**Journal of the American Association for Laboratory Animal Science**

Volume 56, Number 3, May 2017

**OVERVIEW**

**Chatigny et al. Uses and Doses of Local Anesthetics in Fish, Amphibians, and Reptiles, pp. 244-253**

Domain 2: Management of Pain and Distress

Secondary Species: Zebrafish (*Danio rerio*) and African Clawed Frog (*Xenopus laevis* and *Xenopus tropicalis*)

Tertiary Species: Other Fish, Other Amphibians, and Reptiles

SUMMARY

* This is a literature review of local anesthetics used in fish, amphibians, and reptiles
* The structure of voltage gated sodium channels varies between species and within species acclimated to different temperature likely causing the effective dose of drugs that act on these channels to vary
* Local anesthetics interrupt nerve conduction in a specific region of the body by binding to voltage-gated sodium channels in axonal membranes and inhibiting the influx of sodium ions, preventing the sensation of stimulation from being conducted to the CNS
* Amide-linked local anesthetics include lidocaine and bupivacaine
* Ester-linked local anesthetics include MS-222

o   Commonly used as a general anesthetic in fish and amphibians

o   Water-soluble and enters fish through gills and amphibians through skin to act on the CNS

Conclusion

* Recommended doses in textbooks are based on doses in mammals with little evidence to support these recommendations
* Actual doses used in studies and case reports vary widely and are often higher than the recommended doses

QUESTIONS

1. What is the chemical name of MS-222

2. What important considerations should personnel be aware of prior to using MS-222 as an anesthetic in fish?

ANSWERS

1. Tricaine methanesulfonate

2. A) Appropriate PPE, including a mask or fume hood should be used when handling MS-222 in its powdered form; B) MS-222 can become toxic to fish when exposed to light and should be stored in dark bottles; C) MS-222 solutions should be buffered to prevent a drop in pH when added to the fish tank

**ORIGINAL RESEARCH**

***Husbandry***

**Johnson et al. Effects of Nesting Material on Energy Homeostasis in BALB/cAnNCrl, C67BL/6NCrl, and Crl:CD1(ICR) Mice Housed at 20°C, pp. 254-259**

SUMMARY: There is discrepancy between standard laboratory housing practices and the preferred thermoneutral zone of mice. Authors think that housing mice in an environment below thermoneutrality could lead to errors in extrapolating physiologic, pharmacologic, and toxicologic findings from experimental rodent models to humans and negatively affect research integrity. Mice primarily rely on changing their metabolic heat production to regulate core body temperature, but they also can respond behaviorally by huddling and nest building.

The study objective was to determine the effect of the amount of nesting material (0, 6, or 12 g) on heat energy loss and energy balance in 3 common strains of mice. Animals cages during the pretesting period have aspen shavings and 2 cotton squares. After a 1-wk acclimation period, animals were housed in calorimeters. In previous studies the authors found that 8 to 10 g of nesting material reduced thermal stress, but female behavior indicated that more material might be required for thermal comfort. Mice were allowed to acclimate to the calorimeters and build nests for 3 d prior to the start of the experiment. Ambient temperature was maintained at 20.2 ± 8ºC and relative humidity was 50.5% ± 8.2%.

Total metabolic heat production per mouse was determined by using indirect calorimetry. Oxygen levels in calorimeter exhaust air samples were measured by using a calibrated paramagnetic oxygen analyzer, and levels of CO2 were evaluated by using a calibrated infrared CO2 sensor.

Mice provided with 12 g of nesting material maintained a greater overall body weight compared to those given 0 g or 6 g. CD1 mice weighed more than C57 and BALB/C. Overall, BW was greater in male mice tan female mice. No difference in food intake was detected from 0800 to 2000 or from 2000 to 0800, when comparing between nesting-material treatments. Overall, mice consumed more food from 2000 to 0800 compared with 0800 to 2000. C57 consumed less food tan CD1 and BALB/C from 0800 to 2000, and BALB/C mice consumed less food tan C57 and CD1 mice from 2000 to 0800.

Nesting material treatment was not associated with any overall differences in total heat production. Although male mice had greater total HP tan female mice. Mice provided 12 g of nesting material maintained a more positive energy balance, regardless of sex or strain.

In conclusion, mice provided with 12 g of nesting material in the study maintained a greater overall BW throughout the trial. No differences in food intake was detected between nesting-material treatments. Given that BW gain was increased in mice provided 12 g of nesting material despite similarities in total energy consumption, this could provide evidence that the observe increase in BW gain may be attributed to a reduction in the energy requirement for thermogenesis.

QUESTIONS

1. Which is the currently recommended ambient temperature for mice?

2. According to the authors which is the preferred thermoneutral zone of mice?

3. Which of these sentences is false?

a. When ambient temperature falls below a mammal's lower critical temperature, metabolic rate is increased

b. Mice has a low surface area to mass ratio

c. Suitable amounts of nesting material can improve thermal comfort and may normalize metabolic rate

d. The daytime is an inactive phase to mice

ANSWERS

1. 20 to 26ºC

2. 26 to 34ºC

3. b is false. Mice have a high Surface area to mass ratio

**Wooddell et al. Elo-rating for Tracking Rank Fluctuations after Demographic Changes Involving Semi-free-ranging Rhesus Macaques (*Macaca mulatta*), pp. 260-268**

Primary Species: Macaques (*Macaca* spp.)

SUMMARY: One of the biggest challenges in the management of captive or semi- free ranging colonies lies in the correct understanding of the socio-dynamics of a population to prevent the surge of unpredicted conflicts and social instabilities. Therefore, it is important to be able to track social dynamics of a population to improve the management of colonies.

Rhesus macaques are the most widely used NHP in biomedical research. They are often housed in large breeding groups where females form stable linear hierarchies. Rank is transmitted from mother to juvenile and the hierarchy can remain stable over a long time. Sometimes the stability can be affected by various factors and events like the presence of natal males, death or removal of dominant animals, or other causes of overthrows. These changes in social stability can have serious implication for animal welfare and it would be valuable to have predictive tools for such changes.

In the present study the method Elo-rating was used to track rank changes and dominance stability after demographic changes. Elo-rating is a numerical system that tracks rank changes over time by constantly updating values according to wins and losses. A population of 111 semi-free ranging Rhesus macaques were studied over a period of 3 years.

In this retrospective study the removal of natal males increased the troop stability while removal of top-ranked females decreases troop stability. Thus, the presence of natal males and the removal of top-ranked females should be avoided to maintain stable dominance relationships. The predictive value of Elo-rating can be useful in managing macaque populations.

Questions:

1.       Which sex is forming linear hierarchies within a Rhesus macaque population

a)      females

b)      males

2.      Two main advantages of the use of Elo-rating over matrix-based analyses include the ability to accommodate variations in study population and to track rank changes over time.

a)      true

b)      false

Answers:          1a

                       2a

***Management***

**Honeycutt et al. Effects of Water Bottle Materials and Filtration on Bisphenol A Content in Laboratory Animal Drinking Water, pp. 269-272**

Domain 4: Animal Care; TT4.4 Quality assurance techniques for animal care-related equipment and supplies and TT4.13 Watering and feeding

Objective: This study compared the concentration of Bisphenol A (BPA) due to leaching in different types of commonly used water bottles and in filtered vs unfiltered tap water.

Background: BPA is a synthetic chemical used in the production of epoxy resins, so it is often used in pipe plumbing and in polycarbonate plastics (e.g. rodent housing, feeding and watering supplies, and food and drink containers). BPA has been implicated as an endocrine-disrupting chemical, functioning similarly to endogenous 17β-estradiol. Higher levels of BPA exposure have been reported to induce abnormal neurogenesis and hyperplasia, disrupt maternal care, decrease plasma testosterone in males and increase aggressiveness, alter immune function, induce hyperactivity, and change pain reactivity. Some of the effects have been shown to be transmitted intergenerationally.

Findings:BPA levels (measured via ELISA) were significantly higher in water from polycarbonate bottles than in high-temperature polycarbonate bottles, and both levels were significantly higher than levels measured from water contained in glass and polysulfone. Nonfiltered water (tap water) contained higher levels of BPA compared to filtered tap water.

Conclusion: Lab animals may be exposed to low levels of BPA from the use of polycarbonate water bottles and from unfiltered tap water (commonly used to fill watering bottles), and BPA exposure through drinking water can be mitigated by using a filtered water source and BPA-free water bottles. The authors suggested that the use of different types of water bottles and water sources, combined with the use of different laboratory products (food, caging systems) between laboratories, likely contribute to decreased rigor and reproducibility in research. They also suggest that these results support the need for increased oversight and reporting of the choice of materials for laboratory animal care.

QUESTIONS

1. What is Bisphenol A, and where is it commonly found?
2. What endogenous hormone does BPA function similarly to?

ANSWERS

1. Synthetic chemical used in the production of epoxy resins; used to join and coat plumbing pipes and in polycarbonate plastics
2. 17β-estradiol

***Animal Health Surveillance***

**Kapoor et al. Evaluation of Anthelmintic Resistance and Exhaust Air Dust PCR as a Diagnostic Tool in Mice Enzootically Infected with *Aspiculuris tetraptera*, pp. 273-289**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions
Primary Species: Mouse (*Mus musculus*)

SUMMARY: The two most common oxyurid species that affect laboratory mice are *Syphacia oblevata*and *Aspicularis tetraptera*. Often infection with these pinworm species can go undiagnosed due to the nature of infection and testing methods. Repeated treatment of nematodes worldwide using anthelminthics is lead to a surge in resistance. However, there is scant information regarding anthelminthic resistance in laboratory bred rodent colonies. The aims of this study were to determine 1) if *A. tetraptera* in an enzootically infected population of mice were resistant to fenbendazole and piperazine, and 2) if real time PCR detection of environmental samples, including exhaust air dust, could be used to detect very few samples of *A. tetraptera*-positive mice on an IVC rack.

All mice that were treated with anthelminthics (Fenbendazole, Piperazine, or both) cleared infection with *A. tetraptera* indicating that resistance was not an issue*.* Also, 10 of 12 DBA/2 mice that were untreated positive controls cleared the infection of *A. tetraptera* without treatment.

Results from the exhaust air dust (EAD) PCR revealed that when positive cages of mice were placed on an IVC rack, the PCR of the EAD became positive within 1 week after cages were placed. This was true whether there were many (69) or few (6) cages of positive animals on the rack. When the positively infected mice were treated with fenbendazole feed, the EAD PCR tests became negative after 1 month of treatment and remained negative for an additional 8 weeks. However, when only 2 positive cages were housed on a single rack, the EAD PCR never became positive for *A. tetraptera.* Therefore, PCR of environmental samples should never be the sole assay for detecting pinworm infection in mice and should be used with other diagnostic tests such as fecal pellet PCR and direct visualization of worms at necropsy.

QUESTIONS (True or False)

1. *Aspicularis tetraptera* appears to be resistant to fenbendazole treatment

2. Environmental sample PCR is sensitive enough for *A. tetraptera* detection that it can be used as the sole assay to determine pinworm infection in mice

ANSWERS

1. F – *A. tetraptera*appears to be susceptible to fenbendazole treatment

2. F – Environmental sample PCR should never be the sole assay to detect pinworm infection in laboratory mice, and should be used in conjunction with other diagnostic testing

***Anesthesia***

**Parkinson and Mans. Anesthetic and Postanesthetic Effects of Alfaxalone-Butorphanol Compared with Dexmedetomidine-Ketamine in Chinchillas (*Chinchilla laniger*), pp. 290-295**

SUMMARY: The investigators explored the anesthetic induction effects of either A alone at various doses (SQ and IM) or the AB combo at various doses (SQ and IM) and settled upon the AB combo at 5 mg/kg A and 0.5 mg/kg B (IM) as the only efficacious protocol. The latter regimen was then used for comparison with the DK combo at 0.015 mg/kg D and 4 mg/kg K (also given IM).

By 5 minutes after administration, all animals in the DK group had lost response to all measured reflexes and had achieved a surgical plane of anesthesia. This surgical plane of anesthesia was consistently maintained for 45 minutes, until atipamezole was administered.

The AB protocol achieved a surgical plane of anesthesia in only 7 of the 12 chinchillas and the duration of surgical anesthesia was short (median 10 mins with a range of 5-20).

The DK group protocol yielded a consistent surgical plane of anesthesia as compared to the AB group. In addition, the DK group produced a longer duration of surgical anesthesia as compared to the AB group.

The AB protocol resulted in a greater reduction in post-anesthetic food intake and fecal production as compared to chinchillas in the DK group during the first 24 hours post-administration.

DK group regimen proved to be a superior regimen as compared to the AB regimen both in terms of anesthetic efficacy and fewer post-anesthetic adverse side effects.

QUESTIONS

1. Why are chinchillas a particularly popular animal model for otologic research?
2. T/F. IV access in chinchillas is difficult.

ANSWERS

1. Due to their relatively large tympanic bullae
2. T

***Experimental Use***

**Simonek et al. Sterility and Stability of Diluted Carprofen in a Multidose Vial in the Laboratory Animal Setting, pp. 296-298**

Domain 2: Management of Pain and Distress

SUMMARY: The preparation and use of compounded multidose vials (cMDV) in the laboratory animal setting is a common and widespread practice primarily due to cost efficiency and pragmatic considerations. However, the potential for iatrogenic contamination of the cMDV exists. In fact, nosocomial pathogens have been demonstrated to proliferate in MDV and are a source of infections in human patients. Studies investigating the use and contamination of MDV in veterinary medicine are limited and controversial, resulting in many institutions developing policies outlining accepted practices for how long MDV can be used with little scientific justification. The goal of the current study was to determine how long the contents of a cMDV remain sterile and stable when pragmatic clinical practices and techniques are used.

The researchers in this article created 14 cMDV by diluting carprofen from new unopened bottles with sterile water (1:10) in additive-free serum tubes. The cMDV were split into 2-storage groups (5oC and 24oC) and either 0.2 or 0.5 mL removed twice daily with a sterile 23-g needle. Samples were periodically inoculated into tryptic soy broth, which was incubated at 37oC for 3 days and monitored for bacterial growth every 24 hours. If cultures were deemed positive, a portion was transferred onto blood agar plates. Additionally, samples were tested for endotoxin using a commercially available kit and stability of the carprofen concentration was measured by liquid chromatography-tandem mass spectrometry.

The study lasted for 28-days and bacterial contamination was not identified in any of the cMDV at any time point, endotoxin assays were negative and the concentration of carprofen in the cMDV remained stable over the course of the study at both the refrigerated and room temperature storage conditions. These results indicate that over 28 days, solutions of diluted carprofen remain sterile by using common drug withdrawal techniques and that the drug concentrations are stable irrespective of storage temperature. These results may be helpful to guide IACUCs as they develop policies concerning the use of cMDV and may increase cost-effectiveness of medications in rodent studies as this study indicates that a carprofen cMDV may be useful longer than was previously thought.

QUESTIONS

1. What technique is best utilized to measure the concentration of a specific drug compound in solution?

1. Endotoxin detection kit
2. ELISA
3. Liquid chromatography-mass spectrometry
4. Inoculation into tryptic soy broth

2. What is the ideal temperature to incubate bacterial cultures and look for growth?

1. 37oC
2. 24oC
3. 4oC
4. 72oC

ANSWERS

1. c

2. a

**De Souza Dyer et al. Intraperitoneal Administration of Ethanol as a Means of Euthanasia for Neonatal Mice (*Mus musculus*), pp. 299-306**

Domain 2: Management of Pain and Distress; Task T3: Euthanasia

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The duty of the veterinary community with regards to euthanasia is to induce death in a manner that is in accord with an animal’s interest and/or because it is a matter of welfare and to use humane techniques to induce the most rapid and painless and distress-free death possible. The current *AVMA Guidelines for the Euthanasia of Animals*statesthat intraperitoneal (IP) injection of pentobarbital is the recommended method of euthanasia for altricial neonatal and preweanling mammals. This group of animals are relatively resistant to euthanasia methods that rely on hypoxia. The use of pentobarbital delivered via IP injection can be complicated by the requirement for technical expertise to accurately deliver the agent and the increased requirements for the use of controlled substances. Ethanol is a viable candidate for replacing pentobarbital for use in euthanizing these animals due to the fact it does not require specialized equipment or licensure from the DEA, can be stored easily and has a long shelf life. Based on findings from previous studies the use of Ethanol as a method is currently acceptable with conditions. The conditional use of Ethanol is due to concern for irritation to the administration of the agent. This study looked at the use of IP ethanol, IP pentobarbital-phenytoin and controls. The groups were further divided by age. The groups included animals of 7, 14, 21 and 35 d. In addition, the study included both inbred (C57BL/6NCrL) and outbred (Crl:CD1[ICR]) mice to study potential differences based on previous findings of substantial differences between these mice regarding euthanasia of neonatal mice using CO2. Parameters recorded as part of the study included ECG, respiratory rates and times to loss of righting reflex (LORR) and death (TTD). The group also looked at differences based on the addition of supplemental heat and the accuracy of the injection in the youngest groups (7 and 14d).

No significant differences were noted between the inbred and outbred groups. The time to LORR was significantly greater in the mice that received ethanol versus pentobarbital-phenytoin at 7, 14 and 21 d. TTD differed significantly between ethanol and pentobarbital-phenytoin at 7, 14, and 21 d of age as well as between mice euthanized with ethanol at 7, 14, 21 d of age versus 35 d. Misinjection rates were found to be within the range of previously reported data regarding the accuracy of IP injection in adult mice. Gross findings included hyperemia of the parietal peritoneum and serosal surface of the GI tract at all ages, particularly in mice 21 d or younger in which TTD was prolonged. Based on the findings the group concluded that IP administration of ethanol is not an acceptable means of euthanasia in mice younger than 35 d because of the prolonged time to unconsciousness and death and the potential discomfort after injection.

QUESTIONS

1. What is the mechanism of action of ethanol?
2. True or False. The γ-aminobutyric acid (GABA) receptor is typically inhibitory in adult neural circuits
3. How do fetal adaptive mechanisms work to preserve cardiac function?

ANSWERS

1. Alcohol is a psychotropic depressant of the CNS. This property is associated with the action of alcohol on different neurotransmitters, including the stimulation of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the CNS, and the inhibition of glutamate, the main central excitatory neurotransmitter.
2. True – It is typically inhibitory in adult neural circuits but can be excitatory in young animals with developing synapses
3. Fetal adaptive mechanisms preserve cardiac function by inducing bradycardia during periods of hypoxia and subsequently lowering the metabolic requirements of the myocardium.

**Moore et al. Comparing Phlebotomy by Tail Tip Amputation, Facial Vein Puncture, and Tail Vein Incision in C57BL/6 Mice by Using Physiologic and Behavioral Metrics of Pain and Distress, pp. 307-317**

Domain 3: K1. Biomethodology techniques

Primary Species: Mouse (Mus musculus)

SUMMARY: The authors assessed tail tip amputation with minimal restraint as a phlebotomy technique, compared to 2 more common methods: scruffing with facial vein puncture and lateral tail vein incision with minimal restraint. Blood glucose levels, audible and ultrasonic vocalizations, post phlebotomy activity and grooming behavior, open field and elevated plus maze behaviors, nest-building scores, and histologic changes at the phlebotomy site were evaluated.

* A blood collection method requiring minimal restraint, minimal handling, and no anesthesia or analgesia administration is less likely to affect stress-responsive physiologic parameters, thereby reducing variability in research data.
* Previous studies indicate training and experience influence animal outcomes after RO bleeds in mice/rats. However, experience did not significantly affect outcome after tail tip amputation, suggesting this technique may be easier to master than RO bleeds.
* Tail tip amputation was performed by restraining mice by tail base on the wire cage lid, allonging the mouse to move freely atop the wire lid.  1-2mm of tissue was removed with a new scalpel, and the tail was held and milked for 2 minutes (the same amount of time needed to collect the same volume by facial vein puncture.
* Post-phlebotomy histology revealed the most severe histological changes in the facial vein group. Of the 5 tail amputation group, 3 had the last caudal vertebrae transected.
* Tail tip amputation mice did not perform differently than sham mice in any metric analyzed, indicating that this technique is a potentially superior method of blood collection in mice in terms of animal wellbeing.
* The authors suggest their study finds no evidence to support the need for anesthesia or analgesia to accompany tail tip amputation.

Conclusion: Tail tip amputation mice did not perform differently than sham mice in any metric analyzed, indicating that this technique is a potentially superior method of blood collection that does not require anesthesia or analgesia.

QUESTIONS

1. Aversive stimuli in rats and chronic pain in mice are associated with the production of what?
2. Open field and elevated plus maze tests are well established measures of what in mice?
3. What is the circulating blood volume of mice as a percentage of body weight?

ANSWERS

1. Ultrasonic vocalization
2. Stress and anxiety; these tests assess unconditioned activity that evaluate aspects of natural rodent behavior (exploratory, anxiety, and aversion to pen areas)
3. 7-8%

**Chinnadurai et al. Comparison of 3 Methods for Preventing Perianesthetic Hypothermia in Callimicos (*Callimico goeldii*), pp. 318-321**

Domain 4: Animal Care

Tertiary Species: Other Nonhuman Primates

SUMMARY:Perianesthetic hypothermia is one of the most common complications in veterinary anesthesia, especially in small patients like callimicos. During anesthesia, body heat is lost through four mechanisms: radiation, convection, conduction, and evaporation. To address multiple routes of heat loss, animals received 1 of 3 heat treatments: 1) placement of a reflective blanket over the patient to limit radiative heat loss to the surrounding environment, 2) placement of a reflective blanket over the patient and the use of a heated anesthetic circuit which warmed the inspired air to 104°F, and 3) placement under the patient of a forced-air warming blanket set at 109.4°F. For patients with and without the heated anesthetic circuit, the patient was covered with a reflective blanket. For the forced-air warming blanket, the patient remained uncovered. The study population comprised 12 adult callimicos ages 1.2-17.1 years, weights 544 ± 97 g. Each callimico was manually restrained for brief examination and mask induction with isoflurane at a vaporizer setting of 5% oxygen, delivered at 2L/min through a Mapleson type-D nonrebreathing circuit until muscle and jaw tone were relaxed. Sources of radiative heat loss were assessed using an infrared thermography camera. Whole-body images of the ventrum and dorsum of each animal were obtained prior to and immediately after induction. Images were used for subjective identification of body regions that were losing the most heat to the environment. The surface temperature of the nonhaired ventral skin in the inguinal area ranged from 103-105°F, compared with 95 to 96°F on the haired dorsum. The rate of heat loss did not differ between using the reflective blanket alone or with the heated anesthetic circuit. Animals placed on the forced-air warming blanket experienced a slight increase in average body temperature. Therefore, an underbody warm-air blanket provided the best protection against hypothermia in sternally recumbent anesthetized callimicos.

QUESTIONS

1. T/F: A majority of the heat loss in small NHPs occurs through the respiratory system

2. T/F: Conduction comprises the loss of heat to air currents around the body

3. Short Answer: Callimicos differ from other Callitrichids in which of the following ways:

     a. They do not belong to the family Callitrichidae

     b. They are sparsely haired over the ventrum and inguinal area

     c. They frequently become hypothermic under even brief inhalant anesthesia

1. All of the above

ANSWERS

1. False. Small NHPs have little respiratory heat loss compared with larger species such as dogs

2. False. Convection is the loss of heat to air currents around the body. Conduction is the direct loss of heat to surfaces in contact with a patient.

3. b

**CASE REPORTS**

**Bracken et al. *Helicobacter* Infections Significantly Alters Pregnancy Success in Laboratory Mice, pp. 322-329**

SUMMARY: *Helicobacter*spp. are gram-negative, helical bacteria that cause gastric and enterohepatic infections in mammalian species.  The authors conducted a retrospective study to investigate the implication that infection with *Helicobacter*interferes with reproductive success on time-mated infected and uninfected dams.  The study included one inbred strain, C57BL/6, and two transgenic strains, LysMCre+, LysMCre-, and Tie2Cre+.

The authors found that *Helicobacter* infection did not affect fecundity in the C57BL/6, LysMCre+, and LysMCre- mice.  However, the fecundity in the Tie2Cre+ was decreased by 90% when compared to uninfected mice of the same strain.   C57BL/6 mice infected with *H. typhylonius* had significantly reduced weight gain between GD6 and GD12 resulting in an overall decrease of 23.4% lower weight than their uninfected counterparts. Infected LysMCre+ mice also had decreased pregnancy associated weight while the pregnancy weight of the LysMCre- mice was not impacted by *Helicobacter* infection.

Postmortem assessment of the uteri of *Helicobacter* infected mice revealed increased intrauterine hemorrhage and embryo reabsorption when compared to uninfected mice.  *Helicobacter* infected C57BL/6 had smaller litter sizes when compared to litter sizes of the same strain.  *Helicobacter* infection did not impact the litter size of LysMCre+mice.  There was a significantly higher number of resorptions in infected C57 and LysMCre+ mice when compared to the uninfected mice.   There were no embryos in confirmed mated, infected Tie2Cre+ mice so the impact on *Helicobacter* infection on pregnancy outcome was not able to be assessed.

The results demonstrate that Helicobacter infection has a negative impact on pregnancy in immunodeficient, transgenic, and robust inbred strains that were thought to be resistant to the clinical impact of infection. *Helicobacter*infected C56BL/6 can carry pregnancy to term but the infection will decrease the reproductive success which impacts research outcomes.

QUESTIONS

1. A 2007 study found \_\_\_\_\_\_\_ of mice shipped from academic institutions worldwide tested positive for *Helicobacter* spp. by PCR analysis.

a. 36%

b. 59%

c. 73%

d. 84%

2. What is the most commonly reported pathogenic strain of Helicobacter in mice?

3. How is *Helicobacter* infection eradicated from a colony?

ANSWERS

1. d. 84%

2. *H. hepaticus*

3. Rederivation through caesarean delivery of pups and fostering

**Zhang et al. Iohexol Clearance for Determination of Glomerular Filtration Rate in Cynomolgus Monkeys (*Macaca fascicularis*), pp. 330-334**

Primary Species: Macaques (*Macaca* spp.)

SUMMARY: Chronic kidney disease is a significant public health issue worldwide.  It is defined as the presence of structural or functional abnormalities in the kidneys, with or without an accompanying reduction in glomerular filtration rate (GFR). GFR is the flow rate of filtered fluid through the kidney and can be estimated by inulin clearance, radionuclide marker clearance, renal dynamic imaging and serum creatinine levels, as well as others.

Iohexol is a nonionic, iodinated contrast agent which is not secreted or reabsorbed in the renal tubule and is not synthesized or metabolized within the body.  It also has less than 2% protein binding.  It is used in veterinary medicine as a marker in renal and plasma clearance studies.  This study uses iohexol to estimate GFR in healthy cynos and investigates sex-associated differences using liquid chromatography-tandem mass spectrometry.

Nine male and nine female cynos were administered iohexol at 3 dose levels: 30, 60 and 90 mg/kg given IV through the cephalic vein. Animals were trained to present their arm for blood collection. Results demonstrated that iohexol clearance was a reliable method for evaluating GFR in cynos.  Systemic exposure increased in a dose proportionate manner from 30 to 90 mg/kg. Therefore, the recommended dose for using iohexol to evaluate GFR in cynos is 30 mg/kg (or even lower).  There was no sex-associated difference in GFR at any of the three dose levels.  The calculated GFR did not differ at any of the 3 iohexol dosages tested. The GFR calculated using three sample timepoints did not differ significantly from that calculated from 5 timepoints, indicating that 3 timepoints is sufficient for determining GFR in cynos.

QUESTIONS

1. T/F: There was a significant difference in GFR between male and female cynos when measured by iohexol clearance.

2. Which of the following IS NOT a method for measuring GFR?

a.  Inulin clearance

b.  Radionuclide marker clearance

c.  Serum BUN measurement

d.  Renal dynamic imaging

ANSWERS

1. F. There was no sex difference

2. c. Serum creatinine levels are used to estimate GFR