

A preliminary validation of molecular tools for quantifying biomarkers of respiratory pathology

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Respiratory disease is a significant cause of morbidity and economic loss in the cattle industry. Quantifying the nature and severity of respiratory pathology in affected animals is useful in determining prognosis and in selecting optimal therapeutics. The objective of this study was to validate the use of quantitative real-time PCR and Western Blot analysis in measuring gene expression and protein concentration of candidate biomarkers of lung pathology in growing cattle. Bronchoalveolar lavage (BAL) samples were taken from healthy and diseased feed lot cattle and lung tissue samples were taken from healthy slaughterhouse animals immediately postmortem. Protein and RNA were extracted using conventional techniques from the BAL and lung tissue samples respectively. Protein concentrations were evaluated and Western Blot analysis was performed on the BAL samples with the highest protein concentration samples using antibodies against Surfactant protein A, MMP2, and MUC1. Quantitative real time PCR was performed using primers designed for the genes that encode these proteins. Preliminary data suggests that all three proteins are produced in both the healthy and diseased lung. Additional studies will quantify the amount of each protein in the healthy versus diseased lung. It is anticipated that these assays can be used in the diagnosis and treatment of respiratory pathology.

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Student Support: University of Illinois College of Veterinary Medicine

Measurement of IL-1 beta in chelonian plasma by ELISA using anti-human IL-1 beta antibodies

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Interleukin-1 beta (IL-1 beta) is a cytokine produced by monocytes and macrophages that contributes to the acute phase response of inflammation. In mammals, IL-1 beta concentrations in the blood are elevated during inflammatory disease states. In comparison, cytokine activity is poorly

understood in reptiles and reagents specific for detection of reptile cytokines have not been developed. The enzyme-linked immunosorbent assay (ELISA) uses antibodies to detect and quantify proteins and is used frequently for cytokine measurements. The purpose of this study was to determine if a commercial ELISA kit containing anti-human IL-1 beta antibodies could be used to measure IL-1 beta in chelonian plasma. Plasma from red-eared sliders (*Trachemys scripta elegans*) that were experimentally infected with ranavirus (frog virus 3) was used as a positive control for cross-reactivity with human antibodies. Plasma from both infected turtles and uninfected turtles had measurable concentrations of IL-1 beta. The presence of IL-1 beta in uninfected turtles may be due to stress associated with shipment of the turtles or the presence of other inflammatory processes. Plasma from wild Eastern box turtles (*Terrapene carolina carolina*) was also tested for IL-1 beta by ELISA. A better understanding of the activity of cytokines in reptiles can provide insight into the manifestation and course of inflammation in these species. The ability to measure cytokines by ELISA could serve as a valuable tool for wildlife epidemiology by providing a practical and quantitative means of disease surveillance.

Research Grant: Wildlife Epidemiology Laboratory
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Development of a fecal microbiota transplant preparation protocol for dogs

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Fecal microbiota transplants (FMT) are highly effective in humans for treating recurrent *Clostridium difficile*, but have mixed efficacy for other gut dysbiosis conditions. Oxygenation during donor sample processing, and its negative impact on anaerobic bacteria, is a likely cause for this observation. Many processing methods for donor stool samples have been reported, but little is known about their effect on bacterial viability. As there are no established protocols for FMT in veterinary medicine, our goal was to analyze the impact of sample processing on fecal bacteria and create a clinically useful FMT processing protocol. We hypothesize that oxygen-exposed FMT samples will have decreased viability of anaerobic bacteria, and that the addition of L-cysteine, a reducing agent, to fecal homogenate solutions will mitigate the deleterious effect of oxygen exposure. Fecal samples from six healthy dogs were collected and homogenized in normal saline with 10% glycerol or 0.1% L-cysteine in normal saline with 10% glycerol. An aliquot of the donor

homogenate solution was sparged with air to mimic sample oxygenation during homogenization. An aliquot was also treated with propidium monoazide (PMA) to assess bacterial viability. PMA, a membrane-impermeable DNA binding agent, modifies DNA from dead bacterial cells allowing for sequencing of living cell DNA only. Additional aliquots of each buffer were subjected to several freeze-thaw cycles before the addition of PMA. Sample processing is underway and, when completed, genomic DNA will undergo purification and Illumina sequencing of 16S rDNA. Overall, a novel FMT protocol for veterinarians would enhance the treatment options available for animals with intestinal dysbiosis.

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Effect of intracoelomic dexmedetomidine-alfaxalone on righting reflex in garter snakes (*Thamnophis sirtalis*)

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Reptiles often require anesthesia for clinical procedures, but little is known about their response to common anesthetic agents. While alfaxalone has been evaluated in snakes, the effects of alfaxalone combined with sedatives such as dexmedetomidine are currently unknown. A randomized, controlled study was used to evaluate the effects of intracoelomic dexmedetomidine-alfaxalone in 8 common garter snakes (*Thamnophis sirtalis*). Loss and return of righting reflex (LRR and RRR) were examined following the administration of alfaxalone (30 mg/kg) combined with either 0.05 or 0.1 mg/kg of dexmedetomidine (DEX0.05 and DEX0.1) administered through a single intracoelomic injection. If LRR occurred, heart and respiratory rates were monitored until RRR. Loss of righting reflex occurred in 63% (5/8) and 38% (3/8) of snakes following administration of DEX0.05 and DEX0.1, respectively. For DEX0.05, the onset of LRR was variable with a range of 1 to 20 minutes, and duration of 62-124 minutes. Following DEX0.1, the onset of LRR ranged from 3 to 15 minutes, and duration ranged from 15-174 minutes. Following LRR, heart rate ranged between 20-40 beats per minute and respiratory rate ranged between 0-11 breaths per minute for both treatments at recorded time points. The co-administration of dexmedetomidine and alfaxalone in snakes resulted in marked variability in the presence and duration of LRR; however, fewer snakes demonstrated LRR at DEX0.1 compared to DEX0.05. A second phase of the study will evaluate dexmedetomidine and alfaxalone

administered at separate injection sites to determine if the variability is due to pharmacokinetic or pharmacodynamic interactions.

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Effects of intrauterine growth restriction on peripheral inflammatory mediators in a neonatal piglet model

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Infants born small for gestational age (SGA) due to intrauterine growth restriction (IUGR) are more likely to exhibit abnormalities in brain development and greater susceptibility to infection than their average for gestational age (AGA) counterparts. The purpose of this study was to determine if there was a difference in the level of expression of various inflammation mediating proteins between SGA and AGA infants using a piglet model. We hypothesized that the peripheral tissues of SGA piglets would contain lower inflammation-associated gene expression levels, and lower circulating plasma IL-6 levels, than their AGA counterparts. SGA and AGA piglets were weaned at postnatal day (PD) 2, and at PD 14 injected intraperitoneally with *E. coli* lipopolysaccharide (LPS) or sterile saline. Blood was drawn pre- and 4 hours post injection, and ELISAs were performed to quantify plasma IL-6 levels. Four hours after injection, piglets were sacrificed and thymus, liver, and spleen was collected. RNA was isolated for cDNA synthesis and qPCR was performed for gene expression. We found no association between SGA status and plasma IL-6 levels. The thymus samples of SGA piglets showed decreased expression of the genes TMPO and RUNX1. Spleen and liver samples had a main effect of LPS injection with varying expression of several inflammation mediating genes. Only SGA piglet liver had increased levels of pro-inflammatory CCL2 gene after LPS injection. Possible implications of this work include further research targeting the thymus of SGA infants to better determine how hematopoietic proteins like TMPO and RUNX1 contribute to immunodeficiency, in order that effective therapeutics or preventatives can be developed in the future.

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Circadian disruption impacts impulsivity and attention: examining neurochemical mechanisms in Long-Evans rats

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Circadian rhythms are 24-hour cycles that control physiological processes, and their disruption can have negative effects on health. Two forms of circadian disruption in people are working overnight shifts and exposure to artificial light at night. The purpose of this study is to examine the effects of circadian disruption on attention and impulsivity in adult Long-Evans rats using the five-choice serial reaction time task (5-CSRTT). The three circadian conditions were testing in dark phase (control), testing in dark phase with pulse of light (modeling artificial light-at-night), and testing in light phase (modeling shift work). Our results showed that both models of circadian disruption caused decreased attention and greater impulsivity than the control, with the light-at-night group exhibiting greatest impulsivity. This indicates that artificial light-at-night may be more harmful to cognitive functions than overnight shift work. Considering the neurochemical basis for the effects, dopamine modulates impulsive behavior while acetylcholine (ACh) modulates both attention and circadian rhythms. Therefore, we hypothesized that impulsivity resulting from circadian disruption results from an interaction between ACh and dopamine. To explore this, we tested the effects of a nicotinic acetylcholine receptor agonist (nicotine) and a dopamine-1 (D1) receptor antagonist (SCH 23390) on attention and impulsivity. Nicotine increased impulsivity while SCH decreased impulsivity. When administered together, SCH attenuated the effect of nicotine on impulsivity. These results suggest that D1 receptors are involved in impulsivity and that cholinergic signaling interacts with the dopamine system to influence impulsive behavior.

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Evaluating the effect of *Helicobacter pylori* VacA toxin on parietal cell function

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Helicobacter pylori (*Hp*) is a human-specific gastric pathogen, infecting 50% of the world's population and increasing the risk of gastric cancer. *Hp* infection results in peripheral immune cell infiltration, inflammation, and parietal cell proliferation. The vacuolating cytotoxin (VacA), named for its ability to induce vacuole production in human cells, has an unclear role in these processes. Parietal cells are dynamic, gastric-acid-secreting cells that exist in a resting or active state. The resting state sequesters apical membrane proton pumps into the cytosol as tubulovesicles. We hypothesize that VacA targets tubulovesicles to create a microenvironment for *Hp* colonization by maintaining inactive parietal cells. Parietal cells were isolated and cultured from mouse gastric mucosa, then challenged with VacA in a resting or histamine-stimulated active state. The apical and basolateral pH and ion concentrations were measured by metabolomics, and cell morphology and vacuolation visualized by TEM and light microscopy. We predict that VacA-challenged apical media will have an increased pH and concentration of anions, and minimal morphologic changes with co-histamine stimulation compared to controls. The parietal cell tubulovesicles are anticipated to be the observed VacA-mediated vacuoles. Because *Hp* infection induces a prolonged ineffective parietal cell state coupled with proliferation, we propose that VacA targets parietal cells to gather nutrients and create a microenvironment for *Hp* colonization. Understanding the role of VacA in *Hp* infection addresses a fundamental knowledge gap in the study of *Hp* infection, and provides new insight into host-microbe interactions and the importance of secreted microbial effectors.

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The use of SP-10 protein as an acrosome marker in stallion seminiferous tubules

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Optimal breeding stallion fertility is multifactorial; understanding the reasons for infertility would require employment of proper tests to evaluate the progression of spermatogenesis. Previous studies have shown the intra-acrosomal antigen SP-10 as a useful marker for round spermatids and spermatozoa in mice and men. The goal of this study was to evaluate the

usefulness of the SP-10 antibody in the staging of the stallion seminiferous epithelium using immunohistochemistry. Adult stallion testis cross sections were treated with an in-house guinea pig polyclonal antibody raised against the mouse SP-10 antigen at a concentration of 1:750. The Zeiss Axiovert 200M Widefield microscope, the Axiocam 506 Color camera, and ZenPro software were used to capture images. We have found the SP-10 antibody labeling to be highly specific for the acrosome and were able to follow the progression of acrosome development. Historically, staging of cross sections of the seminiferous tubules was done using the morphology of the acrosome as the criterion. In mice, for example, any given cross section of the tubule represents one of the 12 stages of the cycle. Because the SP-10 antibody clearly outlined the acrosome in round spermatids of the stallion, we were able to discern the stage represented in each cross section. In general, we observed more than one stage in a given cross section. Based on observations of 100 cross sections each from three different stallions, 66% had more than one stage represented. In this regard, the stallion exhibited similarities to the human seminiferous epithelium wherein multiple stages are found in a given cross section. These studies provide a reference for staging of the horse seminiferous epithelium.

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Itchy eyes no more: the effects of Zaditor on the healthy canine eye

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The precorneal tear film consists of multiple components that contribute to vision as well as ocular health and nutrition. Previous research demonstrated that the tear film of humans can be negatively affected by certain topical and oral medications; however, such phenomenon has not been determined in dogs. Zaditor is an over-the-counter antihistamine drop frequently prescribed to dogs that have irritated eyes due to allergies. At this time, it is unknown if Zaditor has a detrimental effect on the canine tear film. The purpose of this study is to determine if Zaditor alters the quantity or quality of tears produced in healthy dogs when administered twice daily. We hypothesize that the use of Zaditor will not influence the tear quantity and quality. Healthy dogs were enrolled, and ocular examination and tear production and quality tests performed, including Schirmer tear test (STT), tear osmolality, tear ferning, and meibometry. All parameters were evaluated at baseline, on

days 7 and 14 of treatment, and on day 30 of the study (2-week washout period). To date, baseline values from 25 dogs were obtained. Analysis of variance was used to verify any differences between right (OD) and left (OS) eyes. Mean STT \pm SD (mm/min) were 22.3 ± 2.9 (OD) and 21.8 ± 3.9 (OS). Mean osmolarity \pm SD (mOsm/L) were 305.4 ± 35.3 (OD) and 304.2 ± 33.0 (OS). Mean tear ferning grades \pm SD were 2.1 ± 0.8 (OD) and 2.0 ± 0.8 (OS). Mean meibometry \pm SD (MU) were 115.5 ± 56.7 (OD) and 173.7 ± 139.3 (OS). No significant differences between eyes were observed at baseline day for any test ($p > 0.05$). The results of this work will contribute to better understanding the safety of this topical drug in dogs.

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Making a list and checking it twice – the effects of implementing a surgical checklist in veterinary medicine

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Following development by the World Health Organization, surgical checklists have become an adopted standard in many human hospitals, and studies have shown that they drastically reduce intra-operative and post-operative complications. Despite the proven benefits in human medicine, there has yet to be comprehensive evaluation in veterinary medicine. The aim of this study was to evaluate the impact of adopting a surgical safety checklist in a veterinary medical academic center. We hypothesized that implementing a surgical checklist would decrease the number of perioperative errors and post-operative complications. To begin this study, we looked retrospectively at the orthopedic, neurology, oncology, and soft tissue surgeries that took place in our hospital between March 2017 and May 2017. Of the 301 surgeries that occurred during that time, 21.6% had intra-operative complications and 36.2% had post-operative complications. Next, we observed three weeks of surgeries to observe any perioperative complications prospectively. During this time, a sample checklist was drafted and review by surgeons, anesthesia personnel, and technicians so that a final customized file could be constructed. This final checklist was then implemented for three weeks, during which time surgeries were also observed and perioperative error rates were determined. The number and type of surgical complications associated with the pre-checklist surgeries will be compared to those that used the checklist. We will then extend the study over another three-month period to collect more data and compare results to the retrospective data collected

earlier. Data from this study will assist clinicians in deciding whether to implement use of a surgical checklist.

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How does reproduction affect the personality of female threespined sticklebacks (*Gasterosteus aculeatus*)?

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Evidence has shown that the personality of an animal impacts its life history traits and fitness, but we know little about how life history events like reproduction affect personality. Reproduction is one of the most important events for any individual to experience. The aim of this project is to investigate how personality changes after reproduction in female threespined sticklebacks (*Gasterosteus aculeatus*). We hypothesize that females will be bolder and more risk-prone post reproduction, because the life history theory predicts a trade-off between current and future reproduction. We tested the personality of females before and after reproduction (control: did not reproduce) using three personality assays. The first assay assessed the females' activity level in a new environment, the second assessed how females reacted to conspecifics, and the final assay was a trade-off between resource acquisition (i.e. food) and risk of predation (i.e. wooden bird). All three personality assays were performed on the same experimental and control fish for three consecutive days prior to reproduction. After the final personality assay on day 3, a water sample was obtained for a hormonal assay to evaluate stress and sex hormone levels. Once the "before" assays were complete, the gravid experimental females were placed in a tank with a male for courtship. The females and their controls were then evaluated again using the same assays. The data from the "before" and "after" assays will be compiled and compared to determine if the experience of reproduction changes the personality of females. The results should show that life history could impact personality and provide more evidence about the proximate mechanism for personality change.

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Do the biomechanical differences between the trot and pace affect tarsal osteochondrosis lesions in Standardbred horses?

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Osteochondrosis (OC) is a developmental orthopedic disease that occurs when normal endochondral ossification is disturbed. Standardbred horses are predisposed to OC, particularly in their tarsocrural joint (hock), and it can become a problem when young horses begin race training, as they may show signs of lameness or joint effusion. Both genetic and environmental factors (such as diet and exercise) are known to play a role in the risk of developing osteochondrosis. Previous research has indicated that Standardbred pacers and trotters are prone to developing OC lesions at different locations within the hock. The reason for this difference is unclear but the differing biomechanics of the trot (a two-beat diagonal gait) and pace (a two-beat lateral gait) could play a role. We hypothesize that the amount of time young Standardbreds spend pacing or trotting in the field correlates with the location and severity of the tarsal OC lesions that develop. Our goal is to quantify the activity of these foals in the field and correlate these findings with differences in development and progression of tarsal OC lesions. Horses in the study will be followed from 2 to 12 months of age and observed for a minimum of 2 hours per week with observations made every 30 seconds. In addition, 4 standard views of hock radiographs will be taken and evaluated for OC lesions every two months. By investigating the role of gait biomechanics as a risk factor for OC, we will have a more complete picture of the etiology of this disease and hopefully be able to improve the health of the Standardbred horse.

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Static posturography as a novel diagnostic tool in dogs with suspected thoracolumbar spinal cord disease

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Dogs with spinal cord disease (SCD) are evaluated through the application of subjective scoring systems during serial neurologic examinations. Post-treatment outcome is measured subjectively through the direct observation of an animal's gait, and may overestimate or underestimate a patient's recovery. Static posturography provides an objective, quantitative alternative to subjective gait analysis through measurement of characteristics of an animal's balance in quiet stance. The purpose of this study was to evaluate the utility of static posturography as an objective diagnostic adjunct and prognostic indicator in dogs with suspected thoracolumbar SCD. Control dogs demonstrated a normal neurologic examination and had no known history of neurologic or musculoskeletal disease. Affected dogs presented with clinical signs of thoracolumbar SCD, including pain, weakness and incoordination. All dogs were placed in quiet stance on a Tekscan Walkway™ force and pressure measurement system. Measurements related to each dog's balance were taken during five trials, including aggregate distance traveled by the dog's center of force (COF), maximal cranial/caudal and medial/lateral movement of COF, and weight distribution by limb. The authors hypothesize that these values will be significantly larger in dogs with thoracolumbar SCD. This study has important implications for the development of static posturography as a novel method of evaluating recovery and efficacy of treatment in dogs with SCD.

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Integrating prediction of parturition and calcium supplementation for prevention of subclinical hypocalcemia

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Accurately predicting parturition has the potential to allow for strategic prevention of metabolic disorders such as subclinical hypocalcemia, which affects between 25% and 54% of lactating dairy cows in the U.S. Adequate cytosolic ionized calcium concentration (ICC) is vital for the regulation of neurotransmission and muscle contraction. Cows diagnosed with hypocalcemia can develop problems such as displaced abomasum, uterine prolapse, and retained placenta due to the low ICC suppressing smooth muscle contraction. Moocall is a tail-mounted sensor that uses an algorithm to predict when cows are likely to calve by measuring tail movement patterns triggered by labor contractions. This device sends 2 text messages for

prediction of parturition when tail movements reach a certain level of intensity that generally suggests that parturition will occur within 1-2 hours. We hypothesize that the first message Moocall sends predicting parturition can be used as a time point for strategic prevention of hypocalcemia in dairy cows. Devices will be placed on multiparous Holstein dairy cows. Excel random function will be used to assign cows to treatments (calcium supplemented and control). Calcium-supplemented cows will receive an oral bolus containing 43 g of calcium (Bovicalc) at the time of the first message predicting parturition followed by two boluses at 0 and 24 h after calving. Control cows will not receive calcium supplementation, but will receive a sham bolus administration. By evaluating the accuracy of Moocall and the effects that administering an oral calcium bolus prior to calving has on cows, the welfare of the cow can be improved by regulation of calcium homeostasis at calving and associated health disorders.

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The effect of anal fin color on male mating depth preference in Bluefin Killifish (*Lucania goodei*)

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Bluefin killifish (*Lucania goodei*) populations provide the opportunity to study the population biology of color polymorphism. In most natural systems, one color morph in a species with color polymorphism will go to fixation due to natural selection or genetic drift. However, in *L. goodei* populations found in springs, males display both red and yellow anal fin colors. This polymorphism is controlled by a single locus, and each naturally occurring population has both color morphs. One hypothesis for the maintenance of polymorphisms within populations is that there are different microenvironments where each color morph has a higher level of fitness. The aim of this study was to determine the effect that male *L. goodei* anal fin color has on preferential mating depth in order to determine if mating depth could be a driving factor in the presence of color polymorphism in this species. Red is more vibrant in clear water at shallower depths, so males with red anal fins should display higher preference for mating in shallow water when compared with yellow-finned males. One male and five female *L. goodei* were placed in a tank set up with spawning mops at three different depths. The mops were removed twice daily for five days and the eggs were removed from the mops and counted. The eggs were placed in a solution of methylene blue to determine

whether they had been fertilized. Preliminary data showed that the eggs were most often laid and fertilized at the top of the tank regardless of the coloration of the male. These results indicate that depth is unlikely a major factor in maintaining polymorphism within *L. goodei* populations. Alternative hypotheses to explain maintenance of color polymorphism are being tested.

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Loss of USP18 disrupts brown adipose tissue and causes multi-organ auto-inflammation

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Ubiquitin-Specific Protease 18 (USP18) is a dual-function protein that removes interferon stimulated gene 15 (ISG15) protein modifications and inhibits the activity of the interferon receptor. Necropsy analysis of USP18-null mice revealed immune infiltration of multiple tissues including brown adipose, pancreas, salivary glands and skin. The mice exhibit phenotypes similar to those in autoimmune diseases such as systemic lupus erythematosus and Sjogren's Syndrome. USP18-null mice showed elevated liver glycogen levels, which are associated with liver damage and stress. Gene expression analysis of peripheral blood lymphocytes (PBL), liver, brown adipose tissue (BAT) and white adipose tissue (WAT) were performed using samples derived from USP18-null, heterozygous, and wildtype mice. Interferon signature genes were upregulated in PBL of USP18-null mice and this effect was amplified in BAT where IFIT1, IFR7, OAS1, OASL and ISG15 were each increased over 100-fold. Immunohistochemical analysis demonstrated that both infiltrating immune cells and the brown adipose cells had upregulated ISG15. USP18-null mice cannot maintain body temperature in a cold-tolerance test, suggesting a defect in BAT function. We hypothesize that USP18 reduces responsiveness to type 1 interferon signaling in BAT to maintain its normal thermogenic activity. In the absence of USP18, BAT function is disrupted through amplified interferon signaling causing dysregulation of lipid metabolism, cold intolerance and potentially autoimmunity. These studies demonstrate the usefulness of the USP18-null mouse for studying signaling pathways maintaining normal BAT function and the link between dysregulated interferon signaling and autoimmune disease.

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Rapid identification of *Mannheimia haemolytica* tetracycline resistance in Illinois cattle

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Bovine Respiratory Disease (BRD) is a major health concern in North American feedlot cattle contributing to major economic losses. *Mannheimia haemolytica* is a bacterium considered to be the predominant pathogen associated with BRD. Antibiotics, specifically tetracyclines, have often been used as a feed additive for prevention and treatment of bacterial infections common in feedlot cattle. Because the increased use of antibiotic treatment can select for antibiotic resistance, a method for rapidly identifying resistance can aid in drug selection and treatment success. This study aims to evaluate MALDI-TOF mass spectrometry as a method for rapid identification of *M. haemolytica* tetracycline resistance in Illinois cattle. *M. haemolytica* isolates were collected from tissues taken from cattle with respiratory disease submitted for necropsy to the University of Illinois Veterinary Diagnostic Laboratory. Isolates were subjected to MALDI-TOF, an evolving diagnostic methodology that can provide rapid identification of microbial isolates, as well as information about antimicrobial resistance profiles. Traditional PCR methods were used to detect the *tetH* genotype commonly associated with tetracycline resistance in *M. haemolytica*. Mass spectrometry peaks from tetracycline-resistant and susceptible isolates will be analyzed to detect the presence of unique protein peaks that might identify resistant isolates. Data from these analyses will be combined with information from the PCR assays, as well as clinical and demographic information. A method for rapid detection of tetracycline resistant *M. haemolytica* isolates would improve treatment outcomes for cattle with respiratory disease.

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Does proximity to railroads increase arsenic-related health risks?

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Arsenic-contaminated soil and water pose serious health concerns for both humans and animals. Exposure to arsenic can occur through ingestion of contaminated water or through food grown in arsenic-containing soil, through aerosolized particles or through dermal contact. Railroads have historically transported arsenical-based pesticides and housed arsenic-containing cattle-dipping vats. Moreover, railroad rails are coated in arsenic. We hypothesize that the risk of arsenic exposure increases with proximity to railroads. Escambia and Santa Rosa counties in Florida provided test cases for the characterization of arsenic contamination. These counties were chosen because of past arsenic use on cotton production, the transportation of cattle, and the presence of arsenic-containing superfund sites. A previous study of 89 soil samples from these counties provided data for a spatial statistical analysis of arsenic levels. An additional 25 soil samples and 10 water samples were collected in summer 2017. Samples were collected near railroads and elsewhere in the counties for comparison. Data from this work will help confirm the locally variable presence of arsenic and better equip scientists and public health officials to raise public awareness and prioritize remediation of arsenic-contaminated soil and water.

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Effects of *in utero* exposure to DEHP and high-fat diet on uterine development in mice

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Di(2-ethylhexyl) phthalate (DEHP) is an endocrine-disrupting chemical found in many consumer products. DEHP exposure is related to endocrine and metabolic diseases in humans, and food ingestion is the main source of DEHP exposure. Studies have suggested that DEHP and adipose signaling may act through similar metabolic pathways, specifically PPAR α . It is well-documented that obesity increases the risk of developing endometrial carcinomas, but no link has yet been found to suggest DEHP involvement. In this study, we investigated the effects of prenatal exposure to DEHP and high-fat diet on the development of the uterus. Pregnant dams were orally dosed with either a corn oil control or 20 $\mu\text{g}/\text{kg}/\text{BW}$ DEHP, and fed either a control diet of a balanced maintenance chow or a “western” high-fat diet with 45% of calories derived from fat. Diets were fed from day 0.5 of pregnancy, and continued until parturition. Uterine tissues from post-natal day (PND) 8 and PND 21 pups were collected from all treatment groups. Preliminary

results indicated *in utero* exposure to a high-fat diet resulted in accelerated adenogenesis, uterine gland development, at PND 8. By PND 21 the pups exposed to high-fat diets or DEHP *in utero* showed increased proliferation of the uterine luminal epithelium. These results suggest that *in utero* exposure to DEHP or a high-fat diet may lead to uterine epithelial hyperplasia later in life. Uterine epithelial hyperplasia is associated with implantation failure in women, as well as being a premalignant lesion of endometrial carcinoma.

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Proteinuria in dogs with atopic dermatitis: a retrospective analysis

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Atopic dermatitis is an inflammatory skin condition in dogs, resulting in intense pruritus, self-trauma, and papules, amongst a variety of other cutaneous lesions. These animals are most commonly treated with immunomodulators, such as cyclosporine or oclacitinib, or systemic anti-inflammatories such as glucocorticoids. Given the inflammatory nature of atopic dermatitis, proteinuria is expected to occur in some atopic dogs. In this retrospective study, the records of 77 client-owned dogs with atopic dermatitis were analyzed for evidence of proteinuria. Animals with proteinuria before or at the time of diagnosis of atopy were further studied to determine whether the proteinuria persisted after treatment with oclacitinib, cyclosporine, or glucocorticoids. It was hypothesized that systemic treatment of atopic dermatitis would decrease or eliminate proteinuria in these dogs. Preliminary results of this on-going analysis indicated that most dogs exhibiting proteinuria prior to, or at the time of diagnosis with atopic dermatitis, did not experience lessening or resolution of proteinuria following treatment of atopy. This study revealed that proteinuria is present in a small number of dogs and can be attributed to atopic dermatitis. In addition, proteinuria is unlikely to resolve, even after the atopic dermatitis becomes well-controlled with treatment.

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In vivo gene knockdown approach for validation of gene function in *Cryptosporidium parvum*

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Cryptosporidium parvum is a parasitic protozoan of medical and veterinary significance worldwide. Infections with *C. parvum* occur through ingestion of contaminated water or food, and cause a diarrheal syndrome called Cryptosporidiosis, that may be life-threatening in immunosuppressed individuals. Currently, there is only one approved drug, nitazoxanide, used for the treatment of *Cryptosporidium* infections but it is ineffective in malnourished children and immunosuppressed individuals. In this study, we endeavored to develop an in vivo assay for interrogating gene function throughout the life cycle of *C. parvum* using phosphorodiamidate morpholino oligomers. By targeting the *C. parvum* lactate dehydrogenase (LDH) gene, we achieved over 90% knockdown of its expression during *C. parvum* growth and development in IFN-gamma knockout mice. To analyze the effect of LDH knockdown, we performed real-time PCR to quantify the amount of *C. parvum* oocysts shed by infected mice treated with LDH-target morpholino compared to those treated with control (off-target) morpholino. We then used the oocysts shed by the two groups of mice to infect HCT-8 cells to assess the effect of LDH knockdown on viability. We found that LDH knockdown significantly decreased the growth and development of *C. parvum*, and that the oocysts shed contained daughter parasites with significantly reduced infectivity. These studies have developed an antisense gene knockdown approach for studying gene function in *Cryptosporidium*, and validated LDH as an essential gene for growth, development and viability of *C. parvum*. This technology will be useful in functionally characterizing and validating potential molecular drug targets in *Cryptosporidium*.

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