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**ORIGINAL RESEARCH**

***Biology***

**Day et al.** [**DNA-based Determination of Ancestry in Cynomolgus Macaques (*Macaca fascicularis*)**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00001)**, pp. 432-442**

Domain 3: Research

Primary Species: Macaques (*Macaca spp.*)

SUMMARY: There are significant genetic differences among regional populations of cynomolgus macaques which can influence responses to experimental treatment effects ad confound interpretations of the outcome of experimental research. This study characterized the genetic structure of six regional cynomolgus macaque populations using and comparing panels of 83 single-nucleotide polymorphism (SNP) and 18 short tandem repeat (STR) markers to differentiate the ancestry of animals in these populations. A total of 446 subjects were used and DNA was extracted from blood samples and submitted to two comparative analyses to determine SNP and STR panel’s ability to differentiate populations. STR only differentiated the Mauritian population from all other whereas SNP detected 4 genetically distinct groups (Cambodia, Singapore-Sumatra, Mauritius, and Zamboanga). Mauritius population had the lowest genetic diversity (from small number of founder animals) and the Cambodian population had the highest genetic diversity (in part from admixture with rhesus macaques). The panel of SNP differentiated populations as well as or better than STR (higher overall fixation index, FST values). 45 is the optimal number of SNP for maximizing resources and time efficacy without losing significant discriminating power also 1.6 SNP were necessary to provide the same statistical power as a single STR.

QUESTIONS

1. For which species of *Plasmodium* did cynomolgus macaques from three different populations (Philippine, Malaysian, Mauritian) had a differential response to infection?

a. *P. vivax*

b. *P. falciparum*

c. *P. malariae*

d. *P. knowlesi*

2. Which of the following is NOT an advantage of SNP over STR?

a. Lower false genotyping rate

b. Lower rate of mutation

c. Greater abundance in the genome

d. Higher information content per locus

e. More suitable for automation and standardization in high-throughput sequencing technology

ANSWERS

1. d

2. d (STR has higher information content per locus thus more SNP are required to achieve the same resolution as that provided by fewer STR)

***Husbandry***

**Mohamed et al.** [**Effects of 3 Rodent Beddings on Biochemical Measures in Rats and Mice**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00002)**, pp. 443-446**

Domain 4: Animal Care

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

SUMMARY: This study evaluated the physiologic effects of 3 types of bedding- rice straw, wheat straw, and pine wood shavings- on rats and mice. All three types of bedding evaluated in this study are used at this institution (Cairo University) as the investigators sought to better understand the potential effects as bedding can directly influence animal health and experimental outcome. Groups of 10 animals/sex/species were housed on 1 of the three bedding types- rice straw, wheat straw, or pine wood shavings- at a depth of 2 cm for 4 weeks, changing the bedding weekly. After four weeks, the mice were euthanized and blood was collected for ALT, AST, albumin, urea, and uric acid. Additionally, liver and kidneys were collected, homogenized, and each evaluated for malondialdehyde and catalase- markers of oxidative stress. Significant results of the study are as follows:

Rats: ALT, renal and hepatic malondialdehyde concentrations were higher in the wood-shaving group and lower in the rice straw group as compared to wheat straw. Conversely, the renal and hepatic catalase activity was lower in the wood-shaving group and higher in the rice straw group as compared to the wheat straw.

Mice: ALT and AST were higher in the rice straw group and lower in the wheat straw group as compared to wood-shaving group. Liver and kidney malondialdehyde concentrations were higher in the wood-shaving group and lower in the wheat straw group as compared to the rice-straw group. Liver and kidney catalase activity was higher in the wheat straw group and lower in the rice straw group as compared to the wood-shaving group.

The results were interpreted to show wood shavings can alter rodent physiology as previously suggested. Additionally, mice experienced physiologic effects when housed on rice straw. The authors suggested is may be due to the size of the rice straw, providing additional shelter leading to altered food consumption (though this needs to be investigated further). The authors concluded that rice straw bedding is preferable for rats and wheat straw bedding is preferable for mice.

QUESTIONS

1. Which of the following types of bedding is known to release volatile organic compounds altering hepatic microsomal enzyme concentrations in rodents?

a.  Hardwood chips

b.  Softwood shavings

c.  Corncob

d.  Cellulose bedding

2. Which of the following animal or microenvironment parameters has not been shown to be impacted by the type of bedding used in rodent caging?

a.   Core body temperature

b.   Humidity

c.  Ammonia concentration

d.  Carbon dioxide concentration

e.   Light cycle

ANSWERS

1. b

2. d

**Garner et al.** [**Vibration-induced Behavioral Responses and Response Threshold in Female C57BL/6 Mice**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00003)**, pp. 447-455**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: In this extremely complex study, the investigators attempted to gain some information about the response of female mice to vibration of the cages. Using a platform mounted on a shaker, the cage was subjected to various frequencies and magnitudes of vibration, ranging from 0.05m to 1.0m /sec2 for several minutes. They were videotaped for 6 minutes to cover the pre- and post-vibration time. An ethogram consisting of 2 columns (active behaviors such as locomotion, rearing, sniffing or digging; and inactive behaviors such as sleeping, grooming, feeding or remaining still) was used to record behavioral responses to vibration.

There are many graphs in the paper, but the conclusions were fairly straightforward. Mice show little or no response to 0.05m/sec2, comparable to the human threshold, so this can be used as a threshold in mice. From there on, the response is fairly dose-dependent up to 1.0m/sec2, which is comparable to an earthquake strong enough to move furniture. But the mice respond only transiently, consisting of freezing, hunched posture and occasional startle responses. They return to normal (daytime sleeping) behavior very rapidly. A frequency of 70-100 Hz (cycles per second) is the ‘resonant’ frequency of a mouse, and they respond more in this frequency range than higher or lower ones. For comparison, carrying a cage results in about 2m/sec2 vibration magnitude; transporting on a cart causes 1.25-8.6 m/sec2. A vibration of 0.75m/sec2 increases heart rate and blood pressure.

Future research should examine the response of pregnant or nursing mice, males, and different strains.

QUESTIONS

1. What is the likely threshold of vibration, at or below which mice do not seem to be disturbed?

a. 0.05 m/sec2

b. 1.0 m/sec2

c. 2.0 m/sec2

d. 5.0 m/sec2

2. What is the typical behavioral response of mice to vibration of their cages during the daylight part of the light cycle?

a. Increased locomotion

b. Increased tendency to fight

c. Transient freezing and startling

d. Transient rearing and increased exploration

ANSWERS

1. a

2. c

**Mondloch et al.** [**Hepatic Vitamin A Concentrations in Vervets (*Chlorocebus aethiops*) Supplemented with Carotenoids Derived from Oil Palm**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00004)**, pp. 456-464**

Domain 3: Research

Tertiary Species: Other Nonhuman Primates

SUMMARY: Vitamin A requirements of NHPs have not been precisely defined, and are generally assumed to be similar to the requirements of humans. Vervets are omnivorous and commonly used in metabolism and atherosclerosis research. Because the effects of increased vitamin A stores on biomarkers, metabolism, and physiology are unknown, the subsequent study was carried out in order to determine vitamin A, retinol species, and carotenoid content in vervet livers.

Adult male vervets (n=40) were fed a high fat diet formulated in-house along with 1 of 5 experimental treatments – 1) control, 2) 100mg d-a-tocopherol acetate, 3) 100mg vitamin E from oil palm , 4) 50mg vitamin E from oil palm + 50mg oil palm-derived carotenoid complex (OPC) and free choice oil palm-derived water-soluble antioxidants and 5) oil palm-derived water soluble antioxidants. Animals were fed these diets for 20 months following a 23 week period of high fat diet only. After euthanasia, liver samples from unspecified sites were collected prior to perfusion fixation and frozen at -80C. Samples were collected from 38 of the 40 animals because two of the animals were euthanized for unrelated clinical issues.

Samples were analyzed by HPLC for carotenoids and retinol, and by ultra HPLC for a-retinyl esters. All animals had hypervitaminosis A, which in humans is defined at greater than 1 umol/g or greater. Additionally, all animals lost weight over the duration of the study, although this weight loss did not differ between diet groups. The animals in the OPC group (treatment group 4) had the highest hepatic concentrations of total carotenoids, carotenes, retinol and vitamin A; with the two former being positively correlated with vitamin A content. Four of the animals in this group (n=8) had levels of hepatic vitamin A that exceeded levels considered toxic in humans (10 umol/g). This group also had the lowest concentrations of lutein and zeaxanthin.

Additionally, there was a high degree of variability in hepatic carotenoid concentrations among individuals. Though sample handling and site sampled may have been the cause of the variation, individual variation in the enzyme BCO1 which cleaved carotenoids to retinol may also play a role. Given these findings, the authors believe that hepatic injury and thus compromised liver function were present in some of the animals in the study due to high levels of hepatic vitamin A.

QUESTIONS

1.   When consumed in excess, vitamin A is stored in which organ?

a.   Spleen

b.   Liver

c.   Adrenal gland

d.   Vitamin A is not stored, excess is removed in the urine

2.  Hypervitaminosis A would lead to which of the following serum chemistry changes?

a.  Increased BUN

b.   Increased LDH

c.   Hypokalemia

d.  Hyperbilirubinemia

ANSWERS

1.   b

2.   b

Domain 3: Research

Tertiary Species: Other Nonhuman Primates

SUMMARY: The vitamin A requirements of vervets and other NHPs are based on the estimated daily requirements for humans. Bioconversion of carotenoids to vitamin A is regulated by dietary vitamin A in humans through a negative feedback loop. The authors investigated the vitamin A status of the vervet and how to provide the appropriate amount of vitamin A.

Forty adult male vervets were fed a high fat diet (HFD) for 23 weeks (for a previous study). Following that study, the animals were divided into 5 groups and treated as follows for 20 months; 1: HFD only (control); 2: HFD + commercial vitamin D; 3: HFD + oil-palm derived vitamin E; 4: HFD + oil-palm derived vitamin E and carotenoids; 5: oil-palm derived antioxidants. Two animals were prematurely euthanized (from groups 1 and 2) due to ill health. The HFD was formulated in-house with ingredients calculated from the MRC’s food composition tables. Liver samples were analyzed for vitamin A, α-vitamin A and carotenoid concentrations by HPLC. Data were analyzed using one-way ANOVA.

All monkeys developed hypervitaminosis A (≥1 µmol/g as defined for humans) and in group 4 exceeded 10 µmol/g (potentially toxic in humans). The total hepatic concentration of vitamin A and carotenoids was significantly different among treatment groups and highest in group 4.  Findings in group 4 were surprising because although high carotenoids were fed the negative feedback mechanism did not reduce absorption, as expected, suggesting differences between humans and vervets in the absorption or bioconversion of carotenoids. However, two carotenoids (lutein and zeaxanthin) were lowest in group 4, suggesting that supplemental carotenoids compete with lutein and zeaxanthin for binding sites.

In conclusion, it may be worth considering reducing the vitamin A content of vervet diet.

QUESTIONS

1. Which are the main vitamin A storage sites in the body? (2 answers)

a. Kidney

b. Liver

c. Pancreas

d. Adipose tissue

e. Lung

2. T/F. Vervets in the wild obtain their vitamin A mainly as carotenoids.

ANSWERS

1. b, d

2. True

***Management***

**Miedel et al.** [**Facility-wide Eradication of *Corynebacterium bovis* by using PCR-validated Vaporized Hydrogen Peroxide**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00005)**, pp. 465-476**

Domain 1: Management of Spontaneous and Experimentally Induced Conditions

Primary Species: Mouse (*Mus musculus*)

SUMMARY: *C. bovis* is a resilient lipophilic, opportunistic gram-positive coryneform bacteria associated with scaly skin disease. Clinically affected nude mice shed *C. bovis*-infected keratin flakes that are spread through airborne and fomite transmission and remain resistant to facility decontamination. Environmental monitoring by PCR is encouraged when immunodeficiency strains are housed, especially of exhaust plenums venting IVC rack systems. The threat of organism persistence remains unless a validated facility wide decontamination effort is implemented. Previous efforts of culling PCR-positive mice and decontaminating equipment in affected isolation housing were deemed to have been ineffective for preventing recurrence. Processes of *C. bovis* PCR-based environmental and murine surveillance were implemented in a 12 month study that took place in an SPF murine Cancer Care facility. The prevalence, eradication, and potential for recurrence of *C. bovis* was documented using PCR and aerobic microbial culture as the facility wide decontamination efforts began, progressed, and concluded. From the facility’s opening in 2005-mid 2016, there were no procedures in place to eradicate *C. bovis*. *C. bovis* was initially detected in 13% of the total facility. VHP decontamination efforts were systematic, room by room and facility wide. The entire facility was decontaminated by a combination of OxivirTb (an aHP) and VHP using a commercially available generator and accelerator. Sterile swabs were used to collect environmental specimens from surfaces by tracing a circular pattern for 3 circumferences as its tip was rolled. Samples were analyzed using PCR assays. *S. xylosus* was used as an indicator species to ensure the sterility of VHP-exposed equipment and to serve as a positive control after the return of murine-occupied IVC to the VHP-sterilized rack during monitoring of whether PCR evidence of *C. bovis* recurs in the airways of VHP-sterilized equipment in previously infected housing rooms. During February 2007, culling of all positive mice was complete. The cumulative effect of culling *C. bovis* positives and implementing active-closed VHP decontamination significantly reduced bacterial prevalence during months 6-12 as opposed to months 1-5. Since the conclusion of the 12 month study, none of the 452 additional monthly surveillance specimens tested were *C. bovis* positive.

QUESTIONS (True or False)

1. *C. bovis* is a resilient, lipophilic gram-negative coryneform

2. *C. bovis* has traditionally remained persistent despite culling of PCR-positive animals and decontaminating equipment

3. Vaporized hydrogen peroxide appears to be an effective means of achieving *C. bovis* eradication in conjunction with OxivirTb and culling PCR-positive animals.

ANSWERS

1. False: Gram Positive

2. True

3. True 

***Animal Health Surveillance***

**Dubelko et al.** [**PCR Testing of Filter Material from IVC Lids for Microbial Monitoring of Mouse Colonies**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00006)**, pp. 477-482**

Domain 4:Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY**:**Sentinel mice receiving contaminated bedding from resident animals is a common method for routine colony health surveillance. A limitation of this method is that not all pathogens readily transfer through the bedding to the sentinel animal including lymphocytic choriomeningitis, Sendai virus, mouse norovirus, cilia-associated respiratory bacillus, and murine fur mites. More recently environment sampling has been used to supplement or replace elements of routine bedding sentinel testing. Most commonly this involves the PVC testing of exhaust ducts in IVC racks. This testing is significantly limited if cage exhaust air is filtered prior to entering the plenum. In this study the authors PCR tested the cage top filters from cages holding soiled bedding. They further investigated the idea that periodically shaking a sentinel cage could replace the need of living animals. The idea being that the cage shaking would simulate particulate and air movement normally created by a moving animal. The study has three groups: a cage with 2 mice, a cage with no mice that was shaken for 15 seconds twice weekly and a cage with no mice that was not shaken. Each cage received 30ml of bedding from all other cages on the same side of the rack (maximum 80 cages) every other week. Testing occurred at one and three months after commencing the study. In addition to the three study groups colony mice on the same rack were also tested. The pathogens tested were known to be present in the colony and include *Helicobacter spp*., MNV, *P. pneumotropica*, *E. muris* and *S. muris.*After three months the most effective testing methods were PCR of random colony mice and filter paper from cages without mice that was shaken twice weekly. Cages housing sentinel mice was moderately effective by detecting *Helicobacter spp*., and MNV but not *P. pneumotropica*, *E. muris* and *S. muris*. The least effective cage was the cage with no mice that was not shaken. After one month filter paper from mouse-free cages that were shaken were the most effective at detecting the most pathogens. This paper demonstrates that adventitial pathogens can be successfully detected by testing filter paper directly from cages that have been routinely exposed to contaminated bedding and shaken regularly. This has the benefits of using fewer mice and detecting pathogens that might not normally be detected from the sentinel animal.

QUESTIONS

1. List pathogens that are not easily transferred to a sentinel animal through the transfer of bedding.
2. After three months which of the three test groups demonstrated the most effective testing paradigm?
3. What are two potential benefits of using filter paper from a cage that has been shaken versus a routine sentinel mouse?

ANSWERS

1. Lymphocytic choriomeningitis, Sendai virus, mouse norovirus, cilia-associated respiratory bacillus, and murine fur mites.
2. After three months the most effective testing methods were PCR of random colony mice and filter paper from cages without mice that was shaken twice weekly.
3. This has the benefits of using fewer mice and detecting pathogens that might not normally be detected from the sentinel animal.

**Nashat et al.** [**Ivermectin-compounded Feed Compared with Topical Moxidectin–Imidacloprid for Eradication of *Demodex musculi* in Laboratory Mice**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00007)**, pp. 483-497**

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

Primary Species: Mouse (*Mus musculus*)

SUMMARY: *Demodex*mites are rarely reported  in laboratory mice, but this appears to be due to low detection rates.  *Demodex* mites are microscopic, cigar-shaped, prostigmatid mites that live, feed and reproduce in the pilosebaceous units of mammals. Typically they are present in low numbers in healthy hosts, but can become opportunistic parasites if the host is immunocompromised. Demodicid mites tend to be host-specific, and only *D. musculi* has been reported in laboratory mice.  In human *Demodex* mites, the life cycle is 14.5 days and all life stages (eggs, larvae, nymphs, and adults) are found in the pilosebaceous unit.

The impact of demodicosis on immunocompromised mice has not been studied, but treatment is needed sometimes if clinical disease develops, or prior to importing mice into *Demodex* –free colonies.  Rederivation by embryo transfer or in vitro fertilization can be used to eliminate parasites such as *Demodex*, but it is costly and time-consuming, making other treatments desirable. Treating follicular mites can be challenging due to problems achieving effective concentrations of the parasiticide at the skin’s surface, and difficulty in confirming eradication after treatment. Other challenges include finding a treatment that has limited toxicity, is cost-effective, and easily scaled up to large populations. Treatments must also ensure complete eradication of the mites at all life stages, because parthenogenesis is a common reproductive strategy in demodicid mites.

This study aimed to compare the efficacy of a topical combination of moxidectin and imidacloprid (MI) to oral ivermectin administered in feed.  Several detection methods were also tested: fur pluck (FP), deep skin scrape (DSS) examination, PCR analysis of fur swabs, and skin biopsy. *Demodex* eradication was defined as negative results by all methodologies at the conclusion of the treatment trial. Immunocompromised mice were used for this study (TRP1/TCR and NSG mice). The study had three phases: 8 week treatment with ivermectin (daily feeding with ivermectin feed), once weekly  treatment with MI for 8 weeks, and once weekly treatment with MI for 4 weeks.

Results showed that ivermectin significantly reduced the *Demodex* mite population (FP and DSS mite counts and skin biopsy), but 12 ppm ivermectin did not result in mite eradication.  Higher doses of ivermectin have been reported to cause toxicity and were not considered for this study.

Skin biopsies proved to be highly effective in detecting mites in TRP1/TCR mice. However, skin biopsy analysis is both labor and time intensive, and for best results should be performed postmortem. *Demodex* mites can be found throughout the pelt, however, the interscapular region is more prone to infestation and should be biopsied preferentially.

5/10 mice treated with once weekly MI for 8 weeks were mite-negative at 3 consecutive time points as late as 12 weeks post treatment.  However, in the 5 mice that were evaluated 1 week after treatment ended, a single mite was found on skin biopsy. 2/7 of the 4 week treatment group remained PCR positive (1 skin biopsy positive). Results showed that 8 weeks of weekly MI treatments eliminated mites from a moderately infested immunocompromised strain.

A commercially available PCR assay was highly useful for diagnosis, but FP+DSS or skin biopsy (or both) should be added as additional tests when false-negative results would be problematic.

QUESTIONS

1. T/F: *Demodex* mites can be found throughout the pelt, however, the interscapular region is more prone to infestation and should be biopsied preferentially.

2. T/F: Ivermectin feed at 12ppm for 8 weeks resulted in complete eradication of *Demodex* mites in all tested animals.

3. Treating follicular mites can be challenging for all of the following reasons except:

a.   Problems achieving effective concentrations of the parasiticide at the skin’s surface

b.  Difficulty in confirming eradication after treatment.

c.  Finding a treatment that has limited toxicity

d.  Finding a cost-effective treatment

e.  Lack of parthogenesis makes demodicid mites easy to eradicate.

ANSWERS:

1. T

2. F

3. e

***Anesthesia***

**Nunamaker et al.** [**Evaluation of Analgesic Efficacy of Meloxicam and 2 Formulations of Buprenorphine after Laparotomy in Female Sprague–Dawley Rats**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00008)**, pp. 498-507**

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

Primary Species: Rat (*Rattus norvegicus*)

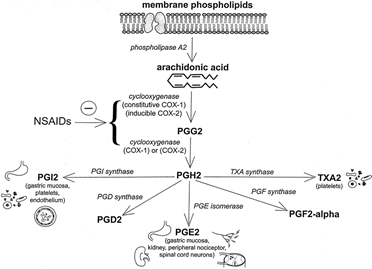
SUMMARY:Using a laparotomy with OVH model of pain the group compared the therapeutic efficacy of clinically relevant doses of two formulations of buprenorphine (buprenorphine HCl and buprenorphine SR) or meloxicam used in combination with ketamine/xylazine anesthesia. The dosing for buprenorphine HCl was low dose 0.05mg/kg and high dose 0.1 mg/kg. The buprenorphine SR was dosed at 1.2 mg/kg. Meloxicam was provided at either low dose 1 mg/kg or high dose 2 mg/kg. The group found all of the surgical groups exhibited average body weight loss ranging from 2.4% to 6.2% depending on the experimental group. Based on this finding the group recommends not utilizing weight loss as a single means of identifying pain in rats. The group utilized an observational scoring system to assess body posture, activity, and coat appearance. Rats receiving either dose of meloxicam, the lose dose buprenorphine and buprenorphine SR all had lower scores using this system. The high dose buprenorphine was not as effective. This is hypothesized to be due to the reported ceiling effect of buprenorphine at 0.03 mg/kg in a previous studied orofacial pain model. Additionally, facial grimace scoring was used to determine the efficacy of the dosing regimens.  Animals receiving meloxicam or either formulation of buprenorphine had lower grimace scores compared to those animals receiving saline. This group also had an incidental finding of injection site ulcers for those animals receiving meloxicam with more ulcers forming in the high dose group versus the low dose group.

QUESTIONS

1. What is the mechanism of action of meloxicam?
2. T or F: In rats, blocking both COX1 and COX2 is required for the formation of gastrointestinal ulcers.

ANSWERS

1. Blocks cyclooxygenase (COX) which is the enzyme responsible for converting arachidonic acid to prostaglandin H2 (the first step in prostaglandin synthesis). Meloxicam selectively inhibits COX-2 over COX-1.



1. True

***Experimental Use***

**Hocking et al.** [**Administering Fixed Oral Doses of Curcumin to Rats through Voluntary Consumption**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00009)**, pp. 508-512**

**Domain 3:** Research and **Domain 4:** Animal Care

Primary Species: Rat (Rattus norvegicus)

SUMMARY

**Goal/Objective:** Develop an alternative method to oral gavage for the administration of curcumin to rats via voluntary consumption of peanut butter to reduce gavage-related morbidity and distress.

**Background:** Curcumin (diferuloylmethane) is a polyphenol derived from turmeric, the rhizome of the plant Curcuma longa. It has been used for centuries in traditional Chinese and Indian medicines to treat various illness (e.g. ulcers, liver disease, wounds and the common cold). It has antioxidant, anti-inflammatory, and anticancer properties. Curcumin is well-tolerated at high doses and is typically administered via oral gavage in the research setting.

Extensive training is required to effectively and safely administer substances via oral gavage. Although effective if done properly by skilled personnel on rats acclimated to handling, it can lead to premature death by accidental administration of fluid into the lungs, serious mechanical damage to the esophagus or stomach, or gavage-related reflux. It may require 2 operators due to difficulties in single-handed restraint. This process (gavage and manual restraint) has been shown to induce stress in animals, which can lead to a wide range of behavioral, biochemical, and physiologic changes, potentially altering experimental outcomes.

**Methods:** Using male and female Fischer 344 rats, pilot studies revealed a preference for smooth PB over hazelnut-chocolate spread. Animals were fasted 3 hours prior to being given the peanut butter + curcumin mixture. They received daily mixtures for 5 weeks. Plasma was tested weekly at 2, 3, and 4 hours post-consumption, and fecal samples were collected 24hr post-administration for the quantification of curcumin (quantified by UPLC- mass spectrometry).

**Results:**

* + All rats ate the mixture within 2 hours after 3 days of acclimation
  + Consumed dose within 5-30min period after a 3hr fast for 5 weeks
  + No adverse effects or apparent signs of stress
  + Similar weight gain compared to control (rodent diet only)
  + Curcumin was detected in the plasma – similar levels measured at 2, 3, and 4hr post-consumption
  + High levels of curcumin was detected in fecal matter

**Discussion/Summary:** Corticotropin-releasing hormone is secreted from the hypothalamus in response to stressors, resulting in the secretion of corticotropin from the anterior pituitary, which stimulates the release of glucocorticoids such as corticosterone from the adrenal gland. Glucocorticoids can lead to changes such as activation of gluconeogenesis, increased hepatic protein synthesis, and downregulation of the inflammatory response, as well as increased heart rate and blood pressure and alter gastric secretions and gut motility. Corticosterone levels are increased in rodents following oral gavage. This noninvasive delivery method eliminates stress caused by daily manual animal restraint and oral gavage and provides environmental enrichment.

Mixing curcumin with peanut butter was cost-effective and a reliable alternative to specialized diets. Other products have also been used – flavored gelatin, various nut pastes, syrup, sugar dough, bacon-flavored dough, and cookie dough. This methodology may not be appropriate when studying models in which appetite is suppressed and incomplete ingestion is likely.

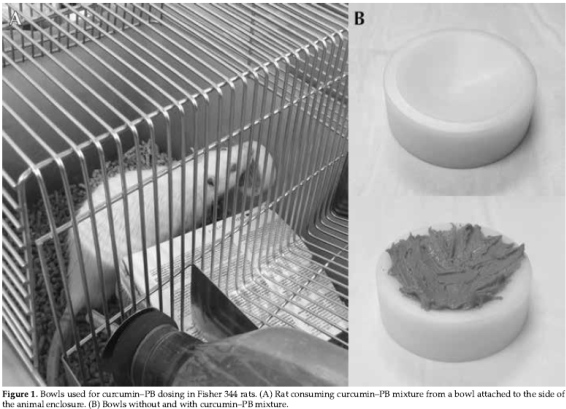
Curcumin has low oral bioavailability, low solubility, poor intestinal absorption, and rapid metabolism. The formulation in used in this study was a phosphatidylcholine formulation shown to be more bioavailable than other formulations. Free curcumin was not detected in the plasma, but total curcumin was detected after deconjugation by β-glucuronidase and sulfatase enzymes. Free curcumin was detected in the feces.

**QUESTIONS**

1. What are several disadvantages to oral gavage?
2. What stress-related glucocorticoid has been shown to be elevated in rodents following oral gavage?

**ANSWERS**

1. Extensive training required; death by accidental administration of fluid into the lungs, serious mechanical damage to the esophagus or stomach, or gavage-related reflux; may require 2 operators due to difficulties in single-handed restraint; stress-related responses
2. Corticosterone



***Creamer-Hente et al.*** [**Sex- and Strain-related Differences in the Stress Response of Mice to CO2 Euthanasia**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00010)**, pp. 513-519**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The 2013 AVMA Guidelines for the Euthanasia of Animals describes a recommended 10-30% volume displacement rate (VDR) per minute for CO2euthanasia of rodents, however the majority of research used to arrive at this recommendation was conducted with rats. Since mice are the most widely used animal in research, the authors sought to conduct a study characterizing the stress response of mice exposed to CO2 as a means of euthanasia. Variables included sex, group or individual euthanasia, strain type, and VDR between 10% to 100%. Measurements of stress included catecholamine levels and behaviors including ataxia, labored breathing, time to recumbency, time to surgical plane of anesthesia, and the number of jumps or paws at the face during the euthanasia process. The results of the study showed that all VDR produced marked catecholamine responses and they did not differ between slow and fast fill rates. Significant differences did occur between individual and group euthanized mice which suggests that the stress response to CO2is tapered with social buffering.  Individual strain-associated differences were observed in the behaviors evaluated but the data is not conclusive in the current manuscript. The current study suggests that 30% VDR is the most ideal in accordance with the current AVMA Guidelines for Euthanasia, however higher VDR for CO2euthanasia with mice should be acceptable also, because no animal welfare concurs occurred with these higher VDR. In addition, a VDR of 10% to 20% should be considered for removal from the euthanasia recommendations in view of animal welfare concerns associated with increased levels of distress.

QUESTIONS

1.   CO2 gas forms what when exposed to moisture which can irritate the nasal mucosa and cause pain when it is inhaled?

2.   Define distress

3.   Which of the following is *not* a hormone response to stress?

a.   Norepinephrine

b.   Corticosterone

c.   Corticotropin releasing hormone

d.   Insulin

ANSWERS

1.  Carbonic acid

2.  An aversive, negative state in which coping and adaptation processes fail to return an organism to physiologic or psychologic homeostasis.

3.   D- insulin

**De Luca et al.** [**Improving the Patency of Jugular Vein Catheters in Sprague–Dawley Rats by Using an Antiseptic Nitrocellulose Coating**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00011)**, pp. 520-528**

Domain 3: Research

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Indwelling polyurethane catheters in the jugular vein in rats provide the ability for frequent sampling over an extended time course to allow for appropriate determination of pharmacokinetic parameters. Anticoagulants are used as locking solutions for these catheters and of all the available anticoagulants, sodium citrate has the fewest limitations for the study of biologics. However, it was observed more frequent loss of catheter patency linked to sodium citrate use. It was noticed that much of the external portion of the catheter was devoid of locking solution and this study hypothesized that loss of patency was due to direct evaporation of the sodium citrate locking solution from the porous polyurethane catheters.

This study tested 5 different catheter materials in vitro (polyurethane, renathane, silicone, polyethylene, and polytetrafluoroethylene) for evaporation of 4% sodium citrate lock solution by measuring displacement of an indicator dye after 24, 48, and 72 h. 2 variables relevant to the evaporation process (environmental humidity an hygroscopicity of the locking solution) were altered and tested. To validate the in vitro findings in a real-world context, the application of liquid bandage in extending the patency of rodent polyurethane jugular vein catheters to intermittent blood draws was tested. Because polyethylene and polytetrafluoroethylene are unsuitable for jugular vein implantation in rodents, and because renathane resulted in the greatest migration of dye among the materials tested, the study proceeded with polyurethane as the preferred catheter material. Several treatments intended to reduce evaporation by acting as barriers to the dry-air environment were tested.: white petrolatum-mineral oil performed better than a polyethylene sheath, which performed better than liquid bandage (topical commercially available nitrocellulose-based polymer that I water-resistant), and all treatments performed better than the control. This showed that coating catheters with different hydrophobic barriers delayed the evaporation process. Although white petroleum-mineral oil performed better at reducing dye migration in vitro, initial testing in vivo showed that the natural movement of the rat in its cage bedding caused the oil to dissociate from the catheter. Therefore, liquid bandage was selected.

This study reports that evaporation of lock solution causes blood to infiltrate into the catheter tip, where it can clot. The in vitro data show that locking solution evaporates from catheters exposed to air, allowing displacement of the lost volume against gravity by a dense indicator dye. The in vivo study validated the conclusions drawn from our in vitro experiments: the application of liquid bandage was able to extend the patency of sodium citrate-locked polyurethane catheters flushed at 4 days intervals, showing that liquid bandage is a safe and effective means of reducing the frequency at which catheter must be flushed to maintain long term patency.

QUESTIONS (True or False)

1. Limiting the number of times a catheter is flushed has benefits, such as preserving the physical integrity of the line and reducing stress on the animal.

2. Liquid bandage doesn’t need repeated applications over time.

ANSWERS

1. True

2. False. Liquid bandage dehydrates over time and requires repeated applications to preserve its ability to prevent sodium citrate evaporation.

**CASE REPORTS**

**Lindstrom et al.** [**Contaminated Shipping Materials Identified as the Source of Rotaviral Infection of Exported Mice**](http://www.ingentaconnect.com/contentone/aalas/jaalas/2018/00000057/00000005/art00012)**, pp. 529-533**

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

Primary Species: Mouse (*Mus musculus*)

SUMMARY: An institution received notification from other institutions that received exported mice, positive rotavirus (MRV) results during quarantine diagnostics.  Sentinel testing of their SPF barrier facilities did not result in positive MRV. The institution concluded that the shipping material, including shipping container, bedding and food were contaminated.  They did not autoclave them prior to usage.

They placed sentinels in clean cages with the bedding and food from the shipping containers they had stored in two buildings and also used material from newly shipped containers.

After 7 d, 14 of the 29 sentinels, were positive for MRV by fecal PCR. After 14 d of exposure, a total of 24 of 29 tested positive by serology. Environmental testing revealed 12 of the 22 filters were positive by PCR and 20 of 22 mesenteric lymph nodes were positive for the virus.

QUESTIONS

1.   Mouse rotavirus (MRV) is a group \_\_\_\_\_\_ rotavirus that replicates in the \_\_\_\_\_\_\_\_\_\_ of the \_\_\_\_\_\_\_\_\_.

2.   Mouse rotavirus is also called \_\_\_\_\_\_\_\_\_\_\_\_\_.

3.   Mouse rotavirus affects what age mice?

4.  Morbidity is high and mortality is \_\_\_\_\_\_\_\_\_.

5.   Mode of transmission is \_\_\_\_\_\_\_\_\_ and is \_\_\_\_\_\_\_\_ contagious.

ANSWERS

1. A, villus epithelial cells, small intestines

2.   EDIM

3.  Infant mice younger than 2 weeks old

4.  Low

5.  Oral-fecal, highly