Can exogenous insulin improve sperm motility in cooled-stored stallion semen?

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Unlike many domesticated large animal species, stallions are selected based on athletic and phenotypic characteristics rather than fertility. Stallion semen is often extended, cooled, and shipped to the location of the mare prior to breeding, and sperm motility inevitably decreases as semen ages despite the addition of semen extender. There is active interest in identifying additives to extend the life and improve motility of sperm, to increase the likelihood of successfully impregnating mares. Recent studies in human males showed that insulin may play a role in sperm metabolism and motility. We hypothesized that addition of insulin to stallion semen would have a positive effect on sperm viability and motility, compared to an insulin-negative control. To test this hypothesis, semen was collected from eight stallions using the Missouri artificial vagina for a total of 24 separate ejaculates. Semen extender was used to dilute each ejaculate to a final volume of 50 ml and a concentration of 25 million sperm/ml. The extended ejaculate was divided into three separate 15 ml aliquots. One was designated as the control, 0.25 IU insulin/ml was added to the second and 1.0 IU insulin/ml was added to the third. Aliquots were further divided into three smaller 5-ml aliquots and stored in a passive cooling device for subsequent examination of motility. A computer-aided sperm analysis machine was used for the testing. Motilities were analyzed at 0, 24, 48, and 72 hours post-collection. Completion of this analysis will indicate whether addition of exogenous insulin aids in preservation of semen quality. Positive results would indicate utility of the method for improving mare breeding and production of new foals for the horse industry.

Research Grant: University of Illinois Department of Veterinary Clinical Medicine
Student Support: Merial Veterinary Scholars Program

Evaluating efficacy of hygiene plans in reducing prevalence of *M. avium ssp. paratuberculosis* in beef cattle

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Johne's Disease (Paratuberculosis) is a chronic, contagious enteric infection that primarily affects cattle and small ruminants. It is caused by infection with *Mycobacterium avium ssp. paratuberculosis* (MAP). Young animals are believed to be most susceptible. Because infected animals typically do not begin shedding the bacterium or showing clinical signs for several years, identifying them is difficult. Current recommendations include improved hygiene practices to minimize disease, particularly in calving areas. The purpose of this study is to determine the longterm effect of improved hygiene interventions on the prevalence of MAP in individual herds. Surveys conducted at multiple beef cattle farms in North Dakota over the course of seven years were gathered and analyzed. Scores that reflected hygiene standards amongst different age groups were tracked over the years and contrasted with the prevalence of MAP in the herd. Preliminary data suggest efficacy of the hygiene interventions because there was a marked decrease in MAP prevalence 2-3 years after the interventions were implemented. Demonstrating that the hygiene interventions are effective would confirm the rationale for their use, and encourage more herd owners to adopt these practices.

Research Grant: Office of Research Infrastructure Programs, NIH Grant Number 8K01OD01968-04
Student Support: Office of the Director, NIH, T35 OD011145

Immersion anesthetics in freshwater stingrays: Comparing the effects of MS-222 and alfaxalone on *Potamotrygon*

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Freshwater stingrays (*Potamotrygon* spp.) are becoming increasingly popular additions to home and institutional aquariums. Consequently, veterinarians may be asked to treat them when medical issues arise. The purpose of this study was to evaluate two different types of immersion anesthetics for freshwater stingrays and develop anesthetic protocols to safely accommodate them for examinations, diagnostic testing, and surgery. Eight

juvenile stingrays were used for this crossover study. The average body weight for these animals was 61.2 g (39.5 – 103.6 g). Two different commonly available anesthetics were used: tricaine methanesulfonate (MS-222) and alfaxalone. Stingrays were randomly assigned to two groups. Group 1 received MS-222 first, followed by alfaxalone, while group 2 received alfaxalone first, followed by MS-222. A 2-week wash-out period was used between trials. Baseline heart rate, respiration rate, and response to noxious stimuli were assessed prior to induction and every 3 minutes until the stingrays were recovered. Blood samples for blood gases were taken before induction (baseline), when a surgical level anesthesia was reached, and after full recovery. Average induction times were 2 minutes for MS-222 and 2.5 minutes for alfaxalone. Both anesthetics successfully anesthetized the stingrays to a level deep enough for surgical anesthesia, with full recoveries being achieved, on average, of 12 minutes for MS-222 and 24 minutes for alfaxalone. By comparing blood gas and electrolyte values, induction rates, response to noxious stimuli, and recovery times, we were able to determine that both anesthetics could be used safely in these animals.

Research Grant: None
Student Support: Merial Veterinary Scholars Program

Promotion of malignant osteolysis via secretion of bone resorptive cytokines in canine osteosarcoma

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Interleukins (IL-1β, IL-6, IL-8, and IL-11) and tumor necrosis factor (TNFα) are inflammatory cytokines that contribute to pathologic bone resorption. Cytokines typically promote bone resorption through RANKL-dependent pathways to enhance osteoclastogenesis. Canine osteosarcoma (OS) is a common bone tumor characterized by focal bone destruction. We hypothesize canine OS cells will produce and secrete IL-1β, IL-6, IL-8, IL-11, and TNFα, and will consequently enhance osteoclastogenesis and bone resorption. The expression and secretion of cytokines were investigated by western blot, immunohistochemistry, and ELISA using four immortalized canine OS cell lines and normal canine osteoblasts. The ability of cytokines to promote osteoclastogenesis is being evaluated by counting osteoclast numbers derived from canine peripheral blood mononuclear cells stimulated with M-CSF, RANKL, and ± cytokine. Our data indicate canine OS cells express IL-1β, IL-6, IL-8, IL-11, and TNFα, however only IL-6 and IL-8 are basally secreted. Furthermore, OS cells secrete markedly greater concentrations of IL-6 and IL-8 compared to normal osteoblasts. The synergies of OS-derived cytokines

with RANKL for promoting osteoclastogenesis are being studied. In vivo studies will be conducted to describe the involvement of IL-6 and IL-8 in dogs with OS through the measurement of circulating cytokine levels and localized tumor expression studies. This study suggests that canine OS cells can secrete IL-6 and IL-8, which could promote unregulated bone resorption within the primary tumor microenvironment. As such, small molecule inhibitors of IL-6 and IL-8 may serve as treatment options for slowing tumor growth and alleviating malignant bone pain in dogs with OS.

Research Grant: University of Illinois College of Veterinary Medicine
Student Support: Merial Veterinary Scholars Program

Effect of combinative treatment with metformin and dichloroacetate in feline carcinoma cell line

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Existing drugs with putative anti-neoplastic activity may be repurposed and brought to clinical manifestation more rapidly than novel drug compounds. Metformin, an anti-diabetic agent that reduces cancer risk in people, shows promise in feline cancer. In cats and people, metformin therapy can result in the development of hyperlactatemia and potential subsequent acidosis. Dichloroacetate (DCA) is one clinical option for treating human hyperlactatemia. Both metformin and DCA have been shown to promote apoptosis in human carcinoma *in vitro* and DCA may also attenuate metformin-induced lactate production with potential additive cytotoxicity. We hypothesize that metformin and DCA have cytotoxic and metabolic effects in feline carcinomas. Two feline cell lines (CAT-MT mammary carcinoma and SCCF1 oral squamous cell carcinoma) were treated with DCA and metformin at various concentrations. Cell viability/cytotoxicity was monitored with a tetrazolium-based assay, lactate dehydrogenase assay, colony formation assay, and Trypan blue exclusion. Western blotting was used to assess protein expression of total and phosphorylated pyruvate dehydrogenase E1α (PDE1α/p-PDE1α), an enzyme involved in lactate production. Effect on lactate metabolism was assessed via supernatant lactate quantification and pH. Preliminary results demonstrate that 1) metformin and DCA decrease cell viability in a dose-dependent manner, 2) feline carcinomas express PDE1α/p-PDE1α *in vitro* with modulation by drug therapy, and 3) DCA ameliorates metformin's decrease in supernatant pH. This study demonstrates anti-neoplastic activity of DCA and metformin in feline cell lines and may lay the groundwork for clinical metformin/DCA combination therapy of feline carcinoma.

Research Grant: J. Wypij, Donor gift funds
Student Support: Merial Veterinary Scholars Program

Effect of pre-plating on tendon-derived progenitor cell enrichment

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Interest in cell-based therapy for tendon injuries in horses has grown vastly in the past decade. Accepting the great promise stem cell (SC) therapy holds for improving tissue repair in many contexts, identifying and enriching the small SC numbers in primary samples for ex vivo expansion is important for clinical applications. Several methods have been developed to select stem/progenitor cells from tissue and body fluids. One of them, the pre-plate technique was originally developed for muscle-derived SC isolation and is based on the differential attachment of non-stem (rapid) and SC (slow) to cell culture plastic surfaces. The objective of this study was to determine whether pre-plating enriches for tendon-derived stem cells (TDSC). Immuno-phenotypic characteristics (fluorescent activated cell sorting-FACS) and tri-lineage differentiation capacities of cells obtained at early, mid and late stages of pre-plating were assessed. Donor-matched bone marrow-mesenchymal stem cells (BM-MSCs) were used as control. There were no differences in immuno-phenotype characteristics among the different pre-plates of TDSC and BM-MSCs. FACS analysis showed that more than 90% of cells from each pre-plate were CD29+, CD44+, CD90+ and CD45-. In addition, phenotypic assays confirmed similar adipogenic, chondrogenic and osteogenic differentiation in all TDSC pre-plates. RT-PCR analyses for lineage-specific gene expression were similar among the pre-plates and about 3-4 fold less than BM-MSCs. These data show that pre-plating does not enrich for tendon-derived progenitors. Rather, un-segregated tendon digests are adequate for purification of stem cells via monolayer expansion.

Research Grant: Animal Health and Disease Research Funds
Student Support: Office of the Director, NIH, T35 OD011145

Glowing guts? Exploring the use of fluorescent *Candida albicans* in a piglet model of oro-GI tract colonization

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Candida albicans (*C.a.*) is a commensal fungus of humans. Disruption of normal microbiota or immune function can lead to candidiasis. In its disseminated form, the disease can be life-threatening. The patient's oro-gastrointestinal tract (oro-GIT) often serves as the source of *C.a.* in these cases. Reducing a patient's fungal burden may prevent disseminated disease. The mouse is commonly used for studying *C.a.* colonization, even though antibacterial pre-treatment is needed for *C.a.* to colonize the murine oro-GIT. In previous work, we developed a piglet *C.a.* colonization model that is established simply by feeding *C.a.* cells to the animals. Quantification of fungal burden is labor-intensive with this model, however. It requires many workers to harvest and homogenize tissues, plate serial dilutions of homogenates, and count the resulting colonies. It is difficult to achieve consistency in methods among individual workers. We hypothesize that use of fluorescent *C.a.* will improve the piglet model because tissue sections can be viewed microscopically, morphology of fungal cells documented, and fungal burden quantified using image analysis software. A *C.a.* strain that produces green fluorescent protein and another that produces a near-infrared fluorescent protein were evaluated. In vitro analyses assessed growth and morphology of the strains, and stability of fluorescence following treatment with common tissue fixatives. In vivo experiments investigated piglet colonization, and provided tissue specimens for developing visualization and quantification protocols. A relevant animal model of *C.a.* colonization will facilitate development of methods to reduce fungal burden in patients at-risk for disseminated candidiasis.

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

Isoliquiritigenin reduces antral follicle growth and estradiol levels in the mouse ovary

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Isoliquiritigenin (ISL) is a phytoestrogen that can be extracted from licorice root. ISL has been used as an anti-inflammatory, antimicrobial, and chemotherapeutic agent, but little is known about the effects of this phytoestrogen on the ovary. Thus, the purpose of this study was to test the hypothesis that exposure to ISL affects ovarian antral follicle growth and estradiol levels. To test this hypothesis, antral follicles (260-400 μM) were mechanically isolated from the ovaries of CD-1 mice, and cultured with vehicle control (dimethylsufoxide) or varying doses of ISL (600 nM, 6 μM, 36

μM, and 100 μM) for 48 or 96 hours. The sizes of the follicles were measured every 24 hours. At the end of the cultures, media were collected and subjected to measurements of estradiol using enzyme-linked immunosorbent assays. Exposure to the lower doses of ISL (600 nM, 6-36 μM) did not significantly affect follicle growth at any time point. However, the highest dose of ISL (100 μM) significantly reduced follicle growth at 72 hours (DMSO: 121.72 ± 6.48; ISL 100 μM: 103.10 ± 1.69 % change, n=3, p≤0.05) and 96 hours (DMSO: 145.75 ± 7.41; ISL 100 μM: 101.67 ± 1.58 % change, n=3, p≤0.05) compared to control. Further, the low doses of ISL (600 nM, 6-36 μM) did not significantly impact estradiol levels compared to the control. However, the highest dose of ISL (100 μM) significantly inhibited estradiol levels compared to controls (DMSO: 1996.28 ± 666.31; ISL 100 μM: 134.81 ± 15.06 pg/mL, n=3, p≤0.05). Collectively, these data indicate that ISL at 100 μM, but not at 600 nM-36 μM, reduces antral follicle growth and estradiol production. These data suggest that high doses, but not low doses, of ISL may be ovarian toxicants.

Research Grant: National Institute of Environmental Health Sciences, NIH, R03 ES023972
Student Support: Office of the Director, NIH, T35 OD011145

Not all soy is digested "equol": equol's adverse effects on ovarian follicular growth and estradiol production

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Equol is a non-steroidal isoflavone derived from the soy phytoestrogen daidzein by gastrointestinal microbiota. Equol is commonly used as a dietary supplement and as a pharmaceutical agent for hormone imbalances. This lab previously demonstrated that equol exposure for 96 hr inhibits growth and estradiol production in intact antral follicles *in vitro*. However, it is unknown whether acute exposure to equol has similar effects. We hypothesize that acute equol exposure causes decreased follicular growth, resulting from lower estradiol production. To test this hypothesis, antral follicles from CD-1 mice were mechanically isolated and cultured in vehicle control (dimethylsulfoxide; DMSO) or equol (600 nM, 6 μM, 36 μM, 100 μM) for 48 or 96 hr. Follicle diameters were measured every 24 hr. Upon culture completion, media were collected to measure estradiol levels by enzyme-linked immunosorbent assays. The results indicate that at 48 hr, equol (100μM) did not significantly affect follicular growth, but it significantly increased the production of estradiol compared to the vehicle control (DMSO: 118±34.4 pg/mL; 100 μM equol: 369.1±35.6 pg/mL; n=3, p=0.001). At 72-96 hr,

equol significantly decreased follicular growth (72 hr DMSO: 138.5±2.6 µm; 72 hr 100 µM equol: 111.9±5.1 µm; n=3; p=0.015; 96 hr DMSO: 156.1±4 µm; 96 hr 100 µM equol: 121.5±3.6 µm; n=3; p=0.002). At 96 hr, equol (100 µM) also significantly decreased estradiol production compared to the vehicle control (DMSO: 6203.9±1050.6 pg/mL; 100 µM equol: 1803.5±265.7 pg/mL; n=3; p=0.033). These results indicate that short-term exposure to equol (24-48 hr) does not inhibit follicle growth, but longer-term exposure (72-96 hr) inhibits follicle growth and estradiol production.

Research Grant: Office of the Director, NIH, T35 OD011145
Student Support: National Institute of Environmental Health Sciences, NIH, R03 ES023972

The effects of metritis on dairy herd reproduction, production and profitability

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Metritis is a postpartum disease that negatively affects the health and well-being of cows and the sustainability of dairy farms. To date, there have not been controlled studies thoroughly evaluating the economic impact of metritis. We hypothesized that an economic analysis taking into account differences in antibiotic costs, mean time to pregnancy, differences in cure rate between treatments, and decrease in milk production would reveal the least costly approach to treat metritis. To test this hypothesis, cows diagnosed with metritis were blocked by parity and within each block allocated randomly to receive either ampicillin (N=259), or ceftiofur (N=269). Additionally, a no metritis control group matching parity and day in milk was enrolled (N=268) to be used as baseline for comparison. Mean time to pregnancy was analyzed using PROC PHREG and LIFETEST on SAS 9.4, while milk production and cull rate were evaluated using PROC GLIMMIX. While there was no significant difference between cull rates, the mean time to pregnancy was 12 days longer for cows with metritis, and those treated with ampicillin produced nearly 700 pounds of milk more than those treated with ceftiofur. We will account for these differences in a subsequent economic analysis using market prices from the time period in which the study was conducted. Results from this analysis will provide practical guidelines for the management of metritis in dairy herds.

Research Support: University of Illinois College of Veterinary Medicine
Student Support: Merial Veterinary Scholars Program

Does mother know best? Factors that influence habitat attractiveness for *Culex pipiens* oviposition

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Management of the mosquito *Culex pipiens* is crucial to public health because it is the main vector for West Nile Virus (WNV) in the Midwest, a common concern not only for humans, but also for populations of birds and animals. The main predictor for risk of WNV infection is local abundance of vectors; the abundance of these vectors is determined by the attractiveness and quality of larval habitats. Leaf detritus present in these larval habitats is an important factor in creating an ideal environment. A previous study found that leaf infusions of an exotic invasive plant, *Lonicera maackii* (Amur honeysuckle), and a native plant, *Rubus allegheniensis* (blackberry), attracted the most female mosquitoes to lay eggs. While honeysuckle provided a high-quality habitat for larval development, blackberry leaves acted as an ecological trap that prevented larvae maturation. This study aims to determine the mechanism that makes these habitats attractive for mosquito oviposition: the microbial community formed by the leaves, or chemical aspects of the leaves. We hypothesized that oviposition behavior is determined largely by the microbial community of the leaf infusions. Binary choice assays were conducted in which field-collected gravid females were put in cages with two cups where each cup contained one of the following treatments: whole leaf infusion of Amur honeysuckle or blackberry leaves, microbes, leaf extract, or deionized water. Egg rafts were counted after 3 days. Findings from this study may allow for effective management of *Cx. pipiens* and WNV infection incidence. Results from this study may also indicate whether mosquito-attracting leaves can be used to lure mosquitos into certain habitats for insecticide application.

Research Grant: U.S. Environmental Protection Agency Science to Achieve Results (STAR) Fellowship
Student Support: Office of the Director, NIH, T35 OD011145

Does prenatal exposure to di-ethylhexyl phthalate affect spleen cytokine levels in mouse pups and their dams?

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Di-(ethylhexyl) phthalate (DEHP) is a plasticizer found in numerous products including food containers, toys, and medical equipment. Unfortunately, DEHP can leach out of plastics and bio- accumulate in fats after absorption. This capability represents a significant health issue because phthalates, including DEHP, are associated with endocrine toxicity and neurotoxicity, as well as immunotoxicity. For instance, DEHP acts as an adjuvant that can disrupt normal and pathological immune pathways. Further, the immune system in young animals appears more sensitive to immunotoxicants, especially with *in utero* exposure. There is also evidence that immune markers and functions can be sex-specific. Yet, very little is known about DEHP developmental immunotoxicity and its potential gender dimorphism. Thus, this project aimed to investigate cytokine levels in the offspring of CD-1 mice that were exposed to DEHP orally throughout pregnancy (0, 20 or 200 ug/kg/day, 500 or 750 mg/kg/day). At various time points (pups at Post- Natal Day 8, PND21, and PND60; dams at PND21), spleens were collected to measure levels of selected cytokines (Th2 markers: IL-4, IL-5, IL-13; Th1 markers: IL-12p70 and IFNγ) using a Luminex assay. Our initial data in the dams suggests an increased inflammatory response towards Th2 > Th1 when exposed to DEHP at 750 mg/kg/day during pregnancy, even three weeks after discontinuation of the toxin. This Th2 imbalance was not observed in pups' spleens. Interestingly, IL-12 levels increased between PND21 and PND60, suggesting a mounting Th1 response in these juveniles. Further work is underway to analyze any sex differences in our selected markers.

Research Grant: University of Illinois Campus Research Board (RB13207)
Student Support: Department of Comparative Biosciences; NIH/USEPA P01 (ES022848-2, RD835434010)

Risky business: Factors that contribute to the presence of human cases of West Nile virus in the Chicago area

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West Nile virus (WNV) is a mosquito-borne pathogen transmitted to humans via *Culex* mosquitoes. Transmission is via a dynamic mosquito-bird-mosquito transmission cycle that sometimes spills over to humans, horses, and other mammals. In humans, fewer than 1% of WNV infections develop into a neuro-invasive disease, but 10% of those cases are fatal, and WNV is an ongoing

public health concern in the United States. Prevention efforts have focused on active surveillance and reduction of the vector mosquito population, monitoring of reported human cases, and promoting preventive practices, including mosquito repellants and avoiding outdoor activities during peak mosquito activity. Previous studies have identified weather conditions, landscape features and several demographic features as the risk factors for human WNV, but this varies spatially. The human illness reported to public health authorities is a primary indicator of risk in an area, but passive surveillance systems can lead to an incomplete picture of risk and be biased toward specific neighborhoods. Using historical data of human cases and mosquito infection rates in northeastern Illinois during two years of high prevalence of human cases (2005 and 2012) this analysis determined spatial associations between human WNV cases and WNV mosquito infection rate, while also accounting for socio-economic, weather related, and land use factors. The resultant model was then used to identify areas of spatial mismatch between human illness and mosquito infection and to determine the factors that are related to this incongruity. Also reported are the preliminary results of the use of canine surveillance to supplement the human case data as a measure of potential exposure to WNV.

Research Grant: NSF Ecology of Infectious Disease Grant 0429124, Mariangela Segre Student Support Research Grant 2015, Department of Pathobiology, University of Illinois, Urbana, IL
Student Support: Office of the Director, NIH, T35 OD011145

Time to return of righting reflex in *Bufo marinus* anesthetized with isoflurane, sevoflurane or desflurane

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Volatile anesthetics are commonly used in mammals for general anesthesia. Isoflurane, sevoflurane, and desflurane induce similar states of anesthesia, however they have different solubilities in various tissues resulting in different recovery times. Potency of inhalant anesthetics in non-mammals is expressed as minimum anesthetic concentration (MAC), the concentration of anesthetic required to immobilize 50% of the subjects exposed. Loss of righting reflex (LRR) is commonly used to demonstrate anesthetic effects in non-mammals. It is generally recognized that 1.5 X MAC is necessary to produce an anesthetic plane in 100% of subjects. We tested the hypothesis that there would be a difference in time to return of righting reflex in toads anesthetized with isoflurane, sevoflurane, or desflruane. In a random, cross-over design, 10 toads

were anesthetized with each of the anesthetic agents at 1.5 X MAC_{LRR}. Time to return of righting reflex was determined from discontinuation of exposure to an anesthetic to when a toad spontaneously returned to sternal position from dorsal recumbency. Median (range) recovery times were 175 min (123-211) for isoflurane, 192 min (116-383) for sevoflurane, and 74 min (52-220) for desflurane. Toads in the desflurane group recovered 2.5 times faster than isoflurane or sevoflurane toads. These findings suggest that the use of desflurane may be a more appropriate choice of anesthetic for amphibian species when a shorter recovery period is desirable.

Research Grant: Clark-Price Anesthesia Laboratory External Unrestricted Gift Funds
Student Support: Merial Veterinary Scholars Program

Battling feline oral squamous cell carcinoma with deoxynyboquinone compounds in combination with radiation

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Oral squamous cell carcinoma (SCC) is the most common malignant tumor in cats. It responds poorly to available therapies including surgery, chemotherapy, and radiation therapy. Novel therapeutics which are effective against this aggressive cancer would be extremely valuable for the treatment of these animals. Additionally, cats are excellent natural models for human head and neck squamous cell carcinoma and treatments that benefit them may also be effective for humans. NQO1, a 2-electron reductase commonly overexpressed in solid tumors, is typically responsible for the detoxification of quinones. However, reduction of certain quinones such as deoxynyboquinone (DNQ) by NQO1 leads to an unstable hydroquinone that generates reactive oxygen species (ROS) resulting in selective cancer cell death. We show here that two out of three feline SCC cell lines were sensitive to treatment with DNQ and IB-DNQ (isobutyl DNQ). Western blot confirmed that the sensitive cell lines overexpressed NQO1 while the insensitive cell line did not. Additionally, studies using NQO1 inhibitors confirmed the dependence of compound activity on NQO1. The resistant cell line was exposed to ionizing radiation in order to induce NQO1 expression and increase its sensitivity to DNQ. Together these results showed that feline SCC was sensitive to the

deoxynyboquinone class of compounds in cell culture and new treatments involving IB-DNQ or DNQ may be effective either alone or in combination with other therapeutic strategies.

Research Grant: University of Illinois Department of Chemistry
Student Support: Office of the Director, NIH, T35 OD011145

Development of a PEDV transmission model for live haul transport at swine lairage facilities

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Porcine Epidemic Diarrhea Virus (PEDV) is an alpha coronavirus that is found globally. It was introduced into the United States in 2013, causing high neonatal mortality. Previous studies showed that live animal transport to harvest plants is a key potential route of transmission during an outbreak. We developed a model to replicate the process of unloading pigs into lairage. The model allowed us to test the effects of several environmental and management variables on PEDV transmission and survival. The trailer was modeled using an aluminum sheet; a plastic tub filled with a mix of manure and shavings was used to simulate the unloading dock. A foot contact event was mimicked using a clean plastic boot to step on the model dock and directly onto the model trailer. Variables such as conditions on the dock (temperature, UV light, scraping, PEDV concentration) and in the trailer (temperature, humidity) were tested. PEDV was detected pre- and post-contamination using real-time polymerase chain reaction (RT-PCR). In trial 1 we investigated the impact of trailer temperature on PEDV persistence over 1 hour. Trial 2 focused on dock conditions including scraping, temperature, UV light intensity and time post-contamination on PEDV contamination in the trailer at 1 hour post contact. Under the conditions of the study, transmission of PEDV was highly efficient as PEDV RT-PCR CT values were similar on the dock, at time 0 and at 1 hour post contact on the trailer. The results of trial 2 are pending. This model confirms that lairage is a significant risk for the dissemination of PEDV between swine production sites.

Research Grant: National Pork Board Grant #14-266
Student Support: Merial Veterinary Scholars Program

The effect of low-dose aspirin on the primary feline urinary thromboxane metabolite

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Thrombus formation is a common, life-threatening sequela to heart failure in cats and is, in part, driven by the arachadonic acid (AA) pathway, where inflammation results in the production of eicosanoids. Thromboxane A_2 (TXA_2), the leading eicosanoid participating in thrombus formation, undergoes rapid, non-enzymatic conversion to thromboxane B_2 (TXB_2) in vivo making it difficult to quantify. TXB_2 is enzymatically metabolized to 11-dehydro-thromboxane B_2 (11-dehyrdroTXB_2) and 2,3-dinorTXB_2. There is a species-specific preference for one metabolite over the other. In humans and cats, 11-dehydroTXB_2 is the primary metabolite whereas in dogs 2,3-dinorTXB_2 is the major metabolite. Urinary concentration of the primary metabolite is representative of platelet activity in humans and dogs. Aspirin is a readily available NSAID known to irreversibly block thromboxane A_2 formation in platelets via the cyclooxygenase pathway. Low-dose (1mg/kg q24h) aspirin is administered as a thromboprophylaxis in humans. The advantage of the dose is that it minimizes systemic side effects. The effectiveness of the 1 mg/kg dose is unknown in cats. Eight male neutered purpose-bred cats were administered approximately 1 mg/kg of aspirin every 24 hours for 2 days, and after a 7 day washout period they were given 1 mg/kg q24h for 5 days. Urine was collected at time 0, 48h, 72h, 120h post administration. Creatinine corrected urinary 11-dehydroTXB_2 concentrations were measured by a commercially available ELISA. Decrease in the concentration of urinary 11-dehyrdroTXB2 with the low-dose aspirin makes this a convenient test for aspirin effectiveness. If identified, this relationship could be important in changing the management of cats with heart failure.

Research Grant: Morris Animal Foundation
Student Support: Merial Veterinary Scholars Program

The effects of perinatal propylthiouracil exposure on novel object and location recognition in Long-Evans rats

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Developmental exposure of rats to higher doses of the anti-thyroid drug, propylthiouracil (PTU), results in persistent neurodevelopmental effects. PTU inhibits enzymes required for the synthesis of thyroid hormone (TH), and is transplacentally and lactationally transmitted to offspring. Therefore, perinatal PTU exposure is a potentially useful model for examining the effects and mechanisms of environmental contaminants that perturb developmental thyroid homeostasis. However, research using more subtle PTU exposures could yield a more accurate model of human developmental hypothyroidism. In this study, we compared cognitive performance of offspring of both sexes from Long-Evans rat dams exposed to PTU prenatally, postnatally, or during both stages, with offspring of unexposed dams. Recognition and spatial memory were evaluated using two well-established tasks: novel-object recognition (NOR) and novel-location recognition (NLR). Over 15 days, rats were placed in an arena with 2 identical objects and removed 3 minutes later. Then one object was either exchanged with a different object (NOR) or moved (NLR), and rats were placed back into the arena after 1, 4, or 24 hours. We measured time spent exploring and number of visits to the novel versus familiar object or location as indices of recognition. We hypothesize that rats exposed to PTU will show recognition and spatial memory deficits, and that these deficits may depend upon age of PTU exposure. Experimental results are pending. These results could identify sensitive periods for the behavioral effects of developmental hypothyroidism, and could improve our understanding of the role of thyroid hormone in neurodevelopment. *Does not reflect EPA policy.*

Research grant: National Institute of Environmental Health Sciences, NIH, K08 ES017045
Student support: Office of the Director, NIH, T35 OD011145

Characterization of immunological gene expression in the intestine of healthy calves

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Gastrointestinal (GI) disease is a cause of morbidity and economic loss in young livestock. Understanding the pathophysiological significance of each element of the gut health 'triad' (immune system, microbiome, epithelium) will be useful in designing strategies to improve GI health. In this study, we

characterized mucosal inflammatory gene expression (IGE) profiles at different biogeographical sites of the healthy calf intestine during the first 3 weeks of life. Twelve calves from second parity cows on a single commercial dairy were removed from their dams upon delivery and fed clean, high quality colostrum. Calves were fed milk replacer and health-monitored daily. A subset of calves (n=3) were euthanized at days 1, 3, 7, and 21, and mucosal tissue samples were taken from the duodenum, jejunum, ileum, and colon. Quantitative PCR analysis was performed to quantify the expression of interleukin 10 (IL10), toll-like receptors (TLR) 2, 4, and 10, and tumor necrosis factor-α (TNF-α) genes. There was a correspondingly low level of IGE at all intestinal locations and time points. The ileal mucosa showed a significantly higher pattern of IGE than all other sites ($p < 0.05$) for IL10 and TNF-α. TLR 4 had a three-fold higher expression than all other genes. As expected, the IGE profile of this calf group was subdued. There appears to be higher inflammatory activation in ileal mucosa, possibly due to the relative abundance of Peyer's patches. In future studies, we will compare the IGE profiles with the intestinal microbiota and epithelial function of sick and healthy calves. Gaining an understanding of the immune system and microbial population could lead to management solutions for GI disease and reduce the use of antimicrobials.

Research Grant – National Institute of Food and Agriculture, U.S. Department of Agriculture, award number 1002450
Student Support – Merial Veterinary Scholars Program

The effect of FOXA2 inactivation on mucus overproduction in canine respiratory infections

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Dogs naturally develop several pulmonary diseases similar to humans. Older dogs, especially of small breeds, develop infectious canine tracheobronchitis (kennel cough), chronic bronchitis (CB), and chronic obstructive pulmonary disease (COPD) with excessive mucus due to chronic exposure to environmental pollutants or infectious agents. Two previous clinical studies involving 766 canine lower respiratory tract infection cases showed that major pathogens included those on the ESKAPE list: *Pseudomonas aeruginosa*, *Staphylococcus*, *E. coli* + other Enterobacteriaceae and *Enterococcus*. FOXA2 is a key transcriptional regulator that maintains airway mucus at healthy levels. FOXA2 is inactivated in human airways with respiratory infection, resulting in excessive mucus. Previously, our laboratory

demonstrated that pyocyanin (PCN), a virulence factor of *P. aeruginosa*, causes goblet cell hyperplasia and metaplasia and mucus hypersecretion by repressing the expression of FOXA2. However, the role of FOXA2 regulation in canine respiratory diseases remains unexplored. In this study, we retrospectively examined cases of canine respiratory infection received by the University of Illinois Veterinary Diagnostic Laboratory. We also used immortalized dog bronchial epithelial BACA cell lines to examine the mechanism of FOXA2 inactivation by PCN, in a time and concentration-dependent manner. Finally, we examined bronchial sections from dogs with CB and COPD by immunohistochemistry to determine whether microbial pathogens found in these airways inactivate FOXA2. The results of these studies will reveal the contribution of FOXA2 inactivation by microbial pathogens to excessive mucus in canine respiratory diseases.

Research Support: American Lung Association DeSouza Research Award (DS-192835-N) and NIH (HL090699) to Gee W. Lau
Student Support: Office of the Director, NIH, T35 OD011145