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**OVERVIEW**

**Denommé and Mason.** [**Social Buffering as a Tool for Improving Rodent Welfare**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00001)**, pp. 5-14**

Domain 4: Animal Care

Primary Species: Mouse (Mus musculus)

**SUMMARY:**This paper discusses how “social buffering”, or the presence of an affiliative companion to an animal at the time of stress, can overall improve animal welfare. The responses to buffering vary depending on species/strain/stock, sex, life stage, exposure to conditioned versus unconditioned stimulus, among other factors. Two subtypes of social buffering exist (that are not mutually exclusive): 1) ‘housing type’ in which animals are co-housed prior to and after, but go through the aversive stimulus alone, or 2) ‘exposure type’ in which animals are exposed to a companion during the aversive stimulus. Social buffering can be modified. For more social species, there are dose-dependent effects of both exposure and housing type buffering with regards to number of companions, and duration/timing of social housing, respectively. The extent of exposure (full contact versus sensory cues) may differ in buffering effects. Familiarity and bonding, as well as the affective state of the companion can influence the extent of buffering. In general, calm induces calm, or vice-versa, in an effect called “emotional contagion” – except in male mice, possibly. The authors provide practical recommendations on applying social buffering in the lab for rodents (see figure). Best practices would include high levels of both social and physical enrichment for maximum resilience to stressful procedures; especially for animals in categories D or E. The companions should be non-stressed, ideally attached individuals that may need to be rotated due to negative emotional contagion. If full contact is unavailable, less effective alternatives include offering certain conspecific sensory cues, or human proxies such as rat tickling.



**QUESTIONS**

1.  Which of the following have been shown to decrease male-male aggression in mice?

a.  Moving soiled bedding at cage changing

b.   Moving used nesting at cage changing

c.   Adding running-wheel igloos to the cage

d.   a and c

e.   b and c

f.    All of the above have been shown to decrease aggression

2.  What is a conditioned stimulus in the context of fear/stress?

**ANSWERS**

1.   b. Moving used nesting at cage cleaning

2.  Use of repeated exposure to a cue that is paired with a negative reinforcement (i.e., shock). The cue becomes a conditioned stimulus that induces fear/stress with or without the negative reinforcement.

**Hedenqvist et al.**[**Toward Global Harmonization of Training and Certification of Specialists in Laboratory Animal Veterinary Medicine**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00002)**, pp. 15-20**

Domain 6: Education

SUMMARY: International Association of Colleges of LAM (IACLAM) was founded in 2005 to improve communication, exchange of information, and globally define the roles of veterinarians in LAM which has been a specialty for over 60 years. There are 5 colleges of LAM (American,  European, Japanese,  Korean,  Indian) which are members of IACLAM. LAM is a broad field and covers many species not covered in general veterinary curriculum. In 2018, IACLAM reviewed training and certification requirements of the colleges. April 2020 an agreement was reached between ICLAM and the 4 colleges for minimal knowledge and skills required for a LAM Diplomate.

Each college has developed its own similar role delineation documents (RDDs) that specify minimal knowledge and skills required for LAM diplomates. All require knowledge of laws,  regulations,  and guidelines. All 5 colleges agree mouse, rat, rabbit are primary species and goats,  sheep,  Syrian hamsters,  and zebrafish are secondary. ICLAM views macaques as secondary and guinea pigs as primary though the other 4 colleges view macaques as primary and guinea pigs as secondary. ICLAM views pigs as tertiary whereas the other colleges consider them secondary.

ECLAM is least similar to the other 4 colleges because it follows the European Directive and emphasizes the 3Rs and animal welfare. IACLAM aims to harmonize recommendations for training and certification by reducing variation versus standardization which tries to eliminate variation.

QUESTIONS

1. What was the first LAM college to establish an RDD?

a. ACLAM

b. ECLAM

c. ICLAM

d. JCLAM

e. KCLAM

2. Which 3 colleges expect more knowledge from veterinary school related to laboratory animal species?

a. ACLAM

b. ECLAM

c. ICLAM

d. JCLAM

e. KCLAM

3. Which colleges consider reptiles a tertiary species?

a. ACLAM

b. ECLAM

c. ICLAM

d. JCLAM

e. KCLAM

ANSWERS

1. a

2. c, d, e

3. a, b, c

**ORIGINAL RESEARCH**

***Biology***

**Dimitrakakis et al.** [**Biochemical and Hematologic Reference Intervals for Anesthetized, Female, Juvenile Yorkshire Swine**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00003)**, pp. 21-30**

Domain 1

Primary Species: Pig (*Sus Scrofa*)

SUMMARY:  There are several uses for swine in a laboratory setting. Particularly for juvenile females. The authors set out to define reference intervals for the species using 120 values from separate individuals as recommended by the National Committee for Clinical Laboratory Standards Institute and American Society for Veterinary Clinical Pathology. The authors used a combination of arterial hematology and blood chemistry in Yorkshire swine and compared the results to published references in baboons and humans.

The authors found that:

1. Statistical comparison of arterial and venous hematologic values showed that only the platelet count was significantly higher in venous samples than in arterial samples.
2. Phosphorous, Na+, total protein, and globulin values were significantly higher in venous samples than in arterial samples.
3. K+ was significantly lower in venous than arterial samples.
4. All swine hematologic reference intervals were significantly different from those for humans and baboon except for platelets, for which swine and human values were similar.
5. All swine biochemical reference intervals were significantly different from those for both humans and baboon except for glucose, for which swine and baboons were similar.

The authors were able to generate reference intervals for 11 hematologic and 14 biochemical parameters and suggest that research involving swine should be done knowing that there are some biochemical and hematologic differences between other species. It is important to note that animals used in this study were on IV fluids, under anesthesia, and ventilated. Therefore, some blood gas values, and renal values may be altered compared to unanesthetized awake pigs.

QUESTIONS

1. T/F: During swine intubation the lateral folds can be easily traumatized/ruptured, and the tube passed into the subcutaneous tissues if forced into the airway.
2. T/F: The platelet values for Yorkshire pigs are similar to the published human reference ranges.
3. Which biochemical reference range do swine and baboons share?
	1. BUN
	2. Potassium
	3. Chloride
	4. Glucose

ANSWERS

1. True
2. True
3. d. Glucose

***Husbandry***

**Stover and Villano.** [**Evaluation of Various IVC Systems According to Mouse Reproductive Performance and Husbandry and Environmental Parameters**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00004)**, pp. 31-41**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Mice are frequently used models for human and animal biomedical research.  Appropriate housing is essential for animal well-being, high-quality research, and personnel health and safety.  There are several different caging choices for mice including static microisolators and individually ventilated cages (IVC).  The latter are becoming more common in mouse research facilities due to their ability to improve husbandry practices, reduce potential exposure of mice to mouse pathogens, reduce allergen exposure in personnel, and biocontainment use.   There is literature suggesting that mouse reproductive performance, husbandry, and environmental parameters are affected by IVC rack systems.  The current study concurrently evaluated mouse reproductive performance, husbandry, and environmental parameters in three different high density IVC rack systems (RS1, RS2, and RS3).  The study used three breeding trios each of Swiss Webster (CFW) and BALB/c mice.  The reproductive parameters evaluated were time to parturition, litter size and pup weight, survivability, and interbirth interval (measured for 3 breeding cycles over 2 generations).  The husbandry parameters evaluated were cage dirtiness, need for spot changing, ease of cage changing, daily health checks, and cage wash processing (evaluated over 18 weeks).  The macroenvironmental parameters evaluated were temperature, relative humidity, noise, and total particulate matter (measured weekly over 14 weeks).  The microenvironmental parameters evaluated were temperature, relative humidity, NH3, CO2, and O2(2 cages each of male and female CFW mice at 6 time points over 2 weeks).  Notable findings included: RS1 had significantly smaller mean litter sizes of CFW mice compared to the other two racks; RS1 was scored as significantly easier to process through cage wash; and RS2 had significantly lower room noise levels but higher humidity (2-week cycle, 8 and 12 days after cage change).  The three high density rack systems evaluated each had different advantages but overall, all were appropriate for housing the mice in this study.  The authors suggest that institutions evaluate multiple factors when considering implementing mouse IVC in their facilities.

QUESTIONS

1. Which of the following is TRUE about IVC?

a. They often differ in ventilation strategies, structural design, and housing capacity

b. Negative pressure ventilation is effective for reducing allergen exposure and biocontainment

c. Negative pressure is advisable for maintaining SPF colonies

d. All of the above are true

e. All but c are true

2. Swiss Webster (CFW) mice are \_\_\_\_\_\_\_\_ and BALB/c mice are \_\_\_\_\_\_\_.

a. Inbred, outbred

b. Outbred, inbred

c. Both are outbred

d. Both are inbred

3. What is the NIOSH recommended exposure limit to minimize occupational induced hearing loss?

a. 85 dBA as an 8-h time-weighted average

b. 90 dBA as an 8-h time-weighted average

c. 95 dBA as an 8-h time-weighted average

d. 100 dBA as an 8-h time-weighted average

4. True or False: A sound meter measuring dBA is relevant for human and mouse noise exposure.

5. What is the OSHA permissible exposure limit for carbon dioxide?

a. 2000 ppm by volume (0.5% concentration) as an 8-h time-weighted average

b. 3000 ppm by volume (0.5% concentration) as an 8-h time-weighted average

c. 4000 ppm by volume (0.5% concentration) as an 8-h time-weighted average

d. 5000 ppm by volume (0.5% concentration) as an 8-h time-weighted average

ANSWERS

1. e; positive pressure for SPF colonies

2. b

3. a

4. False; would need one that measures ultrasonic noise (above 20 kHz)

5. d

**Merley et al.** [**Behavioral and Physiologic Effects of Dirty Bedding Exposure in Female ICR Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00005)**, pp. 42-51**

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Rodent health surveillance monitoring is routinely conducted by utilizing sentinel animals in a laboratory animal vivarium. Dirty-bedding sentinels are regularly exposed to soiled bedding from colony animals to transfer and detect excluded microorganisms in the resident population. However, dirty bedding can also contain other elements, such as pheromones and ammonia, which may act as a source of stress for sentinel mice. The goal of this study was to determine whether acute or chronic dirty bedding exposure causes behavioral and/or physiologic changes consistent with stress to better understand the experience of a sentinel mouse. Therefore, the objective of this current study was to evaluate the behavioral and physiologic effects of exposure to dirty bedding on sentinel mice.

Female ICR mice exposed to pooled dirty bedding from breeding and experimental colony mice were compared with control mice exposed only to clean bedding. Behavioral parameters measured included home cage evaluation of hair coat and behavior (nest building, stereotypical behavior) and behavioral tasks (light-dark box, elevated plus maze). Neutrophil:lymphocyte ratio (NLR) and weight were evaluated as physiologic measurements of stress. The estrous cycle stage was determined at the end of the experiment and analyzed as a covariate. The effects of exposure to dirty bedding were evaluated at 2 time points: 24 h (acute) and 4 wk. (chronic). The hypothesis was that exposure to dirty bedding would result in behavioral and physiologic changes consistent with stress at both time points.

No significant differences in home cage behavior (hair coat, nest score, stereotypical behavior), behavioral tests (light-dark box and elevated plus maze), or NLR were detected between the mice exposed to dirty or clean bedding in the acute or chronic groups. However, the mice exposed to dirty bedding in the chronic group weighed significantly less at days 21 and 28 as compared with the control mice. The chronic dirty bedding mice also had a significantly lower net weight gain over the course of the study.

QUESTIONS

1.  What are the 5 behavioral and physiologic parameters measured in the acute and chronic groups treated with control or dirty bedding in the current study?

* + 1. Nest Scores, Light-Dark Box, Elevated Plus Maze, Neutrophil: lymphocyte ratio (NLR), Weight
		2. Estrous cycle, Glucocorticoids conc, Elevated Plus Maze, Weight, Neutrophil: lymphocyte ratio (NLR)
		3. Nest Scores, running wheel, Morris Water Maze, Neutrophil: lymphocyte ratio (NLR), Weight
		4. Weight, Nest Scores, Light-Dark Box, Elevated Plus Maze, Balance beams

2.  Among the behavioral and physiologic parameters monitored in the current study, which parameter was significantly affected in the chronic group (Dirty bedding animals vs control animals)?

a. Estrous Cycle

b. NLR

c. Weight

d. Nest Scores

ANSWERS

1. a

2. c

**Geyer et al. Establishing and Maintaining an Etruscan Shrew Colony, pp. 52-60**

Domain 4: Animal Care

Tertiary Species: Other Rodents

SUMMARY

Background:The Etruscan Shrew (white-toothed dwarf shrew) are one of the smallest known mammalian species (2-3g in body weight). They have high metabolic rates and HR/RR rates are 1500/900 respectively. These unusual metabolic characteristics and their seasonal variations in cell architecture and neuronal activity make them interesting in neuroscience research.

Housing: Communication via ultrasonic vocalization. Must remove artificial ultrasonic noises and other animals communicating in the ultrasonic range (rats) from the husbandry environment. Housed in light-colored or slightly transparent plastic or glass containers. 1800cm2 is required for breeding pairs. Great jumpers and climbers and can squeeze through gaps only a few millimeter in width. Cage height needs to be 25cm and need stainless steel mesh (2-3mm) with shrew proof cover.

Bedding: Autoclaved soil-sand-mixture 4-6cm deep to allow for burrowing. Enrichment with moss, wood, or bark. Areas of soiled bedding should be replaced weekly with complete substrate change every month. All dead feeder insects must be removed prior to next live feeding to prevent mites. No conventional rodent bedding recommended. Three hideouts per animal in cage including bark, flat stones, brick fragments, small clay flowerpots.

Breeding Cage: They are monogamous breeders that live in family groups and require specific breeding conditions including cave stones, temperature, and light cycles. Porous stones of plaster blocks with a labyrinth of paths and caves are needed for rearing young. Path should orient toward transparent part of cage to allow for observations. One breeding pair per cage and after weaning 3-5 littermates separated by sex can be housed together. Single housing is not recommended. One pup litters can be housed with other pups of the same sex. Breed year round. Gestation period is 27-28 days and have 2-6 young per litter. Can determine sex at 23 days (weaning). Females can get pregnant immediately after giving birth (can have a new litter in 4 weeks) and new mating pairs can be made when the males are 12 weeks and the females are 16 weeks. First litter produces the most progeny and fertility decreases after 1 year.

Light, Temperature, and Humidity: 12:12h light dark cycle with light intensity between 50 to 300lx. Temp between 19-24 C. Keep consistent through the year with no change for the breeding animals. Humidity is between 45-60%.

Behavior and Handling: Circadian activity pattern with frequent bursts of activity over a 24hr period. Sleep for longer periods of time in the day and cannot maintain their high metabolic rate, thus body temp, vegetative, and sensory functions are reduced during sleep, i.e. They can look ill or dead. Afternoon is the best time for observations. Food shortage or low temps can cause them to go into Torpor which is similar yet different than hibernation. Quickly become active to hunt and kill when live prey is introduced. Unknown if saliva of this shrew is venomous or not. Best to handle several hours before or after eating via the tail root using soft silicone padded tweezers. Hand restraint is not possible due to small size and speed of movement.

Prey Species: Live feed with mealworms or crickets from a specialized vendor to avoid contamination with other small mammals. Can eat (8-12 crickets) 6 times their body weight in a day. Prefer live prey over frozen. Cannot feed dog or cat food. Aged animals (>2years) need supplementation with meal beetle larvae. Recommended to dust or gut load insects to ensure proper nutrition (calcium, thiamine, omega 3 fatty acids, vitamins).

Water: Shallow bowl in bottom of cage and a water bottle. Water needs median concentration of 2.52mmol/l calcium and 0.47mmol/l magnesium ions.

Behavioral and Animal Welfare Monitoring: Exploratory, hiding, startling behavior, and escape behavior are all normal. Absence of foraging or escape when manipulating the cage is abnormal and chasing behaviors should be monitored. Aged shrews (>30month) move slower and under groom. Shrews can die suddenly with no evidence of disease due to their high metabolic rate.

Environmental and Microbiological Monitoring:Shrews can be screened for some mouse pathogens via PCR testing but not for ELSIA assays. Recommended to have sentinel mice for the shrew colony. Mice some of the shrew bedding into the cage with the mice and then test those mice for any pathogens.

Natural Life Expectancy and Size: Median life span was 454 days but can live up to 1192 days. Avg torso length was 4.6-4.8cm with females being slightly larger.

QUESTIONS

1. What is the scientific name of the Etruscan Shrew?

a. *Blarina brevicauda*

b. *Sorex araneus*

c.  *Suncus etruscus*

d.  *Sorex vagrans*

2. What is the gestation period for the Etruscan shrew

a. 3 weeks

b.  4 weeks

c.  5 weeks

d.  6 weeks

3. What is the correct temperature and humidity for the Etruscan shrew?

a.  15-20°C and 35-45%

b.  19-24°C and 35-45%

c. 15-20°C and 45-60%

d. 19-24°C and 45-60%

ANSWERS

1.  a.  *Blarina brevicauda* – Northern short tailed Shrew

 b.  *Sorex araneus* – Eurasian/common shrew

c.  *Suncus etruscus* – Etruscan Shrew

 d.  *Sorex vagrans* – American vagrant shrew

2.   b. 4 weeks

3.   d.   19-24°C and 45-60%

**Carlson et al.** [**Assessing Elimination of Mouse Kidney Parvovirus from Cages by Mechanical Washing**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00007)**, pp. 61-66**

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

Domain 4: Animal Care

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Mouse kidney parvovirus (MKPV) and murine chaphamaparvovirus (MuCPV) are individual viruses within the new parvovirus genus *Chaphamaparvovirus*, and the reported prevalence of these viruses in academic mouse colonies is as high as 9.4% in immunocompromised mice and 10.9% in immunocompetent mice.  MKPV is capable of causing significant clinical disease in severely immunocompromised strains of mice, characterized by inclusion body nephropathy that can result in renal insufficiency and mortality. Immunocompetent mice can also become infected and persistently shed virus, although infection remains subclinical and renal pathology is mild. This is significant because MKPV and MuCPV infections in immunocompetent mice are often subclinical and can result in asymptomatic carriers within colonies that can persistently shed virus in their urine. Due to the subclinical nature of infection in immunocompetent mice, the presence of MKPV in immunocompetent colonies can go undetected without routine surveillance screening, but the potential for MKPV to cause significant pathology in immunocompromised mice suggest it is an agent that should be excluded.  Part of that exclusion process requires understanding how spread of MKPV might be interrupted by sanitation practices, et cetera.  Mammalian parvoviruses are unenveloped and, in general, are environmentally stable and resist inactivation by exposure to elevated temperatures, desiccation, and multiple classes of disinfectants.  Evidence suggests that mouse parvovirus (MPV) has thermal resistance and can be transmitted by fomite between cages, although standard cage wash procedures decrease the level of infectious MPV sufficient to reduce transmission of infection.  While genetically distinct from other parvoviruses, MKPV, as a related virus, may have similar thermal resistance, yet little is known about MKPVs infectivity and environmental stability.  This study evaluated whether fomite (cage) transmission of MKPV occurs, and if it does, whether mechanical washing of MKPV-contaminated caging is adequate to prevent fomite transmission to naïve mice or whether the additional step of autoclaving after washing is also necessary to eliminate MKPV on animal caging and prevent transmission.

Using a cohort of MKPV-infected mice, the authors created MKPV contaminated cages (confirmed by PCR) that were used to house 60 naive CD1 mice (4 mice per cage) assigned to 1 of 3 treatment groups, with 20 mice in each group:

1. Control (bedding change only)

2. Sanitization in a tunnel washer (88°C final rinse for 20 s)

3. Sanitization in a tunnel washer followed by autoclave sterilization (121°C for 20 min)

When creating the MKPV contaminated cages, the authors discovered that soiled cages were significantly more likely to test positive by cage swab PCR when the MKPV-inoculated source mice were at a later stage of infection (i.e., 20 weeks). Each group of naive CD1 mice in the three treatment groups was housed in a PCR positive MKPV cage for two, 1 wk. period, with mice harvested at 12, 17, and 20 wk. after the first exposure so that their renal tissue could be evaluated for MKPV infection by PCR.  While MKPV was detected by PCR on the surface of 63% of the pretreatment cages, none of the mice from cages treated by cage wash alone or cage wash and autoclaving tested positive for MKPV. In contrast, most (7 out of 10) mice housed in cages without treatment tested positive by 20 wk. after exposure.  The key revelation of this study is that none of the mice housed in cages sanitized in a tunnel washer with or without sterilization tested positive for MKPV at any time point.  As the author’s institution does not use chemical detergents during the cage wash process, these results suggests that the physical removal of material on the cage surface with pressurized water, as well as the exposure to high temperatures employed during the cage wash process were adequate to prevent transmission of MKPV in caging without the need to use chemical detergents or autoclaving.  It was unclear whether the disruption in MKPV transmission in this study was due to thermal inactivation of the virus, physical removal of the virus by washing, or both, but the absence of any detectable viral nucleic acid on cage surfaces in post-treatment testing suggests that physical removal of the virus from the surface of the cage occurred.  The authors conclude that their results indicate MKPV contaminated caging is a famine that can result in MKPV infection of mice, and the use of a tunnel washer at the temperature and duration evaluated -without the use of chemicals or detergents- was sufficient to remove MKPV nucleic acid and prevent MKPV transmission.

QUESTIONS:

1. Mouse kidney parvovirus (MKPV) is a newly identified parvovirus that causes inclusion body nephropathy in severely immunocompromised mice and is prevalent in research mouse colonies… what genus does it belong to?

a.  *Amdoparvovirus*

b.  *Chaphamaparvovirus*

c.  *Dependoparvovirus*

d.  *Erythroparvovirus*

e.  *Protoparvovirus*

2. What pathognomonic lesion is caused with mouse kidney parvovirus (MKPV) infection in severely immunocompromised mice?

a. Viral enteritis

b. Immune dysfunctions

c. Inclusion body nephropathy

d. Viral myocarditis

e. Reproductive failure and abortion

3. The reported prevalence of mouse kidney parvovirus (MKPV) and murine chaphamaparvovirus (MuCPV) in immunocompetent mice is only 10.9%, and infection causes no significant illness or pathology… why is this still significant?

4. What percentage of study mice housed in untreated cages contained a mouse positive for MKPV by 20 wk. after exposure?

a. 60%

b. 70%

c. 80%

d. 90%

e. 100%

5. T/F: In this study, none of the mice housed in cages sanitized in a tunnel washer with or without sterilization tested positive for MKPV at any time point.

6. T/F: This study indicates that MKPV contaminated caging can result in MKPV infection of mice, and the use of a tunnel washer at the temperature and duration evaluated was sufficient to remove MKPV nucleic acid and prevent MKPV transmission.

ANSWERS

1. b. Chaphamaparvovirus

2. c. Inclusion body nephropathy

3. c. 10.9%

4. MKPV and MuCPV infections in immunocompetent mice are often subclinical, resulting in asymptomatic carriers within colonies that can persistently shed virus in their urine and -potentially- spread to immunocompromised mouse colonies

5. True

6. True

**Wooddell et al.** [**Sex Differences in Hierarchical Stability in a Formation of a Mixed-sex Group of Rhesus Macaques**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00008)**, pp. 67-74**

**Domain 4: Animal Care**

**Primary Species:** Macaques (*Macaca spp.*)

SUMMARY: Social group housing for rhesus macaques is common for breeding, housing arrangements and research purpose, yet trauma and aggression can be a problem. Goal of this study aims to improve the group formation to minimize trauma and maintain animals’ welfare and costs of care. Specifically, this study examines whether female and male rhesus macaques maintain their established same-sex hierarchies during group formation when an established group of females was introduced to an established group of males. Over 2+ year period (2017-2019), 129 adult females were observed for intensity of aggression, initiator and recipients and status interactions). Over 3 year period (2016-2019), 14 males (2 excluded due to health and social reasons) were placed in corncrib structures and were monitored for hierarchical displacements. In 2019, the 12 males were placed in novel outdoor enclosure and 1 week later the females were introduced (during annual mating season to increase likelihood that they would accept the males). The female introductions occurred over a 3 week period (1-4 adult females per day) and introduced in order of dominance rank. Group was monitored by two observers for male-male and female-female aggression and status over about 5months. Weights and statistical analyses used to assess associations and dominance ranks. For the females in their natal group, hierarchy was highly stable over time, since females inherited their maternal ranks. For the males in the natal group, matrilineal rank predicted dominance rank which was stable over time. After the mixed group formation, male hierarchy was stable for 3 weeks. After 20 days, the alpha male was hospitalized for a shoulder laceration, though did not fall in dominance rank, when returned. However, ranks changed increased during the alpha male’s absence, with male-male traumas occurring and resulting in instability for the remaining of the study. Therefore, findings suggest that females are likely to maintain social stability than males, and previous social stability cannot guarantee future social stability for males.

QUESTIONS (True or False)

1. Automated feeders and RFIS tracking can be used to continuously monitor and detect instability in groups.
2. Introducing females to males in order of dominance rank does not promote social stability nor affect rank in rhesus macaques.

ANSWERS

1. True
2. False

***Analgesia***

**Kendall et al.** [**Toxic Effects of High-dose Meloxicam and Carprofen on Female CD1 Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00009)**, pp. 75-80**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: NSAIDs such as carprofen and meloxicam are frequently used as post-operative analgesics across laboratory species. In mice, meloxicam is normally dosed at 0.2-1 mg/kg SID and carprofen at 5-10 mg/kg BID. However, there is pharmacokinetic evidence that these doses are inadequate. Meloxicam at 1 m/kg SC remains at therapeutic levels for 4-8h in mice, and carprofen at 5 mg/kg SC remains at therapeutic levels for 8-12h.  Higher doses would likely provide longer analgesic action, but NSAIDs can also cause gastrointestinal or renal damage. In this study, female CD1 mice were given 20 mg/kg of meloxicam or carprofen (or equivalent volume of saline) SID for 3 or 7 days. Mice were either euthanized the day after the last dose or one week after cessation of dosing. There were no significant findings in serum chemistry across groups, but meloxicam groups had a higher incidence of fecal occult blood when tested the day after 3 or 7 days of treatment. That effect was resolved by 15 days. 20 mg/kg meloxicam causes gastric toxicity in mice over 3-7 days of treatment and should be used with caution, but carprofen at 20 mg/kg appears to have minimal toxic effects.

QUESTIONS

1. Based on this study, which NSAID is preferred for 3 days of treatment at 20 mg/kg in mice?
2. Meloxicam
3. Carprofen
4. How long does 1 mg/kg meloxicam normally stay above therapeutic threshold in mice?
	1. 2-4 h
	2. 4-8h
	3. 8-12h
	4. 12-24h
5. Which two organs are generally of concern for NSAID toxicity?
6. Liver
7. Kidney
8. Brain
9. Heart
10. Stomach

ANSWERS

1. b
2. b
3. b, e

**Alamaw et al.** **Extended-release Buprenorphine, an FDAindexed Analgesic, Attenuates Mechanical Hypersensitivity in Rats (Rattus norvegicus), pp. 81-88**

Domain 4:  Animal Care

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Sustained release buprenorphine (Bup-SR) is widely used for pain management in laboratory rodents as it provides a sustained period of analgesia without the need for repeated dosing and restraint.  Buprenorphine XR, an extended-release buprenorphine, has recently become available as a pharmaceutical-grade, c-GMP compliant, and FDA-indexed post-operative analgesia for use in laboratory rodents.  Seventy 3-month-old male Sprague Dawley rats underwent paw incisional surgery then were tested for both mechanical and thermal hypersensitivity after receiving either sustained release buprenorphine (1.2 mg/kg SC), low dose extended release buprenorphine (0.65 mg/kg SC), high dose extended release buprenorphine (1.3 mg/kg SC), or 0.9% saline (5 ml/kg SC).  Mechanical hypersensitivity was defined as an increase in the frequency of paw withdrawals following application of an 8g von Frey filament to the plantar surface of both the ipsilateral and contralateral hind paws at random locations.  Thermal sensitivity was defined as a significant decrease in paw withdrawal latency after the onset of focal thermal stimuli using a plantar analgesia meter.  Thirty-nine rats were chosen from the larger group to determine plasma drug concentration levels at various time points after drug administration.  Body weights in all groups did not significantly differ throughout the study.  Low dose extended-release buprenorphine effectively attenuated mechanical hypersensitivity for at least 3 days.  The high dose extended-release buprenorphine did not attenuate hypersensitivity more effectively than the low dose. Neither dose of extended-release buprenorphine or the label dose of sustained-release buprenorphine attenuated thermal hypersensitivity.  A buprenorphine plasma concentration of approximately 1 ng/ml was determined to effectively attenuate mechanical but not thermal hypersensitivity for rats in an incisional pain model.

QUESTIONS

1. Which of the following behavioral methods measures thermal hypersensitivity in rodents ?
	1. Von Frey filaments
	2. Randall Selitto test
	3. Hargreaves test
	4. Acetone evaporation test
2. Which of the following behavioral methods is depicted in the picture below?



* 1. Von Frey filaments
	2. Randall Selitto test
	3. Hargreaves test
	4. Acetone evaporation test
1. Which of the following drugs is lipid-bound and suspended in medium chain fatty acid triglyceride oil that is degraded over time with lipase and esterase activity?
	1. Sustained-release buprenorphine (Bup-SR)
	2. Extended-release buprenorphine (XR)
	3. Simbadol
	4. Buprenorphine HCl
2. Which of the following has NOT been observed as a side effect of buprenorphine administration?
	1. Decreased body weight
	2. Emesis
	3. Respiratory depression
	4. Pica
	5. Gastrointestinal tract motility problems

ANSWERS

1. c
2. a
3. b
4. b

***Experimental Use***

**Martins et al.** [**Comparison of Gelatin Flavors for Oral Dosing of C57BL/6J and FVB/N Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00011)**, pp. 89-95**

Domain2: Management of Pain and Distress and 3-Reserach

Primary Species:Mouse (*Mus musculus*)

SUMMARY: Intragastric gavage is typically used to facilitate oral dosing in rodents. This technique is typically performed in awake animals requiring significant handling which can lead to stress and potentially confound study results. Additionally, if the procedure is performed incorrectly, oral gavage may result in serious injuries such as gastric distention, aspiration pneumonia, esophageal and gastric rupture, and death. The current study aimed to evaluate an alternate method of oral dosing using gelatin vehicles. The authors investigated the time taken by two different inbred strains of mice to ingest sugar-free gelatin pellets of varying flavors (unflavored, strawberry, lemon, or diet-flavored).

Male C57BL/6J and FVB/N mice were fasted for 12 hours prior to being fed a gelatin pellet. Each animal was removed from their home cage, placed in a barren cage, and allowed 60 minutes to consume a single gelatin pellet. No prior training or adaptation was used. On the following days, for a total of 8 days, the same procedures were followed; however, the mice were not fasted on subsequent days. Spillage was not taken into account when calculating food and water consumption.

The authors found that C57BL/6J mice ate the unflavored, strawberry and diet-flavored pellets faster than the FVB/N mice. On average, the C57BL/6J mice took 3 minutes to eat the entire pellet. Both strains showed very little interest in the lemon-flavored pellet. The authors conclude that this method of oral administration offers an alternative to the gavage technique.

QUESTIONS

1. Which of the following is a characteristic of the FVB/N mouse?
	1. Albino mouse with large pronuclei ideal for pronuclear injection
	2. White-bellied agouti mouse
	3. Hybrid animal used for pronuclear injection
	4. Poor mothers; used to create ESC lines
2. What is the daily water intake for a mouse?
	1. 1-3 mL/day
	2. 3-5 mL/day
	3. 5-6 mL/day
	4. 6-7 mL/day
3. What is the daily food intake for a mouse?
	1. 1-2 g/day
	2. 2-3 g/day
	3. 3-5 g/day
	4. 5-6 g/day

ANSWERS

1. a
2. d
3. c

**Nicolis et al.** [**Performance and Consistency of Circulating Warm Water Blankets for Rodents**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00012)**, pp. 96-100**

SUMMARY: Providing supplemental thermal support during anesthesia is the primary method of prevent hypothermia. Hypothermia can have negative effects on the cardiac system and the body’s ability to metabolize anesthetic drugs. Providing supplemental thermal support during anesthesia is the primary method of preventing hypothermia. This is typically done with a circulating warm water blanket (CWWB) or a forced air system. This study looked a controlled assessment of a heat therapy system with a CWWB in order to establish a SOP to ensure the equipment would provide an effective and consistent support to lab rodents.  The experiment used 6 Gaymar T/P-500 circulating hot water pumps and a single new MuloToPad water blanket. A calibrated LaserGRip1022 Infrared Thermometer was used to measure the surface temp of the CWWB. Each pump was set up at 30C and let to circulate for 1 hour, then for the experiment the pumps were set to 42C. Temps were measured immediately and then every 10 mins for 60 mins  at 3 locations on the water blanket. The pumps reached a maximal temp ranging from 38.3-42.9 C beginning at time point 20. Comparisons made between timepoints 20, 30, 40, and 60 did not yield significant differences. No significant diff was found between surface temperatures across locations. Based on this study, the current facility now uses a SOP of prewarming CWWB for 20 mins prior to anesthetizing animals.

QUESTIONS

1. T/F: Mice have a body temperature ranging from 37-37.2C and a thermoneutral zone of approximately 29.6-34C. This TNZ is wider than that of other mammals?
2. This study showed that you should prewarm your circulating water blankets prior to using them during anesthesia for how long?
	1. Prewarming is not needed
	2. 10 mins
	3. 20 mins
	4. 30 mins
3. T/F: Hypothermia during anesthesia can have deleterious effects on cardiac function and poses an anesthetic toxicity risk

Answers:

1. False
2. c
3. True

**Wu et al.** [**Acute Normovolemic Hemodilution-assisted Terminal Blood Procurement in Swine for Ex Vivo Organ Perfusion**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2022/00000061/00000001/art00013)**, pp. 101-104**

Domain 3: Research

Primary Species: Pig (*Sus scrofa*)

SUMMARY: Numerous research and clinical applications (such as machine-assisted perfusion of organs) necessitate the use of large volumes of blood. Whereas there are publications detailing maximal terminal blood collection in small animals (such as rodents), the same techniques have not been thoroughly described in swine; furthermore, these techniques (e.g. cardiocentesis) may lead to ischemic organ damage, contraindicating its use in organ procurement procedures. Acute normovolemic hemodilution (ANH) is a perioperative technique used to minimize loss of blood volume in human medicine where whole blood is removed from the patient while simultaneously being replaced with fluids (either crystalloids or colloids). This paper describes the use of ANH as a terminal technique to maximize blood collection in swine while preventing ischemic organ damage. Four male Yorkshire x Landrace swine were anesthetized with ketamine (2.2 mg/kg IM), tiletamine-zolazepam (4.4 mg/kg IM), xylazine (2.2 mg/kg IM) and isoflurane (1-3% inhaled), with isoflurane and fentanyl (0.03-0.1 mg/kg/h) used for maintenance. The right femoral vein was catheterized with a central venous catheter, and the right femoral artery was catheterized with an 14-17 fr arterial cannula. A heparin bolus (30,000 IU) was administered IV. The venous catheter was connected to a pressurized fluid bag (containing balanced crystalloid solution), and the arterial catheter was connected to a blood collection reservoir. Passive ANH was performed for the first 2L blood obtained, after which the crystalloid replacement was discontinued and terminal exsanguination was performed. Phenylephrine was administered when mean arterial pressure (MAP) fell below 65 mm Hg. When the patient failed to respond to this treatment, organs were explanted. The total volume of blood collected was 4.5 + 0.5 L (representing 47 + 4 mL/kg body weight or 73 + 6% total blood volume). The overall hemoglobin concentration was 9.1 + 1.7 g/dL (representing 64 + 8% total intravascular hemoglobin). Phenylephrine was able to maintain blood pressure until 2L blood loss. There were no statistically significant differences in pH, lactate, or pO2 between 0 and 3L blood collected. Given this, ANH-assisted exsanguination appears to be a useful tool to maximize blood and hemoglobin collection with minimal derangement of pH, lactate, or oxygenation. This technique may prove useful in clinical applications or research investigating organ recovery, machine-assisted organ perfusion, surgical blood-loss intervention, or organ-replacement devices.

QUESTIONS

1. Which of the following is NOT a routine venous access site in swine?
	1. Auricular v.
	2. Cephalic v.
	3. Caudal vena cava
	4. Femoral v.
	5. Abdominal v.
2. Which of the following characteristics of the swine circulatory system make it a promising translational model for human medicine?
	1. Coronary arterial blood supply is RIGHT-sided dominant and has little-to-no collateral circulation
	2. Coronary arterial blood supply is LEF-sided dominant and has little-to-no collateral circulation
	3. The aorta lacks a true vaso vasorum
	4. The electrophysiologic system is more myogenic than neurogenic
	5. The right azygous vein drains the intercostal vessels into the coronary sinus
3. What is the total estimated blood volume (in mL/kg) of an adult swine?
	1. 51-58
	2. 61-68
	3. 71-78
	4. 81-88
	5. 91-98

ANSWERS

1. c. Caudal vena cava (LAM Blue Book p697; cranial vena cava may be utilized. Other commonly used sites are auricular, cephalic, external and internal jugular, lateral saphenous, cranial abdominal or mammary, and femoral vv.)
2. a. Right sided dominant coronary arterial blood supply (LAM Blue Book p700, noted as similar to 90% human population. Other answers wrong as aorta has a true vaso vasorum, the electrophysiologic system is more neurogenic, and the left azygous vein drains)
3. b. 61-68 mL/kg (Swine in the Laboratory, p. 9)