Evaluation of the Effect of Transforming Growth Factor Betas (TGFB) on Malignant Osteoblasts

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Transforming Growth Factor Beta (TGFB) plays an important role in malignant osteoblast progression and the development of osteosarcomas (OS). Malignant osteoblasts produce and secrete TGFB and its associated receptors, TGFBRI and TGFBRII, for signaling purposes. TGFB also causes downstream effects like phosphorylation of Smad 2/3 and subsequent activation of target genes via intracellular signaling. The role of TGFB as a protumorigenic cytokine is complex and variable, as evidenced by its mitogenic, angiogenic, and immunosuppressive effects as well as its ability to act in an autocrine or paracrine fashion. Clonally-derived OS cell lines of variant metastatic potential derived from 3 different species (human, murine, and canine) will be utilized to assess differences in TGFB expression. OS production and secretion of TGFB will be demonstrated by Western Blot and ELISA. OS expression of TGFBRI and TGFBRII will be demonstrated by Western Blot and IHC. TGFB induction of P-Smad 2/3 will be demonstrated by Western Blot. Western Blot and ELISA for TGFB suggest the presence of a feedback loop between OS and TGFB in all 3 species. Western Blot and IHC suggest expression of both TGFBRI and TGFBRII by malignant osteoblasts in all 3 species. TGFB, TGFBRI and TGFBRII are present in OS cell lines from various species. We expect to demonstrate the mitogenic, angiogenic, and immunosuppressive effects of TGFB in future studies. Ultimately, we hope to demonstrate that blocking TGFB signaling can have beneficial biologic effects including enhanced survival time and decreased number/size of pulmonary metastases.

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Equine Strangles: Evaluation in Weanlings of Two Novel Modified Live Vaccine Candidates Against Streptococcus equi

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Streptococcus equi, is the causative agent of “strangles,” a common bacterial disease of horses. A novel mutant of S. equi, C3-19, has deletions in genes encoding control of virulences (covS), superoxide dismutase (sodA), and strep
Zooepidemicus-like protein (szp). We hypothesized that C3-19 would induce humoral immunity in weanling horses and safely prevent S. equi infection. Four weanlings (5-11 mo.) received live intranasal C3-19 vaccine. Antibody levels following immune response were evaluated using direct ELISA and Western Blots. C3-19 vaccination was protective against S. Equi. There were no significant fevers following vaccination (p>0.14). Daily lymph node and nasal discharge scores did not exceed 2 on a scale of 0 to 3. Mild increases in lymphadenopathy and nasal discharge were observed following vaccination but no abscessed lymph nodes or purulent nasal discharges were noted. Nasal cultures demonstrated vaccine clearance within 3 days post inoculation. At necropsy, all weanlings were culture negative for the S. equi challenge and vaccine strain. Serum ELSIA titers against C3-19 demonstrated a rise in antibody titer levels following vaccination for 2 weanlings: levels went from undetectable at day 0 to 17-33% of positive control. Titer levels of 2 weanlings showed a decrease in titer, but remained significant at 26-55% of positive control. M-Protein titers performed by Equine Diagnostic Solutions declined during the trial. This study may allow development of a safer vaccine against S. equi, crucial to minimizing economic losses in the equine industry. The testing of another strain, #34, which has a sole gene deletion of covS, is ongoing.

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Does Female Predator Exposure Affect Male Courtship in Three-Spined Stickleback Fish?

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The behavior of an individual often depends on the characteristics of their audience. For example, courtship behavior might vary with the traits of the individuals involved. In three-spined stickleback fish (Gasterosteus aculeatus), males might be particularly choosy in female mate choice since males provide costly offspring care. Previous studies have shown that maternal predator exposure increases cortisol levels in eggs and negatively affects offspring anti-predator behavior and survival. Thus, males might be expected to adjust their courtship behavior based on the previous predator exposure of the female. The aim of this study was to determine whether males change their courtship behavior based on the predator-exposure experience of the female. On one hand, males might prefer to court unexposed over predator-exposed females because high cortisol levels in the eggs induced by predator
exposure result in lower-quality offspring. Alternatively, males might be equally motivated to court both types of females but predator-exposed females might be less receptive to courtship behavior requiring males to compensate by courting them more intensely. To examine these hypotheses, we used a within-subjects design where males interacted both with a predator-exposed and unexposed female in random order. Data was recorded on male courtship behavior for five minutes. Our study will determine whether males can assess the predation exposure or stress level of potential mates and whether they use this information in their courtship decisions. It will also provide insight into how ecologically relevant maternal stressors, such as predation risk, might affect male mating behavior and subsequent reproductive success.

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**Modifications of Airway Mucin Glycoproteins by Pseudomonas aeruginosa Alginate and Flagella**

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Alginate and flagella are two of many virulence factors produced by Pseudomonas aeruginosa during chronic lung infections, as commonly seen in cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD) patients. Alginate characterizes the mucoid phenotype of P. aeruginosa that is most commonly isolated from patients with exacerbated infections. Flagella, usually associated with motility, have been shown in P. aeruginosa to function as an adhesin to mucins found in human lungs. P. aeruginosa infection exacerbations have been linked with structural changes in the glycosylation of mucin glycoproteins that make up the thick mucus obstructing the airways of these diseases. In order to determine if alginate and flagella contribute to these changes, mice were treated intratracheally with 140 ug/treatment of alginate or intranasally with 2 ug/treatment of flagella for 1, 3 and 7 days. Lungs from these mice were examined under a High Iron Diamine/Alcian Blue stain as well as a Periodic Acid-Schiff (PAS) stain. The PAS stain showed evidence of goblet cell hyperplasia around large airways in both the alginate and flagella samples. Additionally, evidence of sialylated mucins was seen in these same locations. Goblet cell hyperplasia was seen at a higher frequency in the flagella sections compared to the alginate. This supports the claim that alginate and flagella induce goblet cell hyperplasia and changes in mucin glycosylation. Knowing which virulence
The Effects of Dopaminergic Drugs on Impulsive Behavior in Long Evans Rats Exposed to Polychlorinated Biphenyls and Polybrominated Diphenyl Ethers

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Impulsivity, a component of neurobehavioral disorders including attention deficit/ hyperactivity disorder (ADHD), is associated with dysfunctional dopaminergic activity in the brain. Differential reinforcement of low rates of responding (DRL), a behavioral test of impulsive action, requires that a subject waits for a predetermined amount of time before pressing a lever to earn a food reward. If a subject prematurely presses the lever, the clock resets with no reward. In this study, Long-Evans rats were dosed perinatally with environmentally relevant mixtures of either polychlorinated biphenyls or polybrominated diphenyl ethers, toxicants that affect dopaminergic signaling in the brain. The offspring were trained on the DRL task and then were treated with the dopaminergic agents amphetamine, flupenthixol, or both in order to allow assessment of the role of perturbed dopaminergic signaling in mediating the effects of toxicant exposure. We hypothesized that toxicant exposure would increase impulsive action manifested as increased lever pressing and decreased food rewards earned. Furthermore, we hypothesized that toxicant exposed subjects, as compared to controls, would be relatively more impulsive after flupenthixol and less impulsive after amphetamine. Preliminary results did not reveal effects of toxicant exposure on DRL performance but the drug challenges changed behavior. Amphetamine increased impulsive behavior as demonstrated by increased lever pressing and less food rewards earned. Flupenthixol resulted in decreased lever pressing and less food rewards earned, suggesting that flupenthixol caused subjects to wait for relatively longer intervals between lever presses, thus failing to maximize the number of rewards earned.

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**Invasion of the Black-Legged Tick in Illinois**

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The black-legged tick, *Ixodes scapularis*, is the main vector of Lyme disease in the United States and is becoming established in woodland areas in Illinois, initially in the northwest part of the state and moving south and southeast. While the purported mechanism of establishment is long-distance movements by wildlife hosts, little is known about the pattern of establishment in novel habitats in relation to landscape features. Predictions from the Theory of Island Biogeography would suggest that migrating hosts are more likely to encounter large habitat fragments than small, and more connected than isolated, while dispersing through an otherwise inhospitable matrix habitat (e.g., row-crop agriculture). The pattern of this invasion was studied and it was predicted ticks would establish in large sites first, while smaller sites farther from these large foci would exhibit the lowest tick densities, contradicting the pattern seen in long-term established areas. Ticks were collected in 4 counties in each of 3 regions of Illinois: northwest, north central, and east central. In each county, 5 sites were sampled: a large site, 2 “medium” sites and 2 “small” sites and 1 of each near and 1 of each far from the large site. There was no significant relationship between invasion history and the abundance of *I. scapularis* nymphs (*P* = 0.983). However, across the entire invasion gradient, there was a significant effect of site type on nymph abundance (*P* = 0.039). Large sites supported significantly more ticks than Small, Far (*P* = 0.042), and there was a marginally significant trend that Large sites supported more ticks than Medium, Far (*P* = 0.066). Understanding the establishment patterns of *I. scapularis* during an invasion are vital for implementing preventative measures not only for inhibiting the establishment of *I. scapularis*, but stopping the spread of Lyme disease into new regions.

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**Microscopic and Ultrastructural Lung Injury Following Intravenous Bolus LPS Administration to Healthy Adult Horses**

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Endotoxemia is a serious complication in several diseases of horses that exacerbates disease severity and may result in death. Endotoxin (LPS) is
released from the cell membrane of Gram-negative bacteria and can induce a pro-inflammatory systemic reaction. Acute Lung Injury (ALI) and Acute Respiratory Distress Syndrome (ARDS) may result. Additionally LPS administration causes significant pulmonary hypertension, known to damage the pulmonary vasculature, creating ‘vascular leak syndrome’. This study investigates the severity of secondary lung injury induced in horses approximately 3 hours after intravenous bolus administration of 100 ng/kg LPS (Escherichia coli O55:B5) to healthy adult horses as part of a larger study evaluating pulmonary changes associated with LPS administration. Percutaneous lung biopsies from a randomly chosen lung field were collected from 8 healthy horses (BASELINE) after sedation with xylazine hydrochloride (0.2-0.3 mg/kg IV) for analysis by histopathology, micro-computed tomography and transmission electron microscopy. Following two weeks of recovery, horses underwent LPS or saline administration via a jugular catheter. LPS resulted in pulmonary hypertension as anticipated. The maximum increase in mean pulmonary artery pressure [128% (+/- 54%)] was noted in the horses at about 28.5 (+/- 2.4) minutes after start of the LPS infusion. This was transient for most of the horses and the pressures had begun to return towards baseline values for several horses near time zero. At this time tissue sample analysis is still ongoing. A better understanding of clinical symptoms and internal tissue damage of infected horses is essential to creating more efficient treatment and management protocols.

Research supported by Merial Veterinary Scholars Program

Influence of wetland flora and avian fauna density and diversity on vector-host interactions and WNV transmission risk

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Urban and suburban environments are known to be favorable for the transmission of West Nile Virus (WNV) by providing both an abundance of competent avian hosts and an advantageous aquatic habitat for mosquito vector development (stormwater drains and other forms of standing water). However, within these environments, differences in vegetative and host density and diversity can greatly affect not only WNV transmission but also disease occurrence. In this study, we compared the diversity and density of plants, birds, and mosquitoes in wetland and corresponding residential environments to determine if the differences in habitat and avian abundance had an effect on the mosquito population. In each of our three study cities (Bloomington, Decatur, and Springfield) we chose wetland sites that were within 200 m of urbanized areas and a corresponding residential site within a
500 m radius of the wetland. Mosquitoes were collected via light and gravid traps at each site over 3 consecutive days each month (collections will continue May through September). Species and blood meal status will be determined for all female mosquitoes collected. Reproductive status and fecundity of females will be assessed and host DNA will be extracted from blood fed females for identification. A subsample of females collected from gravid traps will also be tested for the presence of WNV. Avian surveys (roosting and point count) will also be completed at least twice at every site in July to determine bird diversity and density and to assess roosting behavior. Results from this project will aid in assessing how wetlands influence the risk of WNV transmission in urbanized areas in Illinois.

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**Does Osteosarcoma-Derived-IL-8 Participate in Tumor Microenvironment Bone Resorption?**

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Interleukin-8 (IL-8) is a cytokine which participates in many inflammatory pathologies, and has been recently incriminated as a pro-tumorigenic cytokine. Indeed, breast carcinoma cells that have metastasized to skeletal sites secrete IL-8 which leads to the overexpression of Receptor Activator of NF-kB Ligand (RANKL) by osteoblasts, thereby which promoting osteoclastogenesis and malignant bone resorption. Although metastatic breast carcinoma cells can directly influence malignant osteolysis through IL-8 secretion, whether this resorptive mechanism is conserved across various skeletal pathologies, such as osteosarcoma (OS), remains undetermined. The purposes of this investigation are 1) to assess the production and secretion of IL-8 by canine OS cells; 2) to assess the presence of IL-8 receptors (CXCR1 and CXCR2) on canine OS cells; and 3) to characterize if an autocrine or paracrine loop exists between IL-8 secretion and RANKL/OPG expression in canine OS cells. IL-8, RANKL, OPG, CXCR1 and CXCR2 expressions were assessed by RT-PCR and Western blot in 6 canine OS cell lines. Secreted IL-8 will be measured by ELISA. IL-8 was expressed at varying levels among the 6 OS cell lines (high, intermediate, and low); however, IL-8 expression did not directly correlate with RANKL/OPG ratios. Preliminary data support the expression of CXCR1 and CXCR2 by OS cells; supporting the possible existence of an autocrine or paracrine feedback loop. IL-8 might directly participate in OS-associated bone resorption and understanding osteoclastogenesis induction
though IL-8 secretion by OS cells might lead to new treatment options for attenuating malignant osteolysis in dogs with OS.

Research supported by Merial Veterinary Scholars Program

Bisphenol A Exposure Inhibits Follicular Growth and May Induce Oxidative Stress

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Bisphenol A (BPA) is used commonly as a monomer for polycarbonate products. Previous studies have shown that BPA advances puberty, inhibits follicular growth, and inhibits steroidogenesis by ovarian follicles. However, the mechanism by which BPA causes these effects is unknown. BPA may cause ovarian toxicity by inducing oxidative stress. Thus this study was designed to identify changes in levels of three antioxidant enzymes: superoxide dismutase (SOD1), catalase (CAT), and glutathione peroxidase (GPX) after BPA exposure. We hypothesized that BPA reduces levels of follicular antioxidant enzyme expression, creating oxidative stress. Antral follicles were isolated from post-natal day 31 CD1 mice and cultured with either vehicle (dimethylsulfoxide, DMSO) or BPA (1 ug/ml, 10 ug/ml, or 100 ug/ml) in essential alpha minimum media. Every 24 hours, growth was measured on perpendicular axes. At selected times (6, 12, 18, 96 hours), follicles were collected and subjected to quantitative polymerase chain reaction for measurement of SOD1, CAT, and GPX. The results indicate that BPA 10 ug/ml significantly inhibits follicular growth at 48-72 hours compared to DMSO controls (DMSO at 72 hours = 33% growth +/- 5.52%, BPA 10 ug/ml at 72 hours = 12.40% growth +/- 5.52%; n=43, p < 0.05). Further, BPA 100 ug/ml significantly inhibits follicular growth at 24-96 hours compared to DMSO controls (DMSO at 96 hours = 42.43% growth +/- 10.79%, BPA 100 ug/ml at 96 hours = 1.22% growth +/- 10.79%; n=38, p < 0.05). Collectively, these data suggest that BPA may cause oxidative stress.

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Formation of Drug-Protein Adducts by Dog Keratinocytes Exposed to Sulfamethoxazole

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Drug hypersensitivity (HS) reactions are common in both dogs and humans and frequently target the skin. The drugs themselves are too small to trigger an immune response, but one theory postulates that covalent drug-protein adducts can be formed and induce an immune response in allergic patients. Sulfamethoxazole (SMX)-protein adducts have been detected in vivo and in vitro in humans, rats, and dogs. They have been identified in immune cells, in the blood, and in the liver. In humans, they have also been detected in skin cells, but this has not been evaluated yet in dog cells. We therefore hypothesize that dog keratinocytes will produce drug-protein adducts after treatment with SMX in vitro. Immortalized dog keratinocytes (CPEK cell line) were grown in vitro and treated with SMX at five different doses (2000 uM, 500 uM, 100 uM, 20 uM and 2 uM) for 24 hours. An ELISA assay was then used to determine the relative amount of SMX-protein adducts present in each condition, using a rabbit antiserum raised against a highly immunogenic SMX keyhole limpet hemocyanin (KLH) conjugate (Panigen Inc, IL, USA) and an alkaline phosphatase anti-rabbit IgG (AbdSerotec). We predict a dose-dependent increase in adduct formation. If found in vivo, such SMX-keratinocyte adducts could be the first step towards an SMX-specific immune response targeting the skin. Further work is underway with beta-lactam protein adducts in CPEK cells.

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**Determining Tissue Factor Pathway Inhibitor Release Induced by Unfractionated and Low-Molecular Weight Heparins in the Dog**

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Tissue Factor Pathway Inhibitor (TFPI) is a physiologic anticoagulant responsible for the feedback inhibition of coagulation by binding Factor Xa (FXa) and Factor VIIa-Tissue Factor complex (FVIIa-TF). Heparin therapy, commonly used for prevention of thrombosis, has been shown to induce a 2-4 fold increase in plasma TFPI concentration in humans, due to release from the endothelial surface. Neither the normal physiologic levels of plasma TFPI nor the change induced by heparins have been described in the dog. Fifteen healthy dogs were randomly assigned to receive unfractionated heparin
UFH, n=5), enoxaparin (Enox, n=5), or dalteparin (Dal, n=5). Blood samples were collected at 0, 1, 3, 5, 7, and 9 hours after subcutaneous administration. Plasma was frozen for later analysis. A functional microplate chromogenic assay (Actichrome) performed poorly with canine plasma likely due to lack of ability of canine TFPI to inhibit the human derived enzymes. Dot blots with recombinant human TFPI and canine pooled normal plasma (PNP), using MAb antibodies against TFPI Kunitz 1, or Kunitz 2 indicated cross-reactivity with canine samples. Western blot conditions were then optimized for canine plasma. These conditions will be used to quantitatively evaluate plasma TFPI concentration in the stored samples. The determination of the relative effectiveness of different heparins in inducement of TFPI release may allow for more effective heparin therapy in canine patients.

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The effect of an endocrine disrupter on sexually dimorphic neuropeptide expression in the brain of adult female intact mice

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Di-2-ethylhexyl phthalate (DEPH) is a plasticizer used in polyvinyl chloride (PVC) plastics that has been shown to suppress ovarian hormone production and cause anovulatory cycles in rodents; however, the effects of DEHP on the expression of steroid-dependent expression of neuropeptides is unknown. Adult intact female C57/B6 mice were treated daily orally for at 30-32 days with DEPH (corn oil vehicle, 20 ug/kg/day, 200 ug/kg/day, 20 mg/kg/day, 200 mg/kg/day). Estrous cyclicity was determined daily via vaginal cytology and brains were collected from animals on the day of diestrus. Using immunocytochemistry, the number of cell bodies and density of fibers of tyrosine hydroxylase (TH) and kisspeptin (KISS) were identified in several areas of the brain, including the dorsomedial hypothalamic nucleus (TH, KISS), anteroventral paraventricular nucleus (TH, KISS), zona incerta (TH), periventricular nucleus (TH), and arcuate nucleus (TH, KISS). These are both peptides for which expression is influenced by circulating steroid hormones. We predict that the administration of DEHP will result in a decrease in the number of cell bodies and fiber density throughout the brain for both peptides, with a greater decrease in cell body number and fiber density as the dosage of DEHP increases. Because humans and other animals are regularly exposed to DEHP through plastics, determining DEHP’s estrogenic
effect on the brain is crucial for understanding how oral exposure may impact neural growth and development.

Research supported by the Merial Veterinary Scholars Program
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**Aerobic Exercise Accelerates Extinction of Cocaine-Induced Condition Placed Preference in Nestin-Thymidine Kinase Transgenic Mice**

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C57BL/6J mice readily acquire conditioned place preference (CPP) for cocaine. Wheel running after conditioning can facilitate extinction of CPP, possibly due to increased numbers of new hippocampal neurons from the exercise. Nestin-thymidine kinase transgenic mice (NTK) have a C57BL/6J background with a thymidine kinase (TK) gene inserted downstream of the Nestin promoter. When NTK mice are fed ganciclovir, TK phosphorylates the chemical, exclusively disrupting cell division in the Nestin-expressing neural progenitor cells, thereby selectively reducing neurogenesis. The purpose of this study was to establish whether the NTK mice fed normal chow exhibit accelerated extinction of CPP from running as did C57BL/6J. If so, NTK mice can be used to test the extent to which new running-generated neurons are required for the accelerated extinction. Mice were habituated to the CPP apparatus and then evaluated for a baseline preference of two textures. Subsequently, mice were conditioned to associate cocaine on one texture and saline on the other. After conditioning, half the mice were placed in sedentary cages and half in cages with running wheels interfaced to pedometers to record rotations daily for 28 days. All mice received bromodeoxyuridine (BrdU) injections during the first 10 days of runner or sedentary treatment to label dividing cells. After 28 days, mice will undergo place preference testing without cocaine followed by reinstatement testing with cocaine injections. Three days after testing, mice will be transcardially perfused and hippocampal neurogenesis measured in the extracted brain. These results will set the foundation for future experiments in showing the different facets of learning, memory and neurogenesis.

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The Effects of Experimentally Induced Acute Endotoxemia on Pulmonary TNFalpha and Lung Inflammation in Healthy Adult Horses

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Endotoxemia is a major cause of morbidity and mortality in critically ill horses. The contribution of a specific endotoxin, lipopolysaccharide (LPS), to acute lung injury has been described in several species. Although many systemic effects of LPS have been characterized, little is known regarding the contribution of LPS to acute lung injury in the horse. The objectives of this study were to determine whether systemic administration of LPS is associated with increased TNFalpha concentration in bronchoalveolar lavage fluid (BALF) and sera and to characterize changes in inflammatory cell distribution in BALF and lung tissue of healthy adult horses. Baseline BAL, percutaneous lung biopsy, TNFalpha, and white blood cell (WBC) counts were collected from 6 healthy adult horses. Following a 2-week recovery period, LPS from Escherichia coli O55:B5 (100 ng/kg IV) was administered to each horse. Serum TNFalpha, and WBC counts were determined prior to LPS administration and at 0, 0.5, 1, 1.5, 2, 4, 6, and 24 hours post-LPS infusion. BAL and percutaneous lung biopsy samples were collected at 3 hours post-infusion and were submitted for cytologic and histopathologic analysis. Serum and BAL TNFalpha concentrations were determined using a sandwich ELISA. A Student’s T-Test and Repeated Measure ANOVA were used to determine differences compared to baseline and over time with significance defined as P < 0.05. WBC counts decreased (P=0.000) and serum TNFalpha increased (P=0.000) compared to baseline, with the maximum change occurring at 1 hour post-infusion. No change in BALF cytology was observed. Analysis of BALF TNFalpha concentration and lung histology are pending. This research will expand our understanding of endotoxin-mediated acute lung injury in the horse and will contribute towards improved characterization of systemic effects of LPS. Ultimately this may aid in the management of critically ill adult and neonatal equine patients.

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Loss and return of righting reflex in American Green Tree Frogs (Hylidae cinerea) after topical application of compounded sevoflurane or isoflurane jelly

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Amphibians respire through pulmonary and cutaneous routes, allowing for topical anesthetic delivery. Anesthesia in frogs can be estimated by loss and return of righting reflex. Topical isoflurane or sevoflurane jellies can be used to anesthetize anurans. Sevoflurane is less tissue-soluble than isoflurane resulting in shorter induction and recovery times in mammals. It is unknown if tissue solubility affects topical anuran anesthesia. Loss and return of righting reflex was determined in 8 Hylidae cinerea weighing 6.5 ± 1.7 g in a randomized study. Frogs were placed into a container with 2 mL of isoflurane or sevoflurane jelly spread on the floor. Containers were intermittently inverted until loss of righting reflex. Frogs were then removed, rinsed clean of jelly and placed in new containers in dorsal recumbency. Frogs were observed until return of righting reflex. Four frogs initially tested with isoflurane developed skin lesions. Two died and were submitted for necropsy, histopathology and bacteriology; results pending. Use of isoflurane jelly was discontinued and only sevoflurane jelly was tested on remaining frogs. Data was analyzed after log transformation with t-tests. Data is reported as mean ± SD. Frogs receiving isoflurane jelly lost righting reflex at 90 ± 20 sec and returned righting reflex at 2659 ± 135 sec. Frogs receiving sevoflurane jelly lost righting reflex at 112 ± 33 sec and returned righting reflex at 1085 ± 1204 sec and no skin lesions were observed. There was no significant difference between loss of righting reflex times (p = 0.3); however there was a significant difference in return of righting reflex times (p = 0.04), isoflurane recovery was 4.5 times longer than sevoflurane. Topically applied sevoflurane jelly appears to be a satisfactory anesthetic agent compared to isoflurane which may cause fatal skin lesions in frogs. Options for providing anesthesia should be investigated and identified in order to advance care for this diverse species.

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