Title: **INVESTIGATING CENTRAL AUDITORY DYSFUNCTION IN A MOUSE MODEL OF LATE-ONSET SPORADIC ALZHIMER'S DISEASE**

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Background: Epidemiological studies have established an association between cognitive decline and age-related hearing loss, though further studies are needed to establish whether hearing loss causes an increased risk of the development of Alzheimer's disease (AD). Apolipoprotein ε4 (APOE4) is the strongest genetic risk factor for late-onset AD. This study utilizes APOE4 mice as AD models and APOE3 mice as controls to test whether genetic predisposition to AD accelerates central auditory dysfunction.

Hypothesis: APOE4 mice will display central auditory deficits by 12 months of age.

Objectives: To observe the relationship between central auditory dysfunction and late-onset sporadic AD.

Methods: Central auditory processing was assessed via gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS). Mice were placed into a sound-attenuating startle chamber with a piezoelectric platform, high-frequency loudspeakers, and acclimated for five minutes (55dB broadband background noise). Startle responses were recorded through the SR-LAB startle response system. Sessions introduced two trial types: startle trials, which presented startle stimuli alone (105 dB broadband noise), or gap trials, where startle stimuli were preceded by a gap (0 dB) with gap lengths of 2, 4, 8, 16, 32, 64, or 256 ms and pseudorandomized trial orders. GPIAS were calculated by dividing averaged gap trial responses by averaged startle trial responses.

Results: We anticipate that 12-month APOE4 mice will show decreased GPIAS compared to the age-matched APOE3 mice.

Conclusion: We are hoping to establish a relationship between hearing loss and cognitive decline to potentially influence the development of biomarkers to slow the progression of AD.

Funding Sources and Student Support:
NIH T35OD011145
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Field of Research: Neuroscience

Title: **GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 VIRUS**

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Background: Highly Pathogenic Avian Influenza (HPAI) presents a significant economic and public health threat due to its persistence within wild bird reservoirs, rapid transmission, and high mortality in domestic poultry. A key reagent for detection of viral infection are monoclonal antibodies (mAb). Thus, we generated and characterized specific mAbs against hemagglutinin (HA) of HPAI H5N1 virus for future use in viral diagnostics and research.

Hypothesis: We hypothesized that mice immunized with H5 HA protein would mount an antibody response against H5N1 viral antigens, facilitating mAb production.

Objectives: 1) Generate mAbs against H5N1 virus via hybridoma technology. 2) Characterize mAb reactivity by different immunological assays. 3) Perform epitope mapping to identify relevant binding sites.

Methods: Harvested splenocytes from mice were fused with myeloma cells to generate hybridomas. MAbs were screened by immunofluorescent assay (IFA), then further tested with Western blotting and ELISA. Epitope mapping was performed using a panel of overlapping peptides.

Results: We generated and characterized a panel of mAbs against HA antigen of HPAI H5N1 virus, including mAbs #29-108, 90-26, and 95-62. Epitope mapping reveals distinct binding sites on either the head or stalk domain of H5N1 HA, verified through IFA. MAbs #29-108 and 95-62 were further tested in western blotting and ELISA. MAb #29-108 demonstrated unique cross-subtype reactivity by recognizing HA stalk domains from both H5N1 and H1N1.

Conclusion: The availability of this panel of mAbs provides an important tool for future development of targeted diagnostic assays and identification of vaccine targets against HPAI H5N1 virus.

Funding Sources & Student Support:
United States Department of Agriculture | National Institute of Food and Agriculture (USDA-NIFA), Grant #2025-39601-44645
Center for Zoonoses Research, College of Veterinary Medicine, University of Illinois

Field of Research: Infectious Disease

Title: **THE EFFECT OF ORAL PREGABALIN ON BEHAVIORAL SCORES, CARDIORESPIRATORY PARAMETERS, AND PROPOFOL REQUIREMENTS AT ANESTHETIC INDUCTION IN DOGS**

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Background: Pregabalin binds to voltage-gated calcium channels on CNS neurons and decreases the release of excitatory neurotransmitters. It is commonly used for anxiety, neuropathic pain, and seizures in dogs; however, its effect on behavior, physiologic variables, and anesthetic requirements has not been formally evaluated.

Hypothesis: Administration of oral pregabalin prior to anesthetic induction will induce anxiolysis and reduce the propofol dosage required for intubation without significant cardiovascular effects.

Objectives: Evaluate the sedative and anxiolytic effects, cardiorespiratory effects, and propofol requirements of orally administered pregabalin in dogs.

Methods: Twelve adult research Beagles underwent a randomized, blinded, cross-over study, receiving oral pregabalin (50 mg) or placebo (lactulose). Sedation and anxiety scores, heart rate, respiratory rate, and non-invasive blood pressures were recorded before treatment administration and 100 minutes after treatment administration. Anesthesia was then induced with intravenous propofol administered to effect to achieve orotracheal intubation. Propofol dosages were recorded and physiologic variables were remeasured following orotracheal intubation.

Results: Propofol requirements for intubation did not differ significantly between treatments, with mean ± standard deviation dosages of 6.2 ± 1.6 mg/kg and 8.0 ± 2.3 mg/kg for pregabalin and placebo control treatments, respectively. There was no difference in sedation scores, anxiety scores, or cardiorespiratory parameters between treatments.

Conclusion: Oral administration of pregabalin prior to anesthetic induction did not significantly affect sedation or anxiety scores, cardiorespiratory parameters, or propofol induction requirements.

Funding Sources & Student Support: Boehringer Ingelheim Veterinary Scholars Program

Field of Research: Clinical Medicine

Title: **LONG-TERM EFFECTS OF DI-(2-ETHYLHEXYL) PHTHALATE (DEHP) AND DIISONONYL PHTHALATE (DiNP) ON CD68-POSITIVE MACROPHAGE ABUNDANCE OF THE COLON IN FEMALE ADULT MICE**

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Background: Di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP) are used in plastic food and water containers for durability and flexibility. Recent research has shown evidence that DEHP and DiNP are endocrine disrupters. The primary route of exposure of phthalates is dietary; therefore, the gastrointestinal (GI) tract is a potential target organ of phthalates. The long-term effects of phthalate exposure on immune homeostasis of the colon and macrophage activity remain unknown.

Hypothesis: This study tested that chronic DEHP and DiNP exposure increases CD68-positive macrophage abundance in the colon compared to the control group.

Objectives: The goals of this project are to 1) investigate whether phthalate exposure affects macrophage abundance in the colon and to 2) perform immunohistochemistry (IHC) staining on exposure and treatment colon samples to compare the abundances of CD68, CD3 (T cells), and MUC2 (goblet cells).

Methods: Adult female mice were fed a mouse chow diet containing either control substance, DEHP, or DiNP for six months. After the dosing period, the mice were euthanized during diestrus, and colon samples were collected for IHC analysis.

Results: We anticipate that the colon samples from mice exposed to higher doses of DEHP and DiNP will show greater abundances of CD68 and CD3 staining than the colon samples from control or lower doses of the phthalates.

Conclusion: The hypothesis that long-term DEHP and DiNP exposure increases CD68-positive macrophage abundance in the colon is expected to be supported. This study shows that DEHP and DiNP exposure may contribute to altered colonic immune responses.

Funding Sources & Student Support:
Boehringer Ingelheim Veterinary Scholars Program

Field of Research: Gastroenterology

Title: **DOES MRI MAGNETIC FIELD EXPOSURE INDUCE TEMPORARY STRABISMUS IN ANESTHETIZED CANINE AND FELINE PATIENTS?**

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Background**:** Magnetic Resonance Imaging (MRI) is a non-invasive diagnostic imaging tool utilizing a strong magnetic field. A disproportionate number of patients have exhibited ocular deviation on head MRI images that are inconsistent with awake eye position or lesion localization. This finding prompted investigation into whether exposure to 3T MRI magnetic field itself transiently influences eye position.

Hypothesis**:** 3T MRI magnetic field exposure induces temporary alteration of normal eye positioning in anesthetized pets.
Objectives: The goal of this study is to determine if eye position alters in animals lying in the magnetic field.

Methods**:** 1 feline and 10 canine patients had a brain MRI (including both entire eyes) under general anesthesia. Eye positions were recorded prior to anesthesia, under anesthesia, during imaging using the T2 DORS and T2 TRV series, and directly after the MRI until initial eye positions returned.

Results**:** 11/11 patients had eye position change in the MRI compared to anesthetized pre-MRI baseline position. 11/11 patients showed changes in anesthetized eye position immediately after exiting the MRI. All patients returned to baseline awake eye positions after recovering from anesthesia.

Conclusion**:** 3T MRI magnetic field exposure appears to induce temporary alteration of normal eye positioning in anesthetized pets, regardless of body position, disease type, and lesion location. This is the first report to document the influence of 3T MRI on eye position in canine and feline patients. This temporary condition appears to be common and resolves after recovering from anesthesia.

Funding Sources & Student Support:
Boehringer Ingelheim Veterinary Scholars Program

Field of Research: Clinical Medicine

Title: **EVALUATING THE EFFECTS OF PERFLUOROOCTANOIC ACID IN MOUSE UTERINE STROMAL CELL DIFFERENTIATION**

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Background: Per- and polyfluoroalkyl substances (PFAS) are a broad class of synthetic chemicals used in a wide range of consumer products and applications. Perfluorooctanoic acid (PFOA), a member of the PFAS family, was historically used to manufacture non-stick coatings, textiles, and firefighting foams. Although no longer produced in the United States, PFOA remains a persistent environmental pollutant linked to adverse health effects in humans and animals. However, little is known about its effects on the decidualization process.

Hypothesis: PFOA will alter the expression of genes associated with decidualization, thereby impacting their function.

Objective: This study aims to identify the effects of PFOA on decidualization using a well-established in vitro model.

Methods: Endometrial stromal cells were collected from untreated pregnant mice and subjected to differentiation conditions with or without PFOA. The treatment groups consisted of a vehicle control (methanol) and two PFOA doses (1 and 10 µg/mL). After 72 hours of culture, cells were collected, and RNA was extracted from decidual cell cultures to assess changes in gene expression by qPCR.

Results: Our data show that PFOA treatment exhibited a U-shaped dose-response, with the low dose altering the gene expression of *Esr1*, *Pgr*, *Cebpb*, *Slc2a1*, and *Angpt1*, whereas *Hand2* showed a dose-dependent decline in gene expression.

Conclusions: Exposure to PFOA at environmentally relevant levels alters genes that regulate decidual processes, potentially compromising the differentiation of uterine stromal cells into decidual cells and affecting pregnancy outcomes.

Funding Sources and Student Support: NIH T35OD011145

Field of Research: Other

Title: **METHOD VALIDATION FOR QUANTIFICATION OF CELL-FREE DNA IN EQUINE PERITONEAL FLUID**

Authors - **Kuhlman R.**, McCoy A., Bishop R.

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Background: Colic is a common potentially life-threatening condition in horses. Cell-free DNA (cfDNA), released during cell injury or inflammation, is a promising biomarker for assessing disease severity and predicting outcome. Quantification of cfDNA in peritoneal fluid (PF) may yield more diagnostic information over plasma in horses with colic, but methodology for PF-cfDNA measurement has not been validated.

Hypothesis: Quantification of PF-cfDNa will be reliable and repeatable.

Objective: Validate PF-cfDNA quantification by fluorometry.

Methods: PF was collected from 66 horses (16 healthy, and 50 colic cases) in plain glass (RTT) and EDTA (LTT) tubes. PF-cfDNA was measured by Qubit 4 fluorometer using the 1xdsDNA HS assay kit. Validation steps included inter- and intra-assay coefficient of variation (CV), spike recovery, and dilution linearity. PF-cfDNA concentration was compared between sample types by Wilcoxon test.

Results: PF-cfDNA concentration was greater in LTT samples (median 1.64 ng/uL [IQR 0.949, 3.0) compared to RTT (0.46 ng/uL [0.259, 0.691]; $p<0.001$). Mean inter-assay variability was lower in RTT (18.6%) than LTT (23.1%), as was intra-assay variability (RTT 22.6%, LTT 31.1%). There was a linear correlation between expected and measured concentrations for both dilution and spike recovery series for both RTT and LTT ($p < 0.05$). There was no significant difference in recovery ($p=0.1$) between sample types (RTT 86.8% [82.3, 98.7]; LTT 99.5% [87.7, 115.8])

Conclusion: Measurement of cfDNA in equine PF was more repeatable when collected in RTT vs LTT. Intra- and inter-assay CV were greater at low cfDNA concentrations. Evaluation of clinical utility is warranted.

Student Support - Summer Research Training Program at University of Illinois, NIH T35OD011145

Field of Research - Clinical Medicine

Title: **DOES LUMASON MAINTAIN ITS ECHOGENICITY AND CONTINUE TO HELP DELINEATE VENTRICULAR WALLS DURING CANINE ECHOCARDIOGRAMS UP TO FIVE DAYS POST-RECONSTITUTION?**

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Background: Proper visualization of the ventricular walls during echocardiograms is important for the diagnosis and monitoring of many cardiac diseases. In human medicine, Lumason, a contrast agent, is used in patients when visualization is difficult. However, the requirement for Lumason to be used within three hours of reconstitution has limited its use in veterinary medicine, as it places the cost of one bottle on one patient regardless of the amount used. However, there is little evidence regarding the stability of Lumason past three hours.

Hypothesis: Lumason remains stable longer than three hours post-reconstitution.

Objectives: Determine whether or not Lumason maintains its echogenicity and improves visibility of ventricular walls up to five days post-reconstitution during canine echocardiograms.

Methods: Lumason was reconstituted at the beginning of the week and administered to patients as they presented to the University of Illinois Cardiology Service. Patients were intravenously administered contrast at 0.03mL/kg. Images were taken pre- and immediately post-contrast in Left Apical 4 Chamber view.

Results: Data will be analyzed using OsiriX to quantify the difference in brightness of the echocardiograph images, allowing for comparison of Lumason's echogenicity at different times post-reconstitution. We expect to not see a significant decrease in echogenicity for up to five days.

Conclusions: If the expected results are correct, then Lumason could be used more frequently in veterinary echocardiograms, potentially allowing for better diagnoses and monitoring of cardiac disease.

Student Support: Boehringer Ingelheim Veterinary Scholars Program, College of Veterinary Medicine, University of Illinois

Field of Research: Clinical Medicine

Title: **THE MYC/TFRC/TP53 EXPRESSION NETWORK AS A DETERMINANT OF CHEMOTHERAPEUTIC RESPONSE IN TESTICULAR GERM CELL TUMORS (TGCT)**

Authors: **Lopez S,** [1], Spinella M[1], Singh R[1]

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Background**:** Cisplatin is a commonly used and effective chemotherapeutic drug for treating testicular germ cell tumors (TGCT). However, a significant percentage of patients developed resistance to cisplatin. Previous studies have indicated a connection between this resistance and target genes associated with *MYC*, *TFRC*, and *TP53*, which are related to the proliferation and survival of cancer cells. Further analysis is needed to better understand the mechanisms that lead to cisplatin resistance (CR) in these cells.

Hypothesis**:** In TGCT, resistance to cisplatin results from failure to activate a coordinated p53-dependent transcriptional program, coupled with persistent MYC-TFRC-driven survival signaling that promotes iron-dependent metabolic adaptation and suppresses cell death

Objectives**:** To characterize differential expression of p53-regulated genes and MYC-TFRC signaling components in cisplatin-sensitive and isogenic CR TGCT models.

Methods**:** We analyzed the response of parental and CR TGCT cell lines to cisplatin treatment using qPCR.

Results**:** RT-PCR was performed on p53 target genes and *MYC-TFRC* pathway components to compare cisplatin response in parental and CR cells. Analysis in cells treated with cisplatin revealed that, p53 target genes are upregulated in parental cells (PC), whereas a lack of expression in CR cells. Additionally, *p53,* and *TFRC* target genes are upregulated in PC, whereas in CR the expression does not change significantly. Lastly, *MYC*, *TFRC* and *p53* target genes are upregulated in PC, and no expression in CR cells.

Conclusion**:** The upregulation of P53 in PC supports the p53 pathway leading to apoptosis whereas the lack of apoptosis in CR cells explains their proliferation and survival.

Funding and Student Support**:** Boehringer Ingelheim Veterinary Scholars Program, College of Veterinary Medicine, University of Illinois

Field of Research – Cell Biology

Title: **PREVALENCE OF CHLAMYDIA SPP. IN WILD BIRDS IN CENTRAL ILLINOIS**

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3. Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA.

Background: Chlamydia spp., an obligate intracellular bacteria, is a global threat to public health and safety. Chlamydia psittaci infects birds, is zoonotic, and is transmissible to food animals, horses, and people. The most studied avian species belong to the Psittaciformes order, with the highest prevalence followed by Galliformes, Columbiformes, and Anseriformes. Chlamydia spp. diagnosis is typically confirmed by PCR analysis from conjunctiva, choanae, or cloaca samples.

Hypothesis: The prevalence of chlamydia in passerines will be higher than that of raptor species.

Objectives: Passeriformes are understudied in this disease process, and this project aims to bridge that knowledge gap as well as survey the central Illinois region.

Methods: The 15 most common bird species presenting to the Wildlife Medical Clinic were eligible to be sampled and tested at the Infectious Diseases Laboratory at the University of Georgia. Collection methods utilize sterile swabs from the conjunctiva and choanae, as well as cloaca. When possible, blood was collected for serum titer analysis if patients tested positive by PCR.

Results: To date, the sample size (n = 16) includes individuals of all five avian orders represented by the targeted species. Data is insufficient to evaluate the hypothesis. This study is ongoing.

Conclusion: Further surveillance will be beneficial for public health. Clinical presentation is highly variable between species and compatible signs have not been seen in individuals sampled. Additionally, this bacteria is intermittently spread. This initial study will provide a better understanding of the potential risks with the spread of Chlamydia across central Illinois.

Funding Sources and Student Support: Center for Zoonoses Research, College of Veterinary Medicine, University of Illinois

Field of Research: Infectious disease

Title: **CAN WE USE HIV POINT-OF-CARE TESTS TO ACCURATELY DETECT SIVcpz ANTIBODIES IN CHIMPANZEE FECAL EXTRACTS?**

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Background: Simian Immunodeficiency Virus (SIV)cpz, the precursor to HIV-1, causes AIDS-like immunopathology in chimpanzees. Infection status in wild chimpanzees is monitored using enhanced chemiluminescent (ECL) Western Blot (WB) with fecal antibody extracts and confirmed with sequencing. Due to ECL equipment needs, fecals must be exported outside of Tanzania posing biosafety risks, requiring multiple permits, and delaying results. For field researcher safety, real-time, onsite screening is critical.

Hypothesis: We hypothesized that an HIV POC test is at least 90% as sensitive as ECL-WB for diagnosing SIVcpz in chimpanzee fecal samples.

Objectives: 1) Extract chimpanzee antibodies from fecals and run ECL-WB for SIVcpz. 2) Compare efficacy of commercial HIV-1 with modified WB strips. 3) Compare commercial HIV-1 WB strips with HIV POC assays.

Methods: Antibodies were extracted from chimpanzee fecal samples (n= 114). Extracts (n=27) were tested by ECL-WB using commercial HIV-1 strips and modified strips with HIV p24 protein and human IgG and results compared. Three commercial HIV POC assays were tested using neat and antibody concentrated fecal extracts.

Results: Irrespective of chimpanzee SIV status, POC assays were either all negative or all positive using neat and concentrated fecal extracts. Modified strips with p24 antigen alone had 100% sensitivity and 70% specificity compared to commercial HIV-1 strips.

Conclusion: HIV POC tests cannot be used with chimpanzee fecal antibody extracts for SIV testing. Modified WB strips with only HIV p24 and anti-human IgG controls are sensitive and can be used in place of commercial HIV-1 WB strips that are no longer available.

Funding Sources & Student Support:
Boehringer Ingelheim Veterinary Scholars Program, College of Veterinary Medicine, University of Illinois

Field of Research: Infectious Disease

Title: **The purr-fect solution? The effect of estrogen implant treatment on estrous behavior, ovarian activity, and hormone cyclicity in female cats**

Authors: **Modica G[1]**, Reinacher L[1], Vasetska A[1], Soto S[1], Ko C[1,2]

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Background: Reproductive behaviors and cyclicity in mammals are governed by neonatal organization of the hypothalamic-pituitary-gonadal axis. In female rodents, neonatal treatment with estradiol (E_2)-based implants disrupts these pathways, permanently suppressing sexual receptivity and ovulation despite persistent ovarian function. However, this non-surgical sterilization approach remains unverified in felines.

Hypothesis: Feline non-surgical sterilization via early estrogen exposure is dependent on treatment dose, duration, and timing; specifically, neonatal treatment with a single E_2 implant will permanently suppress estrous behavior and ovulation, despite endocrine cyclicity and follicular development.

Objectives: We evaluated the effects of neonatal E_2 implant treatment on estrous behavior, follicular development, endocrine cyclicity, and pregnancy outcomes.

Methods: Female kittens received a single sustained-release E_2 implant (0.5 or 1.0 mg) at postnatal days 7, 14, 28, or 56, or served as controls (EB oil, untreated, or spayed). At 8 months of age, cats were exposed to an intact male (40 hours/week) for a 1-month study period. Video monitoring quantified rolling, treading, lordosis, and successful intromission, while fecal progesterone tracked ovulation and cyclicity.

Results: While E_2 implants at postnatal days 14, 28, and 56 did not prevent pregnancy or the display of estrous behaviors under the tested parameters, evaluation of the earliest treatment group (postnatal day 7) is ongoing to assess potential sexual developmental disruption.

Conclusion: These findings suggest that successful feline non-surgical sterilization may require optimizing implant timing, dosage, and release duration, or targeting alternative hormonal pathways. The efficacy of early intervention remains pending complete evaluation of the postnatal day 7 cohort.

Funding Sources & Student Support: NIH T35OD011145, NSF SBIR Phase I 2415687

Field of Research: Translational Research

Title: **DIAGNOSTIC UTILITY OF DIFFERENT IMAGING MODALITIES IN AN EQUINE MODEL OF EARLY POST-TRAUMATIC OSTEOARTHRITIS**

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Background: Post-traumatic osteoarthritis (PTOA) is a progressive, degenerative disease that commonly affects horses. Radiographic changes typically occur late in the disease, prompting investigation of other imaging modalities to diagnose this disease in its earlier stages. Early diagnosis would facilitate treatment to slow down the progression of clinical signs.

Hypothesis: Radiographs are not as helpful in diagnosing early PTOA as Magnetic Resonance Imaging (MRI), and arthroscopy is best to evaluate intraarticular health.

Objectives*:* Evaluate the effectiveness of different imaging modalities in detecting experimentally induced early PTOA in the metacarpophalangeal joint (MCPJ) in horses.

Methods: Thirteen Standardbred horses underwent induction of PTOA using an equine MCPJ chip model. Arthroscopic images and MRIs (n=7 horses) were taken of both the injured and sham-operated joints every 16 weeks. Radiographs were taken every 4 weeks. Images were evaluated blindly after the conclusion of the 32-week study period.

Results: Mild joint pathology (synovial tissue thickening and atrophy, superficial cartilage scoring) was seen medially and laterally on arthroscopy images in both operated and sham joints. Radiographs showed mild changes to the joint, and the fragment was inconsistently visible. MRI did not consistently offer a better view of the fragment, though mild changes were also seen in the joint.

Conclusion: Neither MRI nor radiographs are sufficiently sensitive to diagnose very early/mild PTOA. More reliable ways of detecting early changes within the joint are needed to make a diagnosis earlier in the progression of disease. Future work includes evaluation of molecular biomarkers (gene/protein expression) in this PTOA model.

Funding Sources and Student Support*:*
NIH T35OD011145
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Field of Research: Clinical Medicine

***Title*: FUNCTIONAL CHARACTERIZATION OF CANINE MONOCYTE-DERIVED DENDRITIC CELLS TO SUPPORT STANDARDIZED IMMUNOTHERAPY DEVELOPMENT**

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4. Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, IL

Background: Dendritic cells are professional antigen-presenting cells that play a crucial role in mobilizing an immune response. Monocyte-derived dendritic cells (moDCs) are central to dendritic cell-based immunotherapies; however, standardized methods for generating functional canine moDCs remain lacking. Recent work suggests that culture conditions substantially influence canine moDC phenotype, but their effects on antigen processing and activation remain incompletely characterized. Establishing standardized culture conditions will provide a critical foundation for the development of canine dendritic cell-based immunotherapies.

Hypothesis: Culture conditions influence the functional phenotype of canine moDCs, including antigen processing and activation.

Objectives: 1) Compare antigen uptake and processing among canine moDC culture conditions via DQ-ovalbumin (DQ-OVA) assay. 2) Characterize activation-associated cytokine secretion across culture conditions.

Methods: Canine moDCs generated under eight culture conditions (serum versus serum-free media, varying IL-4 and GM-CSF concentrations) were incubated with DQ-OVA to quantify antigen uptake and processing by flow cytometry. Protease inhibition (E-64) and low-temperature (4°C) controls were included to verify assay specificity. Activation-associated cytokine secretion is being evaluated by ELISA to further characterize functional differences among culture conditions.

Results: Serum-free culture conditions demonstrated the greatest DQ-OVA fluorescence, consistent with enhanced antigen uptake and processing compared with serum-containing conditions. Cytokine analyses are being performed to further characterize the relationship between antigen processing and moDC activation across culture conditions.

Conclusion: Culture conditions influence functional characteristics of canine moDCs, with serum-free conditions enhancing DQ-OVA uptake and processing. These findings support continued investigation of serum-free culture systems for generating functionally distinct canine dendritic cells and complement ongoing studies evaluating dendritic cell activation.

Funding Sources & Student Support:
University of Illinois, NIH T35OD011145 Interdisciplinary Biomedical Research Training Program

***Field of Research*:** Immunology

Title: **QUANTIFYING THE IMPACT OF EXTERNAL TANK ENRICHMENT ON ZEBRAFISH (*Danio rerio*) STRESS AND THE GUT MICROBIOME**

Authors: **Sawin C.**, Alvarado D., Gaulke CA.
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Background: Enrichment is understood to increase mental stimulation and reduce stress in mammalian model organisms; while in zebrafish, less is known. Specific enrichment items have been shown to reduce stress while others may increase aggression or have little to no effect. It is also currently unknown whether enrichment causes changes in the zebrafish gut microbiome which may contribute to physiological responses to these enrichment items.

Hypothesis: We hypothesize that enrichment will reduce zebrafish stress and correlate with increased gut microbial species richness and reduction in pathobionts.

Objectives: The objectives of this experiment are to 1) quantify stress in zebrafish with different enrichment using Novel Tank Tests (NTT) and cortisol ELISA, 2) measure fecal microbial diversity, and 3) evaluate associations between stress and gut microbiome composition

Methods: Adult tropical 5D line zebrafish were divided into an enrichment (N = 15) and control (N = 15) groups and individually housed for 4 weeks. Fecal sampling and NTTs were performed weekly to assess stress in each animal. Cortisol was measured in the trunk and skin mucus via ELISA and the microbiome composition of fecal samples will be assessed by 16S amplicon analysis.

Results: Preliminary results suggest a trend of reduced stress behaviors with exposure to external tank enrichment, particularly in males. Cortisol and microbiome measurements are currently ongoing.

Conclusion: Indicated trends of the NTTs suggest lower stress in zebrafish with external tank enrichment. Final analysis along with cortisol and gut microbiome assays will further determine whether enrichment, stress, and the gut microbiome are interconnected.

Funding Sources & Student Support:
NIH, NIEHS R01ES036174
Center for Zoonoses Research, College of Veterinary Medicine, University of Illinois

Field of Research: Other

Title: **INVESTIGATING THE SEX-DEPENDENT ROLE OF SEC16B IN BROWN ADIPOCYTE DIFFERENTIATION IN VITRO**

Authors: **Senthil P.**[1], Yang T.[1], Johnson K.[1,2], Lu W.[1], Zhang X. [1], Wang B.[1,2,3]

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Background: SEC16B has been linked to obesity through genome-wide association studies. Our previous *in vivo* studies showed that female *Sec16b* knockout (KO) mice exhibited greater body weight and fat accumulation than female wild-type (WT) mice under a high-fat diet, whereas minimal effects were observed in males. This suggests that SEC16B regulates adiposity and energy metabolism in a sex-dependent manner. Brown adipocytes differentiated from brown adipose tissue-derived stromal vascular fraction (SVF) cells play critical roles in energy metabolism. Therefore, this study investigated whether SEC16B directly regulates brown adipocyte development in a cell-autonomous and sex-dependent manner.

Hypothesis: *Sec16b* deficiency impairs brown adipocyte differentiation more strongly in female-derived cells than in male-derived cells.

Objectives: To compare brown adipocyte differentiation between female and male WT and *Sec16b* KO SVF cells.

Methods: SVF cells were isolated from WT and Sec16b KO mice, immortalized, expanded, and induced to differentiate into brown adipocytes. Oil Red O staining was used to assess lipid accumulation.

Results: Female *Sec16b* KO cells showed reduced lipid accumulation compared with female WT cells, whereas little difference was observed between male KO and WT cells.

Conclusion: SEC16B may contribute to brown adipocyte differentiation in a sex-dependent manner, with its deficiency exerting a greater impact in female-derived cells. Future studies will evaluate the expression of genes involved in adipogenesis and energy metabolism by qPCR and investigate underlying mechanisms.

Funding Sources & Student Support:
Office of the Director, NIH T35OD011145

Field of Research: Cell Biology

Title: **ROLE OF VAMP8 PROTEIN IN RESTRICTING INTRACELLULAR *TOXOPLASMA GONDII* GROWTH IN RAT MACROPHAGES**

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Background: *Toxoplasma gondii* is a zoonotic parasite causing serious congenital and reproductive diseases, and life-threatening diseases in immunocompromised individuals, with limited therapeutic options. Intracellularly, *T. gondii* evades host defenses by replicating in a nonfusogenic parasitophorous vacuole. By RNA-sequencing, we found that *T. gondii*-refractory Lewis rat upregulates Vesicle-Associated Membrane Protein 8 (VAMP8) in response to infection. VAMP8 is an endolysosomal SNARE that mediates phagosome-lysosome fusion.

Hypothesis: VAMP8 in *T. gondii*-infected cells drives fusion of lysosomes to parasitophorous vacuole membrane (PVM) leading to degradation of parasites by lysosomal enzymes.

Objectives: Determine the role of VAMP8 in restricting intracellular growth of *T. gondii* in macrophages.

Methods: Knockout of VAMP8 in rat macrophages was achieved by CRISPR/Cas9. WT and VAMP8 knockout cells were infected with *T. gondii* at 1:10 multiplicity of infection (MOI). At various time intervals, parasite growth was determined by fluorescence microscopy and qPCR.

Results: VAMP8 knockout macrophages showed significantly higher parasite fluorescence intensity and *T. gondii* genomic DNA than WT cells, indicating that VAMP8 plays a role in restricting intracellular growth of *T. gondii*.

Conclusion: Loss of VAMP8 promotes intracellular parasite proliferation. Since VAMP8 is an endolysosomal SNARE, it can be postulated that expression of VAMP8 facilitates fusion of lysosomes to the otherwise nonfusogenic PVM, leading to lysosomal enzyme-mediated degradation of intravacuolar parasites. Thus, upregulation of VAMP8 expression may be a promising host-directed anti-*T. gondii* therapeutic strategy.

Funding Sources And Student Support: Boehringer Ingelheim Veterinary Scholars Program, College of Veterinary Medicine, University of Illinois

Field Of Research: Cell Biology

Title: **OPTIMIZATION OF AN ENDPOINT PCR ASSAY FOR MOLECULAR IDENTIFICATION OF *BLASTOMYCES* SPECIES FROM ARCHIVED CANINE CYTOLOGY SLIDES**

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Background**:** Canine blastomycosis is a systemic fungal disease caused by *Blastomyces dermatitidis* and *Blastomyces gilchristii*. Although once considered a single species, molecular studies have identified genetic differences between these pathogens. Human studies suggest species-specific differences in disease presentation, but their significance in dogs remains unclear. Reliable molecular identification is needed to investigate species-associated disease patterns in canine blastomycosis.

Hypothesis**:** Optimization of primer design and PCR conditions will improve amplification of *Blastomyces* DNA from archived canine cytology slides.

Objectives**:** To optimize an endpoint PCR assay targeting the ITS2 region for future species identification by Sanger sequencing.

Methods**:** DNA was extracted from archived canine *Blastomyces* culture and cytology slides. Following elimination of laboratory contamination, redesigned primers were evaluated using Phusion High-Fidelity PCR Master Mix. Primer concentration, annealing temperature, agarose concentration, and electrophoresis conditions were optimized to maximize specific amplification and minimize primer-dimer formation.

Results**:** Preliminary optimization identified 0.1 µM as the best-performing primer concentration. A 1.3% agarose gel run at 98 V and 60 mA improved resolution of the expected 451-bp amplicon. Annealing-temperature optimization is ongoing.

Conclusion**:** Optimization of this assay is essential for reliable sequencing and molecular identification of *Blastomyces* species from archived canine cytology slides. The finalized protocol will support future studies of species-specific epidemiology and clinical disease in dogs.

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Title: **HSD11B1 GENE AND PROTEIN EXPRESSION ACROSS MALIGNANT CANINE CELL LINES**

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Background: 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) converts inactive cortisone into biologically active cortisol, providing a mechanism for local glucocorticoid activation within tissues. Intratumoral cortisol production has the potential to promote an immunosuppressive tumor microenvironment, making HSD11B1 an emerging therapeutic target in oncology. However, expression has not been characterized across canine cancers.

Hypothesis: We posit that HSD11B1 expression varies among canine tumor types.

Objectives: To utilize a panel of immortalized canine cell lines (eleven) to measure and compare gene and protein expression of HSD11B1 across various tumor types.

Methods: Eleven cell lines, including ten malignant cell lines representing five different tumor types (hemangiosarcoma, osteosarcoma, soft tissue sarcoma, oral melanoma, and glioma), were cultured in triplicate. HSD11B1 gene expression was quantified by RT-qPCR using three reference genes for normalization. Protein expression was assessed by western blot following total protein normalization by BCA assay.

Results: Six of eleven cell lines exhibit robust gene expression (mean Ct<35) and while two additional lines amplified at lower levels (mean Ct= 35.6 and 36.1). Expression was most consistently detected amongst Hemangiosarcoma (2/2) and osteosarcoma (3/3) cell lines. Preliminary western blot analysis demonstrates HSD11B1 protein expression in multiple cell lines; however, optimization and quantification remain ongoing.

Conclusion: HSD11B1 expression varies among canine tumor cell lines, with the most consistent gene expression observed in hemangiosarcoma and osteosarcoma cell lines. These findings establish a foundation for future studies evaluating relationships among HSD11B1 gene and protein expression, enzymatic activity, and local glucocorticoid metabolism in canine cancers.

Funding Source/Student support:
University of Illinois NIH 2T35OD011145

Field of research: Cell Biology

Title: **COMPARING COGNITIVE FUNCTION AND LEARNING IN TAME AND CONVENTIONAL RED FOXES (*Vulpes vulpes)***

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Background: Domestication and artificial selection have shaped companion animals for docility, yet their effects on cognition remain poorly understood. The red fox provides a valuable model for studying domestication through the populations created in the Russian Farm-Fox Experiment. Beginning in 1959, the experiment selectively bred conventional foxes solely for friendliness, resulting in a tame population that is sociable towards humans. We had the opportunity to study the cognition of these tame and conventional foxes by observing their interactions with a puzzle enrichment toy created for dogs.

Hypothesis: We predict tame foxes to outperform conventional foxes in novel puzzle solving.

Objectives: 1) Determine whether selection for sociability influences cognition and learning. 2) Evaluate the effect of age on performance.

Methods: 25 foxes (16 tame, ages 1.5-9 years; 9 conventional, ages 1.5-11 years) were recorded during 10-minute interactions with a novel food puzzle containing four boxes requiring different strategies. One day later, foxes were presented with a second puzzle, in which two boxes were repeated and two were novel. Behaviors were scored using an ethogram, and statistical analysis was performed in RStudio.

Results: Data analysis is ongoing. We anticipate that tame foxes will approach and solve the puzzles more quickly and exhibit greater improvement on repeated boxes during the second puzzle. We also expect age will not be significant.

Conclusion: This study will clarify whether selection for sociability is associated with differences in cognitive abilities. These findings will inform how artificial selection for docile behavior influences learning, problem-solving, and cognitive performance.

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Field of Research: Other

Title: **UNCOVERING THE GENETIC STRUCTURE OF ANDROGEN REGULATION: INSIGHTS FROM F1 HYBRID MALE THREE-SPINED STICKLEBACK DERIVED FROM ECOTYPES THAT DIFFER IN PARENTAL CARE**

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Background: Parental care is critical for reproductive success, yet the genetic correlations of hormones involved in this behavior remain poorly understood. Nova Scotian three-spined stickleback ecotypes have recently diverged in paternal care: the common ecotype exhibits paternal care, whereas the white ecotype does not. Previous studies showed that common males exhibit a decline in 11-ketotestosterone (11-KT) by four days post-fertilization (d4pf), accompanied by increased parental behaviors, whereas white males do not. Reciprocal laboratory-generated F1 hybrids provide a model to investigate the genetic basis of hormone-mediated reproductive behaviors.

Hypothesis: We hypothesized that F1 hybrids would exhibit 11-KT profiles resembling those of their paternal lineage and that 11-KT concentrations would negatively correlate with nest-oriented reproductive and parental behaviors.

Methods: Male F1 hybrids were housed individually with nesting material. Nest-oriented courtship and parental behaviors were recorded for 15 minutes during nesting phase and at d4pf. Waterborne hormones were collected >24 hours at nesting and at d4pf, steroids extracted, and 11-KT quantified using a competitive ELISA.

Results: Preliminary results show that F1 hybrids with white paternal lineage exhibited stable 11-KT concentrations from nest building to d4pf, along with higher rates of egg cannibalism and reduced parental behavior, whereas hybrids with common paternal lineage exhibited a decline in 11-KT, but lower rate of egg cannibalism.

Conclusion: Reciprocal F1 hybrids may exhibit androgen profiles resembling those of their paternal ecotype, suggesting that genes regulating 11-KT may be Y-linked. These findings provide insight into the evolution of hormone-mediated paternal care.

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Boehringer Ingelheim Veterinary Scholars Program

Field of Research:
Animal Behavior; Genetics and Biochemistry