

Ecological Determinants of Lyme Disease Risk

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Ecological determinants of disease risk play a critical role in the understanding and prevention of Lyme disease, a vector-borne zoonosis caused by the bacterium *Borrelia burgdorferi*, and transmitted to humans by the bite of an infected black-legged tick, *Ixodes scapularis*. Due to human activity, the Midwestern United States has experienced fragmentation of what were once contiguous habitats of deciduous forest and tall-grass prairie. The white-tailed deer, *Odocoileus virginianus*, which has adapted extremely well to habitats fragmented by human activity, also serves as a critical host organism for the black-legged tick. The purpose of this study was to determine the types of habitats colonized first by *I. scapularis* and *B. burgdorferi* in east-central Illinois, a region located at the front wave in the expanding distribution of Lyme disease. Due to the movements of hosts such as *O. virginianus*, we hypothesized that *I. scapularis* will colonize large forest fragments (>40 ha) first, later progressing to smaller fragments (1-2 ha) nearby. Tick density and prevalence of infection with the bacterium *B. burgdorferi* were sampled from 18 forest fragments extending over 3 counties in east-central Illinois. Of these fragments, 6 were large and 12 were small, with half of the small sites being near (4 km) from the large sites. A higher density of infected ticks associated with large forest fragments at the invasion front may indicate that the encroachment and establishment of *I. scapularis* and *B. burgdorferi* are predictable processes associated with host movements through fragmented landscapes. Eventually, this pattern may be used to forecast areas that will be at high risk for the establishment of Lyme disease and targeted preventative measures may be utilized.

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The Effects of Bisphenol A on Steroidogenesis in the Rat Ovary

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Bisphenol A (BPA) is an endocrine disrupting compound frequently used in polycarbonate plastics and epoxy resins. BPA has been declared toxic for

infants in many countries including China and Canada. In the United States, BPA has been banned in products that are intended for infant use in both California and the city of Chicago. The decision to reduce the amount of BPA used in infant products arises from new research demonstrating adverse effects of this chemical on endocrine parameters, specifically those in reproductive systems. Previous studies indicate that BPA exposure inhibits steroid hormone production. The goal of this study was to expand previous studies by determining if BPA affects steroid hormone production by altering the expression of genes encoding the steroidogenic enzymes, steroidogenic acute regulatory protein (Star) and aromatase (Cyp19a1), in rat ovaries. Our hypothesis is that BPA exposure decreases the expression of these steroidogenic genes, decreasing the amount of testosterone and therefore estradiol produced by the ovaries. To test this hypothesis, pregnant adult rats were dosed orally with vehicle or BPA (2.5 – 300,000 µg/kg) from gestation day 11.5 until birth. After delivery, the pups were dosed with vehicle or BPA until ovaries were collected on postnatal day 21. Once the ovaries from the pups were harvested, the mRNA levels were determined by quantitative real-time PCR, specifically investigating changes in gene expression of Star and Cyp19a1. Preliminary results indicate that middle concentrations of BPA affect the amount of Star mRNA found in the rat ovaries. We are currently determining the effect of BPA on the expression of the other steroidogenic gene. These results indicate that BPA may affect the expression of key enzymes required for normal steroidogenesis in rats. The main concern about BPA is that exposure will cause infertility by targeting the ovary.

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Intra- and Inter-Day Variability in Plasma [tCO₂] in Horses

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Sodium bicarbonate and other alkalinizing solutions (“milkshakes”) have been administered to horses to buffer the lactic acid accumulated during high velocity exercise. Governing jurisdictions measure plasma [tCO₂] as a proxy for plasma [bicarbonate] to determine if horses have been administered exogenous bicarbonate illegally. Our hypothesis was that plasma [tCO₂] varies measurably over a multi-day monitoring interval. Our objective was to determine the intra- and inter-day variability of plasma [tCO₂] and other plasma strong ion variables. Eight sedentary horses fed only alfalfa hay (5.3 kg twice daily at 7 AM and 3 PM after sampling) were sampled

3 times daily (7 AM, 11 AM, and 3 PM) for 5 days. Anaerobically-obtained heparinized jugular venous samples were analyzed immediately for plasma [tCO₂], [Na⁺], [K⁺], and [Cl⁻], on an Olympus AU680 chemistry analyzer which was internally calibrated by the manufacturer's recommendations. Mean ± s.e. was determined for each sampling interval with results compared by RMANOVA and other tests where appropriate. P

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Microfilarial Nematodes and West Nile Virus Co-Infection in an Urban Setting

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West Nile Virus (WNV) is an emerging infectious disease posing increasing risks to wildlife populations, domestic animals, and humans. Microfilarial nematodes are thought to potentially increase the risk of WNV transmission by two mechanisms. First, by penetrating the mid-gut barrier of the mosquito, microfilarial nematodes may decrease the time it takes for the mosquito to be able to transmit the virus. This would enable the virus to be spread through a population at a much quicker rate. Second, infection with nematodes may suppress the host's immune system making the host more susceptible to falling ill from WNV. If filarial nematodes play a role in WNV transmission, then we expect the prevalence of microfilaria to be high in important avian hosts. In order to determine the role of microfilarial nematodes in WNV transmission, it is important to understand how common microfilarial nematode infection is in wild bird populations. Five species of important avian hosts for WNV located in the Chicago suburbs were tested for microfilaria and West Nile Virus: the American Robin (*Turdus migratorius*), House Finch (*Carpodacus mexicanus*), House Sparrow (*Passer domesticus*), Mourning Dove (*Zenaida macroura*), and Northern Cardinal (*Cardinalis cardinalis*). Catching the birds was accomplished by mist-netting in the morning and late at night. Blood was collected by jugular venipuncture, and approximately 40 uL of each sample was centrifuged in a capillary tube and examined by light microscope for microfilaria. The remainder of each blood sample was saved for West Nile Virus testing. Birds positive for microfilaria were euthanized and necropsied to recover the adult filaroid nematodes for

species identification. Collecting the parasites and correct identification of the nematodes is crucial to understanding the relationship between WNV and microfilaria.

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Of Mice and Pigs: Optimization of Animal Models of *Candida albicans* Colonization and Disease

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Animal models of human disease are useful to study the interaction between microbes and host. *C. albicans* is a commensal fungus that can cause disease when the normal flora or immune system of the host is compromised. Oral candidiasis is one of the hallmarks associated with development of AIDS, and also affects people who wear dentures. Disseminated candidiasis occurs as the result of immunosuppression associated with chemotherapy or organ transplantation, for example, with the gastrointestinal tract believed to serve as the source of *C. albicans*. This work focused on optimization of two animal models to study *C. albicans*, one in its commensal state and the other in the context of oral disease. Because pigs are used as a model system to study the human gastrointestinal tract, we explored the possibility of stably colonizing pigs with *C. albicans*. We identified a source of pigs that were *C. albicans*-free. Piglets were farrowed normally and left with the sow for three days. On the fourth day, piglets were transferred to an artificial rearing environment and fed milk replacer. Following oral inoculation with *C. albicans*, pigs were monitored daily to detect *C. albicans* using swabs of the oral cavity and rectum, as well as the environment. Pigs were necropsied to assess the relative abundance of *C. albicans* throughout the gastrointestinal tract. Results showed that pigs could be stably colonized with *C. albicans* and the fungus recovered readily. The mouse model of oral candidiasis involved immunosuppression of mice with cortisone acetate and subsequent oral inoculation with *C. albicans*. Variables optimized included inoculum size and length of infection. The model was used to assess the effect of gene deletions on *C. albicans* pathogenesis. Both models will be used in future studies of *C. albicans* colonization and pathogenesis to better understand the interaction of the fungus with its host.

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A Retrospective Evaluation of Long-Term Survival in Dogs Diagnosed with SARDS

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Sudden acquired retinal degeneration syndrome (SARDS) is an acquired syndrome causing spontaneous and irreversible blindness in dogs. The goal of this retrospective study was to determine incidence of mortality and cause of death in dogs diagnosed with SARDS at the University of Illinois Veterinary Teaching Hospital. Medical records were reviewed and data regarding age at SARDS diagnosis, age at time of death, and other systemic illness was collected from the medical record and from both the referring veterinarian and owner via telephone. A total of 51 dogs were included in the study, with mixed breed dogs and Dachshunds representing the most common breeds. Dogs were diagnosed with SARDS at an average age of 8.09 ± 3.20 years. A total of 28/51 (54.9%) dogs were deceased and 23/51 (45.1%) dogs survived. The average age of the surviving dogs was 9.07 ± 3.10 years. The average age at time of death for non-survivors was 11.42 ± 2.89 years. The median interval since diagnosis of SARDS in surviving dogs was 1.38 years (IQR 1.10-4.48). The mean interval since diagnosis of SARDS in non-surviving dogs was 1.72 ± 1.58 years. Of the 28 non-survivors, 7 dogs died spontaneously and 21 were euthanized. The reason for euthanasia was reported for 15/21 euthanized dogs. The most common reasons for euthanasia were cardiac failure, renal failure, and musculoskeletal disease. Mortality rate for dogs diagnosed with SARDS was high, and cause of death often resulted from systemic disease rather than owner-perceived decrease in quality of life from SARDS-associated vision loss. Furthering the understanding of SARDS and its effects on the canine species is fundamental for the development of future treatment and prevention.

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A Comparative Study of Osteogenesis in Equine Mesenchymal Stem Cells from Bone Marrow, Adipose Tissue and Synovium

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Mesenchymal stem cells (MSC) are present in many tissues and are capable of multilineage differentiation, including osteogenesis. Osteogenesis is critical for embryonic skeletogenesis and for the repair of orthopedic injuries. The goal of this study was to determine the relative osteogenic potency of equine MSCs isolated from adipose tissue, synovium and bone marrow. Equine MSCs were isolated from bone marrow [BM], adipose tissue [AD] and synovium [SYN], expanded in monolayer for two passages, then transferred to standard osteogenic medium. At Day 7 and 14, the phenotypic status of the cells was evaluated by measuring alkaline phosphatase (ALP) activity, and staining for mineralized matrix (Alizarin red and Von Kossa). Robust osteoblastic differentiation occurred in cells derived from BM and SYN. Staining of the mineralized matrix of BM MSCs demonstrated a much higher uptake of both Alizarin red (AR) and Von Kossa (VK) stains in treated cells as well as a 4-fold increase in osteogenic nodule formation. SYN cells showed a 2-fold increase in nodule formation between osteogenic cells and control cells. In contrast, there were minimal differences between the control and treated AD cells. There was no nodule formation in either AD group. Osteogenic medium increased ALP activity in both BM and SYN MSCs in comparison to the controls. However, AD MSCs did not show any difference in ALP activity between treated and control groups. These findings suggest that both BM and SYN MSCs are capable of osteogenesis, although BM MSCs exhibit more robust differentiation. In contrast, equine AD cells were poorly responsive to osteogenic stimulation. On the basis of these data, BM MSCs should be preferred for clinical applications involving bone repair.

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Evaluating Impulsive Behavior: Effects of Dopaminergic Drugs on Rat Performance of a Delay Discounting Task

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Studies have suggested that dopaminergic activity may play a direct role in impulsive behavior, which is a component of neuropsychological disorders such as attention deficit/hyperactivity disorder (ADHD). One aspect of impulsive decision making manifests as intolerance for reinforcement delay,

which can be assessed using a delay discounting task that evaluates delay tolerance. Based on a lever-press, normal individuals choose between a smaller but immediate food reward (the more impulsive choice) and a larger, delayed reward, with preference for the larger reward decreasing over longer delays. Decreased dopamine is associated with greater discounting, or higher impulsivity, in this task. The goal of this pilot study was to determine appropriate dosages of dopaminergic drugs that will be used in subsequent studies observing the behavior and performance of rats developmentally exposed to toxicants that may perturb dopamine signaling in the brain. Six male and six female toxicant-naïve Long-Evans rats were trained on the delay discounting task. Their performance in the same task after systemic administration of α -flupenthixol, d-amphetamine, and both drugs combined was then evaluated. Flupenthixol is a dopamine antagonist, while amphetamine increases synaptic concentrations of dopamine. Relative to baseline levels of delay tolerance, impulsivity was found to increase after α -flupenthixol injection, but administration of d-amphetamine did not produce a significant change in performance. The combined effect of these drugs will be determined as the drug trials are completed. These preliminary results indicate that, in normal Long-Evans rats, performance of the delay discounting task may not be sensitive to alterations in synaptic dopamine concentrations, although dopamine does influence performance of the task. These data help us further understand the relationship between dopamine and impulsivity, and may provide further insight into the mechanisms of neurological disorders that affect impulsive behavior.

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Inhibitory Effect of Curcumin on Uterine Leiomyoma Cells Obtained from Aged Laying Hens

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Uterine leiomyomas (fibroids) are noncancerous tumors that form within the myometrial wall of the uterus. The incidence of these benign tumors is 77% in reproductive-aged women in the United States, with the most common symptom being abnormal uterine bleeding. In serious cases, infertility or miscarriages also occur. The specific pathogenesis of human uterine leiomyomas has not been determined. Several types of animals have been suggested as in vivo models to study uterine leiomyomas. Laying hens have been validated as an appropriate model for uterine leiomyoma studies because hens develop smooth muscle leiomyomas on the wall of the oviduct

with age and hormonal cycling patterns similar to humans. Previous studies have shown the polyphenol curcumin has an inhibitory effect on uterine leiomyoma cell proliferation, but optimal dose ranges for treatment were not determined. In the present study, we used three chicken leiomyoma cell lines (2P, 302P and 624P) established from leiomyomas harvested from three different birds. We determined the doubling time for each cell line, which was 0.66, 0.89, and 1.24 days, respectively. We carried out dose-response curves using concentrations of 20 μ M, 50 μ M, 100 μ M, and 250 μ M curcumin on each cell line, with corresponding concentrations of vehicle (DMSO) to determine whether it had any effect on cells. Using titrated-thymidine assays, we assessed the effects that the varying concentrations of curcumin had on DNA synthesis as a measure of cell proliferation at 24-, 48- and 72-hour time points. Preliminary results suggested that curcumin doses ranging from 100-500 μ M had the greatest inhibitory effect on chicken leiomyoma cell lines. It is possible that the optimal dose for a particular cell line depends on doubling time. The results from our study showed that chicken leiomyoma cells can be used to screen compounds and to identify effective therapies for uterine leiomyomas.

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The Effectiveness of PAC-1 on Peripheral and Central Compartment Lymphomas

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Apoptosis evasion is a hallmark of cancer, and studies suggest that proapoptotic compounds maybe promising anticancer agents. The induction of apoptosis can occur extrinsically or intrinsically, and both pathways lead to the activation of protein degrading enzymes called caspases. Procaspase-3 is an inactive zymogen that is cleaved to caspase-3, the key executioner caspase. In 2006, a small molecule named Procaspase Activating Compound 1 (PAC-1) was discovered, which activates procaspase-3 through zinc chelation, and induces apoptosis in cancer cells. Many cancer cells, including lymphomas, express procaspase-3, and should be susceptible to apoptosis induction. Uniquely, PAC-1 rapidly penetrates the blood brain barrier, however, the mechanisms for how PAC-1 may enter the brain remain uncharacterized. Given its CNS penetrant characteristics, the purposes of this study were to demonstrate the effectiveness of PAC-1 as an anticancer treatment for peripheral and central compartment lymphomas and to

further characterize potential mechanisms for how PAC-1 permeates the CNS. Murine lymphoma cells (EL-4) were exposed to different concentrations of PAC-1 at various time points and the activation of procaspase-3 was quantified using flow cytometry, Western blotting, and a colorimetric assay. The in vivo mechanism of PAC-1 action was studied in mice intraperitoneally inoculated with EL-4 cells, and subsequently treated with PAC-1 for varying times. The ability of PAC-1 to traverse vascular endothelial cell barriers was studied using a commercially available vascular permeability assay. In vitro, PAC-1 activated procaspase-3 in EL-4 cells at biologically achievable concentrations (25-50 μ M) and exposure durations (4-8 hours), based upon prior pharmacokinetic and toxicity studies. Significantly, PAC-1 administration to EL-4-bearing mice also activated procaspase-3 in vivo. Finally, PAC-1 did not increase vascular permeability, suggesting that its rapid CNS penetrating effects are unlikely dependent upon direct vascular permeation. These results suggest that PAC-1 maybe a promising anti-lymphoma agent and warrants further investigation.

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Competitive Revision-Small Molecule Activators of Procaspsases as Anti-Cancer Agents: The Effect of Acute Exercise on the Central Immune and Behavior Responses of LPS-Challenged Mice

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Upon pathogen recognition in the periphery, the innate immune system produces pro-inflammatory cytokines which signal the brain to induce sickness behavior, characterized by weight loss, anorexia, fever, and lethargy. Under normal conditions, sickness is an adaptive response aimed at conserving energy while the host clears the infection. However, under certain pathological conditions (e.g. aging, cancer, autoimmune disorders), sickness behavior often progresses toward a depressive behavior phenotype, characterized by helplessness, anhedonia, fatigue, and hyperalgesia. While both long-term moderate exercise training and acute exhaustive exercise have been shown to decrease pro-inflammatory cytokine levels in the periphery (serum & tissues) following innate immune activation with E. coli-derived lipopolysaccharide (LPS), it is unclear if this translates to reduced brain inflammation and attenuated depressive behavior. The aim of this study was to determine if acute exercise attenuates LPS-induced sickness behavior

and depressive-like behavior in adult CD-1 mice. We hypothesized that acute treadmill exercise would decrease peripheral inflammation, which would translate to reduced brain inflammation and behavioral abnormalities. Male CD-1 mice (n=40) were randomized into exhaustive treadmill running and sedentary groups (10/group). After an 80-minute acute exercise or sedentary session, mice were injected intraperitoneally with either LPS at a dose of 0.33 mg/kg or saline. Bodyweight, food intake, and novel cage motor activity were assessed at 3 and 8 hours post-injection. Depressive-like behavior was assessed by the tail suspension test at 9 hours post-injection. If the mechanism of acute exercise's effects on immune function can be clarified, it may have implications for treating the chronic low-grade inflammatory status associated with aging, autoimmune disease, and cancer, which could decrease the likelihood of depressive episodes subsequent to disease onset.

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Effect of Voluntary Exercise on Hippocampal Microglia, Neurogenesis, and Learning in Aged Mice

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Microglia, the brain's primary immune cells, undergo age-related changes that may be responsible, in part, for the cognitive decline seen in older adults. Exercise has been shown to combat cognitive decline and improve brain health by enhancing learning and stimulating neurogenesis in the hippocampus. It was the goal of this study to elucidate what effect exercise has on microglia proliferation and phenotype, as microglia can take on a classic inflammatory phenotype or an alternative neuroprotective phenotype. Additionally, we assessed the potential correlation between changes in microglia, neurogenesis, and learning. In order to do this, aged and young mice were housed with or without access to a running wheel for eight weeks. Mice received daily injections of bromodeoxyuridine (BrdU) for either the first or last 10 days of the study to label cell division. After seven weeks of running, mice underwent auditory and contextual fear conditioning to test learning. Brains were collected, sectioned, and labeled for BrdU, NeuN, Iba-1, and/or IGF-1 using immunohistochemistry to estimate neurogenesis, microglia proliferation, and microglia phenotype. If exercise has an effect on microglia that is correlated with a decrease in neuroinflammation and an increase in neurogenesis and learning, then exercise may be beneficial as a therapy for

disorders such as Alzheimer's disease and may be used to slow cognitive decline.

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Oranges Might Contain the Key for Your Memory Formation

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Ascorbic acid, the reduced form of Vitamin C, is highly concentrated in the central nervous system (CNS), and is able to inhibit glucose consumption and stimulate lactate uptake in neuronal cells. Astrocytes in the CNS produce lactate, which will be preferably used as an energy source by neurons during memory formation. Therefore, it is hypothesized that ascorbic acid functions as a mediator between lactate and glucose during neuronal activity. In this experiment, a biosensor probe able to detect lactate, ascorbic acid or glucose, was inserted in the hippocampus of young Sprague Dawley male rats to determine the concentration of the mentioned molecules during training on hippocampus-dependent place or striatum-dependent response learning tasks. The hippocampus-dependent place learning task was performed by placing a rat in a maze in which a reward will always be in the same location of a cue-rich room. On the other hand, the striatum-dependent response learning task was performed in a maze in which the rat would learn to obtain a reward every time it turns to the same direction in a cue-poor room. It is hypothesized that the levels of ascorbic acid and lactate will be higher during place learning. Future work will consist of inserting a biosensor with the same probes into the striatum to determine the levels of glucose, lactate and ascorbic acid during both hippocampus-dependent place and striatum-dependent response tasks. It is expected that the levels of lactate and ascorbic acid in the striatum will be higher during the response task. These findings suggest a specific role for astrocytes during memory formation in controlling ascorbic acid and lactate release. Such results point to novel targets for treatment of memory loss.

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Effects of Sedation on Standard Equine Coagulation Parameters

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Acepromazine and xylazine are commonly administered to critically ill equine patients to reduce anxiety, provide analgesia and enable safe evaluation of the animal. These medications cause a decrease in hematocrit and may impact platelet function. This study evaluates whether administration of acepromazine or xylazine impacts thromboelastometry (TEM) or other coagulation assays in healthy horses. Using a random crossover design 7 adult horses were administered a single dose of xylazine (1.1 mg/kg IV) or acepromazine (0.1 mg/kg IV) with a 3-week washout period between treatments. Hematocrit, total protein, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), TEM and thrombin antithrombin complexes (TATc) were measured at baseline, 30 minutes and two hours for both medications, and at 6 hours and 12 hours for acepromazine. At 30 minutes post xylazine administration (n=3) hematocrit decreased by 15.7%. Changes in TEM included an 8.0% decrease in clot formation time (CFT) and a 5.3% increase in alpha angle. At 30 minutes, 2 hours and 6 hours post acepromazine administration (n=3), hematocrit decreased by 18.0%, 19.7%, and 14.3%, respectively. Changes in TEM (n=2) included a 9.0% decrease in CFT and a 3.0% increase in alpha angle at 30 minutes. As expected, hematocrit decreased after the administration of acepromazine and xylazine. Changes in TEM parameters at 30 minutes suggested hypercoagulability. Evaluation of additional animals is required to determine statistical significance. Measurement of PT, aPTT and TATc will help determine whether changes in TEM represent true alterations in coagulation or are due to decreases in hematocrit. Coagulation abnormalities

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Prevalence and Spatial Distribution of Frog Virus 3 in Wild Populations of Northern Cricket Frog (*Acris crepitans*) in Western Illinois

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Ranavirus is a lethal and highly contagious disease responsible for mass mortality events in wild amphibian populations. Due to the drastic decline of

amphibian species witnessed in the last three decades, determining susceptible locations and populations is a critical key to slowing down the present extinction rates. This study focuses on determining the prevalence and spatial distribution of Frog Virus 3 (FV3), a species of ranavirus, in western Illinois ponds. The northern cricket frog was chosen due to its unexplained decline from being one of the most abundant frogs in Illinois. Using a sterile technique, each wild-caught frog was physically examined and swabbed for virus particles in the oral cavity and cloaca. DNA was extracted and will be examined using real-time PCR to determine if and how many individuals test positive for this deadly pathogen. A positive result does not prove the individual was infected, but indicates the presence of the virus in the pond. Knowing if and where FV3 exists in Illinois is extremely useful in aiding efforts to save frog populations.

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Is Dermatophyte Morphology Only Skin-Deep? Identification of Veterinary Dermatophyte Isolates Using Molecular Methods

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Dermatophytes are pathogenic fungi that can invade keratinized tissue (skin, hair and nails) of humans and animals. The familiar name for the resulting infection is ringworm. The most common dermatophytes that cause ringworm in animals are *Microsporum canis*, *Microsporum gypseum* and *Trichophyton mentagrophytes*. Identification of dermatophytes traditionally has relied upon the appearance of the colony on an agar plate and microscopic visualization of fungal structures. For example, an isolate can be placed into the genus *Microsporum*, rather than into the genus *Trichophyton*, based upon relative differences in the abundance of microconidia and macroconidia, whereas the species *M. canis* and *M. gypseum* can be differentiated from each other based on the shape of the macroconidia. The use of molecular methods to identify dermatophyte isolates is becoming more developed, demonstrating that previous taxonomic groupings at the species level are actually comprised of more than one dermatophyte. For example, *T. mentagrophytes* is now recognized as a species complex, that includes several distinguishable species. This study applies molecular identification methods to a collection of dermatophyte isolates from veterinary clinical cases. We hypothesize that molecular identification methods will reveal greater taxonomic variation in the collection of isolates than recognized based on morphological identifications. Genomic DNA from

each dermatophyte isolate will be amplified by polymerase chain reaction (PCR) using primers specific to the internal transcribed spacer (ITS) region of the nuclear-encoded ribosomal RNA genes (rDNA), as well as primers specific for the 28S rRNA-encoding gene. DNA sequences will be compared to those in public databases to aid in identification of the dermatophyte isolate. Clinical case records that are available for many of the isolates will be used to evaluate potential associations between specific dermatophyte species and animal hosts. Information from this analysis will further knowledge of the epidemiology of dermatophytosis in animal species.

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Brain-Behavior Changes in Female Three-Spined Sticklebacks (*Gasterosteus aculeatus*) Using Immediate Early Gene Expression as an Indicator of Brain Activation

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The three-spined stickleback (*Gasterosteus aculeatus*) is a model organism for studying behavior. The goal of the study is to determine which regions of the female brain are activated in response to courtship from a male. Females were placed in a flask and then introduced into a male's tank for ten minutes. We compared immediate early gene expression in the brains of females placed in a flask and exposed to a courting male, to females that were placed in a flask (but not exposed to a courting male), and to females that were sampled directly from their holding tank. To detect brain activation, we measured the expression of an immediate early gene, *Egr-1*, in the brain using whole mount in situ hybridization. Briefly, brains were excised and placed into 4% paraformaldehyde. After 24 hours, the dura mater and remaining debris were cleaned from the brains and the brains were placed in 100% methanol. Brains were incubated in a probe specific for stickleback *Egr-1*. Next, the brains were treated with an antibody specific for the created probe and visualized through a chemical reaction using a FITC fluorescent marker. Finally, brains were incubated in DAPI, a neuronal cell marker, to allow all brains to be compared across treatments regardless of activation. This study will enable us to further understand the neuronal mechanisms that mediate the dramatic behavioral shifts seen during the breeding cycle in this male uniparental species.

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Topically Applied Sevoflurane Jelly: an Alternative Method of Anesthesia for *Bufo marinus*

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Amphibians have a unique respiratory system that allows gas exchange by two routes: pulmonary and cutaneous. Studies have shown that anurans can be anesthetized by topical application of isoflurane. Sevoflurane, an anesthetic similar to isoflurane, has not been tested for topical application. We developed and tested a topically applied sevoflurane jelly-mixture on *Bufo marinus* to determine anesthesia induction and recovery times. Eight toads were used for the study. Sevoflurane jelly was made by mixing 3 parts of sevoflurane liquid, 1.5 parts of distilled water, and 3.5 parts of aqueous jelly. The anesthetic mixture was applied to the dorsum of toads at 0.025 ml/g of body weight. Toads were placed in an airtight, custom-built anesthetic chamber that allowed manipulation of the toads and determination of sevoflurane vapor. Toads were turned to dorsal recumbency every 30 seconds until righting ability was lost. During the anesthetic induction phase, gular movement slowed and eventually became absent. After loss of righting ability, anesthetic mixture was rinsed off and toads were placed back in dorsal recumbency. Toads were observed until return of righting ability and normal gular movements. Anesthetic chamber air was measured for sevoflurane vapor throughout. Results from a single toad trial showed an induction time of eight minutes and recovery in 52 minutes. Anesthetic chamber sevoflurane reached a maximum concentration of 0.9% during induction and was 0% during recovery. Topical application of sevoflurane jelly mixture may be a convenient method of anesthesia for anurans, particularly in field settings, eliminating the need for euthanasia.

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Detection and Differentiation of Canine Lymphoma Using Quantitative PCR

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Lymphoma is the most common cancer found in dogs. It arises from a clonal expansion of either B cells or T cells and rarely NK cells. The diagnosis of lymphosarcoma is frequently corroborated by B- and T-cell immunohistochemistry; however, this technique is somewhat subjective and takes time. Quantitative Polymerase Chain Reaction (qPCR) provides the opportunity for a rapid and definitive diagnosis. Using qPCR allows one to differentiate between B- and T-cell lymphomas more objectively, which is important for treatment and prognosis. The common mechanism in the pathogenesis of lymphosarcoma is generally a monoclonal expansion of lymphocytes. Single or double amplicons obtained using qPCR suggest lymphoma, contrary to multiple amplicons that appear in healthy lymphoid tissue. Template DNA was isolated from both formalin-fixed tissues embedded in paraffin and fresh tissue samples. Tissue samples from both positive and negative lymphosarcoma cases were used for optimization of a qPCR protocol. Primers specific to the VDJ region of B- and T-cell receptors were used in each assay. Differences in these joining regions account for different products formed from the qPCR reaction. Melting curve analysis was used to determine whether positive samples had unique amplicon numbers and sizes compared to negative samples. Gel electrophoresis was used to isolate the reaction products for cloning and DNA sequence analysis. Sequence results were compared to gene databases to ensure the correct gene segments were amplified. Having this molecular diagnostic tool will help to specifically treat lymphomas in a timelier manner.

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Effect of Lactoferrin on Piglet Gastrointestinal and Immune Development

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Human breast milk protects the infant by providing maternal antibodies that infant formula does not contain. Lactoferrin (LF), an antimicrobial breast milk-protein, is known to strengthen immunity. By adding LF to formula, we can study its effects compared to infant formula alone. Since neonatal pig gastrointestinal physiology is similar to infants, piglets make an excellent model to study gastrointestinal and immune development. This study used naturally farrowed piglets to study LF's effect on piglet gastrointestinal and immune development. Piglets were colostrum-deprived and therefore lacked

maternal antibody transfer, resulting in immunocompromised animals. Piglets were randomly divided into three groups (n=10-13 per time point): formula without added LF (0.36 g LF/L), formula with 1.02 g LF/L, and formula with 3.62 g LF/L. The piglets were euthanized on day 7 or day 14 and serum samples from the heart, tissue from the middle of the jejunum, and contents of the ascending colon were collected. We hypothesized that LF would increase IgG and IgA antibody concentrations as well as modulate gastrointestinal genes related to LF, proliferation and innate immunity. Total serum IgG concentration was measured by ELISA as was total ascending colon IgA concentration. Intestinal gene expression was measured using jejunal tissue extracts and quantitative real-time PCR. The results of this study will help us to better understand the effect of lactoferrin on human infant gastrointestinal and immune development.

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