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**ORIGINAL RESEARCH**

***Biology***

**Williams et al. Vertebral Heart Score in Rhesus Macaques (*Macaca mulatta*): Generating Normal Reference Intervals and Assessing its Validity for Identifying Cardiac Disease, pp. 347-355**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Macaque (*Macaca spp.*)

SUMMARY

Background:A thorough evaluation of cardiac disease and structural abnormalities often require diagnosis via echocardiography. Unfortunately, echocardiography requires expensive equipment and staff with specialized training, which may not be available at every facility. The vertebral heart scoring (VHS) system is a semi-quantitative tool used in multiple species to assess the presence and severity of various cardiac diseases. VHS uses thoracic radiographs to measure the cardiac silhouette along its short and long axes and compares this with the number of thoracic vertebrae present within the measured distance. Due to its ease of use and accessibility, VHS may offer an inexpensive alternative in detecting cardiac disease. The goal of this study is to establish reference VHS intervals for rhesus macaques and compare the sensitivity of diagnosing cardiac disease via VHS versus echocardiography.

Methods:A total of 150 rhesus macaques were enrolled in the study and divided into 3 groups: control (animals which lacked any significant cardiac abnormalities), animals with hypertrophic cardiomegaly (HCM) diagnosed via echocardiography, and animals with other cardiac disease (OCD). All animals were sedated with ketamine and evaluated for cardiac abnormalities via echocardiography and radiographic examination during the same sedation event or within 2 sedation events, 1 month of each other.  Thoracic radiographs were performed in right lateral (RL), dorsoventral (DV), and ventrodorsal (VD) recumbency.

Results:VHS scoring did not differ between male and female macaques in all radiographic views. Significant correlations were observed between age and VHS, and between body weight and VHS in the DV view only. VHS was significantly greater in the OCD group than in the control group in the RL and VD view. VHS did not differ between HCM and control, or HCM and OCD group in any radiographic view.

Conclusions:VHS may be a valuable screening tool for cardiac disease, but it is not likely useful for detecting occult HCM or other diseases causing concentric left ventricular hypertrophy in rhesus macaques. The use of VHS along with clinical signs of cardiac disease or heart murmurs may improve the sensitivity and specificity of VHS to detect animals with heart disease.

QUESTIONS

1. Which cardiac abnormality is most common in *Aotus sp*.?

a. Ventricular septal defect

b.  Aortic aneurysm

c. Myocardial hypertrophy

d. Myocardial fibrosis

2. True/False – Results from a similar paper, using the VHS system in Japanese macaques found no significant correlations between VHS and weight, age, or sex of the animal

ANSWERS

1. c
2. False - the other study found VHS differences emerged depending on sex, weight, and age in Japanese macaque. See reference below.

Harada M, Koie H, Iwaki S, Sato T, Kanayama K, Taira M, Sakai T. Establishment of vertebral heart scale in the growth period of the Japanese macaque (Macaca fuscata). J Vet Med Sci. 2010 Apr;72(4):503-5. doi: 10.1292/jvms.09-0328. Epub 2009 Dec 15. PMID: 20009429.

***Husbandry***

**Coda et al. Behavioral Effects of Cage Size and Environmental Enrichment in New Zealand White Rabbits, pp. 356-364**

Domain 4: Animal Care

Primary Species: Rabbits (*Oryctolagus cuniculus*)

SUMMARY

Material/Methods:

First part - adult NZW rabbits were housed in 3 different cage sizes (S,M,L). An ethogram was then developed and used to analyze 3 main behaviors: exploring, grooming, and resting.

Second part - analyzed enrichment in standard housing cages. Enrichment included a “hanging toy” (wire ball with a bell containing timothy hay), “digging toy” (plastic bin filled with corncob bedding and timothy hay), “destructible toy” (origami paper box filled with timothy hay), and the last group had no enrichment.

Third part - determine the stress effect of the hanging and destructible toy enrichment via fecal pellets. Fecal pellets were collected 2 hours after exposure to the enrichment devices. Additional fecal samples were also collected from rabbits that underwent a surgical procedure to use as an additional stressor.

Video recording was utilized for all 3 parts of the study. Fecal hormone extraction and glucocorticoid assays were performed by the St Louis Zoo endocrinology department. Statistics utilized a one-way ANOVA with Tukey-Kramer or Dunnett post-hoc test.

Results:Rabbits explored more and groomed less when in the large and medium sized cages. When exposed to the 3 different enrichment devices there was no significant difference in the expressed behaviors between the 3 devices. When compared to the non-enrichment group, the rabbits with enrichment spent more time exploring than grooming. Rabbits spent more time with the destructible enrichment compared to the other 2 options. There was no significant difference in any of the fecal glucocorticoid samples which included the enrichment animals and the surgical animals.

Discussion:Wild rabbits spend a large amount of time performing active exploring behaviors including foraging for food, avoiding predators, socializing, finding mates, raising young, resting, and grooming. The study found that the larger cage setting encouraged more exploring behaviors as well as adding enrichment devices to standard sized cages. Increasing the activity of rabbits has health benefits including decreasing osteoporosis and GI stasis. Subjectively, hanging devices and the larger cages encouraged more sustained rearing activities commonly seen in wild rabbits. Frenetic random activity periods (FRAP or “binkies”) is a phenomenon documented in dogs and rabbits and is described as an expression of excitement. Increasing cage size or including enrichment did not increase the amount of FRAP activities. Digging behavior not commonly seen even with digging enrichment. Rabbits did not lose interest in the same enrichment toy between days 2-6. Glucocorticoid deposition in the GI tract in rabbits occurs around 12 hours after an acute stressor. This data was not meaningful in the study since there were no reliable increases in fecal glucocorticoid samples. Some reasons for this include: decrease appetite and less feces after a stressor, complex rabbit GI physiology limited amount of steroid in the feces, GI transit times vary greatly in rabbits from 4-48hrs and hard to correlate time of stressor to the fecal samples, and rabbits are coprophagic animals. Researcher would not recommend fecal glucocorticoid testing in rabbits.

QUESTIONS

1. What is the GI transit time for Rabbits?

a. 12 - 48hrs

b. 4 - 48hrs

c. 24 - 48hrs

d. 8 - 48hrs

2. What is the recommended housing environment for rabbits? Choose all that apply

a. Standard cage with hanging enrichment

b. Large cage with hanging enrichment

c. Large cage with destructible enrichment

d. Standard cage with digging enrichment

e. Large cage with digging enrichment

3. List 3 reasons as to why Fecal Glucocorticoid analysis is not effective in the rabbit.

ANSWERS

1. b

2. a & b & c

3.   Decrease appetite and less feces after a stressor, coprophagic, and GI transit times vary greatly.

***Management***

**Dell’Anna et al. Room Decontamination Using Ionized Hydrogen Peroxide Fog and Mist Reduces Hatching Rates of *Syphacia obvelata* Ova, pp. 365-370**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Pinworms inhabit the cecum and colon of rodents. Infestation of rodents in the research setting can have adverse effects on behavior, growth, intestinal physiology, and immunology, potentially affecting research data. Traditional methods of eradication include fenbendazole, but ova can remain viable in the environment for long periods of time. Chlorine dioxide, heat, UV light, ethylene oxide, formaldehyde, and chlorhexidine have all been tested for their ability to destroy ova. The goal of this study was to evaluate the efficacy of ionized hydrogen peroxide (iHP) in reducing viability of *S. obvelata*ova.

40 mice were purchased from a local pet store and tape was used to collect ova via anal impressions, and the tape affixed to a slide impression side up. Microscope and PCR was used to confirm *S. obvelata* ova. Slides holding tapes with attached ova were either exposed to iHP fog (experiments 1 and 2) or a surface misting system (experiment 3) in a room. Experiment 1- each room received an initial 15-minute fogging application, then ova were continually exposed to fog and removed after either 15, 30, 90, or 240 minutes of exposure time. Exp 2- slides were continually exposed to fog in the room, with slides removed after 3, 4, or 6 cycles of 15 minutes each. Exp 3- iHP mist sprayed on slides from 20-24 inches away for 5s per square foot. Control slides were maintained at room temperature without fog and mist exposure. Slides were placed in individually in single culture dishes, with hatching medium added, then dishes placed in an ambient air incubator. Slides were then rinsed with sterile water, air dried, then scanned under the microscope to quantify percentage of hatched ova.

            Exp 1 showed 57, 55, 52, and 46% hatching at 15, 30, 90, and 240 min time points (controls 87%); exp 2 showed 49, 42, and 47% hatching for 3,4, and 6 fog application cycles (controls 81%); exp 3 showed 44, 37, and 46% hatching for the 1, 2, or 3 iHP mist application (controls 91%).

            Results showed a significant reduction of ova hatching across all three experiments for iHP exposed groups as compared with non-exposed groups. The authors note that the pinworm ova are very strong, composed of chitin, which may explain why iHP did not achieve complete inhibition of hatching. The authors note this decontamination method is not adequate for eradication of *S. obvelata* ova from the room environment. H2O2, delivered by fog or mist, is a widely used decontamination method in hospital settings and the food industry. It is a potent oxidizing agent, whose killing activity is mediated by the production of free hydroxyl radical. The ionization process allows a high concentration of reactive oxygen species and the addition of an electrostatic charge, improving the dispersive characteristics, allowing it to disinfect hard to reach spaces.

QUESTIONS

1.  What can be used to indicate the presence of H2O2?

2.  T/F: *S. obvelata* is the common mouse pinworm, and can also infect the rat, gerbil, and hamster.

3.   How are *Aspiculuris tetraptera* pinworms different *from S. obvelata*?

ANSWERS

1.  Iodine test papers. These were placed throughout the room and next to the slides to confirm fog penetration via color change.

2.   True

3.  *A. tetraptera* is a mouse pinworm too but are not ordinarily found in tape preparations like *S. obvelata*. They are ellipsoidal and can be found in cecal and colonic contents. *A. tetraptera*takes longer to mature than *S. obvelata*, and eggs are deposited in the feces and not on the host.

**Hotchkiss and Young. Comparative Risk of Human Injury / Exposure While Collecting Blood from Sedated and Unsedated Nonhuman Primates, pp. 371-376**

Domain 3, T3, K1

SUMMARY: Blood collection is a very common research procedure and can be performed in both awake and sedated primates. Historically, physical restraint was used which was stressful and potentially dangerous for both the animal and the human. Over time chemical restraint, such as ketamine, became a common method. Recently, the use of positive reinforcement has been employed to allow for collection of blood samples from unsedated primates that are minimally restrained or unrestrained. Elimination of anesthesia is beneficial as it reduces health risks to the animal and minimizes the potential confounding effects on research. This study explores the impact of this change on human health, specifically in terms of the potential for increased exposure to B virus. Animal records were reviewed over a 10-year period and procedures were divided into ones performed under sedation or without sedation. The university’s Online Accident Reporting System was reviewed to determine if any incidents associated with blood collection occurred as well as an overview of all reported injuries or health concerns related to a nonhuman primate exposure. Overall, exposures and injuries related to blood collection account for a small fraction of the reported incidents.  Statistical analysis performed on this data did show a higher risk of exposure for unsedated animals but shows no practical difference in probability of an incident. Out of 73,626 blood collection procedures performed over a 10-year period, 99.97% of sedated and 99.88% of unsedated blood collections occurred without incident.

QUESTIONS

1.  Experimental infections of B virus are recommended to be housed at what ABSL level?

2.  True or False: Cercopithecine herpesvirus is pathogenic in the natural host and causes 80% mortality.

ANSWERS

1.  ABSL-4

2.  False. It is rarely pathogenic in the natural host but can cause mortality as high as 80% in injected humans without prompt appropriate treatment.

***Anesthesia***

**Rufiange et al. Prewarming Followed by Active Warming is Superior to Passive Warming in Preventing Hypothermia for Short Procedures in Adult Rats (*Rattus norvegicus*) Under Isoflurane Anesthesia, pp. 377-383**

Domain 2

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: General anesthesia impairs thermoregulation in all mammals but is most clinically relevant in smaller animals. Redistribution of blood circulation to the periphery due accounts for much of the decrease in core temperature. Once it has occurred, hypothermia can be difficult to reverse, especially while an animal remains anesthetized or during recovery. This study explored whether pre-warming rats prior to isoflurane anesthesia would promote better thermoregulation during anesthesia and recovery. Male and female Sprague Dawley rats were subjected to pre- and intra-operative warming with two different devices (passive: fleece blanket and active: temperature-controlled heating pad) followed by a 30-minute isoflurane anesthesia event. Both groups showed a 1% (0.4°C) increase in core body temperature during pre-warming. Pre-warming followed by passive warming, hypothermia occurred after 30 minutes under isoflurane during the anesthetic recovery period, but active warming better preserved core body temperatures during and after anesthesia.  In fact, pre-warming prior and active warming during anesthesia prevented hypothermia for 30 minutes under isoflurane and during recovery thereafter. Pre-warming with an active device in addition to heat support during the procedure may decrease or eliminate complications or delayed recovery due to hypothermia from isoflurane anesthesia.

QUESTIONS

1. What is the mechanism of hypothermia related to anesthesia?
2. Depression of central thermal homeostasis
3. Slower blood flow
4. Redistribution of blood flow to the periphery
5. Lower cardiac output

2. T/F: Active heating devices better maintain normothermia in rats than passive heating devices.

3. T/F Larger animals are more susceptible to anesthesia-related hypothermia than small animals.

4. Prewarming rats with passive or active devices may combat hypothermia related to isoflurane anesthesia for \_\_\_\_\_\_\_\_ minutes.

1. 10 minutes
2. 30 minutes
3. 1 hour
4. 2 hours

ANSWERS

1. c

2. True

3. False

4. b

**Eshar and Beaufrère. Anesthetic Effects of Alfaxalone-Ketamine, Alfaxalone-Ketamine-Dexmedetomidine, and Alfaxalone-Butorphanol-Midazolam Administered Intramuscularly in Five-striped Palm Squirrels (*Funambulus pennantii*), pp. 384-393**

Domain 2:Management of Pain and Distress, T3 Administration of anesthesia

Tertiary Species: Other Rodents

SUMMARY: Five-striped palm squirrels are native to Southeast Asia and are adaptable to a wide variety of environments. They weight between 135 and 200 g and have a body weight of 20-30 cm, half of which is the tail. This species of squirrel is used in endocrinology research. The authors site safety concerns with inhalant anesthetics such as waste gas from using a mask delivery system and a fire hazard from the presence 100% oxygen.

The common drug in the three preparations presented here is alfaxalone. Alfaxalone is a steroid molecule that potentiates gamma-aminobutyric acid A receptors resulting in non-reversible muscle relaxation and anesthesia. Adding alpha2 adrenoceptor agonists, such as dexmedetomidine, and opioids, such as butorphanol can improve the quality of analgesia and anesthesia while requiring less volume of alfaxalone. Given a paucity of information in the literature regarding the save anesthesia of palm squirrels the investigators compared three common anesthetic regimes, Alfaxalone-Ketamine (AK), Alfaxalone-Ketamine-Dexmedetomidine (AKD), and Alfaxalone-Butorphanol-Midazolam (ABM).

Squirrels were randomly divided into three groups of eight. At doses previously established as effective, by the researchers, each squirrel received one intramuscular injection. Time zero was the time at which the righting reflex was lost. 40 minutes after induction the AKD group received a single dose of Atipamezole and the ABM group received a single dose of Flumazenil. Heart rate, respiratory rate and temperature were monitored every 5 minutes. At 5-minute intervals the animals were checked and scored for a righting reflex, palpebral reflex, forelimb and hindlimb withdrawal reflex. Surgical plane of anesthesia was defined as the loss of reflexes and recovery was defined as the presence of all reflexes with the presence of purposeful movement.

Anesthetic induction was rapid and uneventful in all groups. Induction times were significantly different with ABM being the fastest (median time 15 s), AK the next fastest (median time 49 s) and AKD being the longest (median time 60 s). Anesthetic duration was 62 minutes in the AK group and the ABM and AKD were similar with respective recovery times of 51 and 48 minutes. After 40 minutes AK squirrels showed the lowest heart rate, ABM was the next lowest rate and the AKD group showed a decline until about 20 minutes and then began to rise for the remaining 20 minutes. Respiratory rates were the lowest but stable in ABM mice. AK mice showed a consistent decline from approximately 125 to 50 and the AKD group showed an initial decline for about 20 minutes and then began to increase for the remaining 20 minutes finishing with a much higher rata around 125. All. Mice had a progressive, predicable decline in rectal temperature from about 38 degrees (C) to just above 32 degrees (C) after 40  minutes. Responses across the groups were similar with a notable exception where the righting reflex returned much faster in the AK group versus the AKD and ABM groups.

Overall the AKD and ABM groups improved anesthetic parameters compared to the AK group which had variable reflexes.

Alfaxalone was not given intraperitoneally due to concerns of inconsistent anesthesia and adverse events in other rodent species. All squirrels suffered from profound hypothermia and under normal procedures should have external thermal sources to support to correct the hypothermia. Only the AK group showed adverse reactions during recovery including vocalization and twitching.

QUESTIONS

1. What is the mechanism of action of alfaxalone?
2. Which drugs are used to reverse dexmedetomidine and midazolam respectively?
3. Of the three anesthetic regimes which one should the least consistent response to reflexes.

ANSWERS

1. Alfaxalone is a steroid molecule that potentiates gamma-aminobutyric acid A receptors resulting in non-reversible muscle relaxation and anesthesia
2. Atipamezole and flumazenil
3. AK

**Katz et al. The Stability and Efficacy of Tricaine Methanesulfonate (MS222) Solution After Long-Term Storage, pp. 393-400**

**Domain 2: Management of Pain and Distress**

Primary Species: Zebrafish (Danio rerio)

SUMMARY: Tricaine Methanesulfonate (MS222) is the most commonly used anesthetic agent in fish and other aquatic species. It is an ester type local anesthetic agent that acts systemically when absorbed through the gills or skin. It is highly lipid soluble and readily crosses the cell membrane, where it blocks sodium channels. Tricaine-S (Western Chemical) is the only FDA approved formulation of MS222 for aquaculture. Western Chemical recommends storing 10% solutions for up to 3 days in a cool place protected from light. Cloudiness, darkening or the presence of an oily substance on the stock solution indicates that the solution should be replaced. The objective of the study was to determine whether MS222 was stable after long term storage and to identify specific storage parameters for the stock solutions. Chemical analysis using liquid chromatography and mass spectrometry with subsequent anesthetic testing and histological examination of zebrafish was performed to assess stability. Fish (n=50, 30 males, 20 females) were randomly assigned to 5 groups for anesthetic evaluation: 0 months, 2 months at 4C, 2 months at -20C, 6 months at 4C and 6 months at -20C. Fish were placed in an induction tank were evaluated for initial opercular rate, time to stop swimming, loss of equilibrium, lack of response to von Frey (VF) monofilament, and the final opercular rate. After a light plane of anesthesia was achieved, fish were placed in a recovery tank and monitored for neutral buoyancy, return of righting reflex, the ability to maintain normal posture with the dorsal fin up and time to start swimming. All fish were euthanized via rapid cooling at either 24h or 2 weeks after anesthesia to evaluate acute and chronic effects.  Chemical analysis of the stock solutions showed that all solutions were within 6% of their initial concentrations and were considered stable and safe. All 50 fish were safely anesthetized to a light plane of anesthesia and recovered without complications.  The time to stop responding to 2VF tests in the 2m4 group was significantly longer than that of the 2m-20 and 6m-20 group. The time to neutral buoyancy was significantly longer with MS222 stored for 2m4 than 6m-20. (In the discussion the author alludes that this difference is likely due to the fact that these animals were tested and scored first, and later groups received the benefit of an improvement in researcher’s skill levels to identify stages of anesthesia and performing VF testing ) No statistically significant differences were seen in water temp, pH, conductivity or RDO in the tanks. No fish exhibited histological lesion in the gills, skin, kidneys, muscle, or CNS, which would mean toxicity of MS222.  Various degrees of hepatic megalocytosis were seen across all groups and euthanasia time points. Of the female fish, across all anesthetic groups 13/14 had egg associated inflammation of the ovary. Naïve animals experienced both of these histological changes and the author stated that these histological changes were not due to MS222.

The data showed that MS22 was stable and an effective agent for anesthesia when stored at concentrations of 100mg/ml in opaque glass jars at 4C and -20C for up to 6 months. Fish were safely and effectively anesthetized with all stored MS222 solutions without evidence of histologic toxicity. The authors found no clinically significant differences between naïve zebrafish and those anesthetized with fresh or stored MS222.

QUESTIONS

1. According to AVMA Euthanasia Guidelines, what is an acceptable means of euthanizing Zebrafish?
   1. Immersion of adult zebrafish in MS222 for 10 mins following loss of rhythmic opercular movement
   2. Rapid chilling (2-4C) until loss of orientation and cessation of opercular movements
   3. Immersion in diluted sodium or calcium hypochlorite solution for adult zebrafish
   4. Immersion into preservative concentrations of ethanol (70%)
2. How is MS222 absorbed? What organs metabolize MS222? What is its target organ?
3. What is the lethal dose(LD50) of MS222 for zebrafish?

ANSWERS

1. b
2. Absorbed in gills and skin, metabolized in kidney and muscle, target organs: muscle and CNS
3. 171 ± 7mg/L to 216 ± 4mg/mL

***Experimental Use***

**Litvinova et al. Modifications of Fecal Bacteria Counts and Blood Immune Cells in Offspring of BALB/c and C57BL/6 Mice Obtained through Interstrain Mouse Embryo Transfer, pp. 401-410**

Domain 3

Primary Species: Mouse (*Mus musculus*)

Summary:There is some evidence showing gut microbiota can shape the immune system and metabolism of the host. Additionally, the proportion of immune cells in the blood in a strain is specified by genetics, however, can be altered by changes in microbiota caused by different factors, including antibiotic treatment and diet. The current study aimed to evaluate the transmission of microbiota and immune cell blood phenotypes to the C57BL/6JNskrc and BALB/cJNskrc offspring from surrogate dams of different genotypes. Interstrain embryo transfer was used to determine the effects of surrogate dam on the microflora and immune cell blood phenotype of the offspring.

The experiment was carried out on 12-to 14-wk-old male mice obtained by interstrain embryo transfer and 5 interstrain breeding pairs of each substrain. Four groups were used: C57BL/6JNskrc mice born of C57BL/6JNskrc dam (B6>B6), C57BL/6JNskrc born of BALB/cJNskrc (B6 > C), BALB/cJNskrc born of BALB/cJNskrc (C>C); and BALB/cJNskrc born of C57 BL/6JNskrc (C > B6). Blood samples were collected followed by collection of 3 fecal boli from the distal proximal colon in all mice; plus, spleen samples. Fecal samples were used for detection of *Bacteroides spp*., *E. coli*, *Lactobacillus spp*., *Enterococcus spp*., and *Bifidobacteria spp* by PCR analysis. Also, blood and spleen samples were used for flow cytometry analysis.

Flow cytometry analysis found differences in blood cell count among interstrain embryo transfer groups. Male mice born to dams of the 2 substrains (B6>B6 and C>C groups) differed in blood leukocyte and B-cell (CD19+) counts; leukocytes and B cell counts were significantly higher in male mice of the B6>B6 group compared with C>C group. However, the number of splenic T and B cells did not differ between the male offspring of the B6 > B6 and C > C groups. Moreover, the number of leukocytes in the B6 > C and C > B6 groups was different than the B6> B6 and C> C groups. The percentage of blood immune cells in the male offspring of the B6 > C and C > B6 groups depended on their genotypes and not on the strain of the surrogate dam. A shift in the microbiome was also observed, the counts of *Enterococcus spp* and *L. murinus* were altered in the fecal contents of male offspring that were born to surrogate dams of different genotypes (B6>C compared with B6>B6 and C>B6 compared with C>C). The shift in the normal gut microbiota can affect blood immune cells and possibly those in the intestine.

The study demonstrated that shifts in the microbiota and immune parameters occurred in offspring that were embryo-transferred to surrogate dams of a different genotype. However, the determination of causality in these events and the role of embryo transfer and mother-fetus genotype differences require more investigation.

Question

1. Which of the following statements is true?

a.  The number and percentage of WBC in blood are affected by various genetic, sex, and environmental factors.

b. The genotype of carrying mother can change the quantitative composition of blood leukocytes.

c.   The strain of surrogate dam used for embryo transfer can affect the microflora of the offspring.

d.  All of the above

Answer

1. d

**Terashvili et al. Effect of Nearby Construction Activity on Endothelial Function, Sensitivity to Nitric Oxide, and Potassium Channel Activity in the Middle Cerebral Arteries of Rats, pp. 411-422**

Domain 1: Animal Care; Task T3. Manage or provide indirect management/oversight of laboratory animals; Knowledge K11. Environmental causes of physiological alterations in animals and their effects on research (e.g., sound, light, temperature, humidity, housing systems)

Primary Species – Rats (*Rattus Norvegicus*)

SUMMARY: To assess the effect of construction activity on rat middle cerebral arteries (MCA) to acetylcholine and sodium nitroprusside and the activity of MaxiK potassium channels in the MCAs from male Sprague-Dawley rats,  a commercially available system and Raspberry –Pi-based vibration monitoring system were used to monitor vibration levels. During construction, vasodilator responses to acetylcholine and sodium nitroprusside were abolished, the MaxiK single-channel current opening frequency and open-state probability in cell-attached patches of isolated MCA myocytes were dramatically decreased. The rats housed during the construction period did not recover MCA responses completely after completion of construction, however, animals purchased, housed, and studied after construction had normal responses to the aforementioned drugs. These results show that vibration associated with construction can be disruptive to physiology studies in rodents.

QUESTIONS (True or False)

1. True or False: Construction-related noise and vibration levels that occur in the vivarium and affect animals may not be perceptible to humans working in the area.
2. True or False: Vibration levels should be monitored in facilities during construction activities and phenotypes measured in animals exposed to construction activities prior to, during, and after the construction period.
3. True or False: Investigators engaged in animal-related research projects should be notified prior to the start of construction activities so they can take steps to protect their research animals from potential influence of construction-related stress factors.

ANSWERS

1. True
2. True
3. True

**Lee et al. Effects of *Giardia lamblia* Colonization and Fenbendazole Treatment on Canine Fecal Microbiota, pp. 423-429**

Domain 1

Primary Species: Dog (*Canis familiaris*)

SUMMARY: Giardia lamblia (also known as Giardia duodenalis) is known to have a significant impact on public and veterinary health. Infections with the flagellated parasite can result in subclinical infection or soft to watery feces. Although testing for the parasite occurs often in the veterinary setting, little is known regarding interactions between Giardia lamblia and the canine gut microbiota (GM). In recent years the gut microbiota has been implicated as vital factors that influence host physiologic and immune responses. Authors of this study hypothesized fenbendazole (FBZ), a widely used treatment for Giardia in dogs, would have minimal impact on the GM of clinically normal dogs, regardless of their Giardia colonization status. For this study, fresh fecal samples were collected noninvasively from dogs housed in three different facilities with confirmed cases of Giardia. A small number of dogs in each facility developed diarrhea and tested positive for Giardia using a SNAP test. FBZ (50mg/kg) was used to treat all the dogs in all the three facilities, regardless of infection status for 10 consecutive days.  Fresh samples were collected 2 days prior to FBZ treatment, on the last day of treatment, and 2 weeks after treatment. Samples were processed for DNA extraction and targeted 16rRNA amplicon sequencing. Statistical comparisons were made for both pre and post treatment GM and for the GM of Giardia infected and non-infected samples. The study found no consistent difference in β-diversity, α-diversity and richness of GM based on Giardia status or FBZ use, but significant differences were found in GM between the 3 facilities from which samples were obtained. Diarrhea is likely associated with changes in microbiota and gut barrier function, however, their results indicated that Giardia does not preclude a normal GM. Their findings also demonstrate the importance of genetic, environmental, and husbandry factors that potentially influence the gut microbiota. Differences in GM composition can be a confounding factor in research and may negatively affect the reproducibility of studies. Researchers should be mindful of potential inconsistencies and reduced reproducibility. Results showed that in contrast to metronidazole, FBZ has minimal effect on the canine GM and that FBZ can be safely used in dogs without compromising the richness and diversity of their GM.

QUESTIONS

1.  Giardia lamblia exists in what life stages?

a.  Merozoite and trophozoite

b.  Sporocyst, Shizont, and Merozoite

c.  Oocyst, Sporocyst, Merozoite, and male and female gametes

d.  Trophozoite and cyst

2.  What is Fenbendazole’s mode of action?

a.  Acts by binding to tubulin, an essential structural protein of microtubules.

b.  Promotes the release of acetylcholine, inhibiting cholinesterase, and stimulation of ganglionic neurons, serves as a depolarizing neuromuscular blocking agent which results in paralysis

c.  Binds directly and selectively to muscle membrane GABA receptors, presumably causing hyperpolarization of nerve endings, resulting in flaccid paralysis.

d.  Inhibits protein synthesis by interacting with DNA and causing a loss of helical DNA structure and strand breakage leading to cell death.

ANSWERS

1. d

2. a

**Ober and Geist. Assessment of a Noninvasive Chronic Glucose Monitoring System in Euglycemic and Diabetic Swine (*Sus scrofa*), pp. 430-437**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Pig (*Sus scrofa*)

SUMMARY: Diabetes can occur in a couple forms, type 1 which occurs through loss of insulin producing beta cells, and type 2 which is due to cellular resistance to insulin. Pigs have frequently been utilized in the study of diabetes, but there are challenges including their size, and lack of cooperation when obtaining daily blood samples for glucose monitoring. Training, and gentle handling can improve comfort and minimize distress, but ideally blood glucose can be monitored continuously in a minimally invasive way. For this study, 4-6 month old American Yorkshire pigs and STZ-induced diabetic minipigs (4 per group) were utilized. Insulin was administered twice daily to diabetic animals. Blood glucose was monitored twice daily prior to feeding using point-of-care glucometers and blood obtained via ear prick. Additionally a central line was placed to allow for serial blood collections for chemistry analysis. Each pig had a FreeStyle Libre monitor placed posterior to the base of the ear for continuous glucose monitoring. There was a strong linear correlation between time-matched blood chemistry values and a point-of-care glucometer. Likewise, the continuous glucose monitor had a strong linear correlation with the point-of-care glucometer and the blood chemistry analysis. However, 64% of measurements made with the continuous glucose monitor did not meet ISO standards (within 15% of actual chemistry value). One of the point-of-care monitors (validated for use with dog and cat blood), overestimated blood glucose which could lead to negative clinical outcomes following insulin administration. When choosing a point-of-care monitor, one must consider the species for which that monitor has been validated. While the continuous monitor can contribute to management of diabetes in swine, it is not as accurate as the point-of-care machines and therefore is not a good substitute for data collection.

QUESTIONS

1. Which of the following substances can be used to induce diabetes in animal models?

a. Dextran Sulfate Sodium

b. Streptozotocin

c.   Lipopolysaccharide

d. Bleomycin

2. Blood sampling from the cranial vena cava in pigs, should only be done from which side?

a. Right

b.   Left

ANSWERS

1. b

2. b

**Zuo et al. Measurement of the Luminal Diameter of Peripheral Arterial Vasculature in Yorkshire×Landrace Swine by Using Ultrasonography and Angiography, pp. 438-444**

Domain 1 - Management of spontaneous and experimentally induced diseases and conditions

Primary Species: Pig (*Sus scrofa domesticus*)

SUMMARY: Swine are commonly used in cardiovascular research and there is limited information on swine peripheral artery dimensions and means by which to pre-select swine that have particular arterial dimensions for the purposes of research. The aim of this study was to use ultrasonography to measure the size of superficially located carotid and femoral arteries, as well as angiography to evaluate deeper iliac arteries to more closely correlate animal size to vessel size. Angiography is suggested to not generally be a useful pre-procedural system for measuring arteries given it is invasive and may cause vasoconstriction and inaccurate measurements (secondary to passing guidewires and catheters).

Landrace x Yorkshire animals (n=38) were divided by weight (i.e. < 40 kg, 40-50 kg, etc. up to >70 kg). Ultrasound of L+R common carotid and femoral arteries were performed as well as angiography (n=11) of the iliac arteries. No difference in luminal diameter was detected between the L and R sides of the same arteries.

When assessing weight and luminal diameters, a general trend of increasing luminal diameter with high correlation between weight and those diameters in animals that weighed under 70 kg. For both carotid and femoral arteries significant differences in mean artery diameters were discerned among the weight categories. Regression analyses found quadratic regression of best fit indicating moderate (R=0.53) correlation between carotid artery diameter and weight while the correlation between diameter and animal weight for the femoral artery was strongly correlated (R=0.75). Artery diameter was found to plateau in animals heavier than 65 kg.

QUESTIONS

1. Which of the following is one of the features of the swine cardiac circulatory system that distinguishes it from most other mammalian species?

a.  The pulmonary artery consists of a triplet of vessels as opposed to a paired (R+L) configuration

b.  The azygous vein drains directly into the coronary sinus

c.  The musculature of the heart consists predominantly of smooth as opposed to true cardiac muscle

d.  The pig heart is generally less electrically conductive than human hearts

2. Which portion of the heart is more susceptible to ischemia -- the epicardium or the endocardium?

3. (T/F) Swine, like humans, receive the majority of the cerebral blood flow from via the carotid arteries.

ANSWERS

1. b

2. Endocardium

3. T