Differentiation of early and late lytic gene expression using a Marek's disease alphaherpesvirus model

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The Herpesviridae family of viruses establishes a latent infection in hosts that can last for life, reactivating to produce infectious virions. Little is known about the genes involved in latency and reactivation. We are addressing these questions using Marek's disease virus (MDV), an alphaherpesvirus that affects poultry. Lymphocytes are targeted for latent infection and can be transformed to tumors. We constructed a recombinant MDV (v2001) that expresses monomeric red fluorescent protein (mRFP) inserted in frame with the early lytic RLORF4 gene (RLORF4mRFP) and enhanced green fluorescent protein (eGFP) inserted in frame with the late lytic UL47 gene (UL47eGFP). We hypothesize that viruses in the early lytic phase express only RLORF4mRFP and viruses in the late lytic phase express both RLORF4mRFP and UL47eGFP. Our goal is to develop transformed cell lines in which we can separate cells at early and late phases of lytic replication following reactivation. This approach will allow us to identify viral genes important for reactivation. Seven-day-old chickens (n=11) were infected with v2001. At 7, 14, and 21 days post-infection, the spleen, bursa of Fabricius, and thymus were collected from infected chickens and used to prepare cell suspensions of infected lymphocytes. Flow cytometry was used to detect viral antigens and host surface markers. B and T lymphocytes could be differentiated with this technique, but infection could not be verified. We are also developing ex vivo cell lines from tumors induced by v2001. The long-term goal of this project is to identify proteins important for reactivating herpesviruses from latently infected lymphocytes that could be used in the development of drug or antibody targets.

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Adverse azole antifungal effects: a retrospective study of fluconazole, itraconazole, ketoconazole

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Fluconazole, itraconazole, and ketoconazole are the most-common medications prescribed for canine fungal diseases, such as blastomycosis, aspergillosis, and dermatophytosis. These medications may have adverse effects with long-term usage. The most-common, serious adverse effect is hepatotoxicosis, usually characterized by an increase in alanine transaminase activity (ALT). The goal of this study is to use retrospective analysis of University of Illinois Veterinary Teaching Hospital records to identify factors that predispose dogs to azole-induced hepatotoxicity. Records were included for dogs i) at least 6 months of age receiving systemic fluconazole, itraconazole, or ketoconazole, ii) with at least 3 months of therapy, and iii) monitoring with serial biochemistry profiles. Exclusion criteria included i) systemic illness other than fungal infection, ii) medications known to increase liver enzyme activity, iii) steroid use at the initiation of antifungal therapy, and iv), steroid use for > 14 days at or after antifungal therapy initiation. Hepatotoxicity was defined as an ALT increase above the reference range. Medical records will be separated into groups according to whether or not the patient developed hepatotoxicity. Possible predisposing factors will be compared between groups. Fisher’s exact test or Chi Square test will be used for non-continuous data, such as type of drug. Depending on normality, a Student’s t test or Mann-Whitney U test will be used for continuous data including drug dose and patient age. Identifying predisposing factors for azole-induced hepatotoxicity will help clinicians choose antifungal medications for individual patients with the goal of reducing the incidence of adverse drug reactions.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

Can IS711 be used as a surrogate for MLST genotyping of *Brucella ceti* in wild cetaceans?

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*Brucella ceti,* identified in dolphins, whales, and porpoises beginning in the 1990s, has 3 main sequence types (ST): ST23, ST26, and ST27, which vary by species and geographic region. *B. ceti* produces a range of pathologic lesions and can induce abortions, negatively affecting already-declining wild
cetacean populations. Additionally, as the human-cetacean interface increases, so does the need for a rapid genotyping assay to determine the zoonotic risk to exposed individuals, as at least ST27 has been identified as zoonotic. Although the ST of \textit{B. ceti} isolates can be differentiated using the nine-gene multilocus sequence typing (9-MLST), the method is cumbersome for diagnostic testing. An insertion sequence called \textit{IS}711 can be used to differentiate between STs of many \textit{Brucella} species, but it is unknown if it can differentiate among \textit{B. ceti} STs. Here, we investigate the use of \textit{IS}711 genotyping for differentiation of ST23, ST26, and ST27 in \textit{B. ceti} isolates. We hypothesize that \textit{IS}711 genotype will be predictive of 9-MLST genotype, allowing for the identification of \textit{B. ceti} type by a single PCR. Both the \textit{IS}711 and 9-MLST methods were used to genotype \textit{B. ceti} isolates (n=56) that were collected from cetacean tissues on necropsy. 9-MLST genotyping identified the isolates as ST23 (n=3), ST26 (n=25) and ST27 (n=28). Preliminary comparisons indicate that the \textit{IS}711 genotyping may provide a more-rapid method for distinguishing among \textit{B. ceti} STs of pathogenic importance.

Research Grant: University of Illinois College of Veterinary Medicine
Student Support: University of Illinois College of Veterinary Medicine

**Variation in cytosine arabinoside protocols based on dosage and route of administration in canine patients**

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Cytosine arabinoside (CA) is one of many chemotherapeutics used as an immunosuppressant or anti-neoplastic agent to treat a variety of diseases in veterinary medicine. It is particularly useful in the treatment of diseases of the central nervous system due to its ability to cross the blood brain barrier. However, given its side effects (myelosuppression, GI disturbance, hepatotoxicity), it is critical to monitor patients that receive CA. The goal of this study is to collect and compare data about CA treatment protocols in veterinary medicine. Delegates of the American College of Veterinary Internal Medicine were offered a 26-question survey via email about the administration, monitoring, and side effects in dogs being treated with CA. The University of Illinois Web Services Toolbox was used to collect and collate responses. Survey data were tabulated for each response and for selected cross-tabulations. Compiled survey data were compared statistically to similar data extracted from records of dogs treated with CA at the University of Illinois Veterinary Teaching Hospital. Results of this study will provide insight into best practices for use of CA in veterinary medicine.
Essentiality and functional analysis of Calcium Dependent Protein Kinase 1 (CDPK1) in Cryptosporidium parvum

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Cryptosporidium parvum, a protozoan parasite, is a leading cause of diarrheal disease in young children and agricultural animals. There is no effective drug to treat cryptosporidiosis, which makes C. parvum highly relevant to veterinary medicine and research. The development of novel therapeutics against C. parvum has been challenging since there is limited understanding of the parasite biology. With new molecular genetic tools now available for C. parvum, it is possible to dissect gene function. One of the leading drug targets in C. parvum is the Calcium Dependent Protein Kinase-1 (CDPK1). The ortholog of CpCDPK1 in other protozoan parasites is essential for parasite growth. However, there is no information available regarding the essentiality of CDPK1 for C. parvum. To determine essentiality of CDPK1, we used the genome-editing CRISPR/Cas9 tool and immunocompromised mouse model system to create a transgenic CDPK1 knockout strain. To create this strain, we attempted to delete the cdpk1 gene and replace it with a sensitive luciferase reporter fused to a drug-resistance marker. Fecal luminescence measurements were performed to monitor the emergence of drug-resistant parasites. When compared to our positive control, there was no rise in luciferase readings for the CDPK1 knockout strain, indicating that the CDPK1 was essential for C. parvum. We are repeating our experiments to confirm our results. We also created another transgenic strain to append an epitope tag to the cdpk1 gene. Efforts are underway to purify these tagged transgenic oocysts and determine the localization of CDPK1 in the parasite. Discovery and validation of new drug targets will aid in development of effective treatments against cryptosporidiosis.

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Student Support: Office of the Director, NIH, T35 OD011145
Effect of dose rate on expression of immunologic mediators in irradiated tumor cells

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Radiation therapy can elicit a systemic immune response against tumor cells and improve the effectiveness of cancer treatment. Extended radiation treatment times at low dose rates can allow for repair of DNA damage and we hypothesized that this could impact the interaction between the cancer cell and the immune system. The goal of this study was to compare the effect of different radiation dose rates on two cell lines representing tumor types that have been treated with immunotherapy in dogs: osteosarcoma (OSA) and melanoma. Cells (400,000/well in 6-well plates) were irradiated with 8 Gy using a Co-60 at 25 cGy/min or a Linear Accelerator (Linac) at 20, 400, or 1000 cGy/min, then harvested at 2 h and 24 h. RT-PCR was used to quantify changes in gene expression of immunologic endpoints including MHC-I, Fas-L, PD-L1, and HMGB-1. Comparisons to untreated control at 24 h post treatment showed the greatest differences. Low-dose-rate treatment had similar results of gene downregulation, except for MHC-I where Co-60 showed upregulation. Inhibition of PD-L1 is a popular strategy for immunotherapy, however, our results revealed no consistent pattern in its expression and thus an insensitivity to dose-rate. Unexpectedly, HMGB-1 (marker of DNA damage) appeared to be downregulated at the 24-hour time point. The most remarkable finding was Fas-L, with a 75-fold increase in the OSA cell line at the highest dose rate and over a 3-fold increase in the melanoma cell line at the standard dose rate (400 cGy/min). Expression of genes related to the response of canine melanoma and OSA cells to the immune system can vary with dose rate of radiation therapy. These findings could impact the use of radioimmunotherapy in cancer treatment.

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Metformin is a biguanide oral antihyperglycemic agent used in the treatment of type 2 diabetes. Metformin intoxication can occur in pet dogs that ingest the prescription drug that is intended for human use. Most cases will resolve with fluid therapy and the prescription of gastrointestinal (GI) protectants. The Animal Poison Control Center (APCC) is a 24-hour consultation service that provides support to poisoned animals through advising both animal owners and licensed veterinarians across North America and collecting case information to be stored in the AnTox database. In this study, the AnTox database was searched for calls regarding metformin monointoxications in dogs to specifically search for common characteristics in previous cases that led to the development of hypoglycemia. Between the years 2001 and 2017, the APCC received 1,833 calls regarding metformin monointoxication and 4,281 regarding multiple-point intoxications with metformin. Twenty-four dogs ingested only metformin and developed hypoglycemia. Doses ranged from 29.76 mg/kg to 859.80 mg/kg, with a mean of 259.02 mg/kg. The other 1,809 dogs developed signs of GI upset, lactic acidosis, or no clinical signs. In these cases, the average metformin dose was 202.67 mg/kg, with a range of 1 to 3,000 mg/kg. Dogs that developed metformin-induced hypoglycemia were generally young, smaller breeds (0.2 – 2 years of age). There did not appear to be a link between metformin dosage and the development of hypoglycemia. Identifying the variables associated with metformin-induced hypoglycemia would improve monitoring of cases. This information is important because severe hypoglycemia can lead to a worse prognosis and more lethal sequelae, such as seizure and death.

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Student Support: University of Illinois College of Veterinary Medicine

Predicting West Nile: a spatial analysis of human activity and environmental factors in Cook County, Illinois

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In 2002, northeast Illinois experienced a large outbreak of West Nile virus (WNV) with higher-than-average human caseloads. WNV continues to be a significant human infectious disease in the area. The WNV transmission cycle is dependent on a mosquito vector, Culex sp., which feeds on birds preferentially over mammals, and is most active during the dusk hours. Using multisource data, a model was developed to predict human cases within
1000-meter hexagon sites in the Cook and DuPage counties of northeastern Illinois. This model ran its predictions from the years 2005 to 2016 while creating residual values to describe prediction accuracy against recorded human cases. In this study, 30 hexagon sites within the Northwest Mosquito Abatement District of Cook County, with varying residual values, were selected for human observation and interview. These data were collected to determine differences in outdoor activity between sites during the hours from 5 pm to 9 pm which may account for differences between predicted and observed human WNV illness. Mosquito infection rate, catch basin location, open water bodies, and percent forest data will be evaluated, as well, to further describe differences between sites. Results from this study may allow researchers to further increase accuracy of the prediction model and improve public health in the region. With over 46,086 cases and 2,017 deaths from WNV in the U.S. since its introduction in 1999, there is an urgent need for improved preventative practices and tools.

Research Grant: Centers for Disease Control and Prevention
Student Support: Office of the Director, NIH, T35 OD011145

A retrospective study of prognostic indicators for survival in eastern grey squirrels (Sciurus carolinensis)

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The eastern grey squirrel (EGS), Sciurus carolinensis, is a tree squirrel native to the eastern United States. This species commonly presents to wildlife medical clinics for a variety of human-related injuries including road traffic and pet predation. The purpose of this study was to determine prognostic indicators for survival in young or orphaned EGS. Retrospective data were collected from January 1, 2012 to June 12, 2018 for all EGS weighing less than 300 g that presented to the Wildlife Medical Clinic at the University of Illinois Veterinary Teaching Hospital. Information regarding each animal’s predefined weight class, month of the year the animal presented, overall health status (sick or healthy), presence of diarrhea, necessity for assisted feeding, and presence of respiratory, integumentary, and neurologic signs was collected. Outcome factors (survivor or non-survivor) were modeled using a logistic regression model and all single factors were considered individually. A total of 872 EGS were included in this study. Factors that significantly predicted a non-survivor
status included EGS that presented to the clinic in any type of diseased state (OR 4.8, p < 0.0001), specifically those with respiratory signs (OR 1.54, p < 0.0001), or neurologic signs (OR 2.85, p < 0.001). The month the animal presented was also significant because EGS that presented during the second quarter were more likely to be non-survivors (OR 1.54, p = 0.019). These findings can be used by wildlife clinicians to identify EGS with negative prognostic indicators, thereby informing treatment decisions. Further research will reveal how these negative indicators are associated with EGS non-survivor status.

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Student Support: University of Illinois College of Veterinary Medicine

**Comparison of axillary and inguinal temperature to rectal temperature in healthy guinea pigs (Cavia porcellus)**

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Core body temperature is an essential health parameter. Temperature aberrations can indicate certain disorders, influence clinical management decisions, and serve as a prognostic indicator for patient recovery. Historically, rectal temperature measurements have been utilized in companion animals, however there is growing interest in less-invasive methods including auricular and axillary measurements to determine core body temperature. In this study, triplicate temperature measurements (axillary (AT), inguinal (IT), and rectal (RT)) were performed in 19 healthy guinea pigs (7 male, 12 female). Paired sample t-tests were utilized to evaluate differences in the mean body temperature measurements between each body site. There was no agreement in body temperature measurements between the body sites. The IT (36.3 C – 39.7 C) was significantly lower than both AT (36.8 C – 39.9 C; t = -3.401, p = 0.003) and RT (38.2 C – 40 C; t = -5.068, p < 0.0001), and AT was significantly lower than RT (t = -3.485, p = 0.003). These results indicate that AT, IT, and RT cannot be used interchangeably to represent core body temperature. However, these data provide a basis for clinicians to infer RT from AT and IT (RT = IT + 0.83 C, RT = AT + 0.44 C). Additional investigation is planned to assess these temperature relationships in animals suffering from disease.

Research Grant: None
Student Support: University of Illinois College of Veterinary Medicine
The effects of acute exposure to di(2-ethylhexyl) phthalate and diisononyl phthalate on hormone levels in mice

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Di(2-ethylhexyl) phthalate (DEHP) is a synthetic chemical used ubiquitously in the environment in a variety of plastics, medical equipment, and children’s toys. Individuals can absorb DEHP via dermal contact, inhalation, and ingestion. Previous studies show that DEHP affects fertility and causes endocrine disruption in both males and females, however, most of these studies focused on males. This information led to a shift to replace DEHP with other plasticizers. One such replacement is diisononyl phthalate (DiNP). Unfortunately, little is known about the reproductive effects of DiNP. Thus, the purpose of this study was to fill the knowledge gaps of how DEHP and DiNP affect female reproduction, and to test the hypothesis that acute exposure to DEHP or DiNP has negative, long-term effects on female reproductive health. Adult female CD-1 mice were orally dosed with either vehicle control (corn oil), DEHP (20 or 200 µg/kg/day, or 20 or 200 mg/kg/day), or DiNP (20 or 100 µg/kg/day, or 20 or 200 mg/kg/day) for 10 days. Mice were euthanized three months post-dosing and blood was collected to measure sex steroid hormone levels via enzyme-linked immunosorbent assays. Treatment with 100 µg/kg DiNP significantly decreased progesterone and testosterone levels compared to controls (n=5-6; p≤0.05). DiNP also moderately decreased progesterone (20 µg/kg, p=0.082) and increased estradiol (200 mg/kg, p=0.083) levels compared to controls. DEHP exposure did not significantly affect hormone levels compared to controls at any time. In conclusion, these data show that DEHP does not alter hormone levels significantly and that DiNP, a common replacement for DEHP, alters hormone levels in mice three months after exposure has stopped.

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Genomics of methicillin-resistant Staphylococcus pseudintermedius

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The gram-positive bacterium, *Staphylococcus pseudintermedius*, is a major cause of canine skin and soft tissue infections (SSTIs), pyoderma, ear infections, otitis externa and urinary tract infections (UTIs). Because methicillin and multidrug-resistant *S. pseudintermedius* cases are becoming more frequent, it is important to understand the evolution and mechanism of acquired antibiotic resistance. This project utilizes whole-genome sequencing to identify genetic changes associated with antibacterial resistance. *S. pseudintermedius* genome sequences from public databases will be analyzed using a pipeline established in our laboratory. The multilocus sequence type (MLST), phenotype profile, antibiotic resistant genes, and virulence genes of each strain will be identified. We expect that an isolate exhibiting elevated minimum inhibitory concentration (MIC) against an antibiotic will have a corresponding mutation or gene associated with phenotypic resistance. The strains with high MICs to multiple antibiotics are expected to bear a greater number of resistance mutations. In addition to known mutations, we expect to discover some novel mutations that may be associated with high MIC and multidrug resistance. Information from this study will provide insight for better management and control of the pathogen, and future development of treatment options.

Research Grant: College of Veterinary Medicine
Student Support: Office of the Director, NIH, T35 OD011145

**Ticking all the boxes: bureaucracy, research, and reality in reports of tick-borne diseases**

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To address the increasing public-health concern about tick-borne diseases it is necessary to know where people are exposed to ticks. This study addresses the efficiency of the disease-reporting process and comparisons between five states: Illinois, Iowa, Michigan, Minnesota, and Wisconsin. Data were collected from discussions with public-health officials and by assessing each state’s reporting documents. Currently, each state uses a different methodology to report tick-borne diseases, with particular differences in how geographic exposure location and travel data are collected. One important variation between states was the number of follow-up reports to gather additional information about the illness from either the patient or healthcare provider. This value also varied by disease. Another discrepancy between states was who does the follow-up, varying from public health officials to a team of student employees. Additionally, some states follow up cases with the
provider and others follow up with the patient. This process results in varied
detail regarding information where the tick may have been acquired. Data for
Wisconsin from 2007 to 2016 revealed that at least half of the reported cases
of Lyme disease every year had no information about the location the tick
was encountered. Overall, these disparate results reveal a need for improved
surveillance methods of tick-borne diseases in the Midwest. Increased
funding and manpower, a universal form for each disease, and an equal
emphasis on all tick-borne diseases would improve information collected in
the future and provide increased information about tick exposure.
Understanding where people are exposed can allow for improved public
health knowledge and control efforts.

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Student Support: Office of the Director, NIH, T35 OD011145

K9 officer down: lifesaving field training for working-dog handlers

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With the increase in K9 officers, and state legislatures proposing to exempt
responders providing emergency care to working animals from the
Veterinary Practice Act, veterinary educators need to be ready to train first
responders. Veterinarians, emergency medical service paraprofessionals,
medical doctors, and law-enforcement officers evaluated the most common
emergency conditions that affect working dogs in the field and created a
hands-on training program for K9 handlers. The training program focused on
recognition and care of common medical conditions in working dogs
including gastric dilatation and volvulus, heat stroke, blunt force trauma-
pneumothorax, CPR, vital signs and oxygen supplementation, fracture
stabilization, wound care, opioid overdose, toxicity, dental trauma/care, and
ophthalmic injury. Thirty-six K9 handlers from the Illinois State Police and
local municipalities completed a pre-training survey that was followed by
hands-on training. Before training, approximately 70% of handlers were least
comfortable with gastric dilatation and volvulus, administering apomorphine,
recognizing signs of pneumothorax, and knowing how to place a chest seal. A
similarly worded post-training survey indicated that 100% of respondents
were somewhat or very comfortable recognizing clinical signs and providing
care for the covered conditions. This study demonstrates that K9 handlers
benefit from hands-on education in providing emergency care. This study is a
template for training all first responders in states that allow, or propose to
allow, emergency care for K9 officers. Veterinarians should initiate collaboration with K9 handlers and provide the medical information needed to save lives.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Phytocannabinoid metabolism by canine cytochrome P450s

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Phytocannabinoids (pCBs) are natural compounds found in Cannabis plants (marijuana). Recent popularity of pCB use in human and veterinary medicine, combined with an insufficient understanding of pCB interactions on animal homeostasis, warrants investigation of pCB metabolism and pharmacokinetics. Cytochrome P450s (CYPs) are a class of enzymes involved in phase 1 drug metabolism playing a significant role in pCB biotransformation. In this study, metabolism of selected pCBs by canine CYPs 3A12 and 3A26 enzymes was explored and compared to metabolite formation by the human ortholog CYP3A4. We focused on two medically relevant pCBs found in Cannabis: Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD). These enzymes may produce similar metabolites due to conserved substrate and protein structures. To analyze human metabolism, we recombinantly expressed and purified CYP3A4 and its redox partner, cytochrome P450 reductase (CPR) in E. coli. Due to differences in expression between CYPs 3A12 and 3A26 in canine liver and peripheral tissues, native canine liver microsomes (containing both enzymes) and CYP3A12-specific bactosomes were obtained. Experiments will involve incubation of rCYP3A4, canine liver microsomes and bactosomal CYP3A12, with Δ9-THC and CBD. Resulting metabolites will be extracted prior to high performance liquid chromatography-mass spectrometry (HPLC-MS), so that fragmentation analysis can enable structure identification. Since dogs are commonly used in drug trials and pCBs are becoming increasingly popular in veterinary medicine, the comparison and discovery of human-versus-canine-derived pCB metabolites will create a foundation for future studies supporting Cannabis-based therapy.

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Student Support: Office of the Director, NIH, T35 OD011145
Effects of Nerve Growth Factor-β on ovulatory cascade factors from follicular fluid of pre-ovulatory follicles

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Nerve growth factor-β (NGF) is a seminal plasma protein that induces ovulation in camelids. Although NGF is not responsible for ovulation in the bovine species, previous results revealed that it enhances luteal development and markers of maternal recognition of pregnancy. There is, therefore, a critical need to understand what components of the ovulatory cascade are affected by NGF. We hypothesized that administration of NGF to Holstein heifers stimulates ovulatory cascade factors that can help understand the mechanism of action of NGF on pre-ovulatory follicles. Virgin Holstein heifers (n = 24) had their estrous cycle synchronized using a 5-day CIDR Synch program. On day 8 of the cycle, heifers with pre-ovulatory follicle (> 12 mm) and no corpus luteum noted by ultrasonography were deemed eligible for the study. Eligible heifers (n = 17) were randomly assigned to one of two treatments: CON (n = 7), intramuscular injection of 12 mL phosphate buffer solution (PBS, pH=7.4), or NGF (n = 10), intramuscular injection of 296 μg of NGF-β diluted in 12 mL of PBS. Follicular fluid samples were obtained from pre-ovulatory dominant follicles 8 hours after treatment via follicular aspiration, added to TRIzol (Invitrogen, Carlsbad, CA) and flash-frozen in liquid nitrogen. Samples were evaluated for gene expression of estrogen receptor 1 (ESR1) and Prostaglandin E Synthase (PGES) using quantitative real-time PCR. Data were analyzed with the GLIMMIX procedure of SAS. While no differences were revealed for ESR1 (P = 0.99) and PGES (P = 0.89), additional genes associated with the ovulatory cascade will be analyzed in the near future to provide a further understanding of the mechanism of action of NGF.

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Student Support: Boehringer Ingelheim Veterinary Scholars Program

Increasing the diagnostic utility of canine cerebrospinal fluid

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Cerebrospinal fluid (CSF) is used to characterize neurologic disease. Increased CSF protein concentration (PC) can be due to changes in permeability of the blood brain barrier secondary to a variety of infectious, inflammatory, compressive, or neoplastic processes. While this change indicates pathology, it is not specific for etiology. We are conducting several lines of investigation to increase the diagnostic utility of CSF. One aim evaluates the relationship between specific disease processes and CSF-PC. Medical records for dogs that underwent CSF analysis at the University of Illinois Veterinary Teaching Hospital (UI-VTH) were examined retrospectively. Information gathered included the CSF-PC (mg/dl), site of collection (cisternal or lumbar), and diagnosis (presumptive or confirmed). Data analysis will evaluate the relationship between specific disease processes, CSF-PC, and site of collection. Prospectively, CSF was collected from UI-VTH clinical patients. CSF-PC measurements made with a urine dipstick will be compared to data from a Beckman Coulter AU Analyzer to determine whether the dipstick can provide quick, reliable readings of PC. Liquid chromatography/mass spectroscopy (LC/MS) will identify proteins in canine CSF. Discriminant analysis will identify proteins that are associated with specific disease processes. This combination of approaches may expand the diagnostic information that can be derived from canine CSF and aid in neurodiagnostics and treatment of canine patients.

Research Grant: None
Student Support: Boehringer Ingelheim Veterinary Scholars Program

Effect of cocoa flavanols on endothelial nitric oxide synthase (eNOS) expression in brains of aged mice

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Cocoa flavanols improve peripheral vascular function in humans, both acutely and chronically, through increases in the bioavailability of nitric oxide (NO) in circulation. Recent work in aging populations suggests that such beneficial effects on the vasculature might extend into the central nervous system (CNS). Our preliminary work showed that feeding a high-cocoa-flavanol diet in aged mice starting at approximately 12 months old for 6 months prevented age-associated declines in blood perfusion throughout the brain, compared to a control (low-cocoa-flavanol) group. Our current study aimed to
determine the mechanism for these beneficial effects. We hypothesized that improved blood perfusion in the high-cocoa-flavanol group was due to higher levels of endothelial nitric oxide synthase (eNOS), the enzyme responsible for NO production. Brains from the original study (n=5 cocoa and n=5 control) were sectioned in the coronal plane at 40 microns. A 1-in-6 series (with 240 microns separating adjacent sections) was stained using an anti-eNOS primary antibody with Diaminobenzidine (DAB) as the chromogen. Area fraction covered by eNOS-positive labeling was quantified using stereological methods in the granular and molecular layer of the dentate gyrus and CA1 regions of the hippocampus using Image J software. Results showed similar levels of eNOS expression between control and cocoa treated mice for all 3 brain regions. Taken together, results suggest that increased eNOS expression is an unlikely mechanism for increased blood perfusion in the high-cocoa-flavanol group.

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The effect of simvastatin on CD44 shedding in canine osteosarcoma

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Osteosarcoma (OS) is the most common primary bone tumor in dogs. Despite multimodality therapy with surgery and chemotherapy, 90% of dogs develop distant metastases and die from disease progression. Thus, there is a need to identify novel therapeutics that slow metastatic progression and increase survival time. Simvastatin belongs to a drug class called statins and is used primarily for controlling hypercholesterolemia in people. Some studies suggest that statins also exert anticancer effects. These effects may be mediated through shedding of CD44, a transmembrane receptor that normally mediates cell adhesion by interacting with hyaluronan and also contributes to tumor progression and metastasis. Simvastatin stimulates CD44 shedding in human cancers and decreases cell migration, but this effect has not yet been evaluated in veterinary oncology. We hypothesize that simvastatin stimulates CD44 shedding in canine immortalized OS cells. Following simvastatin treatment for 48 hours at concentrations of 100 nM, 1 uM, and 10 uM, flow cytometry was used to assess CD44 expression on the canine OS cells. Exposure to simvastatin decreased median fluorescent intensity of detectable CD44 in treated cells compared to untreated controls. ELISA will be used to quantify the amount of CD44 that is shed, and a
transwell assay will be used to assess the effect of simvastatin on cell migration. In addition, an outcome-linked tissue microarray will be used to evaluate the relationship between basal CD44 expression and patient clinical outcomes. Demonstrating that simvastatin induces CD44 shedding would make it a promising candidate for future *in vivo* studies.

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**Cloning and expression of feline calicivirus P domain protein**

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Feline Calicivirus (FCV), a single-stranded positive sense RNA virus of the family *Caliciviridae*, is a highly infectious and genetically diverse virus. FCV mainly causes respiratory disease in cats. Outbreaks of the related virulent systemic FCV, which causes disease of greater severity, occur throughout the world. Our recent sequencing data indicated that currently circulating FCV strains showed only 80% nucleotide identity to those deposited into GenBank. The high percentage of nucleotide disparity will possibly contribute to false negatives in conventional molecular diagnosis, and vaccination failure in cats. The viral P protein forms a protruding domain of the FCV capsid and is responsible for immune recognition and host receptor interaction. The goal of this project is to clone and express P domain protein of FCV. We produced two different constructs: one with the hinge region that connects the S and P domain of the capsid, and one without the hinge region. The constructs were expressed via the pGEX-4T-1 vector in *E. coli* strain BL21. Recombinant proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis. The recombinant protein will be purified using chromatographic methods. This work provides the foundation for FCV diagnosis and perhaps disease prevention through vaccination.

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