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**OVERVIEWS**

**Divincenti Jr and Rehrig. The Social Nature of European Rabbits (*Oryctolagus cuniculus*), pp. 729-736**

Domain 4: Animal Care

Primary Species: Rabbit (Oryctolagus cuniculus)

SUMMARY:  The 8th edition of the Guide’s emphasis on social companionship for social species and subsequent changes in regulator and accreditor focus are forcing reexamination of long-held beliefs regarding animal housing in laboratories.   Following widespread success with transitioning singly housed NHP into social pairs or groups in larger, more enriched cages and pens and the resulting dramatic improvement in wellbeing, the conversation has broadened to consider all species found in social groups in the wild but often housed singly in research facilities.  European rabbits (Oryctolagus cuniculus) are the third most widely used USDA-regulated laboratory animal species in the United States, yet they are commonly individually housed in research facilities despite the occurrence of social groups in the wild.  The gregarious nature of rabbits in the wild seemingly suggests that they, like NHPs, would benefit from social companionship in the laboratory setting, but forced group housing of rabbits in captivity may have deleterious effects on their wellbeing, evidenced by numerous reports of aggression and injury during socialization attempts in the laboratory.  Numerous authors have suggested a variety of factors - based on anecdote and personal experience rather than true evidence- that influence successful group housing of rabbits factors, including cage size, prior social experience, age of the animals, relatedness, and the presence of visual barriers.  The authors of this article reviewed the current literature (mostly based on observation of wild populations of O. cuniculus or captive populations of NZW rabbits) and discussed the social behaviors and preferences of rabbits in the wild and in captivity.

Understanding of the social preferences of rabbits in the wild remains incomplete, but resource limitation seems to be a major stimulus for group formation in this species.  With adequate resources, being social does not appear to be a necessity for and may actually be avoided by wild rabbits.  With laboratory rabbits, single housing in cages that do not allow normal exploratory and locomotor patterns such as rearing and hopping clearly is deleterious to wellbeing, but socially housing adult lab rabbits in a constricted or inadequate settings also creates challenges to wellbeing, especially for subordinate animals that appear to suffer chronic stress.  In wild populations, rabbits demonstrate submission by fleeing or retreating, and the cages and pens currently used for social housing of lab rabbits likely do not provide adequate space for expression of species-typical dominance–submission displays, thus preventing the establishment and maintenance of stable social groups.  Floor space itself may not be as important as is the configuration of the space available, especially if escape distances are narrowed.  Successful group housing of adult rabbits might be improved through early social housing of young, perhaps related and castrated, animals in very large, enriched pens.  During group housing, neutral locations with multiple hide and escape opportunities are generally recommended.  Runs originally designed for large animals, or whole rooms when possible, may be ideal.  The pen should have multiple sources of food and water as well to avoid conflict over resources. Some authors have used a protected contact introduction period as an attempt to predict compatibility in full contact housing, and rabbits may need to be introduced and separated daily over a period of up to 2 wk before a group is stable.  However, consistent with the observations of intense fighting after social instability in wild rabbits, daily separation has not been helpful in the authors’ experience because the animals have to reestablish the hierarchy each day.  Along those lines, once groups are stable, manipulations and disruptions of the group should be minimized. Because removal of the dominant buck precipitates severe aggression, research strategies in which the subordinate animals are used first may contribute to ongoing success.  As with all socially housed animals, socialized rabbits require close, continued monitoring to ensure all animals in the group remain compatible. Just as in other species, some individual rabbits may not be good social partners with one another, and alternative options, including single housing, may be necessary.  Frequent supervision of the animals and communication among all stakeholders is required to ensure that social housing of lab rabbits actually improves animal wellbeing.

QUESTIONS:

1. According to this study, which of the following factors seems to be a major stimulus for group formation in wild rabbits?

a. Resource abundance

b. Resource limitation

c. Favorable environmental conditions

d. Unfavorable environmental conditions

2. According to the authors of this study, which of the following practices seemed least beneficial for successful group housing of adult lab rabbits?

a. Use of very large, enriched areas, such as large animal runs or whole rooms

b. Use of a protected contact introduction period to assess compatibility prior to full contact housing

c. Provision of neutral locations with multiple hide and escape opportunities

d. Provision of multiple sources of food and water as well to avoid conflict over resources

3. According to this study, in order to maintain the stability within socially housed groups of lab rabbits, researchers should remove and use which social group member last whenever possible?

a. Dominant buck

b. Dominant doe

c. Subordinate buck(s)

d. Subordinate doe(s)

ANSWERS:

1.  b. Resource limitation

2.  b. Use of a protected contact introduction period to assess compatibility prior to full contact housing

3.   a. Dominant buck

**Clemmons and Taylor. Booklice (*Liposcelis* spp.), Grain Mites (*Acarus siro*), and Flour Beetles (*Tribolium* spp.): ‘Other Pests’ Occasionally Found in Laboratory Animal Facilities, pp. 737-743**

Domain 5: Regulatory Responsibilities

|  |  |  |  |
| --- | --- | --- | --- |
|  | Booklice | Grain Mites | Flour Beetles |
| Taxonomy | *Non-Parasitic Lice*  Order: Psocoptera (Psocids)  Genus: *Liposcelis*  Species: *bostrycophila, decolor, entomophila, paeta*    Most Common: *L. bostrycophila* | Subclass: Acari  Superorder: Acariformes (Actinotrichida)  Order: Acaridida (Astigmata)  Family: Acaridae  Genus/Sp: *Acarus siro*    Other storage mites:  Family: Acaridae  *Lepidoglyphus destructor, Tyrophagus putrescentiae*  Family: Glycyphagidae  *Glycyphagus domesticus* | Class: Insecta  Order: Coleoptera  Family: Tenebrionidae  Genera: *Tribolium*  Species: *T. castaneum* (red flour beetle) and *T. confusum* (Confused flour beetle) |
| Food Source | Cellulose, book bindings, fabric, glue, grain, mold, mildew, algae, and plant material | Grain, flour, cereal, vegetables, cheese, and animal feed | Grains,seeds,spices,flour,cereal products, animal matter, wood, vegetables, and various drugs |
| Physical Descript. | * <3mm long * Visible without magnification * White to light colored soft bodies * Long filiform antennae | * 0.5mm long * Not visible to naked eye * Colorless, oval bodies and brown legs * Pungent “minty” smell | * 3.5mm long * Visible without magnification * Small and reddish-brown with well-developed but rarely used wings |
| Eggs | * Simple smooth elongate ovoids or cylinders w/o micropyle * May be bare, covered in fecal material, and or a silk webbing * Found singly or in groups | * Shorter than 165µm * White, glossy, symmetrical, and ellipsoid * Rounded ends and no micropyle | * 0.35mm wide and 0.6mm long * White to colorless * Oblong or ovoidal * Nearly transparent * Sticky surface membrane * *Wolbachia*: ↑male fertility, ↓ female fecundity |
| Repro. | Parthenogenetic – asexual repro | Not mentioned in paper | Not mentioned in paper |
| Optimal Environ. | Temp: 30 ± 2.5ºC  Humidity: 70-80% | Temp: 25 ºC  Humidity: 90% | Temp: 35 ºC  Humidity: 70%   * Cryptonephridial organ to allow for survival in dry environments |
| Life Cycle /Span | 21 days / 72-144 days | Unlikely to occur w/o a temp range of 2.5 ºC - 32 ºC | * Life span of >3yrs * Male: fertile entire life span * Female: fertile until 1 yr of age |
| Significant to Animal Health | * Intermediate host for ruminant tapeworm: *Thysanosoma actinioides* * Possible transmittal of fungi and bacteria * Can carry: *Rickettsia felis*, *Wolbachia, Cardinium* | * Fungal vector * Can be found in rodent feces and can be confused with Mycoptes musculinus, Myobia musculi, and Radfordia. This could lead to unnecessary diagnostic tests and inappropriate treatment and control methods. * Increase in fetal mortality if fed to pregnant mice | * Have benzoquinone-secreting defensive glands. Potentially toxic and is readily absorbed from GI and sub cutaneous tissues. Topically can cause discoloration, papules, and necrosis * Potentially causes cancer * Intermediate host of rat tapeworm (*Hymenolepis diminuta*) |

Human Occupational Health Risks

1. A. Siro can cause intestinal, pulmonary, and urinary acariasis in humans

2.  Sensitizing allergens

a.  Liposcelis spp. identified as allergens in house dust

b.  25% of house dust samples collected contained mites

c.   Storage mite species may pose an occupational health risk in animal facilities

Management

1.   The Guide: Implementation of a regularly scheduled and documented program of control and monitoring

2. AWA: Effective pest control program that addresses insects, ectoparasites, avian, and mammalian pests.

3.  Food storage pests are not routinely considered in pest control program design

4.  Psocid – can survive after permethrin treatment

5. Monitoring – performed using direct visual sampling or traps (pheromones, food odors, light), ELISA, near infrared detectors, and electronic nose gas sensors. Most common: visual inspection, and extraction with Tullgren-Berles funnels and filth flotation.

6.   Food bins should be disinfected prior to refilling. Cleaning outside of food bags not really helpful since some mites are present in unopened bags of food.

7.  Autoclaving is most effective method available in animal facilities

QUESTIONS

1.   What is the scientific name for a Booklice, Grain mite, and Flour beetle?

2.   Which insect cannot be seen with the naked eye?

3.   What is the most effective way to rid food of mites?

ANSWERS

1.  *Liposcelis* *bostrycophila, Ascarus siro, Tribolium castaneum*

2.  Grain mites

3.  Autoclaving food

**ORIGINAL RESEARCH**

***Reproduction***

**Yan et al. Cryopreservation of Cynomolgus Macaque (*Macaca fascicularis*) Sperm by Using a Commercial Egg-Yolk-Free Freezing Medium, pp. 744-748**

Domain: 1

Primary Species: Macaques (Macaca spp.)

SUMMARY: This study compares the effects of a commercial egg-yolk free freezing medium designed for human sperm cryopreservation to a conventional TRIS-egg yolk freezing medium for the cryopreservation on sperm obtained from non-human primates (NHP) on sperm motility and acrosomal integrity after freezing of NHP sperm.

Cynomologus macaques provide excellent translational validity in preclinical studies because of their genetic, physiologic, behavioral, and neurological similarities to humans. Meanwhile, the maintenance and breeding of NHP are extremely costly, sperm banking provides an effective way to preserve genetic resources with a lower financial burden. Though sperm cryopreservation of cynomologus macaques with egg yolk freezing medium is widely used and egg yolk provides cryoprotection to sperm, egg yolk still serves as a source of widespread zoonotic diseases such as avian influenza. Because of its animal origin and potential contamination with bacteria, fungi, viruses, and prions, egg yolk carries the risk of pathogen introduction into cryopreserved sperm samples.

For this study 4 sexually mature cynomologus macaques were collected for semen samples by using electric penile stimulation. A part of each ejaculate was used as a non-frozen control. Sperm motility was assessed by light microscopy, sperm acrosomal integrity was determined by using Alexa Fluor-488-peanut agglutinin assays. Cryosurvival of sperm was assessed after undergoing different procedures: effect of different cooling rates, different holding time in liquid N vapor, dilution ratio in egg-yolk free medium, and finally cryosurvival of sperm in egg-yolk free medium was compared to sperm in egg-yolk medium. All data obtained were statically analyzed. The results showed that the post-thaw motility and acrosomal integrity of sperm cryopreserved with either egg-yolk or egg-yolk free mediums were decreased significantly compared with those of fresh sperm. Post-thaw motility was higher for sperm cryopreserved with egg-yolk medium than in egg-yolk-free medium, but acrosomal integrity did not differ between mediums.

In conclusion the cryosurvival rate, and holding time for cryopreservation of cynomologus macaque sperm frozen with egg-yolk free medium was equal to that of human sperm with the same freezing medium providing and alternative method for the genetic preservation.

QUESTIONS

1.  True/False: Meanwhile, the maintenance and breeding of NHP are extremely costly, sperm banking doesn’t provide an effective way to preserve genetic resources with a lower financial burden.

2.  True/False: The results showed that the post-thaw motility and acrosomal integrity of sperm cryopreserved with either egg-yolk or egg-yolk free mediums were decreased significantly compared with those of fresh sperm. Post-thaw motility was higher for sperm cryopreserved with egg-yolk medium than in egg-yolk-free medium, but acrosomal integrity did not differ between mediums.

3.   Sperm cryopreserved with egg-yolk medium can serve as a source for

a.       Bacterial pathogens

b.      Zoonotic pathogens

c.       Fungal pathogens

d.      a + b

e.      a + b + c

ANSWERS

1.  False

2.   True

3.   e

***Husbandry***

**Uarquin et al. Effect of Overcrowding on Hair Corticosterone Concentrations in Juvenile Male Wistar Rats, pp. 749-755**

Domain 1: Management of Spontaneous Disease and Experimentally Induced Diseases and Conditions; K2 - Physiology with Emphasis on Normative Data and Characteristics, Metabolic Differences, or Metabolic Induced Disease

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: The aim of this study was to determine whether a chronic stress condition (overcrowding) induced changes in plasma and hair corticosterone concentrations, overall growth, and organ weights in Wistar rats.  There were two groups of rats, controls (n=9, 3 per cage, 155 cm2 per rat) and overcrowded cages (n=9, 3 per cage, 48 cm2 per rat).  The overcrowding conditions occurred from post-natal day 38-65. Blood, hair samples (shaved fur), and terminal organ weights were taken.  The researchers found that plasma and hair corticosterone concentrations were higher in overcrowded rats compared to control subjects.  In addition, overcrowding reduced body and organ weight gains.  Overall conclusion, measuring the concentration of corticosterone in hair samples is an effective, noninvasive method for monitoring chronic stress in rats.

QUESTIONS

1. Chronic stress was measured by what method in this study?

a. Concentration of corticosterone in urine

b. Concentration of corticosterone in plasma

c. Concentration of corticosterone in hair samples

d. All of the above

e. Only A and C

2. In this study, which organs sizes were seen in overcrowded rats cages compared to control cages:

a. Decrease in absolute heart weight

b. Decrease in absolute kidney weight

c. No change in absolute adrenal weight

d. Increase in relative weight of the lungs

e. All of the above

3. True/False. In this study, using hair samples to obtain corticosterone levels is an effective way to measure acute stress levels.

ANSWERS

1. c. They looked primarily at steroid levels in fur as an indicator for chronic stress

2. e. All of the above

3. False. Corticosterone levels should only be used to interpret chronic stress levels. Corticosterone levels in plasma or urine are better diagnostics indicators for acute stress.

**Crast et al. Behavioral Effects of an Enhanced Enrichment Program for Group-Housed Sooty Mangabeys (*Cercocebus atys*), pp. 756-764**

Domain 4: Animal Care; K2 - Environmental Enrichment

Tertiary Species: Other Nonhuman Primates

SUMMARY: The authors evaluated the effects of an enhanced environmental enrichment program on a colony of group housed sooty mangabeys. In the wild, these animals spend up to 63% of their time foraging from the forest floor. Behavioral observations collected included locomotion, feeding and foraging, manipulating items in the enclosure, social affiliation, aggression, and abnormal behavior. The enhanced enrichment program included the addition of a substrate (timothy hay), widely distributed small pieces of produce and a forage mixture in the hay, adding structures and perches, and giving an increased variety of food items, foraging devices, and other manipulable items. Study design was ABA of two week phases. A1: baseline (Standard), B, (experimental), A2 baseline. Results showed statistically significant increases in feeding, foraging and manipulation, and decreases in self-grooming, affiliative behavior and aggression. Total enrichment time increased from 9.5% to 21.2 % during the enhanced phase.

QUESTIONS

1. T/F:  Locomotion increased under enhanced enrichment.

2. T/F: The sooty mangabeys in this study chose substrate seeded with food or manipulable foraging devices over toys.

3. Name the primary research use for the sooty mangabey.

ANSWERS

1. False, locomotion did not increase

2. True

3. HIV-AIDs research.  They are the natural host of the simian immunodeficiency virus.

***Management***

**Saren et al. Developing a Performance Standard for Adequate Sanitization of Wire-Bar Lids, pp. 765-768**

Domain 4

Primary Species:  Mouse (*Mus musculus*)

SUMMARY: The article explores the possibility for creating a performance standard for sanitization of laboratory animal housing. The latest editions of the *Guide for the Care and Use of Laboratory Animals* do not describe performance standards for such sanitization of animal housing.  The authors conducted a pilot study to demonstrate, if any, difference between changing wire-bar lids (WBL) every two weeks versus every 4 weeks for mice housed in conventional caging. Evaluation of sanitization efficacy was measured using microbiologic culture or the use of organic material detection systems, which detect small amounts of ATP and is expressed as relative light units (RLU). Two studies suggest that a reasonable cutoff value for laboratory animal facilities is approximately 1000 RLU. The average RLU in the study was found to be 250-RLU or below and no significant changes in bacterial growth nor ATP concentration from one to four weeks. Thus, changing the WBL every four weeks instead of every two weeks will minimally affect mouse wellbeing.

QUESTIONS

1. What are two methods of testing the level of sanitization of laboratory animal housing?

2.  Was there a significant difference between, in respect to bacterial growth or ATP concentrations in housing that was sanitized every two weeks versus every four weeks?

ANSWERS

1.  Microbiologic culture and organic material detection systems

2.  No

**Boivin and Markert. Factors Affecting the Vocational Calling of Laboratory Animal Care and Research Employees, pp. 769-774**

Domain 6: Education

Tertiary Species: Other Mammals

SUMMARY:The authors conducted a survey in the field of biomedical research to see what factors were important in determining overall longevity and happiness in a laboratory animal care and research position. The questions were organized into four categories; passion, job stability or happiness, work volition, and demographics. Older personnel, those who worked in the field for a longer time, and ones with higher educational levels, at higher organizational levels, and those involved in AALAS were all hypothesized to represent individuals who believe their job choice was a calling. Moreover, those who did report that their work was a calling and had increased job satisfaction were in theory suspected to be related to organizational support and work volition. Of the 44% that responded to the survey, at least partially, work was classified as a calling. Those working at a higher level of laboratory animal technician in the organization were more likely to see describe their job as a calling. Overall, significance was seen when vocational calling and higher pay were analyzed, but technicians were the only subgroup where calling and higher pay were significantly related. The survey results reported in this paper may help leaders in the biomedical field look at other factors to strengthen employee workplace perception in addition to wages.

QUESTIONS

1.   What are Likert-type responses?

2.  Maslow’s hierarchy of needs theory points out a non-monetary factor in workplaces that can guide leaders to seeing more employee happiness—what is that factor?

ANSWERS

1.  6-point scale ranging from strongly agree to strongly disagree with no neutral choice but with a ‘not-applicable’ option

2.  Recognition and appreciation for good work

***Animal Health Surveillance***

**Miller et al. Exhaust Air Dust Monitoring is Superior to Soiled Bedding Sentinels for the Detection of *Pasteurella pneumotropica* in Individually Ventilated Cage Systems, pp. 775-781**

Domain 4: Animal Care

Task T1 - Develop animal husbandry programs

Task K1 - Pathogen-free barriers (K9);

Primary Species: Mouse (*Mus musculus*)

SUMMARY: *Pasteurella pneumotropica* is one of the most prevalent bacterial pathogens isolated from mice. Sentinel bedding detection often fails for this organism.  Exhaust air dust (EAD) samples were used in association with a sensitive and specific real-time PCR assay to detect this organism. EAD samples were able to detect this organism at every time point, with a prevalence of 5 infected mice per cage, with 7 infected cages on a rack containing a total of 63 cages.  Sentinel samples failed at every time point to detect this organism. The minimum prevalence required to detect this organism using the EAD method was one infected cage per 63 cages. Reliable detection was achieved after only 1 week of exposure.

QUESTION

1. What was the minimum time period of exposure to reliably detect *Pasteurella pneumotropica* via the Exhaust air dust sample technique?
   1. 1 week
   2. 2 weeks
   3. 3 weeks
   4. 4 weeks

ANSWER

1. a

**Bauer et al. Influence of Rack Design and Disease Prevalence on Detection of Rodent Pathogens in Exhaust Debris Samples from Individually Ventilated Cage Systems, pp. 782-788**

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

Primary Species: Mouse (Mus musculus)

SUMMARY

Introduction: Typically and historically open-top, dirty-bedding, bottom-row sentinel cages have been used for monitoring the presence of adventitious rodent pathogens in large mouse colonies. The current use of microisolater and individually ventilated cages have allowed disease containment at the cage-level, but also reduced the efficiency of disease transmission to sentinel mice and reduces the chance of detection for many pathogens.  To address this deficiency in detection of pathogens, adjunct methods of sampling from the environment are being investigated. Previous studies have suggested that exhaust debris that accumulates in the exhaust plenums of IVC racks may provide ideal sampling material for detection of adventitious agents.  This study was designed to evaluate the effectiveness of exhaust air debris samples for microbiological monitoring (by PCR) in IVC racks with or without filtered exhaust air at the cage level.

Methods: HSD:ICR mice were infected with MHV, MPV or MNV.  To mimic natural infection the infected mice were then co-housed with 3-4 naïve mice for 1 week.  The contact exposed mice were then separated into clean cages and housed on one of 3 IVC test racks.  Sencar mice that are naturally colonized with *P. pnuemotropica, Helicabacter ganmani, H. typlonius, Myobia musculi, Mycoptes musculinus, S. obvelata A. tertraptera, E. muris and T. muris,* were also moved to clean cages and placed on one of three IVC test racks.

Rack 1 contained 5 cages per group (MHV, MNV or MPV infected, or Sencar). Rack 2 contained a single cage per group of infected mice.  Rack 3 used disposable cages and also had 5 cages per infected group, like Rack 1.  The racks were then filled with uninfected cages of mice and empty cages to represent a 7-8% infection rate (racks 1 and 3) or 1% infection rate (rack 2).

Diagnostic fecal or fur samples were collected and assayed by real-time PCR from infected mice at the beginning of the study, weekly for the first 4 weeks, then monthly until the end of 12 weeks, to assure shedding of the infectious agents. Blood was collected at the end of the study to screen for antibodies.

Racks 1 and 2 are configured such that there is no filter between the individual cage exit port and the exhaust manifold.  Filter material was placed at the rack exhaust before the pre-filter or HEPA filter to capture particles returning from the individual cages.  A 2cm square of this capture filter was removed at each sampling point during the study for PCR analysis.  Rack 3 is designed such that return air from individual cages is filtered upon direct exit from the cage.  Samples from this rack were collected from specially made sampling ports that allowed for swabs to be inserted and rubbed along the horizontal exhaust air plenums.  Samples were collected from all racks at day 0, each week for 4 weeks and then monthly until the study ended at 12 weeks.  Lastly, debris was collected from tubing connecting the rack to the blower and tested at 3mo, 6mo and 12mo after the end of the study.

Results: Testing of exhaust debris by PCR analysis corroborated the MHV, bacterial and parasite infection status of mice housed on the rack. MPV and MNV were not reliably detected in exhaust debris despite documented shedding of the virus by infected mice. Rack 2 which had fewer infected mice had prolonged detection of the pathogens in the exhaust debris. The material collected from the connecting tubing remained PCR positive for 1 year past the end of the study, emphasizing the need to thoroughly disinfect and test the racks prior to housing animals. Testing of exhaust debris from racks with unfiltered exhaust from individual cages reliably detected many agents that are difficult to detect by dirty bedding sentinel monitoring.

QUESTIONS

1.  True or False. Mouse norovirus is shed persistently in the feces whereas mouse hepatitis virus and mouse parvovirus are shed transiently.

2.  Which of the following are inefficiently transmitted to sentinel animals through contact with soiled bedding?

a.  Helicobacter spp

b*.  Pasturella pneumotropica*

c. Fur mites

d.  Sendai virus

e. All of the above

ANSWERS

1.  True

2.   e. Helicobacter, Pasturella and mites lose viability outside the host, so don’t linger the bedding long enough to transmit efficiently.  Sendai is transmitted by respiratory route, not fecal-oral, so not easily transferred by bedding.

***Anesthesia***

**Smith et al. Pharmacokinetics and Paw Withdrawal Pressure in Female Guinea Pigs (*Cavia porcellus*) Treated with Sustained-Release Buprenorphine and Buprenorphine Hydrochloride, pp. 789-793**

Secondary Species: Guinea Pig (Cavia porcellus)

Domain 3 Research

SUMMARY: This study compared the pharmacology and efficacy of Bup-HCl and Bup-SR in guinea pigs. Females were randomized into two groups of 7. One group received Bup-HCl (0.05 mg/kg) twice daily for 60 h and the other group received one injection of Bup-SR at 0.3 mg/kg with sterile water at the same intervals as Bup-HCl. Paw withdrawal was tested using an RS electronic algesimeter. Plasma concentration of Bup-HCl decreased from 2331 pg/ml at 1h to 165 pg/ml by 12 h while Bup-SR remained therapeutic even after 48 h (429 pg/ml). Paw withdrawal of Bup-HCl was greatest at 674 g at 1 h and declined to 402 g at 6 h while Bup-SR levels were 555 g at 6 h and peaked at 680 g at 12 h. This significant difference may have indicated that Bup-HCl may require TID or even QID administration although the authors suggest further studies to determine the dose-response curve.

QUESTIONS

1. Buprenorphine is

a. Full agonist at the u opioid receptor and an antagonist at the k opioid receptor

b. Partial agonist at the u opioid receptor and an antagonist at the k opioid receptor

c.  Partial agonist at the u opioid receptor and an agonist at the k opioid receptor

d. Partial agonist at the u opioid receptor and an antagonist at the d opioid receptor

2. What is this device?



ANSWERS

1. b

2. Randall-Selitto electronic algesimeter from IITC Life Sciences

(<http://www.iitcinc.com/pdf/Digital%20Paw%20Pressure.pdf>) 

**Levin-Arama et al. Subcutaneous Compared with Intraperitoneal Ketamine-Xylazine for Anesthesia of Mice, pp. 794-800**

Domain 2; T3 - Administration of Anesthesia

Domain 3; K12- Refinement

Primary Species:  Mouse (Mus musculus)

SUMMARY: Authors compared anesthetic efficacy and adverse consequences of administering ketamine-xylazine either intraperitoneally (IP) or subcutaneously (SC). This study found that both routes were effective, and recommended considering SC administration as a refinement to IP administration. Female mice were: 1) more likely to die following IP administration and 2) less likely to reach effective surgical anesthesia with IP administration. Therefore the therapeutic margin in female mice for IP ketamine-xylazine may be narrow. Additionally, time to onset and duration of anesthesia varied by strain (strains tested included HSD:ICR, BALB/cO1aHsd, and C57BL/6JOlaHsd).

QUESTIONS

1.   How does ketamine prevent spinal sensitization (‘wind-up’)?

2. What is xylazine’s mechanism of action?

ANSWERS

1.  Ketamine inhibits N-methyl-D-aspartate (NMDA) receptors resulting in a pronounced analgesic effect.

2.  Xylazine is an alpha-2 adrenergic agonist that produces species-dependent dose-related CNS depression and has powerful sedative, hypnotic, and analgesic effects.

**Romagnoli et al. Constant-Rate Infusion of Dexmedetomidine to Manage Thiopental Anesthesia during Intracranial Surgery in Cynomolgus Macaques (*Macaca fascicularis*), pp. 801-804**

Primary Species: Macaques (Macaca spp.)

Domain 2; Task 2 - Minimize or Eliminate Pain and/or Distress

SUMMARY: The goal of this study was to evaluate the use of dexmedetomidine CRI as an adjunct to thiopental CRI during intracranial surgery in cynomolgus macaques. 5 healthy male cynomolgus were used. Initial sedation was performed using IM ketamine (8 mg/kg) and dexmedetomidine (0.02 mg/kg). Anesthesia was induced with thiopental (3 mg/kg IV) and maintained using CRIs of thiopental (3 mg/kg/h) and dexmedetomidine (0.012 mg/kg/h).  This combination provided adequate anesthesia and analgesia in all 5 animals; one animal required a higher dose of thiopental (8 mg/kg/h) to eliminate movement in response to surgical stimulation. Cardiovascular parameters remained within normal limits besides the dexmedetomidine-induced bradycadia (although systolic blood pressure remained normal throughout).

QUESTIONS

1.  What kind of drug is dexmedetomidine?

a.  Dissociative agent

b.   Benzodiazepene

c.  Alpha-2 adrenoreceptor agonist

d.  Opioid

e.  Neuroactive steroid

2.  In a recent study in cynomolgus macaques undergoing intracranial surgery, a constant rate infusion of dexmedetomidine was found to be effective alongside which anesthetic agent?

a. Isoflurane

b. Thiopental

c. Propofol

d.  Diazepam

e.   Alfaxalone

3.   Which is true regarding dexmedetomidine use in cynomolgus macaques?

a.  Does not provide analgesia, therefore, other agents must be used in combination

b.   Causes tachycardia

c.  Causes hypotension

d.  Cannot be reversed

e.   Reduces cerebral blood flow

ANSWERS

1. c

2. b

3. e

***Experimental Use***

**Jones et al. Evaluation of Mice Undergoing Serial Oral Gavage While Awake or Anesthetized, pp. 805-810**

Domain 3: Research

Primary Species: Mouse (Mus musculus)

SUMMARY: Oral gavage is the most straightforward approach to achieve precise enteric administration in rodents; however, it is associated with potential adverse consequences.  The authors compared the effects of serial oral gavage in awake compared with anesthetized mice.  Mice were assigned to 1 of 3 treatment groups (control, awake gavage, or anesthetized gavage) and gavaged daily with 0.2 ml of saline for a total of 18 treatment days.  Body weight and clinical appearance were monitored; endpoints evaluated included adrenal gland weight, plasma corticosterone, lymphocyte:neutrophil ratio, and esophageal histopathology.  Mean body weight did not differ between groups.  The awake gavage group had more mice removed prior to study completion due to body weight loss greater than 10% with corresponding gross and histopathologic lesions attributed to the procedure.  The awake gavage group had an over 20-fold higher incidence of incomplete saline retention than did the anesthetized group.  Esophageal inflammation was not apparent at necropsy regardless of treatment, with the exception of one mouse in the awake gavage group.  Although WBC and lymphocyte counts were lower in the anesthetized gavage group, none of the measured endpoints to evaluate stress differed.  The results support use of brief isoflurane anesthesia when performing serial oral gavage in mice.

QUESTIONS

1. Which of the following is a potential adverse consequence of oral gavage in rodents?

a. Esophageal trauma

b. Aspiration pneumonia

c. Weight loss

d. All of the above

2. Which of the following is a possible refinement to oral gavage?

a. Coating the gavage tip in a palatable solution

b. Using a flexible catheter instead of a rigid gavage needle

c. Use of brief inhalant anesthesia

d. All of the above

ANSWERS

1. d

2. d

**Fisher et al. Interstrain Differences in CO2-Induced Pulmonary Hemorrhage in Mice, pp. 811-815**

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

Primary Species: Mouse (Mus musculus)

SUMMARY: Carbon dioxide is the most commonly used gas for the euthanasia of rodents.  The current AVMA guidelines recommend slowly filling the container after the animals are placed inside as opposed to previous guidelines which recommended placing animals in a container that has been prefilled with CO2. This study was conducted to evaluate whether the air-displacement rate influenced the development in of pulmonary or nasal hemorrhage in two commonly used mouse strained.  The investigators also investigated the prevalence of pulmonary hemorrhage and nasal hemorrhage in mice euthanized with isoflurane overdose.

The study was conducted in 79 mice, both male and female, in two age groups (6 week old and 6 month old).  The euthanasia method were altered sequentially in the mice.  In addition, 7 male 6 month old mice were euthanized by an overdose of isoflurane.  Of the 23 BALB/c mice euthanized by the slow fill method, 21 had severe pulmonary hemorrhage as did 8 of the 19 BALB/c mice euthanized by the prefill method.  The prevalence of severe nasal hemorrhage in BALB/c mice did not differ between the two methods.  Of the 19 C57BL/6 mice euthanized by the slow fill method, 2 had severe pulmonary hemorrhage as did 1 of the 18 C57BL/6 mice euthanized by the prefill method. The prevalence of severe nasal hemorrhage in C57BL/6 mice did not differ between the slow fill and prefill methods.  None of the 6 month old BALB/c mice euthanized by isoflurane overdose had pulmonary or nasal hemorrhage.   The severity of hemorrhage did not differ according to mouse age or sex.

The slow fill and prefill methods induced significantly more severe pulmonary hemorrhage in BALB/c compared to C57BL/6 mice.   This study demonstrated that the method of euthanasia may need to be altered depending on the mouse strain used.

QUESTIONS

1. What are three advantages and three disadvantages of CO2 euthanasia?

2. What is the current recommended air displacement rate with CO2?

3. T/F. The filling rate of the chamber is based on the time required to rapidly and successfully render animals unconscious with limited aversive stimuli.

ANSWERS

1. Advantages:  easily accessible, inexpensive, does not cause toxic tissues residues

Disadvantages:  substantial and unpredictable differences in susceptibility according to species and age of the animal, requires a secondary method of euthanasia to ensure death, often results in pulmonary hemorrhage as a background lesion.

2. Chamber should be filled at a rate of 10%-30% per minute.

3. T

**Collymore et al. Lidocaine Hydrochloride Compared with MS222 for the Euthanasia of Zebrafish (*Danio rerio*), pp. 816-820**

Domain 2:  Management of Pain and Distress

Task 4 - Euthanasia

Secondary Species:  Zebrafish (Danio rerio)

SUMMARY: MS 222 is the most commonly used chemical for euthanasia of zebrafish in a laboratory setting.  However, its use involves potential safety concerns for humans and requires it be mixed freshly before each use.  Furthermore, it has sometimes been observed to be ineffective.  Both MS222 and lidocaine have similar mechanisms of action, blocking sodium ion channels.  Therefore, due to the ease of access and safety associated with handling lidocaine it was desirable to compare the efficacy and impact lidocaine euthanasia in zebrafish with MS222.

Groups of adult zebrafish were exposed to 250 mg/L MS222 or 400 mg/L, 500 mg/L, or 600 mg/L of lidocaine and observed for time to death, mortality rate, and display of aversive behaviors.  The effect of these compounds was not evaluated in larval zebrafish at this time.  Both MS222 and lidocaine were effective at causing fish death at all concentrations tested.  Fish exposed to 500 mg/L of lidocaine had a significantly longer time to loss of righting reflex as compared to other treatment groups, although this may have been due to differences in animal size.  Otherwise, lidocaine performed better than MS222 when evaluating time to death (loss of opercular movement) and the number of fish that displayed aversive behavior.  Therefore, lidocaine may be considered a superior alternative to MS222 for the euthanasia of adult zebrafish.

QUESTIONS

1.  What is the mechanism of action for both lidocaine and MS222?

2.  Are larval zebrafish more sensitive or less sensitive to the anesthetic effects of MS222?

ANSWERS

1.  Ion (sodium) channel blockers.  Prevents neuron depolarization.

2. Larval fish are less sensitive than adults to MS222.

**ACLAM POSITION STATMENTS**

**Turner et al. ACLAM Position Statement on Pain and Distress in Research Animals, p. 821**

Domain 2

SUMMARY: Appropriate measures must be taken to eliminate or reduce pain and distress in all research animals. ACLAM supports the premise that animals perceive pain in similar ways to humans. Personnel caring for and using research animals must be knowledgeable of species-specific and individual behavioral, physiologic, and psychologic indicators of well-being. The professional judgment of an experienced laboratory animal medicine veterinarian should be consulted for all experiments involving research animals. Procedures anticipated to cause more than slight or momentary pain require the use of analgesics and requests for exceptions must be approved by the IACUC. Any conditions that result in animal distress should be alleviated by pharmacologic or nonpharmacologic methods. All research involving pain or distress should be designed and conducted such that endpoints are quickly identified and the time to reach scientific objectives minimized.

QUESTIONS

1. What are examples of nonpharmacologic interventions?

2. What is the definition of pain?

3. What is the definition of distress?

ANSWERS

1. Examples include positive reinforcement training, provision of a comfortable environment, fluid therapy, thermal support, tasty food supplements, and habituation to procedures.

2. As an unpleasant sensory and emotional experience associated with potential or actual tissue damage.

3. As an aversive, negative state in which coping and adaptation processes in response to stressors fail to return an organism to physiological and/or psychological homeostasis. Distress is synonymous with suffering, and includes both physical and mental pain as well as negative emotional feelings such as fear.

**Foley et al. ACLAM Position Statement on Rodent Surgery, pp. 822-823**

Domain 1

SUMMARY: ACLAM recommends that institutions develop guidance documents on rodent surgery under the guidance of specialists and in line with the 3Rs.  These documents should be approved by the IACUC and should cover training, consulting with the AV, aseptic technique, anesthesia/analgesia, peri-operative care, and record keeping.

It is recommended that a formal training program be established, and post-approval monitoring be implemented to ensure practices are consistent with policy and protocols.  Appropriate veterinary care should be followed to minimize pain and distress according to regulations.  Aseptic technique must be used and should include instrument sterilization, skin prep, creation of a sterile field by use of drapes, surgeon prep, and disinfection of the surgical area.  Appropriate anesthesia, analgesia must be provided, as well as any other nursing care and treatments as appropriate.  The use of pre-emptive analgesics should be considered.

Following the guidelines in this document are essential for ensuring the health and well-being of rodents used as surgical models, as well as to safe-guard the validity and reproducibility of research results.

QUESTIONS

1.  At a minimum, what five aspects should be covered in an institution’s guidance documents on rodent surgery?

2. What are the recommended components of aseptic technique with rodent surgeries?

3. True or False: The “tips only” method of sterilization is no longer recommended for rodent surgeries.

ANSWERS

1.  (1) Qualifications training, (2) consulting with the AV, (3) aseptic technique, (4) anesthesia/analgesia, (5) peri-operative care, and (6) record keeping

2.   Include instrument sterilization, skin prep, creation of a sterile field by use of drapes, surgeon prep, and disinfection of the surgical area

3.  False

**Lloyd et al. ACLAM Position Statement on Reproducibility, pp. 824-825**

Domain 3

SUMMARY: Ideally, published studies should include methodological and procedural descriptions, environmental conditions and meta-information, which should be readily accessible and provided in sufficient detail to enable a knowledgeable and capable researcher to replicate experiments and achieve equivalent results. Numerous reports have identified multiple causes of lack of reproducibility, including faulty experimental design, inconsistent technique, missing or incomplete descriptions of experimental details, and a lack of insistence by funding agencies and peer-reviewed journals on well-established criteria for conduct and publication of good science. Consequently, a public and scientific crisis of confidence in the veracity and reliability of scientific discoveries, including but not limited to biomedical research, is occurring.

It is important to note that although characterized by observation and experimentation, advances in science greatly depend upon peer-based communication and evaluation to ensure that new information is analyzed, verified, and confirmed.

Humane laboratory animal care, including consistent, efficient and effective practices in husbandry, staff and investigator training, careful technique development and validation, and both preventive and clinical veterinary medicine, can enhance data reproducibility by providing scientists with quality animals and providing those animals with appropriate food, water, bedding, caging, and environmental and social conditions.

Experimental reproducibility is impacted by differences in laboratory animal use and institutional oversight. Animal studies should be designed in the spirit of the ARRIVE (Animals in Research: Reporting *In Vivo* Experiments) guidelines, thus encouraging the reporting of more detailed methodologies that will greatly aide other laboratories in their attempts to recreate the same model systems

Another critical influence on reproducibility, and one that is gaining both scientific and administrative attention, is the role of the microbiome in data variability and study outcomes.

ACLAM states it is incumbent on laboratory animal veterinarians and the scientific community to define elements of study design that affect experimental reproducibility. Scientific progress relies on rigor and reproducibility, particularly for advances made possible by comparative medical research with animals.

QUESTIONS (True or False)

1. With respect to reproducibility, scientist should include comprehensive descriptions of details of animal care and husbandry in their manuscripts and supplemental materials.

2. Experimental results that cannot be replicated and result in the use of additional animal subjects violate Russell and Burch’s Principles of the replacement of animals, reduction to the lowest numbers of animals needed, and inclusion of refinements for improved animal use practices.

ANSWERS

1. True. No detail of this sort should be deemed too small for sharing.

2. True

**Hankenson et al. ACLAM Position Statement on Adequate Veterinary Care, pp. 826-828**

SUMMARY:Adequate veterinary care is an integral component of humane animal care and use to ensure animal well-being, which is essential to reliability of results from investigations involving animals.

Educational Requirements and Competency

Veterinarians should have academic degrees with competency as measured by passage of national certification and clinical competency exams or comparable foreign assessments.  Advanced training may be necessary to gain expertise by postdoctoral training programs or extensive instruction in the field with mentored guidance.  High-level competence is best indicated by board-certification and participation in LAM continuing education.

Authority of the Veterinarian

Key aspects of veterinary authority include:

1)  Oversight to ensure the program meets applicable standards

2) Knowledge of the current and proposed use of animals at the institution

3)  Application of appropriate treatment or control measures

4)   Consultation with researchers

5)   Selection of appropriate agents to alleviate pain or distress

Complexity of the Veterinary Care Program

Veterinary care program specifics depend on several factors.  In all situations, arrangements for provision of veterinary oversight and medical care must be made with a minimum of regularly scheduled visits and readily available veterinary services.

Considerations for Adequate Veterinary Care

Primary areas of responsibility for adequate veterinary care include:

1) Animal procurement and transportation – acquired lawfully and evaluated for suitability of use with transportation in accordance with applicable laws/regulations

2)   Acclimation – health assessment, recovery from shipment, and time to adapt to the new environment; quarantine procedures may be needed

3)   Preventative medicine – processes to protect animals from exposure to disease; disease surveillance to monitor for overt or in apparent disease

4)  Animal well-being and clinical care – adherence to refinement, and evaluation for all aspects of animal’s wellbeing; requires daily observations of animals by qualified personnel with timely and accurate notification to veterinarian; prevention/treatment of noninfectious diseases that may disrupt research or adversely impact animal health/well-being; access to diagnostic services

5)  Surgical procedures

a.  Institutional responsibility: ensure adequate facilities for surgical procedures and adequate training/competency of staff (may be delegated to veterinarian or IACUC)

b.   Veterinarian responsibility: assess adequacy of surgical monitoring; recommendations for pre-surgical procedures, surgical techniques, personnel qualifications; and provision of peri-operative care

6)  Anesthesia, analgesia, and euthanasia – provision of written recommendations for anesthetics, analgesics, sedatives, and euthanasia practices in compliance with AVMA Guidelines for the euthanasia of animals for all species; should be reviewed/revised periodically

Adherence to Regulations

At least one veterinarian must be a voting member of the IACUC and be actively involved in review of proposed animal care and use regardless of USDA humane use categorization and species selection.

QUESTIONS

1. True/False. Diagnostic laboratory services should be available within the animal facility

2. All veterinarians are expected to adhere to a progressive code of ethical conduct known as what?

a.  Principles of Animal Welfare

b. Priorities of Animal Well-being

c.  Principles of Animal Well-being

d.  Priorities of Veterinary Medical Ethics

e.  Principles of Veterinary Medical Ethics

ANSWERS

1. False. It is not necessary for diagnostic laboratory services be available within the animal facility if laboratories with appropriate capabilities are available and utilized

2. e. Principles of Veterinary Medical Ethics (promulgated by the American Veterinary Medical Association)