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**ORIGINAL RESEARCH**

***Husbandry***

**McCullagh et al.** [**Effects of an Extended Cage-change Interval on Ammonia Levels and Reproduction in Mongolian Gerbils (*Meriones unguiculatus*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00003)**, pp. 713-717**

Secondary Species: Mongolian Gerbils (*Meriones unguiculatus*)

Domain 4: Animal care

SUMMARY

Background

* Gerbils natural habitat is in eastern Mongolia which is generally very arid

o   Have adapted to environment by producing very concentrated urine

o   Gerbils produce 0.1-0.2mL of urine per day

§  It is unknown if gerbils in a laboratory setting with free access to water produce this amount of urine

o   Mice produce 0.5-2.5ml of urine per day

* All Mongolian gerbils used in research are descendants from the 20 pairs trapped in eastern Mongolia in 1935.

Study

* What is the effect of an extended (6 week) cage change-out schedule on ammonia level and breeding parameters (average pup weaning weight, number of litters, and number of pups) on pair housed gerbils in IVC caging

Results

* Ammonia did rise over the 6-week period but remained <5ppm.
* No significant change was seen in breeding parameters

Conclusion: An extended, 6-week, cage change out interval can be used for gerbils.

QUESTIONS

1. What is the thermoneutral zone and dry bulb temperature recommended for gerbils in the guide?

2. What pathology is associated with high humidity in gerbil’s primary housing?

3. Name three important husbandry considerations for gerbils according to the BBB

ANSWERS

1. Thermoneutral zone: 28-32°C; Dry bulb temperature: 20-26°C

2. Nasal dermatitis

3. (A) Can be fed commercial rodent diets but develop high blood cholesterol concentrations on diets containing more than 4% fat; (B) require sand bathing to keep their coats from becoming oily; (C) Gerbils often stand erect on their hind limbs, so it is important that cages have a solid bottom and that the floor-to-lid height is tall enough to allow for this behavior.

**Bardi et al.** [**Physiologic Correlates of Interactions between Adult Male and Immature Long-tailed Macaques (*Macaca fascicularis*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00004)**, pp. 718-728**

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

Primary Species: Macaques (*Macaca* spp.)

SUMMARY:Relative to other species male primates have a higher (40%) involvement in the social life of infants and juveniles. This is logical in species where paternity is known as a high investment in paternal care is directly related to the individual’s fitness. However, in long-tailed macaques where paternity is uncertain the close connection between paternal care and fitness is harder to explain. One theory is that by a male increasing its interactions with infants and juveniles this will create a more stable relationship with their mothers and increase the chance of future breedings. Another theory is that these interactions with young animals leads to a reduction in social stress. This study seeks to confirm that male affiliative interactions with infants and juveniles reduces male social stress through positive social reinforcement. Biochemical markers of stress reduction in fathers include higher levels of plasma oxytocin and prolactin. Biochemical markers of increased stress include high levels of cortisol on its own and with respect to levels of dehydroepiandrosterone (DHEA) identified as the DHEA:corisol ratio. This study hypothesizes that males spending more time with infants and juveniles would be characterized by lower physiological and behavioral stress indices. In two large groups of semi-free ranging troops, animals where videotaped for a minimum of 5 hours over three consecutive summers. Periodic blood collection sampled for oxytocin, cortisol and DHEA.

The overall frequency of male-immature interactions was 5% of all social behavior displayed by males. Overall grooming was much higher in the high-ranking males which was predominantly directed to adult females. Overall aggressive behavior was highest in the high-ranking males which was predominantly directed towards other high-ranking males. Self-grooming (an indicator of anxiety) was highest in the low-ranking males. Cortisol levels (a measure of stress) was highest in the low-ranking males. Mid-ranking males had the highest levels of DHEA and DHEA:cortisol ratios. Mid-ranking males had the highest levels of oxytocin and low-ranking males had the lowest levels of oxytocin. Overall mid-ranking males were characterized by high DHEA:cortisol levels, high affiliative behavior and high grooming behavior towards juveniles. Overall low-ranking males were characterized as having high cortisol levels, high vigilance levels and high self-grooming – all measures of social stress. Overall high-ranking males were characterized as having high affiliative behavior towards other adults and were intermediate in their social stress levels, higher than mid-ranking but low than low-ranking males. The results suggest that affiliation with immature conspecifics may benefit adult males by reducing physiologic stress. Low-ranking males were observed to be in a high level of vigilance and that self-grooming behavior is likely a better form of stress reduction compared to interacting with juveniles. High-ranking males are far more engaged with other adult males/females to benefit from interacting with juveniles. This leaves the mid-ranking males with the most to benefit from interacting with juveniles. They get the benefit of reduced social stress and increase interactions with the juveniles mothers increasing matting opportunities.

QUESTIONS

1. What are two theories as to why non-parental males would benefit from interacting with infants and juveniles?
2. An increase in which two hormones is continent with stress reduction?
3. How do DHEA and cortisol levels relate to stress?
4. Which rank of males appears to benefit the most from interactions with infants and juveniles?

ANSWERS

1. One theory is that by a male increasing its interactions with infants and juveniles this will create a more stable relationship with the mothers and increase the chance of future breedings. Another theory is that these interactions with young animals  leads to a reduction in social stress.
2. Oxytocin and prolactin
3. Biochemical markers of increased stress include high levels of cortisol on its own and with respect to levels of dehydroepiandrosterone (DHEA) identified as the DHEA:corisol ratio.
4. Mid-ranking males

**Truelove et al.** [**Two Methods of Social Separation for Paired Adolescent Male Rhesus Macaques (*Macaca mulatta*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00005)**, pp. 729-734**

Primary Species: Macaques (*Macaca* spp.)

SUMMARY: Most of the NHPs in a laboratory setting are housed in pairs or in groups since social housing gives many animal welfare benefits. Sometimes animals need to be separated due to experimental or veterinary interventions or for other reasons. Separation of NHPs is stressful and it causes behavioral and physiological changes to the animals. Apart from having bearing on animal well-being separation can also affect experimental data. Therefore it would be desirable to find ways to mitigate the negative effects of separation.

In the current study two methods for social separation of paired adolescent male rhesus macaques were compared. Twelve pairs were included in the study; six pairs were separated immediately with a solid divider and six pairs had a transition of one week before they were separated completely. During this one week period they were provided with limited social contact through a perforated divider. This process is also referred to as stepwise separation. Behavioral data were recorded before separation, during limited contact (stepwise separation) and after separation. The behaviors most likely to change after social separation were grouped into four groups; protest behaviors, agitation, tension-related behaviors and withdrawn- or self-directed-related behaviors.

Separated NHPs showed more withdrawn or self-directed-related behaviors than when pair housed. Stepwise separation gave more agitation behaviors during the limited contact period and it could not mitigate the negative effects of separation. Based on the results of the current study NHPs should be pair housed until individual housing is required. More studies on stepwise separation is needed for female rhesus macaques and for other and both sexes of other species.

QUESTIONS

1.  Which immunological and physiological changes can be noticed in NHPs due to social separation?

a. Reduced lymphocyte proliferation

b. Antibody response inhibition

c. Elevated plasma cortisol levels

d. Reduction in survival within immunologically challenged populations

e. Elevated heart rate

f. Sleep disturbances

g. All of the above

2.  True or False. The biphasic response to mother – infant separations seen in human infants, known as protest- despair response, is mirrored by a biphasic active-passive response in rhesus macaque infants.

3.  Which behaviors are withdrawn or self-directed behaviors?

a. Inactivity

b. Self-directed stereotypies

c. Huddle

d. All of the above

4. Which behaviors are agitation behaviors?

a. Manipulation of cage

b. Stereotypic locomotion

c. Both of the above

ANSWERS

1. g

2. True

3. d

4. c

**Backus et al.** [**Relationship between Environmental Enrichment and the Response to Novelty in Laboratory-housed Pigs**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00006)**, pp. 735-741**

Domain 4, K2

Primary Species: Pig (*Sus scrofa*)

SUMMARY: Environmental enrichment can reduce pain perception, anxiety, and agnostic behaviors and is protective against stressors, thus potentially improving an animal's overall welfare. This objective of this study was to determine whether rearing pigs in an environmentally enriched laboratory research setting improved the animals' welfare and reduced stress responses to novelty. Pigs were housed 4 pigs to a pen in either standard home pens or environmentally enriched pens. The daily behavioral repertoire was recorded in the home pen for 48 hours at the end of the 2 week treatment period. Additionally at the end of the 2 week treatment period, pigs underwent two behavioral tests: the Novel Object test and the Human Interaction test. In this study, enriched pigs spent more time interacting with enrichment objects and the environment but tended to interact fewer with a novel object or person. Conversely, the control pigs tended to interact more times with the novel items.

QUESTIONS

1. According to the guide, what is the required floor space for 1 pig weighing less 15 kg?

2. According to the guide, what is the required floor space per pig for 4 pigs weighing up to 50kg?

ANSWERS

1. 8 feet squared

2. 10 feet squared

***Management***

**Ragland et al.** [***Staphylococcus xylosus* PCR-validated Decontamination of Murine Individually Ventilated Cage Racks and Air Handling Units by Using 'Active–Closed' Exposure to Vaporized Hydrogen Peroxide**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00007)**, pp. 742-751**

SUMMARY: Vaporized Hydrogen Peroxide (VHP) is used to decontaminate biocontainment facilities and empty animal housing rooms.  VHP is safe on many surfaces including electronics, metals, plastics, and elastomers.  The authors investigated the use of VHP decontamination to eliminate opportunistic pathogens in individually ventilated cage (IVC) racks, air handling units (AHU), and other secondary housing equipment (i.e.- connecting hoses).  The efficacy of VHP sterilization was assessed by using chemical and biological indicators, and PCR testing for *Staphylococcus xylosus* which is a commensal organism of murine skin.  *S. xylosus* was present in 93% of specimens from occupied IVC racks and the interior surfaces from in-use AHU.

To determine efficacy, the authors assessed 7 static-open VHP cycles, in which a total of 28 IVC racks and 17 AHU were exposed, and 5 VHP active-closed cycles in which a total of 20 IVC racks and 11 AHU were exposed.  To document the VMP levels in the room, 3 chemical indicators and 1 biological indicator were placed in the room during each of the 12 VHP cycles assessed.   The authors also assessed the VHP levels inside the hoses connecting the AHU to IVC racks that had been VHP-exposed in an active-closed setting and inside the cages that were docked on the treated IVC racks after room levels had returned to acceptable levels.  Sterile swabs were used to collect specimens from soiled, washed, and VHP-exposed equipment surfaces to evaluate for *S. xylosus* by PCR analysis.

All 21 chemical indicators placed in the room demonstrated that VHP was capable of inducing at least a 6-log reduction in microbial counts and all 7 biological indicators placed in the sealed room demonstrated growth inhibition of *G. stearothermophilus* spores. The static-open VHP exposure method proved ineffective inside the plenums, hoses, and AHU prefilters with the 77% of the chemical indicators achieving less than a 6 log reduction and 73% of the biological indicators yielding growth of *G. stearothermophilus*. In the active-closed VHP protocol, all 4 chemical indicators and all 3 biological indicators placed inside the open end hoses, plenums, and manifolds of IVC racks and in an AHU prefilter chamber detected sterilizing levels of VHP.    The presence of *S. xylosus*was detectable on the interior surfaces of IVC exhaust plenums approximately 7 days after mice returned to VHP-exposed equipment.  The authors concluded that supplementing steam sterilization of the primary enclosure with VHP sterilization of the secondary housing equipment may help mitigate opportunistic agents in immunodeficient strains.

QUESTIONS

1. Which surface sterilant has the best safety profile with a lower required use concentration, a higher permissible exposure limit, and a higher threshold defined as immediately dangerous to life or health?

a. Formaldehyde

b. VHP

c. Chlorine dioxide

2. What is the safe long term exposure limit 8-h time-weighted average of VHP?

3. What is the concentration of H2O2used in VHP?

ANSWERS

1. b

2. Less than 1 ppm

3. 35%

***Animal Health Surveillance***

**Gerwin et al.** [**PCR Testing of IVC Filter Tops as a Method for Detecting Murine Pinworms and Fur Mites**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00008)**, pp. 752-761**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY

Aim: In this study, authors compared parasite detection methods in sentinel cages exposed to soiled bedding collected from cages housing mice infested with fur mites and infected with pinworms using 2 contact bedding types, aspen chip and α cellulose. Authors hypothesized that PCR testing of cage lid filters would be a viable alternative for IVC systems in which air is filtered at the cage level. Further, authors postulated that differences in the amounts of particulates (that is, dust) would affect the ability to detect parasite nucleic acid on the filter tops. Specifically, team compared detection rates of nucleic acid extracts from a section of filter media collected from the filter top, nucleic acid eluted from adhesive swabs applied to the filter top, and samples collected directly from the animals and tested by using both PCR analysis and traditional testing methods, including fecal floatation, the anal tape test, direct examination of intestinal contents, and skin scraping.

Study Design: The study population was composed of 144 female (age, 3 to 5 wk), Swiss Webster (Tac:SW) mice (Taconic Biosciences). All mice were housed in a quarantine facility under ABSL 2 conditions in solid-bottom, polysulfone cages (model no. 9, Thoren Caging Systems, Hazelton, PA) and housed on an IVC system (model no. 9-140-10-14-1-4-5TM, Mobile Maxi-Miser PIV System, Thoren Caging Systems) using intracage supply–intracage exhaust (indirect) with the supply and exhaust blowers located on the bottom of the rack and exhausted into the room (HEPA- filtered). Animals were housed on autoclaved aspen chip bedding or autoclaved α-cellulose bedding. Below listed five parasites were investigated, 2 pinworms and 3 fur mites.

1.   *Syphacia obvelata*(SO)

2. *Aspiculuris tetraptera*(AT)

3. *Myocoptes musculinus*(MC)

4.  *Myobia musculi*(MB)

5.  *Radfordia affinis*(RA)

Six, traditional (non-molecular) and molecular (PCR testing of filter top media; PCR testing of filter top swabs; Direct PCR testing Fecal float; Anal tape; Intestinal contents; Skin scrape), methods were explored for the identification of the above parasites and parasites were monitored at day 30,60,90 and until end of the study. Two different type of bedding material were compared for their sensitivity to the detection method

Findings/Results:For mice exposed to aspen chip bedding, PCR analysis of filter top media had the highest detection rate, detecting 100% of all parasites on days 30, 60, and 90 and overall. For mice exposed to α-cellulose bedding, PCR analysis of filter top media had the highest detection rate, detecting 100% of all parasites on days 30, 60, and 90 and overall, except for AT. Comparison of PCR methods for bedding type (aspen chip bedding vs. autoclaved α-cellulose bedding), aspen chip bedding provided better detection of some of the parasites

Summary: Among the methods tested for the detection of above ecto and endo parasites, PCR testing of filter top media was the best method to monitor the cages and aspen chip bedding provided better detection of some of the parasites.

QUESTIONS

1. Which method among molecular and non-molecular (traditional) methods used in the present study is the best detection method to monitor parasites in the sentinel cage?

a. Anal tape

b. Intestinal contents

c. Skin scrape

d. PCR testing of filter top swabs

e. Fecal float

2. What is the best detection method among the molecular PCR method used in the present study to monitor parasites in the sentinel cage?

a. PCR testing of filter top swabs

b. Direct PCR testing Fecal float

c. PCR testing of filter top media

d. PCR testing skin scrape

ANSWERS

1. d

2. c

***Anesthesia***

**Pugh et al.** [**Plasma Concentration of Meloxicam in Pediatric Rats**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00009)**, pp. 762-767**

Domain 2: Management of Pain and Distress

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: The goal of this study was to determine whether a common adult dosage of meloxicam in pediatric rats would meet or exceed the plasma concentration in adults. The researchers wanted to determine a dose of meloxicam in pediatric rats that met or exceeded the plasma concentration in adult rats given a common dosage. The peak plasma concentration in adult rats was assumed to be the therapeutic level. Pediatric and neonatal patients have immature organ function and rapid metabolism, compared with adults. Pediatric rats were grouped by age (7, 14, 21, and 28 days) and given the same dose as used in adult rats to determine CMax in the younger animals. Young adult Sprague-Dawley rats received meloxicam at 1.34mg/kg subcutaneously as close to the interscapular region as possible. Whole blood was collected via the lateral tail veins and ventral tail artery in PND 70 and PND 28 rats. Blood samples were collected from adult rats at 0, 5, 10, 15 and 30 minutes and 1, 1.5, 2, 2.5, 3, 6, 12 and 24h, with a minimum of 4 samples collected at each time. Once the mean CMax was determined in adult rats, it became the target CMax for all other age groups. If the CMax in a pediatric group was significantly lower than the target CMax, the experiment was repeated for that age group with higher doses of meloxicam until the target CMax was reached. Meloxicam assays were performed. Livers and kidneys were sampled and graded for signs of acute toxicity. The mean CMax of meloxicam in adults was 11.5 +- 2.7 micrograms/ml at 60 min after a single subcutaneous injection of 1.34mg/kg meloxicam. In pediatric rats, PND 28 rats had a mean CMax of 13.7 +- 2.6 micrograms/ml 30 min after injection. PND 21, 14 and 7 rats had CMax of 16.5 ± 4.3, 16.2 ± 3.3, and 17 ± 5.1 micrograms/ml, respectively at 15 minutes after injection. At 24 hours after injection, the mean plasma concentration of meloxicam in the pediatric age groups exceeded that of the adult rats. There were no significant differences in plasma concentration between sexes across time. Overall CMax was higher in younger animals than adult animals but the difference was not significant between adult and PND 28 rats. No changes consistent with acute renal toxicity were identified in any of the samples.

QUESTIONS (True or False)

1. At 24 hours after injection, the mean plasma concentration of meloxicam in the pediatric age group was lower than that of the adult rats

2. There was a significant difference in the plasma concentration of meloxicam among male and female rats

3. There were no signs of acute renal toxicity identified in any of the sample groups post-meloxicam administration

ANSWERS

1. False. The mean plasma concentration in the pediatric age group exceeded that of the adult rats

2. False. The data showed no significant differences in plasma concentration between sexes

3. True

**Zanetti et al.** [**Pharmacokinetics and Adverse Effects of 3 Sustained-release Buprenorphine Dosages in Healthy Guinea Pigs (*Cavia porcellus*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00010)**, pp. 768-778**

Domain 2: Management of Pain and Distress

Secondary Species: Guinea Pig (Cavia porcellus)

**SUMMARY:** Study assessed the pharmacokinetic profiles of 3 SRB dosages (0.15mg/kg (low), 0.3mg/kg (medium), 0.6mg/kg (high) for 72 hrs after a single subcutaneous injection to 4 male and 4 female guinea pigs. Body weight, fecal output, and cortisol levels were monitored and compared with a sham group. After 72 hours, levels of the drug were checked for high dose males and females and the medium dose males.

**Results:** 64.3 ± 9.2 ng/mL (males) and 71.3 ±  3.7 ng/mL (females) in the High group; 11.5 ±  3.2 ng/mL (males) and 6.9 ± 0.9 ng/mL (females) in the Medium group; and 2.3 ± 0.8 ng/mL (males) and 2.0 ± 0.5 ng/mL (females) in the Low group. Fecal output and body weight were significantly lower in the SRB groups compared to the sham group, with the High group showing larger reductions. The guinea pigs lost less than 10% of their body weight. Baseline cortisol in healthy females were significantly greater than in males, but independent of sex, the SRB administration lowered those levels.

**Conclusion:** All 3 SRB dosages can be safely used in guinea pigs and resulted in no significant physiologic changes during anesthesia. Therapeutic levels of buprenorphine >1ng/ml were found for 72hrs in the high dose group in both sexes and the medium dose in males. A higher dose maybe required for female post-operative guinea pigs. A common reported adverse effect was injection site reactions, however, the manufacturer of the drug changed its formulation to be less inflammatory. In this study, none of the animals developed injection site reactions. Opioid-induced bowel dysfunction (OIBD) is a common adverse event reported with constipation and anorexia as clinical signs.  Weight loss was observed but the animals were able to regain weight 7 days after administration of the SRB. This weight gain was comparable to the baseline weight during the acclimation phase. Fast weight recovery was due to the animals need to eat to maintain the high metabolic demands of the species. TTC or time to consumption could be used to help monitor pain response in post-op guinea pigs by monitoring how long it takes them to eat after anesthesia. Fecal output/distribution at baseline found that stool was in the lower quadrant of the cage near the lixit. After SRB, it is hypothesized that the animals loss environmental awareness and thus there was a change in the fecal distribution in the cage to a more random distribution into other quadrants. This can also be used to help monitor pain after a procedure. Further dosing studies need to be done to determine if these dosages can alleviate pain in guinea pigs.

**QUESTIONS**

1. What is the standard dose of immediate release buprenorphine for guinea pigs?

2. Buprenorphine is metabolized in what organ?

3. What doses had therapeutic levels of buprenorphine?

4. What methods can be used to monitor pain after a procedure?

**ANSWERS**

1. 0.05 mg/kg SC BID

2. Liver

3. High dose (0.6mg/kg) for both sexes  and medium (0.3mg/kg) dose for males

4. Time to consumption and fecal distribution

***Experimental Use***

**Hubbard et al.** [**Effects of Repeated Intraperitoneal Injection of Pharmaceutical-grade and Nonpharmaceutical-grade Corn Oil in Female C57BL/6J Mice**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00011)**, pp. 779-785**

Domain 3: Research; Task 2. Advise and consult with investigators on matters related to their research

Primary Species: Mouse (*Mus musculus*)

One-Line Summary: The use of nonpharmaceutical grade corn oil did not result in adverse clinical consequences and may be used for intraperitoneal injections in mice.

SUMMARY:The authors of this article sought to determine the differences in animal wellbeing associated with the use of Pharmaceutical Grade (PG) and non-pharmaceutical grade (NPG) corn oil.  Ninety (90) female mice were injected with either PG corn oil, NPG corn oil or saline all intraperitoneally.  The injections were administered every 48h for a total of 4 injections.  Mice were then evaluation by using body weight, body condition score, visual assessment score, CBC and serum chemistries.  Animals were euthanized at 24h and 14d after the final injections**.**

The results showed that the saline-dosed groups had lower pathology scores at both time points.  At day 21, PG corn oil had a significantly higher pathology score compared with NPG corn oil.  The use of NPG corn oil intraperitoneally did not result in adverse clinical consequences.  However, the differences in inflammation between the PG and NPG corn oil group suggest that their use in studies should be consistent.  They also went on to conclude that NPG products in animals should still be justified and approved on a case-by-case basis.

QUESTIONS

1. Define Pharmaceutical Grade (PG) compounds.

2.  Compare and contrast Pharmaceutical Grade Compounds with Non-Pharmaceutical grade compounds.

3.  Under what two conditions can NPG substances be used in research?

ANSWERS

1.  Pharmaceutical grade (PG) Compounds are substances that are either approved by the FDA or have an established chemical purity standard published by a recognized pharmacopeia.

2.

|  |  |
| --- | --- |
| **Pharmaceutical Grade Compounds** | **Non-Pharmaceutical Grade Compounds** |
| Approved by FDA | Not FDA approved |
| Use in research ensures both purity and sterility | Not guaranteed to be sterile. |
| Established chemical purity standard | No established chemical purity standard |
| Promotes reproducible data between studies | Increased variability in batches |

3.  NPG substances can be used only when 1) there is scientific justification or 2) when a PG alternative is not available.

**Zhang et al.** [**Heating Pad Performance and Efficacy of 2 Durations of Warming after Isoflurane Anesthesia of Sprague–Dawley Rats (*Rattus norvegicus*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00012)**, pp. 786-791**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*) and Mouse (*Mus musculus*)

SUMMARY: General anesthesia disrupts thermoregulation and leads to hypothermia. The hypothalamus normally regulates core body temperature to within +/- 0.3 degrees C, but depression of the hypothalamus due to general anesthesia allows body temperature to fluctuate as much as 2-4 degrees C before triggering control mechanisms. Hypothermia sets in quickly after general anesthesia begins making it important to start thermal support early.

Perioperative hypothermia has been associated with adverse effects such as infection, altered drug metabolism, clotting alterations, and delayed anesthetic recovery.  Maintaining appropriate core temperature relies on accurately measuring body temperature. Abdominal telemetry capsules are considered the gold standard for rodents, but they are impractical for many studies. Therefore, skin, tail and rectal temperatures are used.  Local tissue perfusion can affect skin and tail temperatures and rectal temperature measurements can vary depending on insertion depth.

The purpose of this study was to assess whether warming rats during recovery from anesthesia prevented hypothermia during recovery.  Heating pad performance was also assessed by measuring temperatures at various sites on the surface of the pad. Rats were anesthetized with isoflurane and maintained on a heating pad for the 40-minute duration of anesthesia. The rat was then allowed to recover to sternal on the heating pad. Then the heating pad was transferred under a recovery cage for either 30 or 60 minutes of recovery.

The results showed that 30 minutes of warming after isoflurane anesthesia was sufficient to maintain normal core body temperature (measured via rectal thermometer) in Sprague-Dawley rats.  The 60-minute warming period offered no advantage over the 30-minute period. It was also noted that when a heating pad was placed under the recovery cage there could be a delay in the recovery cage floor achieving the desired temperature (the cage floor took approximately 30 min to reach a plateau).  The heating pad was also shown to have a variable temperature across different areas of the surface. No histological evidence of thermal burns was noted at necropsy, but that is a risk when heating pads are used for warming.

QUESTIONS

1. T/F: 60 minutes of postoperative warming was shown to maintain core body temperature better than 30 minutes of postoperative warming.

2. The best method for measuring core body temperature in rodents is:

a. Rectal thermometer

b.  Tail surface temperature

c. Skin surface temperature

d.  Abdominal telemetry capsule

ANSWERS

1. F

2. d

**Dawson et al.** [**Using Telemetry Data to Refine Endpoints for New Zealand White Rabbits Challenged with *Bacillus anthracis,***](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00013) **pp. 792-801**

Domain 2: Management of Pain and Distress K8: Humane endpoint criteria

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY: Biotelemetry devices were implanted in New Zealand White rabbits in order to track body temperatures in response to exposure to *Bacillus anthracis.* Telemetry transponders were placed surgically in the abdominal cavity and affixed to the lateral body wall. Animals were allowed a minimum recovery time of 2 weeks prior to exposure to *Bacillus anthracis.* Baseline temperatures were read via telemetry a minimum of 48h prior to exposure to *B. anthracis,*then temperatures were recorded continuously until rabbits either died or were euthanized*.* A significant increase in body temperature (SIBT) was defined as 3 standard deviations above the mean baseline temperature. Rabbits were exposed to *B. anthracis*via either inhalation or subcutaneous injection. The rabbits were then monitored for clinical signs of infection twice daily for 10d. Clinical observations were recorded in three categories: appearance, natural behavior, and provoked behavior. Each of these categories were scored on a numerical scale from 0 (normal) to 4 (severe). Animals that had a total score of 7 or more across all 3 categories were euthanized. At death or euthanasia, blood samples were taken and plated for *B. anthracis* culture to establish bacteremia. Spleen samples were also taken at necropsy to determine bacterial load in the spleen.

Results: For rabbits exposed via aerosolized *B. anthracis*, all animals that died or were euthanized due to infection exhibited SIBT approximately 30 to 40h post exposure. All animals that died or were euthanized due to infection also had bacteremia. None of the control animals or survivors exhibited SIBT or had bacteremia. For rabbits exposed via subcutaneous injection, results were similar to those in the inhalation group in that SIBT was exhibited by all animals that eventually died or were euthanized due to infection.

Discussion: There was 100% correlation between development of SIBT and death from infection with *B. anthracis* in this New Zealand White rabbit model via both routes of inoculation (inhalation or subcutaneous injection).  Because of this strong correlation, SIBT has been incorporated into the authors’ clinical score sheet for this infection model. The authors propose that SIBT should be included as an endpoint indicator in conjunction with a clinical checklist to set pre-determined criteria for humane euthanasia. The addition of SIBT is useful for determining endpoint criteria for this infection model because oftentimes progression to death occurs very suddenly, before clinical signs can be used to determine a humane endpoint.

QUESTIONS

1. True or False: Significant increase in body temperature (SIBT) was strongly correlated with death from infection with *B. anthracis* in New Zealand White rabbits

2. Which of the 3R’s would incorporate SIBT into the endpoint criteria for humane euthanasia in this model be considered?

a. Reduction

b. Refinement

c. Replacement

 ANSWERS

1. True. In this study, there was 100% correlation between SIBT and death from infection with *B. anthracis*.

2. b. Refinement

**Scott et al.** [***Evaluation of Best Practices for the Euthanasia of Zebra Finches (Taeniopygia guttata*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00014)**, pp. 802-806**

Domain 2: Management of Pain and Distress

Tertiary Species: Other Birds

SUMMARY: This study looked at euthanasia methods of zebra finches, an area of relatively little previous research. The study compared intracoelomic sodium pentobarbital with or without isoflurane anesthesia, and CO2 at 20, 40, ad 80% volume displacement rates. To evaluate the differences in response to various methods, the researchers looked at behavioral response in terms of head shaking, open-mouth breathing, wing flapping, total wing movement, tail bob, and head retroflexion. They also looked at time to recumbency to identify unconsciousness and time to respiratory arrest to identify death.

In comparing the use of sodium pentobarbital in anesthetized and unanesthetized finches, those anesthetized too longer and showed more behavioral  changes. This included wing flapping and total wing movement, as well as some open mouth breathing. For birds exposed to CO2 there was more headshaking and open mouthed breathing when compared to those receiving sodium pentobarbital. With the higher displacement rate, the duration of open mouth breathing and time to head retroflexion and respiratory arrest were shorter. Using headshake, total wing movement, tail bob, and recumbency were less clear in evaluating behavior and timing due to ability to measure accurately.

Based on the results of this study, the researchers recommend sodium pentobarbital as an option in conscious, well-retrained zebra finches, and higher flow rate CO2 as another option to limit higher rates of behavioral response.

QUESTIONS

1. What are possible reasons for zebra finches headshaking?

2. What are some of the restrictions/downsides associated with sodium pentobarbital for euthanizing zebra finches?

ANSWERS

1. Possible reasons include an alerting response, reducing stress, a reaction to mucosal acidification, or in response to aversive or distressful stimulus. It is unclear if it is really reflective of pain or distress.

2. It is a controlled substance, requires training on the technique, and is not an AVMA approved euthanasia method in conscious animals. There is also some distress associated with capturing the animal.

**ACLAM POSITION STATEMENT**

[**ACLAM Position Statement on Adoption of Research Animals**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2017/00000056/00000006/art00015)**, p. 807**

SUMMARY: ACLAM supports the adoption of healthy, post-study research animals into homes/farms that can provide appropriate care for such animals as pets. The temperament of the animal should be a consideration. Animals having had major operative procedures or having received experimental compounds do not necessarily preclude an animal's candidacy for adoption but such are factors for the institution to consider. The institution should formulate their adoption policy with input by their Attending Veterinarian (AV) and legal counsel. Each adoption should require approval by the AV. Adopters should be carefully screened. In order to prevent post-adoption transfer to other individuals, the adopter should accept (in writing) the life-long care of the animal to include appropriate veterinary care. The institution should consider appropriate vaccinations and spay/neuter prior to adoption. Genetically modified animals can be considered for adoption so long as applicable guidelines are followed.

QUESTION

1. Regarding the consideration of adopting a genetically modified animal, what guidelines should be reviewed when considering the candidacy of adopting such an animal?

ANSWER

1. NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules