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**ORIGINAL RESEARCH**

***Biology***

**Trumel et al. Hematologic and Biochemical Biologic Variation in Laboratory Cats, pp. 503-509**

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

Secondary Species: Cat (Felis domestica)

SUMMARY: Currently available reference intervals for hematology are population based and may confound the intra-individual variability that could be an important factor in detecting significant changes in a specific animal.  The prospective study design used 14 cats (7 males and 7 females) over 7 time points (days 1, 2, 7, 14, 31, 42, and 100) to determine the biologic variation and reference change values of hematologic and biochemical variables in each individual animal.  For each variable, analytical, intraindividual, and inter-individual coefficients were estimated prior to calculating the index of individuality and the reference change value.  The results demonstrated that red blood count variables and five biochemical analytes (cholesterol, creatinine, triglycerides, ALP, and calcium) all had marked individuality.  Population based reference intervals were adequate for glucose and sodium.  The authors concluded that the reference change value determined in the study was a valuable tool for monitoring hematologic and biochemical variables in healthy laboratory cats.

QUESTIONS

1. What is the reference change value?

2. T/F.  The reference change value can be used on all animals regardless of health status.

3. T/F.  A previous study on biologic variation with client owned cats had similar results as this study which used laboratory cats.

ANSWERS

1. The reference change value is the lowest difference that cannot be accounted for by the combined effects of intraindividual and analytical variability.

2. F - It cannot be applied to clinically unstable diseased animals.

3. F - The results of the client owned cat population were very different than those of the laboratory bred cat colony.

***Reproduction***

**Lerch et al. Effects of Embryo Transfer on Emotional Behaviors in C57BL/6 Mice, pp. 510-519**

Domain 3: Research

Primary Species: Mouse (Mus musculus)

SUMMARY

Background:

* Embryo transfer
  + Considered the ‘gold standard’ for inducing genetic alteration in newly generated mouse lines
  + The survival rate of the transferred embryo is more dependent on the genotype of the recipient dam than the embryo itself.
* Cognitive impairment has been noted after use of isoflurane anesthesia
* Cryopreservation induces enhanced birth weights in mice (and humans)

Study Design and Results:

* Investigated the effects of several components of embryo transfer (anesthesia, surgery, recipient strain) on behavior in the dam (Nest building & exploration) and offspring (nest building, exploration, anxiety, and social and depressive-like behaviors)
* Results:
  + Female offspring showed effects on weight gain and corticosterone levels following anesthesia
  + ET-derived female offspring showed a decrease in exploration and nest building, enhanced levels of anxiety, and increased social interest.

QUESTIONS

1. What does the Novel-cage test asses?
   1. Anxiety
   2. Depression
   3. Exploration
   4. Stress
2. What does the Forced swim test assess?
   1. Anxiety
   2. Depression
   3. Exploration
   4. Stress

ANSWERS

1. c

2. b

Domain 3:Research; K3 – Animal Models Including Normative Biology Relevant to the Research

Primary Species: Mouse (Mus musculus)

SUMMARY: Embryo transfer (ET) is a commonly used technique in mice, including for the control of microbes and for the generation of transgenic mice. This study focuses on the emotional behaviors of offspring born from standard breeding and ET. In addition, investigators also tested ET in two different recipients C57BL/6N (ET-C57) and NMRI (ET-NMRI).

Embryo C57BL/6 donor females were superovulated with PMSG and HCG, mated, and euthanized via CO2 24h after copulatory plug confirmation. These 2-cell embryos were transferred into either C57 or NMRI recipients via oviduct transfer.

Offspring derived from these ETs were used in behavioral studies at approximate 8-10 weeks of age. Maternal behavior was also assessed.

Behavioral tests includes. Pup retrieval test. Nest building at 5h and 24h after new nest placed. Novel-cage tests the number of number and latency to rear. Open-field tests the amount of time in center field. Novel-object tests the latency and number of approaches to novel object. O-maze measures the amount of time in closed arm. Dark-light box measures the amount of time in light, entrance/exit. Social recognition tests the amount of time in proximity with mouse. Forced-swim test latency to giving up. Hotplate and fear-conditioning measures latency to licking paws. Serum corticosterone measured.

There were no changes caused by surgery itself on maternal behaviors. However, there ET affected maternal behaviors by decreased exploration and nest building behaviors. There were no major differences between the behaviors of offspring between the different groups. There was an increased anxiety in the male offspring of ET-C57 compared to females of the same group via light-dark test. NMRI mothers explored more and retrieved pups later than C57BL/6. C57 recipient dams appeared to show increased maternal care than NMRI. However, offspring from NMRI dams had higher weights than did C57.

The study authors concludes that ET can modulate the emotional phenotype of the offspring. In summary, female progeny seemed to be more sensitive to ET-associated effects than were male mice. Therefore biotechnological procedures currently considered to be routine practices, such as ET, likely exert long-lasting effects on offspring behavior and potentially on their physiologic state as well.

QUESTIONS

1.  Which of the following tests are NOT used for anxiety testing in mice?

a.  Light-dark test

b.  O-maze

c.  Forced-swim test

d.  Elevated plus maze

2.  Which of the two following groups of mice are NOT needed for a successful transgenic mouse operation?

a.  Donor females

b.  Recipient females

c.  Founder females

d.   Donor males

e.  Infertile males

f.    Founder males

3.  A recent study demonstrates that pups derived from ET are

a.  Heavier when the recipient is NMRI compared to B6 recipients

b. Heavier when the recipient is B6 compared to NMRI recipients

c.   Less fertile when the recipient is NMRI compared to B6 recipients

d.  More fertile when the recipient is B6 compared to NMRI recipients

ANSWERS

1. c,

2. c, f

3. a

**Moran et al. Cryotolerance of Sperm from Transgenic Rhesus Macaques (*Macaca mulatta*), pp. 520-524**

Domain 3: Research

SUMMARY: Transgenic Huntington disease (HD) rhesus at Yerkes have recently reached puberty, enabling semen collection and banking. Viability of cryopreserved and fresh sperm from WT and HD rhesus were compared. A modified TEST extender (egg yolk, TES, and Tris buffers + iodixanol and the detergent Equex Paste) was found to increase acrosome and membrane integrity after thawing. There was no difference in motility between fresh WT and HD sperm. WT and HD sperm both experienced increased damage after freezing, but this damage was not significantly different between groups. Though motility was decreased almost 50%, cryopreserved HD sperm successfully fertilized WT oocytes.

QUESTIONS

1. What stains can be used to determine sperm motility and damage?

2.  How does a macaque model of HD differ from other HD models?

ANSWERS

1.  Hoechst 33342 and propidium iodide stains damaged membranes (dead sperm) red; fluorescein isothiocyanate-peanut agglutinin stains damaged acrosomes bright green.

2.  Fresh sperm from HD minipigs experience a 75% motility loss compared to WT (no difference between WT and HD fresh sperm in this study).

***Husbandry***

**Nicolaus et al. Effect of Ventilated Caging on Water Intake and Loss of 4 Strains of Laboratory Mice, pp. 525-533**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus)*

SUMMARY: Food availability, temperature, humidity, strain, and caging type all affect water consumption by mice. Measurement of transepidermal water loss (TEWL) is a new technique for the quantification of water turnover in mice. To understand water turnover in common strains of adult mice, male and female SCID, SKH, C57BL/6, and FVB mice were housed in same-sex groups of 5 animals in static cages or IVC. Body weight, TEWL, urine osmolality, and water consumption of mice and intracage temperature and humidity were measured every 48 h for comparison. Static cages were monitored for 7 d and IVC for 14 d before cage change. Female SCID, FVB, and C57 mice drank less water than did their male counterparts. Male and female SCID, SKH, and FVB mice in IVC drank less water and had higher urine osmolality than did those in static cages. In SCID and SKH mice, TEWL paralleled water consumption. C57 mice in static cages drank less water, had lower urine osmolality, and had less TEWL than did those in IVC. Temperature and humidity within the cage was higher than the macroenvironmental levels for all housing conditions, mouse strains, and sexes. These data demonstrate that mouse strain and housing conditions significantly influence water balance and indicate that macroenvironmental measurements do not always reflect the intracage environment.

QUESTIONS

1.   Where does sensible water loss occur in mice?

2.  How is TEWL quantified?

3.  Where does the majority of insensible water loss occur in mice?

ANSWERS

1.  Through the urine

2.  By an evaporimeter which measures the rate of water exchange across the dermis.

3.  Through the respiratory tract

**Pan et al. Effects of Transportation on Antioxidant Status in Cynomolgus Macaques (*Macaca fascicularis*), pp. 534-540**

Domain 4: Animal Care

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: The present study investigated the effects of transportation on oxidative stress damage in cynomolgus macaques. To evaluate the effects of transportation on oxidative stress, the authors measured serum levels of reduced glutathione (GSH), malondialdehyde, and protein carbonyl (PC) and the activities of total antioxidant capacity (TAOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase before transportation (day 0), on the day of arrival (day 1), and on days 7, 14, and 21 after transportation. On day 0, TAOC and catalase activities on days 1, 7, and 14 after transportation were significantly decreased, reached their lowest level on day 7, and increased thereafter to reach their pre-transportation levels by day 21 after transportation. Compared with day 0 levels, mean SOD activity and GSH concentration were decreased significantly on day 1 and thereafter increased to reach their pre-transportation measures by day 7 after transportation. In contrast, the authors results of PC and malondialdehyde concentrations in serum and the activity of GSH-Px were increased on day 1 compared with day 0 and thereafter decreased to reach their pre-transportation levels by day 14 after transportation. The authors concluded that increases in the activity or level of malondialdehyde, PC, and GSH-Px and decreases in GSH, SOD, catalase, and TAOC occurred after transportation of cynomolgus macaques. These parameters returned to basal levels after approximately 21 d of recovery. The authors’ results suggested that transportation might imbalance oxidant and antioxidant levels to create excess oxidative stress in cynomolgus macaques. Therefore cynomolgus macaques should have at least 21 days to recover after transportation and regain their healthy status.

QUESTIONS

1. What are the main antioxidant enzymes in the body?

a. Superoxide dismutase

b. Catalase

c. Glutathione peroxidase

d. Glutathione reductase

e. All of the above

2. In the study what day did levels of malondialdehyde, protein carbonyl, glutatthione peroxidase, gluthatione, superoxide dismutase, catalase, and total antioxidant capacity returned to basal levels?

a. 21 days of recovery from transport

b. 14 days of recovery from transport

c. 7 days of recovery from transport

d. 1 month of recovery from transport

e. 6 weeks of recovery from transport

ANSWERS

1. e

2. a

***Animal Health Surveillance***

**Gooodroe et al. Guidance Regarding Sample Collection and Refinement of Fecal Flotation Exam for the Isolation of *Aspicularis tetraptera*, pp. 541-547**

Domain 1, T3

Primary Species: Mouse (*Mus musculus)*

SUMMARY: *Aspiculuris tetraptera*is a common finding in rodent colonies, historically due to difficulties in parasite detection. PCR testing is very sensitive but is expensive and does not provide immediate results. This paper provides guidance on sample collection and the fecal flotation exam to improve diagnostic efficiency in isolating *A. tetraptera.*This study suggests improvements to the protocol for collecting and processing fecal samples by using the passive fecal flotation exam and soaking the fecal pellets in zinc sulfate for 30 minutes prior to a 15-min flotation period. The findings also suggest collecting at least 20 fecal pellets per cage when investigating a suspected infection to reduce false negatives. Additionally, when selecting a cage to test during a suspected outbreak, priority should be given to younger mice, as this study indicated that older mice have lower worm burdens.

QUESTIONS

1. What is the prepatent period of *A. tetraptera?*

a. 5 to 8 days

b.  10 to 14 days

c.  20 to 24 days

d.   21 to 25 days

2. What is the life cycle of *A. tetraptera?*

3. Why is *A. tetraptera* the only rodent pinworm that is readily detectable through fecal flotation?

4. What is the lifespan of adult female *A. tetraptera*?

a. 15 to 20 days

b. 30 to 35 days

c. 45 to 50 days

d. 55 to 60 days

ANSWERS

1. d

2. Direct

3. Because adult female worms shed eggs in the mucous covering of rodent fecal pellets

4. c

***Anesthesia***

**Erickson et al. Intraperitoneal Continuous-Rate Infusion for the Maintenance of Anesthesia in Laboratory Mice (*Mus musculus*), pp. 548-557**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus)*

SUMMARY:Ketamine-xylazine (KX) and ketamine-xylazine-acepromazine (KXA) combinations are 2 common injectable anesthetics mixtures used in laboratory mice when gas anesthesia is not an option due to research aims. The surgical plane of anesthesia with these combinations is typically between 40-50 minutes, however the duration is quite variable among individual animals. Bolus redosing of ketamine alone or in combination has been used to extend the duration of anesthesia, however this method is associated with high mortality rates. Due to this complication, the authors sought to characterize intraperitoneal continuous rate infusion of KX or KXA as a possible means to extend anesthesia time without increasing mortality rate. The authors found that IP CRI maintained a longer duration of continuous surgical anesthesia with comparably lower mortality. The most successful regimens were 1) induction with KXA (0.1) and CRI of 100% initial ketamine dose hourly and 2) induction with KXA (0.5) and CRI of 50% of both the initial doses of ketamine and xylazine. IP CRI dosing maintained a longer duration of continuous surgical anesthesia compared with bolus redosing after reemergence from surgical anesthesia. Mortality was associated with higher anesthetic induction doses, inclusion of acepromazine in the anesthetic induction, and higher CRI doses. Deaths were most likely related to respiratory depression and hypoxia.

QUESTIONS

1.  True/False: This study concludes that IP CRI of KXA (0.5) replaces isoflurane anesthesia as the anesthetic regimen of choice in laboratory mice.

2.  Which of the following is a reversal agent for xylazine?

a. Flumazenil

b.  Atipamezole

c. Naloxone

d.  Saline

3.  True/False: Ketamine is not shown to have any analgesic effect.

4.  Under what DEA Scheduled Drug Class is ketamine classified?

5.   Which DEA form is required for reporting lost or stolen controlled substances?

ANSWERS

1. False, isoflurane is still the anesthetic of choice

2.  b

3.  False

4.   Schedule III

5.   DEA Form 106

**Pan et al. Effects of Ketamine on Metabolomics of Serum and Urine in Cynomolgus Macaques (*Macaca fascicularis*), pp. 558-564**

Domain 3: Research; K15 - Genomics, Metabolomics, and Proteomics

Species Primary: Macaques (Macaca spp.)

SUMMARY: This paper studied the effect of Ketamine on metabolic markers in cynomolgus macaques using nuclear magnetic resonance (NMR) spectroscopy. Ketamine is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist that binds the phencyclidine receptor thus blocking the NMDA receptor channel. Commonly used for sedation and anaesthesia ketamine has known side effects including interference with recall and recognition memory, working memory and executive memory, transient dissociation, induction of psychotomimetic symptoms, the stimulation of Prolactin release and hemodynamic changes such as an increase in blood pressure. Metabolomics has been used to characterize drug metabolic profiles and biomarkers. In this study NMR was used to profile serum and urine metabolites. Under normal housing conditions 12 animals were separated into 2 groups; one for controls (received a single intramuscular injection of saline) and one for the test group (received a single intramuscular injection of ketamine at a dose of 10mg/kg). Blood samples were collected 5 minutes and 24 hours after injection. All animals were maintained in metabolic cages for the collection of urine over a 24 hour period. A large number of parameters were evaluated and the results are reported in the journal. Briefly, in the ketamine group serum levels of lactate, alpha-glucose, and myoinositol were lower than in controls and arginine was higher than in controls. In the ketamine group urine levels of leucine were higher and lactate, pyruvate and succinate were lower. All of the parameters reported as being lower in serum and urine are compounds in the glycolysis-gluconeogenesis pathway suggesting interference of this pathway by ketamine. The authors suggest that the increase levels of serum arginine and urinary leucine may reflect metabolic derangements involving either protein synthesis or amino acid degradation.

QUESTIONS

1. Briefly describe the mechanism of action for ketamine.
2. List common side effects of ketamine
3. T/F.  ketamine interferes with the glycolysis-gluconeogenesis pathway

ANSWERS

1. Ketamine is a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist that binds the phencyclidine receptor thus blocking the NMDA receptor channel.
2. Interference with recall and recognition memory, working memory and executive memory, transient dissociation, induction of psychotomimetic symptoms, the stimulation of Prolactin release and hemodynamic changes such as an increase in blood pressure.
3. T

**Roberts and Syme. Effects of Using Tricaine Methanesulfonate and Metomidate before Euthanasia on the Contractile Properties of Rainbow Trout (*Oncorhynchus mykiss*) Myocardium, pp. 565-569**

Domain 2: Management of Pain and Distress

Tertiary Species: Other Fish

SUMMARY: Anesthesia of fish used in research is common practice. The mechanism of action of many anesthetics involves depressing cell excitability, which may be contraindicated in studies that rely on the excitable nature of tissues (for example, nerve and muscle tissue). The anesthetic tricaine methanesulfonate (TMS) works by binding to sodium channels and reducing depolarization current.  The anesthetic methyl 1-(1-phenylethyl)-1H-imidazole-5-carboxylate (metomidate) binds to central GABAA receptors and enhances the receptors’ function, thus producing hypnotic and immobilizing effects. Previous studies have shown TMS to reduce twitch force in isolated myocardium from Chinook salmon in a dose-dependent manner, as well as to reduce blood pressure and heart rate in rainbow trout experiencing prolonged immersion. The authors of this study were interested in evaluating if these effects could be effectively and rapidly reversed by rinsing excitable tissues in saline. If so, this might allow for refinement of fish euthanasia to include anesthesia under appropriate circumstances (particularly in conjunction with/prior to physical methods of euthanasia). Fish were euthanized either with or without anesthetic treatment. The fish were anesthetized with either TMS or metomidate for 2-3 minutes until they reached stage 2 anesthesia; control fish were placed in water without anesthetic for 2-3 minutes prior to euthanasia. All fish were euthanized using the same physical methods performed by experience personnel. Hearts were removed after euthanasia and placed in anesthetic-free saline while they were dissected and prepared for experimentation. Four measures of contractile performance were assessed, including the ability to perform mechanical work, maximal isometric force production, rates of force rise during activation and force fall during relaxation, and the maximal rate at which the muscle could contract consistently with repeated stimulation. Subsequently, myocardial tissues were exposed directly to TMS and metomidate at the same doses used for anesthesia, and work and force were measured to determine if these drugs have a direct effect myocardial contractile performance. Direct exposure to buffered TMS significantly affected both work and twitch force, while direct exposure to metomidate did not impair output. None of the measures of contractile performance differed significantly between any of the 3 euthanasia treatments. Any negative inotropy associated with TMS and metomidate exposure during anesthesia appears to be relatively easily reversible with a simple saline rinse after brief anesthesia. Studies involving the contractile properties of isolated and saline-perfused cardiac muscle from fish might be refined to include the use of anesthetics TMS and metomidate prior to euthanasia. This could allow more secure physical restraint of the fish during physical methods of euthanasia and may improve efficiency of effective euthanasia.

QUESTIONS

1.  Which of the following methods of euthanasia is unacceptable for rainbow trout used in research, according to the 2013 AVMA Guidelines for the Euthanasia of Animals?

a.  Immersion in TMS at 5-10 times the anesthetic dosage

b.  Metomidate overdose

c.   Decapitation followed by pithing

d.  Immersion in CO2-saturated water

2.  Which of the following anesthetics is/are GABA receptor agonists?

a.  TMS

b.  Ketamine

c. Metomidate

d. Eugenol

ANSWERS

1. b

2. c

***Experimental Use***

**Regan et al. Comparison of Submental Blood Collection with the Retroorbital and Submandibular Methods in Mice (*Mus musculus*), pp. 570-576**

Domain 3: Research

Primary Species:Mouse (*Mus musculus*)

SUMMARY:Large (>100 μL) nonterminal volumes of unhemolyzed or unclotted blood are typically collected from the retroorbital sinus or submandibular plexus. In this study, the authors described a third method—submental blood collection. Forty-five CD1 female mice (30 g, age - 23 wks) were randomly assigned to three groups (*n* = 15 per group, submental, submandibular, and retroorbital). Phlebotomists attempted to collect approximately 150 μL of blood from each mouse/group, for a total of 3 collections and with a 2-wk recovery period between collections. For the submental and submandibular groups, phlebotomists were allowed to perform no more than 2 attempts per collection with either 4- or 5-mm mouse phlebotomy lancet. For each method, the locations were restricted to the right side (collection 1), the left side (collection 2), and the right side (collection 3). For the retroorbital group, the phlebotomist was allowed to make only one entry into the orbit of the right eye (collection 1), left eye (collection 2), and right eye (collection 3) in isoflurane-anesthetized mice. The authors concluded that submental method can also be used to obtain large, nonterminal volumes of blood from mice.

QUESTIONS

1. Blood sample quality can be negatively affected by:

1. Hemolysis
2. Clotting
3. Hemolysis and Clotting
4. None of above

2. True or False. The use of the retroorbital method is universally acceptable in mice.

3. Submandibular blood collection is associated with:

1. Stress
2. Local hemorrhage
3. Lethargy
4. Stress, lethargy and local hemorrhage
5. None of the above

Use the following figure to answer questions 4 and 5:

4. The dot area in the picture above indicate the region where the \_\_\_\_\_\_\_\_\_\_\_ and \_\_\_\_\_\_\_\_\_\_\_\_ veins converge in the mouse.

1. Mandibular and facial
2. Maxillary and submental
3. Facial and submental
4. None of the above

5. The dot area in the figure above indicate the venipuncture location for the blood collection method.

1. Submental
2. Submandibular
3. Retroorbital
4. None of the above

6. As a general guideline, the total circulating blood volume in an adult mice ranges from:

a. Approximately 35 to 40 mL/kg

* 1. Approximately 125 to 240 mL/kg
  2. Approximately 15 to 25 mL/kg
  3. Approximately 55 to 80 mL/kg
     1. As a general rule, variations in individual skill with a particular blood collection technique can have a substantial effect on:

1. Animal welfare, experimental outcome, and reproducibility
2. Animal welfare
3. Experimental outcome
4. Reproducibility

ANSWERS

1. c

2. False

3. d

4. c

5. a

6. d

7. a

**Gilbertson and Wyatt. Evaluation of Euthanasia Techniques for an Invertebrate Species, Land Snails (*Succinea putris*), pp. 577-581**

Domain 2

Tertiary Species: Invertebrate

SUMMARY:  There are many advantages to using invertebrates in research (cost, space, bioindicators, etc.).   Despite the long history of use of invertebrates in research there is relatively little known about the best method of euthanasia.  In the AVMA guidelines there are acceptable methods of euthanasia (2 step, anesthesia flowed by euthanasia) for aquatic invertebrates, but only conditionally acceptable methods for land based invertebrates.  The authors had the goals of identifying a method of euthanasia that preserved cellular structure, had minimal negative impact on wellbeing and a method that did not require chemicals that were controlled or difficult to come by.  The snail that was used was the land snail *Succinea putris*.  There were 5 solutions tested (RO water, 5%, 70% and 95% ethanol, and beer – 4.74% ethanol).  Snails were placed in each solution for 2 hours and then assessed for behavior, aversion maneuvers, and ability to regain consciousness.  Histology of tissues was assessed after euthanasia.  Beer, 5% Ethanol and RO water had no effect on behavior when first submerged, 70% and 95% ethanol caused aversive behavior.  Beer, 5% Ethanol and RO water snails recovered anesthesia where as those in 70% or 95% ethanol never did.  Because the body did not contract the histology was truer for those anesthetized with 5% ethanol or beer.  The authors recommend that for these snails the best anesthesia is 5% ethanol or beer (which is readily available in the field).  To euthanize these animals after they are anesthetized they can be placed in 10% formalin.

QUESTIONS

1.  Based on a recent paper what percent ethanol would be more appropriate for anesthetizing some recently collected *Succinea putris*?

a.  10% formalin

b.  4.74-5% ethanol

c.  70% ethanol

d.  95% ethanol

2.  The AVMA Guidelines for the Euthanasia of Animals: 2013 Edition describes one method of euthanasia for terrestrial invertebrates that is considered acceptable.  What is this method?

a.  Rapid freezing

b.  Blunt trauma

c.  Injectable agents

d.  Submersion in Ethanol

3.  What behaviors of *Succinea putris* have not been described as demonstrating aversive behavior?

a. Retraction of body into shell

b.  Bubble release

c.  Mucous production

d.  Excretion of feces

e.  Seizures

ANSWERS

1.  b

2.   c

3. e

**Burton et al. Evaluation of Fecal Microbiota Transfer as Treatment for Postweaning Diarrhea in Research-Colony Puppies, pp. 582-587**

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

Primary Species - Dog (*Canis familiaris*)

SUMMARY: At approximately the age of weaning (6-8 weeks old), puppies in research settings often develop diarrhea, which may be partially due to an immature and unstable intestinal microbiota permissive to opportunistic pathogens. The objective of this study was to assess whether fecal microbiota transfer (FMT) allowed for transmission of  stable maternal microbiota to pups and decreased the incidence of postweaning diarrhea. Puppies were designated by litter and treated (FMT) with fecal inoculum from their respective dams orally for 5 consecutive days during weaning or sham-treated. Diarrhea was evaluated according to a published Purina scoring system for 11 days during weaning. Fresh feces were collected from dams and puppies at 3 days before weaning and 3, 10, and 24 days after weaning for analysis of the fecal microbiota by using 16S rRNA amplicon sequencing. No diarrhea was reported in either group during the study period, so clinical comparison of the two groups was problematic. However, 16S rRNA gene analysis revealed microbial variability across time in both groups. The fecal microbiota of neither group of puppies mirrored the respective dam at any of the designated time points. However, the data provide new information regarding maturation of the fecal microbiota of puppies at and after weaning.

QUESTIONS (True or False)

1. Fecal microbiota transfer (FMT) has been proven to be efficacious in decreasing incidence of diarrhea at weaning in puppies raised in a research setting.

2. Fecal microbiota transfer (FMT) from dams to pups causes pups' gastrointestinal microbiota (GM) to more closely mimic that of the dam over time.

3. Comingling of litters at weaning may cause stress that precipitates diarrhea during that time.

ANSWERS

* + 1. False
    2. False
    3. True

**Jara et al. Comparison of Microchip Transponder and Noncontact Infrared Thermometry with Rectal Thermometry in Domestic Swine (*Sus scrofa domestica*), pp. 588-593**

Primary Species: Pig (*Sus scrofa*)

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

T1. Prevent spontaneous or unintended disease or condition

T2. Control spontaneous or unintended disease or condition

T3. Diagnose disease or condition as appropriate

T4. Treat disease or condition as appropriate

SUMMARY: Core body temperature is one of the most useful indicators of disease, yet accurate measurement can be challenging. Despite advances in swine health management and production, rectal thermometry continues to be the *gold standard* for measuring core body temperature. Some of the challenges in this species involve safe handling, restraint, and accurate readings.

The authors compared core body temperature of 24 male castrated piglets using rectal thermometers, subcutaneous microchips, and an inexpensive handheld infrared thermometer. All 3 methods showed significant variation [rectal temp—39.3 +/- 0.5 degrees Celsius; microchip—39 +/- 0.7 degrees Celsius; and infrared—34.3 +/- 1.0 degrees Celsius], but although, the infrared metric was the lowest, if a baseline is established, this method for obtaining fast and safe body temperature data, may be the best when used as a screening device.

QUESTIONS

1.       What challenges did the authors cite regarding tympanic infrared thermometry?

2.       List factors that can confound body temperature despite the method used.

ANSWERS

1.       A clean ear canal and a sedentary pig during temperature measurement

2.       Environmental temperature, humidity, and air flow

**Ansel et al. The Effect of Anesthesia on Blood Pressure Measured Noninvasively by Using the Tail-Cuff Method in Marmosets (*Callithrix jacchus*), pp. 594-600**

Domain 2: Management of Pain and Distress

Secondary Species: Marmosets/Tamarins (Callitrichidae)

SUMMARY

Aim:The study aimed to evaluate the safety and validity of measuring blood pressure (BP) noninvasively in marmosets by using the tail-cuff method. The animals were anesthetized, in an attempt to reduce the variability associated with the measuring awake marmosets. To support future longitudinal studies, authors also evaluated the potential side effects of measuring BP in anesthetized marmosets, established the test-retest repeatability of this method, and calculated the number of measurements required for a repeatable reading.

Study Design: Authors evaluated 12 marmosets (7 females and 5 males) ranged from 44 to 534 days in age, 87 to 450 gm in weight. For evaluating the BP, authors used the CODA surgical monito system (Kent Scientific), which provides measures of systolic (SBP), diastolic (DBP) and body temperature. The CODA system used in the current study follows the principle of oscillometry and measures changes in the tail blood volume. Alphaxalone (15 mg/kg, IM) was used for general anesthesia. To calculate the adequate number of measures needed, authors measured each of 10 selected animals 5 times in succession. To assess the validity of BP measured noninvasively, the within- and between-session repeatability and reproducibility were calculated by measuring 12 marmosets twice at the same time of day (±1 h) 1 wk apart.

Summary:Results from study confirmed that CODA system is a safe and reliable noninvasive instrument. The minimum number of measurements required for accurate BP (both SBP and DBP) reading was found to be 4 readings. The readings were less variable when they were taken within 30 min after anesthetic injection. In addition, the device used yielded data that were repeatable within and between sessions, with a larger range of variability between sessions.

QUESTIONS

1. The existing gold standard for the assessment of blood pressure measurements in Marmosets

a. Direct intra-arterial BP recording via Radiotelemetry (Invasive)

b. Doppler technology (Non-invasive)

c. Oscillometry (Non-invasive)

2. Minimum BP readings required for the accurate readings for tail cuff method

a. 2

b. 3

c. 4

d. 5

ANSWERS

1. a

2. c

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

K1 – Diagnostic Procedures

Secondary Species: Marmoset/Tamarins (Callitrichidae)

SUMMARY: In this study, the validity of measuring blood pressure (BP) noninvasively in marmosets by using the tail-cuff method was evaluated. The number of measurements needed for a valid reading was calculated by plotting the average SD of 5 consecutive readings in 10 naïve marmosets; the SD for both systolic and diastolic BP readings plateaued after 4 readings. To evaluate how anesthesia (alphaxalone, 15 mg/kg IM) affected BP in marmosets, 4 animals were measured every minute for 60 min after injection. The average length of anesthesia was 47.3 ± 13.2 min. The variability in the systolic and diastolic BP was the smallest at 10 to 30 min after injection (systolic SD, 6.29 mm Hg; diastolic SD, 5.27 mm Hg) and almost doubled at 30 to 60 min after injection (systolic SD, 13.5 mm Hg; diastolic SD, 12.3 mm Hg). The within- and between-session repeatability and reproducibility were calculated by measuring 12 marmosets twice at the same time of day (±1 h) 1 wk apart. The coefficients of repeatability and reproducibility were 1.98% and 14.5% for systolic BP and 3.37% and 16.2% for diastolic BP, respectively. Results indicate that using the volumetric tail-cuff method to measure BP noninvasively in anesthetized marmosets is safe and feasible. The measures are least variable within 10 to 30 min after the injection of anesthetic, and variability increases slightly between sessions.

Important Points:

* Blood pressure is a physiologic measurement
  + Used as a screening measure
    - Evaluation of medical treatments and interventions
    - Assessment of cardiovascular risk
    - Monitoring of various aspects of blood perfusion
* Common marmoset: *Callithrix jacchus*
  + New World monkey
  + Little to no sexual dimorphism
  + High frequency of twinning (80%)
  + Various research uses
    - Group in paper mentions studying “spatial and temporal characteristics of visual experience that control eye growth and refractive development”
    - Also the “interactions between the visual and vascular functions at both the ocular and systemic levels”
* Multiple techniques to measure BP
  + Direct/invasive
    - Direct intraarterial recording with radiotelemetry
    - Most often used with marmosets
    - Continuous measurements not altered by normal activity
  + Non-invasive
    - Doppler technology
      * Changes in blood flow due to cuff compression
      * Estimate of mean arterial pressure only
    - Oscillometry
      * Measures oscillations in the arterial wall in response to compression
      * Measures both systolic and diastolic pressures
* Volumetric tail-cuff method
  + Used in this paper
    - <https://www.kentscientific.com/products/productView.asp?productID=6361&Mouse_Rat=Physiological+Monitors&Products=CODA+Surgical+Monitor>
  + Measures changes in tail blood volume
    - Similar to oscillometry measurements
  + Established method in mice
    - Agrees with invasive telemetric measures in mice
  + Tail is well-developed even in young animals
    - Allows for longitudinal studies
* Alfaxalone/alphaxalone – “Alfaxan”
  + Steroid anesthetic with no analgesic properties
  + Binds to GABA-ergic receptors
  + Not available in the US (UK, Australia, New Zealand only)
  + Causes dose-dependent hypotension
  + Can produce apnea upon induction
  + Produces myoclonic twitches just before awakening in marmosets, dogs, and cats

QUESTIONS

1. Which of the following would be inappropriate to use in combination with Alfaxan?
   1. Buprenorphine
   2. Flunixin meglumine
   3. Acepromazine
   4. Morphine
2. Identify this type of plot:



ANSWERS

1. Flunixin meglumine – NSAID
2. Bland-Altman plot – evaluates the within-session repeatability and between-session reproducibility for data



**CASE REPORT**

**Henze et al. Cross-Training Laboratory Animal Care Personnel in Physically Separate Animal Facilities at a Land-Grant Institution, pp. 601-605**

SUMMARY: Some laboratory animal departments have multiple facilities. The University of Illinois at Urbana-Champagne assessed cross-training their care staff to balance the workload with the available staff. The first step was to identify the problem areas, which they found to be “higher-level duties, less common and USDA-covered species, and weekend shifts.” They then identified who to cross-train and determined that they would cross-train animal care technicians (midlevel staff) to cover technical work (such as working in the ABSL2 facilities)  and train those in closest physical proximity to cover less common and USDA-covered species. There was also an effort to make sure that the weekend staff was well cross-trained.

There was some staff resistance to cross-training of employees, but that lessened as the process continued. Resistance was also decreased by ensuring that the trainees received complete enough training to feel competent, and that there was a high degree of standardization between facilities. Other complications included logistics such as parking between facilities and ensuring that Occupational Health and Safety requirements were satisfied between facilities.

Cross-training occurred by allowing the trainee to shadow the employee, then be supervised by the employee. When this was satisfactory, the trainee was observed by the supervisor of the facility. Refresher training is available as needed to cross-trained employees.

By implementing this program, this department was able to decrease employee overtime and improve standardization between facilities. Standardization was most successfully improved by implementing standard operating procedures that could be applied across the facilities.

QUESTIONS

1. T/F: By cross-training employees, overtime hours of care staff was increased.

2.  Cross-training of care staff led to

a.  Better standardization of procedures between facilities

b. Better distribution of workload

c.  More travel time needing to be worked into schedules

d.  All of the above

ANSWERS

1. F

2.  d