a vaccine candidate for turkeys that showed protection against CD. Here, we evaluated the protective efficacy of recombinant ntATX-D2 immunization (via subcutaneous route) of broiler chickens against a *C. septicum* challenge. The results showed that the immunized chickens had significantly higher body weight gain and reduced gross pathology (disease severity) when compared to unimmunized birds, indicating protection against CD. Additional investigations into protective mechanisms showed that the ntATX-D2 immunization led to; 1) A significantly higher levels of antigen-specific serum IgY antibodies, and 2) modulation inflammatory responses in the skin and muscle tissues, as indicated by the significant transcriptional downregulation of proinflammatory cytokine (IL-1β, IL-6, and IFNγ) and upregulation of anti-inflammatory cytokine (IL-10 and TGFβ) genes, when compared to the unimmunized control. Furthermore, innate and adaptive cellular mechanisms of protection are currently underway. Collectively, our findings indicate that ntATX-D2 vaccination in broiler chickens can provide protection against CD and the mechanisms of protection seem to operate through anti-ATX antibodies coupled with modulation of local and systemic inflammatory responses.

Funding Source: US Poultry and Egg Association and the USDA-NIFA

Subject Category: Immunology

Title: HERITABILITY AND GENOME WIDE ASSOCIATION STUDY OF ORTHODROMIC ATRIOVENTRICULAR RECIPROCATING TACHYCARDIA IN LABRADOR RETRIEVERS

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Abstract: Orthodromic atrioventricular reciprocating tachycardia (OAVRT) is a supraventricular tachycardia (SVT) caused by an accessory pathway most frequently diagnosed in Labrador Retrievers (LRs). Dogs with OAVRT demonstrate severe tachycardia, syncopal episodes, and tachycardia-induced cardiomyopathy. We hypothesize that OAVRT in the LR is a heritable disease caused by variant(s) in cardiac ion-channel genes. We aimed to determine pattern of inheritance and mean heritability. We further aimed to identify associated genetic variants through whole genome sequencing.

47 LRs with OAVRT were included. 7 geriatric LRs free of OAVRT served as controls. OAVRT was diagnosed by electrophysiologic mapping. Control dogs were determined to be free of any identifiable SVT via 72-hour ambulatory electrocardiograms, absence of cardiovascular clinical signs, and normal echocardiograms.

Using 3-5 generation pedigrees of 41 OAVRT-affected LRs, extended pedigrees were constructed and evaluated for suggestive mode of inheritance. Mean heritability was calculated from using the MasterBayes and MCMCglmm packages in R Studio for all dogs and a subset of 10 affected dogs with identifiable founder effects. DNA was isolated and submitted (N=20 affected; N=7 control) for whole genome sequencing at 30X coverage.

Pedigree constructs from 41 OAVRT affected LRs supported an autosomal recessive mode of inheritance. Mean heritability was calculated as 0.06 +/- 0.08 for all dogs and 0.14 +/- 0.15 for the extended family subset. Variant effect prediction and whole genome association analysis is underway.

OAVRT in LRs appears heritable due, at least in part to genetic variants inherited in an autosomal recessive mode. Continued genetic discovery efforts are warranted.

Funding Sources: American College of Veterinary Internal Medicine, Cardiology Resident Research Grant, 2024; Fluoroscience Endowment of the Veterinary Scholars Program, College of Veterinary Medicine, North Carolina State University

Primary Subject Category: Genetics

SURVEY OF U.S. BOVINE PRACTITIONERS' UTILIZATION, TECHNIQUES, AND SUCCESS RATES OF PARALUMBAR FOSSA ANESTHESIA IN CATTLE

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Abstract:

Background: Standing laparotomies under locoregional anesthesia are routinely performed in bovine practice. To understand the challenges associated with locoregional anesthesia in cattle, we surveyed private and academic bovine practitioners.

Methods: 116 complete responses to a mixed quantitative and qualitative survey were collected. Participant demographics, preferred techniques, perceived success rates, consequences of failed paralumbar fossa anesthesia, and practitioner satisfaction with current described methods were assessed. Relationships between frequency of performing surgery, length of time in practice, caseload type, and type of practice were assessed using Spearman correlations, Kruskal-Wallis, Mann-Whitney, chi square or Fisher exact tests depending on data type.

Results: The most utilized techniques among participants were the line block, inverted L, and distal paravertebral blocks. Dairy cattle practitioners preferred the distal paravertebral block (46.2%) and beef cattle practitioners preferred the inverted L (47.8%). 57.7% of practitioners found their patients sometimes needed additional administration of local anesthetic to create adequate paralumbar fossa anesthesia. 11.2% of all participants indicated having experienced a complete failure of paralumbar fossa anesthesia. As a result of inadequate or failed locoregional anesthesia, 17.2% of participants reported having been injured. 87.1% of participants indicated they would be interested in additives that prolong anesthesia and analgesia to the paralumbar fossa and 91.4% were interested in new locoregional approaches.

Discussion: More research is needed to identify ways to produce consistent, prolonged, and adequate paralumbar fossa anesthesia and analgesia to improve both patient and clinician welfare.

Funding Source: N/A

Subject Category: Clinical Medicine

ASSIGNING PURPOSE OF USE FOR ANTIMICROBIAL DRUG PRESCRIPTIONS USING NATURAL LANGUAGE PROCESSING METHODS IN COMPANION ANIMAL MEDICINE

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Understanding veterinarians' antimicrobial choices for specific conditions is crucial for effective antimicrobial stewardship strategies.

Our objective was to employ natural language processing and machine learning methods to analyze patterns between same-day antibiotic prescriptions and diagnostic problems listed by the clinician in medical records at 16 North Carolina veterinary clinics over a 24-month study period from 2019-2020. 42,419 antimicrobial drug prescriptions for 19,721 patient records were analyzed through a natural language processing pipeline capable of processing and identifying trends of prescribing antibiotics within patient-associated free-response clinician notes. In parallel, our pipeline also utilized the BioBert large language model to develop an initial quantitative classification framework for categorizing urinary, respiratory, dermatological, and a general category that reflect the most recent International Society for Companion Animal Infectious Disease (ISCAID) guidelines for antimicrobial stewardship.

Our pipeline and literature review expanded the original ISCAID classifications to four systemic categories: Gastrointestinal, respiratory, integumentary, urinary, with observations falling outside of these categories assigned to the other category and providing additional subclassifications of ear concerns, dental disease and acute injury. Overall, we identified 2,700 unique character strings within 77,878 patient observations. Our pipeline was able to categorize 77% of the patient observations within systemic categories: Gastrointestinal (32%), Respiratory (5%), Integumentary (30%), Urinary Tract Infections (10%), and Other (23%). Observations not classified as systemic disease and identified as “Other” were further analyzed, classifying 86% of the remaining categorizable observations into five sub-categories of Ear Concerns (47%), Dental Disease (29%), Acute Injury (2%), Healthy (<1%) and Patient History (10%).

Commented [MOU1]: What are notes? undefined terms like “history” ?

Commented [SH2R1]: Updated…basically this is when the clinician/tech made a note to look in the patient history for detail.

Research Grant: US Food and Drug Administration (FDA) U01FD007057

Student Support: National Institutes of Health Interdisciplinary Biomedical Research Training Program T35OD011070

Infectious Disease

THE OCCURRENCE AND IMPACT OF INGESTED PLASTICS ON RECREATIONAL MARINE FISHES IN NORTH CAROLINA

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The presence of both macro and microplastics in the oceans are an increasing environmental concern. Ingested plastics have been observed in many aquatic animal species, including marine fishes. However, the amount of microplastics in the digestive tracts of fish caught off of the North Carolina (NC) coast by head (party) boats, intended for human consumption, is not well documented. This project quantifies microplastics ingested by common NC marine fishes through analysis of their gastrointestinal tract contents at the time of recreational harvest. We hypothesize that every fish harvested will contain microplastics within the GI tract and our intent is to quantify the amount present. To obtain samples, benthic and pelagic fish were caught recreationally off the NC coast from head and personal recreational boats. State laws and regulations were followed regarding bag limits, size, and fish species caught and kept. The fish were humanely euthanized and stored on ice until returning to shore. Samples were collected by extracting the entire GI tract from the esophagus to the anus using methods to minimize external microplastic contamination and stored at -20°C until time of analysis. To prepare for microplastic analysis, samples were chemically digested to remove all organic materials. Plastic particles are then stained using Nile Red and analyzed via epifluorescence microscopy to quantify microplastic particles. Microplastic analysis and quantification is in progress. Future directions of this project may include additional analysis' using micro-fourier-transform infrared spectroscopy (FTIR) and the plastics may be identified and categorized.

Funding source: NC State University Herbert Benjamin Endowment
Primary subject category: Clinical Medicine

A NOVEL KIRKOVIRUS MAY BE ASSOCIATED WITH EQUINE GASTROINTESTINAL DISEASE

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Funding Sources: North Carolina Horse Council, NCSU CVM Equine and Companion Animal Research Endowment
Primary Subject Category: Infectious disease, gastroenterology
Abstract:

Colitis (inflammation of the colon) is a common presenting complaint among horses admitted to referral hospitals. Many cases are suspected to be infectious in origin, but the etiology is often undetermined. The objective of this study was to identify viral pathogens that may be associated with equine colitis through metagenomic sequencing and subsequent targeted analysis via qRT-PCR.

Samples from adult horses with inflammatory gastrointestinal disease (IGiD), including colitis, (n=65, divided into 13 pooled samples) were submitted for next generation viral sequencing, which revealed a novel kirkovirus, most similar to a kirkovirus previously identified in donkeys. Kirkoviruses are CRESS DNA viruses; other CRESS DNA viruses (including circoviruses) can cause diarrhea in calves, dogs and swine. Using this novel kirkovirus as a reference, significant reads were identified in 5/13 pools.

Primers were developed for qRT-PCR targeting the highly conserved replication-associated gene. PCR results were compared between horses with IGiD, horses with colic and clinically-healthy horses. Results indicated that there were significantly more horses positive for kirkovirus in the IGiD group (26%) than in the colic (6%) or clinically-healthy (9.5%) groups ($p<0.0001$, Kruskal-Wallis followed by Dunn's post-hoc).

Nearly all of the positive cases came from 3 farms, 2 of which experienced an outbreak of IGiD in 2021. However, when the 2 IGiD outbreak farms were removed from analysis, it appears that kirkovirus is uncommonly associated with isolated cases of IGiD. There also may be an association between kirkovirus and small colon impactions, with 37.5% of kirkovirus-positive cases also having a small colon impaction.

INVESTIGATING HOW PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) AFFECT NEUTROPHIL METABOLISM

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Abstract:

Per- and polyfluoroalkyl substances (PFAS) are widespread and persistent pollutants and can be detected in the serum of an overwhelming majority of people in the U.S. There is substantial evidence that PFAS alter immune function. Two particular PFAS, perfluorohexanoic acid (PFHxA) and ammonium perfluoro(2-methyl-3-oxahexanoate) (GenX), have been found to suppress the neutrophil respiratory burst, a key neutrophil function and critical process in the innate immune response. However, it is not yet known which aspect of neutrophil biology the PFAS are disrupting. Compared to some other innate immune cells, the characteristic metabolic profile of neutrophils is primarily glycolytic, in part because glycolysis supplies the cells with the burst of energy necessary to rapidly produce the reactive oxygen species (ROS) used to perform the respiratory burst. In addition, there is emerging evidence that mitochondrial metabolism plays an important role in many neutrophil functions. Therefore, it is possible that PFAS suppress neutrophil function by altering cellular metabolism. Real-time cell metabolic analysis with an Agilent Seahorse Analyzer was used to investigate if neutrophil-like HL-60 cells exposed to PFHxA and GenX had altered cellular metabolism. The Glycolytic Rate Assay and Cell Mito Stress Test measured changes in glycolysis and mitochondrial respiration. Uncovering changes in the metabolism of neutrophils exposed to PFAS will improve understanding of the mechanisms behind PFAS-induced suppression of immune cell function and how this can contribute to increased susceptibility to infection.

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

LGR5 STEM CELL RESEARCH IN PIGS: GENERATION OF A NOVEL TRANSGENIC MODEL FOR CELL TRACKING AND ABLATION THROUGH CRISPR- CAS9

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Funding source: NIH

Category: Comparative medicine

Abstract

The leucine-rich repeat-containing G-protein coupled receptor 5, LGR5, present in stem cells across various tissues, relies on niche-specific signaling for cell fate determination not only in tissue homeostasis but also regeneration and even cancer. Our lab has previously generated a transgenic *LGR5-H2B-GFP* pig model, which has yielded important insights for regenerative medicine. However, the drawback of this model is its inability to track LGR5 progeny cells. To overcome this limitation, the goal of this project is to develop a novel transgenic LGR5 pig model that will enable the study of LGR5 stem cells and their progenitors, using CRISPR-Cas9 technology. To this end, a *STOP-LOXP-mCherry-2A-hCD59* was added, by non-homologous repair, at the pig *ROSA* locus, due to its generally acceptance as a safe harbor. The mCherry is widely used for cell tracking, is easily identifiable and has no overlap with GFP emission wavelengths. hCD59/ILY was chosen as a cell ablation system, which can be used in future organoid experiments, in order to understand LGR5+ cell contributions to the formation of other lineages. The next step was to insert *CRE-ERT2* into the *LGR5-H2B-GFP* locus, by homologous recombination, to activate the mCherry reporter by excision of loxP-stop-LoxP. To achieve this, we used 100.000 male swine fetal fibroblasts from a *LGR5-H2B-GFP* animal and performed nucleofection with 3 µg of guide *RNA* and 5 µg of Cas9. The next step will be to generate pregnancies using this cell line via somatic cell nuclear transfer, which will enable the establishment of a transgenic pig model carrying a LGR5+ cell lineage tracer and ablation system. This pig model will have a broad impact in both basic and translational sciences and will greatly facilitate clinically significant discoveries due to this large animal model having greater similarity to humans in anatomy, physiology, and ability to mirror human disease processes, surpassing the current limitations of rodent models.

INVESTIGATING THE ASSOCIATION BETWEEN HEAT TOLERANCE ADAPTATIONS AND ANTIFUNGAL GENES IN *CANDIDA AURIS* IN THE CONTEXT OF CLIMATE CHANGE

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Candida auris (*C. auris*) is a fungal pathogen first isolated in 2009 that has rapidly spread across several countries causing severe infections, particularly in healthcare-associated facilities with immunocompromised patients. This yeast has become notorious for increasing resistance to most antifungals commonly used to treat *Candida* infections such as triazoles, micafungin, and echinocandins. Recent evidence has shown increased *C. auris* heat tolerance, growing at temperatures up to 42°C. These thermotolerance adaptations have not been previously observed in other phylogenetically related species and have been hypothesized to have facilitated their emergence as a human pathogen by reducing the mammalian thermal barrier. This increased heat tolerance coupled with its growing multidrug resistance makes *C. auris* a particularly concerning threat in the context of global warming, highlighting the need for a better understanding of its genetic adaptations and the environmental factors that contribute to its spread. Using over 1,700 whole genome sequences from the U.S., this project aims to identify genes within *C. auris* that confer adaptation to heat tolerance and antifungal resistance, and explore their association with climate change-associated variables in relation to their prevalence over time. By integrating genomic data with a wide variety of climatic factors, this study seeks to provide insights into how *C. auris* might adapt to and spread in different environmental conditions. This could inform public health strategies and interventions to mitigate the risk of *C. auris* infections.

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

Primary subject category for presentation: Genetics & Infectious Disease.

EFFECTS OF REGIONAL DIVERSITY ON ANTIMICROBIAL PRESCRIBING IN COMPANION ANIMALS IN NORTH CAROLINA FROM 2019-2020

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Data on antimicrobial use (AMU) in companion animals is lacking in the United States, along with information regarding drivers of such prescribing. The objectives of this study are to 1) describe spatiotemporal trends in AMU for dogs and cats in North Carolina (NC) from January 1, 2019 – December 31, 2020, and 2) investigate whether social, economic, and demographic factors and the COVID-19 pandemic influence prescribing practices. North Carolina State University acquired AMU data for 389 practices in NC from IDEXX Laboratories, Inc. (IDEXX) for 1,974,323 dogs and cats from 2019-2020. Entries were sorted into strata by their NC region (mountain, piedmont, coastal plain) and urbanization (rural, urban). Variables from the Center for Disease Control and Prevention's (CDC) Social Vulnerability Index (SVI) were summarized for each stratum. Data were classified as occurring before (January 1, 2019 – March 14, 2020) or during (March 15, 2021 – December 31, 2021) the COVID-19 pandemic. Poisson family models were used to identify associations between the prescription predictors and the total number of patients prescribed per the number of patients seen by strata monthly. Beta-lactam combination agents, fluoroquinolones, and 3rd-generation cephalosporins were prescribed to more dogs in urban areas, and to fewer cats in more vulnerable areas. These classes were prescribed less overall in the Mountain region. 3rd-generation cephalosporins were prescribed more during the COVID-19 pandemic. This study indicates that region, urbanization, social vulnerability, and the COVID-19 pandemic impacted companion animal AMU prescription in NC.

National Institutes of Health Interdisciplinary Biomedical Research Training Program (T35OD011070) and the U.S. Food and Drug Administration (U01FD007057)

Pharmacology

PARTICULATE MATTER (PM2.5) FROM WILDFIRE SMOKE IN NORTHERN COLORADO APPEARS TO BE ASSOCIATED WITH CONJUNCTIVITIS IN DOGS.

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ABSTRACT:
Objective. To evaluate ocular surface parameters in dogs with normal eyes when exposed to three different AQI categories corresponding to levels of normal air pollutants (0-50 'good' and 51-100 'moderate') and wildfire smoke (101-150 'smoke').
Animals. 15 privately owned dogs.
Methods. A prospective cohort study with dogs living in northern Colorado. Ocular surface parameters (conjunctival chemosis and hyperemia, STT-1, TFBUT, fluorescein stain, conjunctival microbiology etc.) were evaluated when the AQI was reported in one of the three categories (good, moderate, smoke) for three consecutive days. AQI and air pollutant levels (particulate matter [PM2.5], ozone, etc.) were retrieved from the AirNow database.
Results: Due to scheduling conflicts only seven dogs were examined during the smoke category. Average AQI in the three categories were: good = 44.1, moderate = 73.7, smoke = 103.7. The odds for more severe hyperemia and more severe chemosis for smoke were 5.39 and 7853.02 times the odds, respectively, when compared to good AQI. Additionally, the odds for more severe chemosis were 34656.62 times the odds for smoke when compared to moderate AQI. A significant relationship was found between chemosis and PM2.5.
Conclusion: Exposure to increased AQI related to wildfire smoke caused a significant increase in conjunctivitis. The significant relationship between chemosis and PM2.5 could indicate that PM2.5 in wildfire smoke is associated with an inflammatory factor.
Clinical Relevance: Preventive measures (e.g., use of eyewash, artificial tears, or eye protection), for dogs that are exposed to wildfire smoke should be instituted to decrease the risk of ocular irritation.

Funding: Funding was provided by the Kenneth W. Smith Professorship in the Center for Companion Animal Studies (CSU, Fort Collins, CO).
Subject category: Clinical Medicine.

RACIAL AND SOCIODEMOGRAPHIC DISPARITY IN HEALTHCARE ASSOCIATED INFECTIONS

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Abstract:

Healthcare-associated infections (HAIs) are a significant source of morbidity and mortality, with extensive research identifying modifiable risk factors (ie: comorbidities, length of stay, and antibiotic use). However, the influence of racial and sociodemographic variables on HAI incidence remains underexplored. This study evaluates the impact of racial and sociodemographic variables on HAI incidence, accounting for well-established risk factors. We analyzed clinical records and microbial culture data from over 58,000 patients across four hospitals in St. Louis, focusing on five pathogens groups: (*Clostridioides difficile, Enterobacter spp, Enterococcus spp, Pseudomonas aeruginosa,* and *Staphylococcus aureus)*. Social vulnerability indices (SVI), race, age, sex, antibiotic use, and Elixhauser groupings for comorbidities were included as determinants of HAI incidence and examined. Two decision tree methods were employed to determine the influence of the aforementioned variables: Gradient Boosting Machine and Adaptive Boost Decision Trees. Utilizing these methods, the relative information gain provided by each variable in relation to HAI diagnosis was calculated and ranked. Our analysis revealed that racial and sociodemographic factors were important predictors for HAI incidence, even in the presence of other established risk factors. Our findings indicate Caucasian individuals and those with high socioeconomic status were key decisions in discovering HAI, but exact quantification and methodology for infection needs to be further investigated. Future work will be tailored to identifying the mechanisms through which these variables influence HAI and exploring potential interventions to address health disparities.

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Infectious Disease

FUNCTIONAL REPERTOIRE OF MACAQUE INTERFERON-INDUCED TRANSMEMBRANE (IFITM) PROTEINS AGAINST DIVERSE PATHOGENIC VIRUSES

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Interferon-induced transmembrane (IFITM) proteins serve as innate cellular sentinels, restricting the entry of various enveloped viruses, including HIV-1. As entry-targeting restriction factors, IFITMs can act as barriers to cross-species viral transmission, influencing viral evolution. Hosts and viruses engage in an evolutionary "arms race," where gene duplication allows hosts to explore beneficial mutations in restriction factors. Our recent study identified multiple duplications of the IFITM3 gene in macaques, which are absent in humans, revealing canonical IFITMs (IFITM1, IFITM3, IFITM3A) and non-canonical retrocopies (IFITM3-R1, IFITM3-R2). The antiviral breadth and potency of canonical and non-canonical macaque IFITMs remains unknown. Here, we investigated the antiviral potential of macaque IFITMs against HIV-1, vesicular stomatitis virus (VSV), Sendai virus (SeV), and influenza A virus (IAV). Our findings showed that IFITM3 specifically inhibits IAV entry, while IFITM3A has a broader inhibitory effect against HIV-1, VSV, and IAV. Chimeric constructs of IFITM3 and IFITM3A pinpointed single amino acid mutations in IFITM3A responsible for its antiviral activity against VSV, and HIV-1. Additionally, the PPxY motif in human IFITM3 binds the E3 ubiquitin ligase NEDD4, promoting ubiquitination of lysine residues in IFITM3. Our investigation revealed that mutations in the PPxY motif and a single lysine residue of the IFITM3-R1 retrocopy enhance its expression and antiviral efficacy against VSV and IAV, suggesting that ubiquitination governs IFITM3-R1 stability and function. We also found that mutating the PPxY motif in IFITM3-R2 results in a gain-of-function, inhibiting VSV and SeV entry but not IAV. The mechanistic exploration of IFITM3-R1 stability elucidated that the PPxY motif plays a crucial role in the degradation of IFITM3-R1 through the endosomal-lysosomal pathway. Additionally, we observed that macaque IFITMs show differential incorporation into HIV-1 virions, with antiviral efficacy not correlating with incorporation levels. These findings highlight the functional antiviral breadth of macaque IFITMs against various enveloped viruses. Understanding the mechanisms underlying IFITMs' breadth and specificity could provide valuable insights into their interactions with viral proteins.

EFFECT OF DIETARY VITAMIN D_3 ON PRRSV DISEASE SEVERITY AND IMMUNE RESPONSE IN NURSERY PIGS

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Recent studies have reported anti-inflammatory effects of vitamin D_3 across multiple species characterized by decreased IFN-γ production and increased IL-10 production. Moreover, a metabolite of vitamin D_3, 25-hydroxyvitamin D_3 (25-OH D_3), has been shown to modulate immune function in weaning pigs. Porcine reproductive and respiratory syndrome virus (PRRSV) is a prevalent pathogen of pigs that causes severe systemic inflammation. The objective of this study was to evaluate the effect of dietary vitamin D_3 on PRRSV disease outcomes. Three-week-old pigs were designated to one of four treatment groups: marginal dietary vitamin D_3+no challenge (Negative), marginal dietary vitamin D_3 + PRRSV challenge (Low), industry dietary vitamin D_3 + PRRSV challenge (Standard), or industry dietary vitamin D_3 with 25-OH D_3 supplementation + PRRSV challenge (Supplemented). Following dietary acclimation, PRRSV challenged groups were inoculated with a lineage 1 PRRSV-2 isolate at $5x10^5$ TCID50/pig. Significant differences in serum 25-OH D_3 levels were observed between groups two weeks following diet assignment, though levels dropped in challenged groups following PRRSV inoculation. Histological evaluation revealed pigs in the Low group had higher necrotic macrophage scores compared to those in the Standard or Supplemented groups. PRRSV-specific serum IgM and IgG trended higher in the Supplemented group compared to the Low and Standard groups. No prominent differences in body weight, fever, gross pathology, or viremia were observed between the challenged groups. Present findings suggest 25-OH D_3 supplementation improves antibody production, with ongoing sample analysis to provide additional insight on the host adaptive immune response and viral clearance.

Funding Sources: dsm-firmenich

Subject Category: Infectious Disease

Title: COMPARISON OF INTRARENAL (IR) AND INTRAVENOUS (IV) INJECTIONS OF SODIUM PENTOBARBTAL FOR EUTHANASIA IN RABBITS

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Abstract: Peripheral venous access in rabbits can be difficult to obtain. When failure occurs, the dire need for alternative vascular access routes must be available. The American Veterinary Medical Association (AVMA) categorizes intrarenal (IR) injection of pentobarbital as an acceptable with conditions alternative but minimal studies using IR administration have only been conducted in felines. There are no current studies measuring the efficacy, efficiency, and validity in rabbits. Using 27 New Zealand White rabbits, this study evaluated the time to cardio-pulmonary arrest (TCPA) following an injection of 3ml sodium pentobarbital and comparing the routes of IR (n=13) to IV (n=14) injection. Timing started at the beginning of the injection and ended when cardiac and respiratory arrest were observed. Cardiac and respiratory arrest following IV injection of pentobarbital was significantly quicker (7s-24s, median 10.5s) than the IR route (40s-900s, median 625s)(Mann-U, p=0.001). This study demonstrates that the IR approach is a feasible alternative to IV as it can be reliably performed and was not associated with any observed animal distress or alterations in organ pathology. The overall information from this study can be used to help guide both laboratory and practicing clinicians that are considering performing this technique but factors such as variable times to cardiopulmonary arrest and technical skill should be considered.

Funding: Joint funding from NCSU LAR (Laboratory Animal Resources) and NCSU EAMS (Exotic Animal Medicine Service)

Primary Subject: Clinical Medicine

GFAP-EXPRESSING ENTERIC GLIAL CELLS PROMOTE DNA INTEGRITY IN INTESTINAL EPITHELIAL PROGENITOR CELLS TO PROMOTE REGENERATION AFTER CHEMOTHERAPY INJURY

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Preserving the intestinal epithelium integrity is critical to maintain nutrient absorption while preventing toxins and pathogens from penetrating the body. The epithelial barrier is kept intact in homeostatic conditions through the fast production of intestinal epithelial cells derived from multipotent intestinal stem cells (ISC) and progenitors residing in intestinal crypts. Chemotherapy disrupts the intestinal epithelium barrier due to DNA damage-induced cytotoxicity affecting both ISC and progenitors. Considering that ISC and progenitor functions are regulated by extrinsic signals including those from the gut intrinsic nervous system, and in particular enteric glial cells (EGC), we asked if and how EGC impact intestinal epithelial regeneration after chemotherapy-induced injury. To investigate this, we used the chemogenetic GFAP-hM3Dq mouse model, in which GFAP-expressing EGC were activated by administering Clozapine-N-oxide (CNO) twice daily, intraperitoneally, at 0.5mg/kg. Chemotherapy-mediated genotoxic injury was induced by a single intraperitoneal injection of 5-fluorouracil (5-FU) at 150mg/kg. We uncovered that EGC activation promoted DNA integrity and decreased apoptosis in cells located in the progenitor region of the crypts at 12h and 24h after 5-FU. In addition, EGC activation significantly increased the number of intestinal epithelial progenitors in S-phase as early as 24h after 5-FU. EGC activation for 72 hours after 5-FU administration led to a marked increase in epithelial regeneration as demonstrated by increases in the proportion of cells in S-phase and crypt density. Altogether, this study indicates that GFAP-expressing EGC might have a major role in promoting intestinal epithelial regeneration after genotoxic injury. Follow-up investigations will identify the mechanisms involved.

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

Subject category: Gastroenterology, Neurosciences.

USE OF COMPUTED TOMOGRAPHY TO DETERMINE BODY SURFACE AREA AND K-CONSTANT IN ATLANTIC STINGRAYS (*DASYATIS SABINA)*

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Abstract: The objective of this study was to use body surface area (BSA) obtained via computed tomography (CT) to calculate a species-specific K constant and provide a formula for BSA based on body weight (BW) in Atlantic stingrays (*Dasyatis sabina*). Six deceased Atlantic stingrays of unknown age and a range of sizes were collected during a natural mortality event and underwent CT scans. Following the scans, 3-D surface models were created from the imaging data to measure BSAs and derive a BSA formula based on BW. Non-linear regression analysis of BSA versus BW was performed, and a species-specific formula derived. A two-sample t-test of pooled variance and T-distribution was used to compare the K constant between sexes. BW ranged from 390 – 4,540 grams and BSA ranged from 844.2 – 4043.12 cm^2. The calculated K constant was 14.9 and the CT-derived BSA formula was as follows: BSA in cm^2 = 14.9 X (BW in grams)$^{2/3}$. There was no significant difference in K constants between sexes. These results provide a method to calculate BSA in Atlantic stingrays. The CT-derived BSA formula can be used for allometric dosing of chemotherapeutic agents among other drugs in a clinical setting and in a broader sense can be applied to studies of nutrition, metabolic rate, and physiology.

Funding Source: NCSU CVM Exotic Species Cancer Research Alliance

Primary Category: Clinical Medicine or Other

Title: METAGENOMIC, METABOLOMIC, AND LIPIDOMIC SHIFTS ASSOCIATED WITH FECAL MICROBIOTA TRANSPLANTATION FOR RECURRENT *CLOSTRIDIOIDES DIFFICILE* INFECTION

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Category: Infectious Disease

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Recurrent *C. difficile* infection (rCDI) is an urgent public health threat for which the last resort and lifesaving treatment is a fecal microbiota transplant (FMT). However, the exact mechanisms which mediate a successful FMT are not well understood. Here we use longitudinal stool samples collected from patients undergoing FMT to evaluate changes in the microbiome, metabolome, and lipidome after successful FMTs. We show changes in the abundance of many lipids, specifically acylcarnitines and bile acids, in response to FMT. These changes correlate with Enterobacteriaceae, which encode carnitine metabolism genes, and Lachnospiraceae, which encode bile salt hydrolases and *baiA* genes. LC-IMS-MS revealed a shift from microbial conjugation of primary bile acids pre-FMT to secondary bile acids post-FMT. Here we define the structural and functional changes in successful FMTs and generate hypotheses that will require further experimental validation. This information is meant to help guide development of treatment for rCDI.

INVESTIGATING DOG AND HUMAN FACTORS INFLUENCING CANINE BEHAVIOR AT THE VETERINARY CLINIC

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Fear and avoidance behaviors are observed in up to 69% of dogs during veterinary visits. In children, similar behaviors seen during pediatric visits are partially explained by the phenomenon of parental pain catastrophizing: a negative cognitive and emotional response to a child's real or anticipated pain. Parental catastrophizing leads to parental distress and oversolicitous behavior, which can result in poorer health outcomes for the child. Given parallels in qualities of the relationship, dog owners could be experiencing a similar phenomenon and influencing their dog's fear at the vet. No studies have yet explored pain catastrophizing among this population. We aim to investigate pain catastrophizing in dog owners and its associations with owner affect and their dogs' chronic pain behaviors. To assess this, we adapted the parental Pain Catastrophizing Scale for use in dog owners. The Pain Catastrophizing Scale-Dog Owners (PCS-DO) was administered alongside demographic questions and validated questionnaires measuring dogs' pain and owners' emotional states. The PCS-DO fit the three-factor model of the original survey: rumination, magnification, and helplessness. We found significant positive correlations between PCS-DO scores and owner anxiety ($p<0.05$) for two separate owner populations (painful dogs vs. pain-free dogs). These findings mirror those of parental pain catastrophizing studies, suggesting that similar cognitive processes occur when catastrophizing about a child's pain or a dog's pain. Discovering parallels between pain catastrophizing in pediatric and veterinary medicine could inform interventions to mitigate these effects in both dogs and their owners, thereby improving health outcomes and owner quality of life.

Funding Source: American Kennel Club Canine Health Foundation (AKC-CHF Grant Number 67627)
Primary Subject Category: Pain

INVESTIGATING THE PHARMACOKINETICS AND EFFICACY OF INTRAMAMMARY CEFTIOFUR HYDROCHLORIDE IN NON-LACTATING DAIRY CATTLE

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Mastitis is the most burdensome concern for the dairy cattle industry, affecting animal welfare and economics. Antimicrobials are often prophylactically administered to dairy cows at dry-off to reduce the risk of intramammary infection during the dry period and subsequent lactation. Mastitis incidence has increased in dairy heifers after calving, leading to prohibited extra-label drug use of various dry-cow products, including intramammary ceftiofur hydrochloride. However, the pharmacokinetics and efficacy of this application have yet to be studied. This study aimed to compare the pharmacokinetics and efficacy following no treatment, a teat sealant, or a single dose of intramammary ceftiofur given at 21 or 14 days prior to expected calving. We hypothesized that milk collected following dosing would contain drug residues below the FDA tolerance of 0.1 ppm by calving and heifers within the ceftiofur treatment groups would have lower somatic cell counts than heifers in the teat sealant and non-treatment control groups. Following treatment or no treatment to 24 prepartum heifers, milk samples were collected until 21 days after calving. Somatic cell counts and ceftiofur concentrations were assessed utilizing a cell counter and UPLC/MS detection, respectively. Ceftiofur administration showed the greatest efficacy 96 hours after calving. However, there were no significant differences between groups by 7, 14, or 21 days. Milk had a maximum ceftiofur concentration of 0.02 ppm 24 hours into lactation. The low ceftiofur concentrations in milk collected from these heifers indicate that administration of ceftiofur 14 or 21 days before calving is unlikely to lead to violative residues.

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Primary Subject Category: Pharmacology

Detection of *Dirofilaria repens* and *Mansonella* species in the United States by *Wolbachia* Surveillance

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Wolbachia are intracellular Gram-negative bacteria that colonize insect and filarial species. In mammals, detection of *Wolbachia* is considered a tool for filaria diagnosis, while elimination of *Wolbachia* via antibiotic treatment is utilized to weaken and eliminate filarial infection. Due to their close phylogenetic relationship, 16S qPCR primers designed for detection of *Anaplasma/Ehrlichia* species non-specifically amplify *Wolbachia* DNA. Therefore, we analyzed *Wolbachia* DNA amplified by this technique, from animal blood samples submitted to the North Carolina State University-Vector Borne Disease Diagnostics Laboratory (NCSU-VBDDL) between 2017-2023. Filarial infection was confirmed by filarial 28S gene qPCR. Phylogenetic analysis was performed utilizing filarial *cox1, myoHC,* and *hsp70* gene sequencing. In total, *Wolbachia* was detected in 57 domestic dogs (*Canis familiaris)* and 3 raccoons (*Procyon lotor)* from 23 states and Puerto Rico. Compared to GenBank sequences, a majority of the *Wolbachia* 16S sequences from the domestic dogs were *Dirofilaria immitis*-associated (89%, 51/57), with other *Wolbachia* aligning with insect-associated *Wolbachia* (9%, 5/57) or *Dirofilaria repens*-associated *Wolbachia* (2%, 1/57). *Dirofilaria immitis* infection was confirmed by filarial 28S qPCR for all *D. immitis*-associated *Wolbachia* samples that were available for re-testing (n = 41). *Dirofilaria repens* infection was confirmed by 28S and *cox1* PCR in the one dog with *D. repens*-associated *Wolbachia*; the animal was imported from Slovakia. All insect-associated *Wolbachia* infected dogs were negative by filarial 28S qPCR. The *Wolbachia* detected in raccoons most closely aligned with *Wolbachia* from *Mansonella ozzardi.* Filarial 28S sequencing confirmed the raccoon filaria was a *Mansonella* spp., but the latter has not been previously phylogenetically described in GenBank based on analysis with *cox1, myoHC,* and *hsp70*. These findings indicate that surveillance for *Wolbachia* in domestic and wild animals has the potential to identify novel filarial species in the United States, including potential zoonotic species (*D. repens, Mansonella* spp.).

BIOMARKERS OF BRAIN INJURY IN FOALS WITH NEONATAL MALADJUSTMENT SYNDROME

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Neonatal maladjustment syndrome (NMS) is a common disease of foals resulting in neurological dysfunction and increased mortality. Plasma biomarkers of brain injury, such as brain-derived neurotrophic factor (BDNF), glial-fibrillary-acidic protein (GFAP), and astrocytic-protein-S100B may be used for diagnosis and monitoring of foals with NMS. The goal of this study was to measure plasma concentration of BDNF, GFAP, and S100B in foals with NMS, foals presented for other diseases (sick-foals), and healthy foals, and determine their association with the severity of disease and outcome. A total of 34 foals <7 days of age were included. Foals were classified based on clinical signs into 3 groups: healthy foals (n=8), NMS foals (n=10), and sick-foals hospitalized for other diseases (e.g. diarrhea, n=16). Of the NMS and sick-foals, 20 survived and 6 did not. Blood biomarker concentrations were determined in all foals on admission and at 24h, 48h, and 72h of hospitalization in this prospective, longitudinal study. Plasma concentration of biomarkers was measured with ELISA and single-molecule-array technology. Data were analyzed with parametric methods. Results showed that GFAP concentration was decreased in NMS and sick-foals compared to healthy foals at time 0 (0.9±0.52, 0.6±0.21, 3.7±1.2 ng/mL), 24h (0.84±0.22, 0.73±0.25, 3.6±1.03), and 48h (0.5±0.1, 0.56±0.12, 3.1±0.8) ($P<0.05$), respectively. Non-survivors had a decreased concentration of GFAP (0.4±0.12 ng/mL) compared to healthy foals (3.7±1.2) and survivors (1.2±0.21) over the first 24h of hospitalization ($P<0.05$). BDNF and S100B concentrations were not different between groups of foals or time points ($P>0.05$). We concluded that reduced GFAP concentration in NMS and sick-foals suggests astroglial dysfunction or delayed postnatal astrogliogenesis. GFAP may be used as a prognosticating factor in critically ill foals.

Funding sources: Morris Animal Foundation, CVM Research Grant

Category: Clinical Medicine

LIPOXAZOLIDINONE NATURAL PRODUCT ANALOG DISPLAYS POTENT *IN VITRO* ACTIVITY AGAINST *STAPHYLOCOCCUS AUREUS* BIOFILMS

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Abstract: Bacterial biofilms are major contributors to the rise of multi-drug resistance and are implicated in the incidence of hospital-acquired infections. *Staphylococcus aureus* biofilms can tolerate anywhere from 10 – 1000x the concentration of frequently prescribed antibiotics required to kill planktonic cell growth in vitro, warranting further investigation into antimicrobial treatments that target biofilms. The 4-oxazolidinone family of natural products, particularly lipoxazolidinone A, displays a high degree of antimicrobial activity against *S. aureus*. Despite this, there has been no significant evaluation of the lipoxazolidinones' activity against bacterial biofilms. Our objective was to further characterize the *in vitro* antimicrobial and antibiofilm activities along with the mechanism of action for a leading 4-oxazolidinone analog (SYNX_0001) against pathogenic bacteria in both their planktonic and biofilm states.

We performed minimum inhibitory concentration (MIC) and minimum biofilm eradication concentration (MBEC) assays against a panel of biofilm-forming pathogens from the CDC's ESKAPE group. We further assessed SYNX_0001 in a series of time-dependent inhibition experiments against *S. aureus* in both planktonic and pre-formed biofilm states. The authors also sought to investigate potential differential expression response in *S. aureus* JE2's transcriptome by RNA-seq analysis at subinhibitory concentration.

Our results indicate that SYNX_0001 is a potent inhibitor of both planktonic and biofilm-associated cells for the gram-positive bacterial species evaluated and warrants further study. Future investigations into the mechanism of action will be driven by the differentially expressed genes resulting the RNA-seq analysis.

Funding source: N/A

Primary Subject Category: Infectious Disease

A NOVEL *CARDIAC TROPONIN-I* MISSENSE VARIANT (C.593C>T) IS ASSOCIATED WITH FAMILIAL HYPERTROPHIC CARDIOMYOPATHY IN GOLDEN RETRIEVERS

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Abstract: Hypertrophic cardiomyopathy (HCM) is a naturally occurring cardiac disorder that afflicts humans, cats, rhesus macaques, pigs, and rarely dogs and is characterized by abnormal left ventricular wall thickening. Over 1,500 sarcomere-coding genetic mutations explain the disease in humans, whereas only three have been reported in specific cat breeds. To date, no genetic mutations have been described in dogs. HCM in a nuclear family of Golden Retrievers (GRs) was identified following the sudden cardiac death of four puppies less than two-years-of-age from two dam-offspring repeat matings. Full gross and histopathologic evaluations on three available HCM-affected puppies revealed hallmark features of the disease including cardiomyocyte hypertrophy, interstitial fibrosis, and left-sided congestive heart failure. Given the rarity of canine HCM and the identical clinical and pathological presentations between affected puppies, ~30X whole-genome sequencing (WGS) on the three affected puppies, along with nuclear family members (i.e., sire, dam, four unaffected littermates, four unaffected half-siblings), and one distantly related, geriatric, cardiovascularly normal GR was performed (n=14). A single segregating missense mutation (c.593C>T) in the *cardiac troponin-I* (*TNNI3*) gene was identified according to postulated autosomal recessive mode-of-inheritance. This variant was not observed in a unphenotyped cohort of WGS dogs (n=2,771) and in 42 additional GRs. This variant represents the first-ever reported pathogenic HCM variant in any canine species and its identification holds promise for genetic screening and early prevention within the breed.

Primary Subject Category: Genetics

PREDICTING COMMERCIAL SWINE FARM LOCATIONS AND DEMOGRAPHIC DATA IN THE U.S. USING DEEP LEARNING AND AERIAL IMAGERY

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Accurate farm geolocations are essential for disease monitoring and surveillance, and for developing spatial epidemiological models. Here, we developed a convolutional neural network (CNN) model workflow to i) identify commercial swine barns from non-barn features, ii) create a rule-based filtering method that combines the calculated commercial swine production barn area to iii) predict farm types (e.g., sow and finisher) and population size.

High-resolution aerial imagery of a 300 square kilometer extent was obtained from the National Agriculture Imagery Program (NAIP). It was used to generate 87,800 raster tiles with dimensions of 256 x 256 meters. Binary masks of 2,584 swine barns were created for the corresponding NAIP raster tiles. Image and mask tiles were split for training (70%) and testing (30%) a ResNet50-UNet CNN image segmentation model, and farm-type classification model. Model performance was evaluated by examining the accuracy, mean Intersection over Union (IoU), and the F1-score.

The CNN image segmentation model accurately identified swine barns from non-barn features with a mean IoU of 92% and an F1-score of 84%. Model predictions of the “barns” class also provided valuable contextual information regarding the structural properties of swine barns and the surrounding areas. The classification model predicted different farm types with an accuracy of 96%.

Results obtained may enable researchers and policymakers to understand better the spatial distribution and arrangement of swine farms and aid in developing spatially explicit epidemiological models. Moreover, farm geolocations are vital in identifying high-risk areas and potential sources of disease dissemination.

Funding: This project was funded by the Foundation for Food & Agriculture Research (FFAR) award number FF-NIA21-0000000064.

Category: Infectious Disease

MULTIVARIATE LINEAR REGRESSION IN THE ESTIMATION OF WITHDRAWAL INTERVAL IN GOAT EDIBLE TISSUES FOLLOWING THE EXTRALABEL ADMINISTRATION OF FLUNIXIN MEGLUMINE AT A SINGLE DOSE OF 2.2 MG/KG

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Abstract: Flunixin meglumine, a nonsteroidal anti-inflammatory drug (NSAID) is most used in an extra-label manner in goats. Determining an extended withdrawal time is therefore necessary to lower the possibility of any violative residues during slaughter times. The United States Food and Drug Administration (US FDA) fits univariate linear regression model using the ordinary least squares method (OLS) by considering the concentrations above the limit of detection (LOD) and defines the time point as the withdrawal time when the upper 99% tolerance with 95% confidence falls at or below a specified tolerance. As the tissues collected from the goat namely, liver, kidney, muscle, and fat are correlated, therefore multivariate linear regression model is an appropriate choice to address this high dependency among the tissues. In addition to this, the presence of censored observations can also impact the correlations or covariance pattern among the tissues and can result to biased estimators. Thus, the goal of this study is to use the multivariate linear regression model to estimate the withdrawal interval (WDI) in goat tissues in both censored and uncensored scenarios. To accomplish, twenty goats were enrolled and randomly five goats were euthanized at four time points and tissues were collected. This multivariate data was analyzed using three methods namely, Ordinary least squares (OLS), Generalized least squares (GLS) and Expectation-maximization (EM) algorithm. To estimate the WDI, the upper 99% tolerance with 95% confidence was derived using the multivariate non-central t distribution. A simulation study was also performed to check the performance of the models.

Funding Source: Food Animal Residue Avoidance Databank (FARAD)

Primary Subject Category for Presentation: Pharmacology

MAGNITUDE AND DURATION OF PLACEBO EFFECT IN SENIOR DOGS WITH MILD-MODERATE COGNITIVE IMPAIRMENT.

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The placebo effect is a well-documented phenomenon in human medicine where positive effects are observed in a patient after treatment with an inert substance. We recently performed a randomized controlled trial (RCT) in senior dogs with mild - moderate cognitive impairment, allowing us to examine placebo effect in an elderly canine population. Sixteen dogs in the placebo group of this RCT trial were included and eighteen dogs participating in an observational longitudinal study to distinguish placebo effect from effect of trial participation. Cognitive outcome data from two owner assessments (Canine Cognitive Dysfunction Scale (CCDR) and Canine Dementia Scale (CADES)) and three in-house assessments (Cylinder Task, Detour and Sustained Gaze) were analyzed. We hypothesized that the placebo effect would be larger in caregiver-reported outcomes and wane over time, like human dementia trials. A matched pairs t test assessed the difference between baseline and month 3 or month 6 scores. The placebo group improved across all outcomes at 3 months, but significantly so ($p=0.001$) between baseline CADES scores (mean: 32.12 (14.32)) and month 3 scores (mean: 20.31 (3.40)). A significant improvement from baseline ($p= 0.03$) was also observed after 6 months (mean: 25.38 (16.02)). Conversely, the observational study group showed a non-significant decline in both CADES and CCDR score and non-significant improvements in the in-house cognitive assessments after 6 months. We conclude that there is a placebo effect at 3 months when using CADES that wanes by 6 months but does not disappear, and this is larger than trial participation effect alone.

Funding Source: Animal Bioscience, Dr. Kady M. Gjessing and Rahna M. Davidson Distinguished Chair in Gerontology
Primary Subject Category: Neurosciences

OPTIMIZATION OF THE X-RAY IRRADIATION DOSE TO ACHIEVE NEAR-COMPLETE RECONSTITUTION OF ALVEOLAR MACROPHAGES WITH DONOR CELLS WHILE MINIMIZING ANIMAL DISTRESS.

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Lethally-irradiated mice engrafted with bone marrow of genetically disparate donors, are an important tool to investigate the biological contributions of hematopoietic versus non-hematopoietic compartment. Limitations of this technique are 1) graft-versus-host diseases (GvHD), which results from immune-mediated attack of recipient tissue by donor T cells contained in the transplant, and 2) high radiation dose-induced toxicity, symptoms include gastrointestinal injury, inflammation, and mucosal edema. To determine an effective and recipient-friendly irradiation dose that will result in the complete reconstitution of hematopoietic compartments with minimal animal distress, we exposed 8-week-old-C57BL/6 male mice to 1050, 900, 750, and 600 cGy (n=4) using the X-RAD320 irradiator. The irradiated mice were intravenously administered bone marrow cells from donors that ubiquitously express mTomato fluorescence reporter. Body weight, activity, fur texture, skin texture, and posture data were collected twice weekly to monitor GvHD status. After eight weeks, bronchoalveolar lavage (BAL), blood and bone marrow cells were assessed with either fluorescence microscopy or flow cytometry for engraftment efficacy. Additionally, intestine, lung, spleen, and liver were collected to compare dose-response immunopathology. While 1050, 900, and 750 cGy groups showed complete reconstitution with donor cells in BAL, blood and bone marrow, 600 cGy group exhibited mixed chimerism. However, GvHD scoring revealed that mice from 1050 cGy group exhibited reduced weight gain, reduced activity, skin lesions, fur ruffling/loss, skin lesions, and abnormal posture. Based on these data, 900 cGy appears to be the optimal dose, achieving complete depletion of host alveolar macrophages, followed by complete reconstitution with minimal and transient distress.

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Primary subject category: Immunology

OCULAR TOXICITY, DISTRIBUTION, AND SHEDDING OF INTRAVITREAL AAV-EQIL-10 IN HORSES

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Non-infectious uveitis (NIU) is a painful, recurrent disease that affects humans and horses. Adeno-associated virus (AAV) equine Interleukin 10 (eqIL10) was shown in rats to inhibit experimental autoimmune uveitis. This study evaluated ocular toxicity, distribution, and viral shedding following a single intravitreal (IVT) injection of AAV8-eqIL10 in normal horses. Horses were injected in both eyes with vehicle (n=1), AAV8-eqIL10 (3.75e11vg, n=2) (LD), or AAV8-eqIL10 (3.75e12vg, n=2) (HD). Ocular examinations (OEs) were performed routinely until euthanasia (d86). Viral shedding was evaluated in tears, urine, and feces (PCR). Eyes were processed for histology or used to determine transgene distribution (qPCR). Aqueous (AH) and vitreous (VH) eqIL10 levels were measured (ELISA). Multiple tissues were evaluated for systemic distribution (qPCR). OE revealed mild conjunctival hyperemia and chemosis on d1 and d3. One LD horse had mild aqueous flare and conjunctival hyperemia from d28-49. Both HD horses developed keratic precipitates at d70 and d77. Intraocular pressure was lower in the HD eyes (d28-84). EqIL10 was detected in the AH and VH in high levels (>7 ng/ml). Histologically, LD eyes had slight lymphoplasmacytic (LP) infiltrate in the iris and ciliary body, while HD had moderate in the iris, ciliary body, and choroid. Viral genomes (vg) were detected in the iris/ciliary body, retina, AH and VH in treated eyes. In HD only, tear shedding of vgs was detected on d1 and in the liver of one animal. AAV8-eqIL10 (3.75e11 vg) is a promising potential therapeutic that was well-tolerated with high transgene expression constrained to relevant tissues.

Funding from North Carolina Biotechnology Center Translational Research Grant (project funding) and NIH T32OD011130 (support for KSY)
Subject category: Clinical Medicine

DESCRIPTION OF AN ULTRASOUND-GUIDED PUDENDAL NERVE INJECTION IN DOGS: A CADAVERIC STUDY

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The aim of this study was to develop an ultrasound-guided (USG) locoregional technique for perineural injection of the pudendal nerve (PdN) in dogs. Gross anatomy, sonography, and computed tomography (CT) were employed to develop an USG transgluteal approach to the PdN. Fifteen cadavers, nine males and six females, weighing 25.2 ± 6.3 kg were randomized to receive bilateral USG transgluteal injections of ropivacaine-dye solution, either high [(HV) 0.2 mL kg^{-1}]) or low [(LV) (0.1 mL kg^{-1}]) volume. Following injection, cadavers were dissected. Successful nerve staining (>1 cm) and inadvertent staining of the sciatic nerve, or rectal, urethral or intravascular puncture were recorded. Volumes were compared using a mixed-effects ordinal logistical regression model (p = 0.05). The PdN and landmarks for its associated fascial plane (ischium, pelvic fascia and rectum) were defined on CT. Sonographically, landmarks were identified and dye solution was injected into the fascial plane. Complete staining of the PdN was achieved in 69.2% (HV) and 58.3% (LV) of injections. There was no significant difference in nerve staining between groups (p = 0.864), however there was a higher likelihood of success with HV than LV (OR = 1.161). There was no significant difference in sciatic nerve staining between HV (7.7%) and LV (8.3%) (p = 0.719). No rectal, urethral or intravascular puncture were observed. Perineural PdN injection was successful in the majority of canine cadavers using an USG transgluteal approach and 0.2 mL kg^{-1} injection volume. Further studies are needed to investigate the use in live animals.

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Category: pain, clinical medicine