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

Volume 58, Number 6, November 2019

**POSITION STATEMENT**

[**Association of Primate Veterinarians' Guidelines for Assessment of Acute Pain in Nonhuman Primates**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00001)**,** **pp. 748-749**

Domain 2

SUMMARY: Addressing acute pain in non-human primates requires being able to adequate recognize pain in the species as well as being able to select and administer the appropriate analgesic agent(s). Any procedure considered to be painful in a human should be considered painful in animal models and appropriate monitoring and analgesia instituted.

Recognizing and assessing pain is the first step towards managing it. Non-human primates can be difficult to assess pain in due to having limited conscious exam opportunities. Cage-side assessment is a frequently used but somewhat limited tool to assess pain. An observer familiar with the normal behaviors of both the species, and the individual animal, will enhance accurate pain assessment. Signs such as changes in eating and drinking, abnormal body postures or positions in the cage, and changes in social interactions may all be indicators of pain across species. Physiologic parameters are difficult or possible to assess in conscious non-human primates.

Direct cage-side monitoring or indirect video monitoring can be utilized. Cage-side monitoring can be skewed due to animals’ behavioral changes in the presence of an observer. Video monitoring removes the presence of the observer and may allow for more accurate assessment but can be technically challenging to set up. No validated facial grimace scales have been established for non-human primates. The frequency of monitoring should be based on the procedure performed, the analgesics in use, and the results of observer assessment. All observations and analgesics administered should be recorded in the medical record.

QUESTIONS

1.  How is direct palpation used in assessment of non-human primate post-procedural pain?

a.   Palpation of the painful site in a conscious non-human primate can elicit signs of pain that are otherwise masked and help direct appropriate analgesic treatment.

b.   Palpation of the painful site in a conscious non-human primate is inhumane and should never be attempted

c.  Palpation of the painful site in a conscious non-human primate is unsafe unless the animal is trained to participate in such an examination

d.  Palpation of the painful site in a conscious non-human primate should be performed prior to making any modifications in the analgesic regimen

2.   Which of the following is not a limitation of cage-side observation for assessing pain in non-human primates?

a. Animals may change their behavior due to the presence of a human observer

b.  An untrained observer may interpret submissive behaviors as indicators of pain

c.  An extended observation period is often necessary

d.   It is technically challenging and labor intensive

3.  Facial grimace scales for non-human primates are…

a. Useful as a sole means of assessing pain in non-human primates

b.  Not validated for any non-human primate species

c.   Only useful in conjunction with other clinical parameters

d.  Broadly applicable across multiple species of non-human primate

4. Which of the following should be considered when creating a plan for the frequency of pain assessment monitoring

a.   Severity/invasiveness of the procedure performed

b.   Specific type and method of analgesic

c. Pharmacokinetics of analgesic agents used

d.  Clinician experience

e.   All of the above

ANSWERS

1. c

2. d

3. b

4. e

[**Association of Primate Veterinarians' Guidelines for Laparoscopic Reproductive Manipulation of Female Nonhuman Primates in Biomedical Research**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00002)**,** **pp. 750-752**

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

SUMMARY

Purpose:Provide NHP researchers, IACUC members, and veterinarians with guidelines for considering and conducting research involving laparoscopic manipulation in female NHPs.

Background:Common reproductive manipulation performed in NHPs laparoscopically include ovarian follicle aspiration for oocyte collection, ovariectomy, uterine biopsy, diagnostic laparoscopy, and embryo transfer. Laparoscopy can also be used for induction of reproductive disease models and therapeutic interventions. It is considered a refinement and thus fulfills one of the components of the 3Rs because procedures are considered minimally invasive and reduce the need for major surgery. Given smaller incision size compared to an open laparotomy there is less tissue inflammation and decreased post-operative incision related pain. There is also reduced risk of hemorrhage and reduced post-operative risk of infection when compared to a laparotomy. Appropriate training and proficiency is essential and should be reviewed by the IACUC.

IACUC Considerations:Laparoscopic surgeries may be classified as major or minor surgeries depending on the impact on the animal. No matter major or minor, appropriate aseptic technique, instruments, anesthesia, and analgesics are necessary. Whether a laparoscopic surgical procedure is deemed major or minor should be evaluated on a case by case basis by a veterinarian and the IACUC.

Animal Selection:Female NHPs selected for protocols are generally sexually mature with or without a history of active reproduction or reproductive manipulation. The animal’s future use in research should be considered if reproductive impairment is expected. Screening of candidates should include: complete medical history, physical exam with reproductive evaluation, CBC/Chem, history of previous births, reproductive manipulations with special attention to any abnormalities previously noted (i.e. aberrant anatomy, adhesions, cysts), and menstrual cycles.

Procedure Considerations:Procedures may be modified for certain species or animals with previous manipulations. An example of this would be using a para-midline laparoscope port to allow appropriate visualization. Complications may be associated with entry into the abdomen, creation of pneumoperitoneum, positioning, and visualization or manipulation. Placement of ports with the aid of trocars may result in mechanical trauma, abdominal wall hematoma, perforated bladder, hernia, and/ or ureteral injury. Pneumoperitoneum as a result of insufflation with CO2 gas used to improve visualization and manipulation of abdominal contents has been associated with respiratory acidosis, deep vein thrombosis, subcutaneous emphysema, gas embolism, reduced dynamic lung compliance, and increased inspiratory and plateau pressures. The Trendelenburg position (supine position in a 15 to - degree incline with feet elevated above the head) is a standard position for female laparoscopic procedures that along with CO2insufflation has been associated with increased mechanical impedance to lung inflation. If used, mechanical ventilation is recommended.

Post-Surgical Considerations:Post-operative monitoring should be provided until the patient is able to maintain an upright position. Post-operative monitoring including evaluation of basic NHP physiologic and behavioral parameter conducted daily for 3-7 days post-procedure. Post-operative analgesia for routine laparoscopic procedures should be provide for a minimum of 48-72 hours with opioids, NSAIDS or a combination based on veterinary discretion. Short term complications are typically associated with surgical site incisions but may include abdominal discomfort and rarely fascial dehiscence and herniation. Long term complications include impaired fertility, endometriosis, and adhesion formation.

Record Keeping: Detailed records should include surgical procedure, any perioperative findings and/or complications, anesthetic and analgesics agents, and administration routes, Records should also include the animal’s behavior and objective (e.g. body weight shifts, fluid volumes, biscuit consumption, urine and fecal output) and subjective (e.g. activity and alertness) clinical parameters.

QUESTIONS

1.   According to the Animal Welfare Act and the 8th edition of the Guide for the Care and Use of Laboratory Animals, a major surgery includes all of the following except \_\_\_\_\_\_\_.

1. Penetrates and exposes a body cavity
2. Produces substantial impairment of physiologic functions
3. Involves extensive tissue dissection or transection
4. Produces permanent of physical functions
5. All of the above are included in the definition of a “major surgery”

2.  The gestion period for Cynomolgus macaques (*Macaca fascicularis*) is \_\_\_\_\_\_\_.

* 1. 155 days
	2. 163.5 days
	3. 160.5 days
	4. 180 days
	5. 140.5 days

ANSWERS

1. e

2. b

[**Association of Primate Veterinarians' Socialization Guidelines for Nonhuman Primates in Biomedical Research**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00003)**,** **pp. 753-754**

Domain 4: Animal Care

SUMMARY: The purpose of this article was to provide guidelines for social needs of nonhuman primates for psychological well-being while maintaining consistency with objectives of the research.

QUESTIONS (True or False)

1. The AWA, the Guide and the EU Directive all mention the need to provide social housing for nonhuman primates.

2. Animals with tethers or jackets and animals on infectious disease studies cannot be considered for social housing programs.

3. Animals in protected contact housing situations are considered socially housed.

4. If the individually housed animal has been approved by IACUC, the veterinarian needs to review the continued approval every 30 days.

ANSWERS

* 1. True
	2. False
	3. False
	4. False

**OVERVIEW**

**Stillman and Whittaker.** [**Use and Efficacy of Analgesic Agents in Sheep (Ovis aries) Used in Biomedical Research**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00004)**, pp. 755-766**

Secondary Species: Sheep (Ovis aries)

Domain 2:Management of Pain and Distress

SUMMARY:Despite sheep (Ovis aries) being a widely used model in biomedical research, literature regarding the use of analgesic agents in sheep in biomedical research is limited. This limits references for analgesia in sheep to agricultural reports; however, the type of procedures for which these drugs are administered are likely quite different from the surgical models used in biomedical research. Pain assessment is difficult in this species due to their stoic nature. Pain assessment techniques described include voluntary leg withdrawal from stimulus, skin/deep muscle pricks, changes in behavior and movement.

This review article summarizes analgesics used in sheep involved in biomedical research. 29 peer-reviewed publications were evaluated from 1995 to 2018. Analgesics discussed in these publications included opioids, alpha2 agonists, NSAID, NMDA receptor antagonists, local anesthetics, and calcium channel blockers. In summary, additional studies focused on the use of these agents in biomedical research would be beneficial.

New methods of administrating opioid agonists (buprenorphine, butorphanol, fentanyl, methadone, morphine, piritramide, and tramadol discussed in this article) like transdermal patch and adjunct therapies show promise in providing effective analgesia (especially with visceral pain). Adverse effects associated with opioid administration include excitation/behavioral changes, pruritus, nausea, vomiting, urinary retention, and respiratory depression.

A2-agonists have become the drug class of choice for effective sedation and analgesia in sheep due to being short-acting, and a2 adrenoceptors being widely distributed throughout the body and densely expressed in the laminae I-II in the sheep spinal cord. (Xylazine, clonidine, detomidine, medetomidine, xylazine discussed in this article) Side effects include pulmonary edema, peripheral vasoconstriction, and hypoxemia.

NSAID may only be effective when inflammation is present and not be beneficial for acute pain models. (Carprofen, ketoprofen, meloxicam, phenylbutazone, salicylic acid, and tolfenamic acid discussed in this article) Side effects including prolongation of clotting time and gastric ulceration.

Local anesthetics (bupivacaine, lidocaine) when combined with other analgesics increase efficacy and duration while being dose-sparing. This article mentions combining lidocaine/bupivacaine with epinephrine and MgSO4 in nerve blocks/epidurals and a2 agonists when administered IM/IV.

Ketamine, an NMDA receptor antagonist, provides decent analgesia and may be underutilized in sheep.

QUESTIONS

1. T or F: A direct spinal analgesic effect with ketoprofen administration may only occur in the presence of inflammation.
2. Short answer: How might the addition of epinephrine to lidocaine increase the duration of analgesia?

ANSWERS

1. T
2. Epinephrine produces vasoconstriction at the injection site slowing the rate of systemic absorption leaving lidocaine bound to sodium channels in the area for longer, increasing the analgesic effect.

**ORIGINAL RESEARCH**

***Husbandry***

**Doerning et al.** [**Assessment of Mouse Handling Techniques During Cage Changing**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00005), **pp. 767-773**

Domain 4:Animal Care; T2. Manage or provide indirect management/oversight of animal husbandry programs; K4. Knowledge of quality assurance techniques for animal care-related equipment and supplies

Primary Species:Mouse (Mus musculus)

SUMMARY:This study evaluated alternative methods of rodent handling during cage changing and their potential implications for efficiency, biosecurity, and animal welfare.

Traditionally, using padded-tip forceps to grasp mice at the base of the tail or the loose skin around the nape of the neck has been used based biosecurity recommendations to decrease the potential for pathogen exposure between animals. As a highly repetitive action for animal care personnel performing cage changes, it creates a high risk for musculoskeletal injury.

The goals of the study were to provide animal care personnel with alternatives to using forceps for mouse handling during cage changing, to assess the biosecurity implications, with a particular focus on sanitization efficacy as an indicator of the potential for disease transmission, and to determine any effects of handling techniques on reproductive performance and the caretaker’s ability to perform health examinations

Study groups included plastic cups, gloved hands, 2 methods of tunnel handling, and forceps. In the home tunnel group, mice were transferred by using a tunnel from their home cage. In the novel tunnel group, mice were exposed to tunnel handling only during cage changing. In the cup group, mice were transferred by using a 60-mL polypropylene plastic food-service cup. In the gloves group, the caretaker used gloved hands to transfer mice by grasping the tail. In the forceps group, the caretaker used stainless steel rubber-tipped forceps to transfer mice by grasping the tail.

Evaluations included speed of cage change, ATP-based assessment of sanitization, and retrospective analysis of colony health and breeding data.

The time to change cages was significantly faster for the gloved hands and forceps groups. The speed of cage changing did not increase consistently for any of the handling methods. Tunnel and cup handling both increased the time for cage-changing. The novel tunnel was the slowest method overall.

ATP levels after sanitization with hydrogen peroxide–peracetic acid mixture differed significantly between gloves and forceps; with forceps having a significantly lower RLU (relative light units) than did gloves after sanitization. Sanitization assessment showed that all handling methods resulted in ATP levels that did not vary significantly. Gloves displayed the highest ATP level after sanitization overall.

Breeding performance and colony health were similar among groups. No significant differences were found between home tunnel, novel tunnel, cup, or gloves handling when comparing the average number of pups per litter born during the study. Health records did not reveal an increased frequency of animals found sick or dead at cage change or during daily cage-side observations among handling groups

Using gloved hands to directly handle mice during cage changing is efficient and avoids the ergonomic strain associated with forceps. Forceps-handled male mice have shown increased levels of aggression compared with tunnel and gloved tail-handling, Precautions should be taken when handling mice with gloves, given that the increased contact area carries an increased load of organic debris.

QUESTIONS

1. Traditionally, which method has been used for cage changing mice?

a.  Gloved hands

b.   Home tunnel

c.   Novel tunnel

d.   Forceps

e.  Cup

2. Of the 5 methods, the slowest was:

a.  Gloved hands

b.   Home tunnel

c.   Novel tunnel

d.   Forceps

e.  Cup

3. Of the 5 methods, the highest ATP level was found with (even after sanitization):

a.  Gloved hands

b.   Home tunnel

c.   Novel tunnel

d.   Forceps

e.  Cup

4. The problem with the use of forceps to change mouse cages:

a.  Sanitization

b.  Speed

c.   Increased frequency of sick or dead animals

d.  Decreased breeding performance

e.   Repetitive action injuries to animal care personnel

5. True or False: The use of gloved hands as an alternative to forceps when handling mice during cage changeout should be considered an acceptable practice so long as gloves are changed between rack sides and before proceeding to the animals of a different investigator

ANSWERS

1. d

2. c

3. a

4. e

5. True

**Shelton et al.** [**Effects of Transportation and Relocation on Immunologic Measures in Cynomolgus Macaques (*Macaca fascicularis*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00006)**,** **pp. 774-782**

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

Primary Species: Macaques (Macaca sp.)

SUMMARY: Most NHPs are imported or raised at vendor facilities/primate centers then moved to other facilities for research purposes. The effects of stress from transportation/relocation is well-established and can effect almost all aspects of an animal’s physiology. To try to prevent the potential for these effects to confound research data, periods of acclimation are used. The recommended periods of acclimation can vary but ideally the animals should receive enough time to have a return to physiologic homeostasis. The information available to determine the appropriate acclimation period in NHPs is lacking. This group used the published study to address this gap. They looked specifically at lymphocyte subsets, selected T-cell functional immune parameters, and plasma cytokine levels in Cynos before transport and at 24h and 30d post transport.

The group noted that even after 30d of acclimation, multiple immune parameters in singly housed Cynos were significantly different compared with baseline after routine domestic transport (ground). They also found a number of short-term (2d) changes in circulating lymphocyte numbers and function. They noted increased CD4+ and CD3+ T cells which is consistent with the recovery phase after a stressful stimulus. Additionally, they noted increased NK and NKT cells initially but then a rapid decrease likely reflecting a redistribution of those cells into tissues. The group noted a failure to return to baseline at 30d post stressor based on finding monocytosis and decreased circulating B-cells compared to baseline. However they feel that they were at that point detecting signs of acute stress due to the phlebotomy. This is of note because this potentially illustrates that animals that are acclimated to “routine” animal handling procedures like blood collection may not acclimate within a 30d period to those same procedures in a new institution. The data provided by the group could help in determining the appropriate acclimation period for their study. The determination should include consideration of the intensity and duration of stress, the species, sex, age, genotype, health status, previous life experience, allometric differences, and even the time of year. Additionally, it is important to remember that social housing impacts immune response and disruption of social groups is an additional source of stress for the animals. It is possible to mitigate by socially housing the animals during transport or soon after arrival in the new facility. The conclusion was that a 30d period of acclimation after transportation and relocation is not a sufficient amount of time for return to baseline of all measures of immune function. This should be taken into consideration for animals used on studies involving immune outcome measures.

QUESTIONS

1.  What role do nonclassic monocytes (CD14++, CD16+) play?

2.  True or False Decreased B cell counts are associated with a response to chronic stress.

3.  What is the difference in a type-1 versus type-2 cytokine response?

ANSWERS

1.   They are thought to play a role in tissue repair and the response to bacterial and viral infections through production of high amounts of TNFα and IL1β and by patrolling the vascular endothelium.

2. False – Decreased B cell counts are found in response to acute stress

3.   The cytokine profile is influenced by the nature and dose of the antigen, route of infection, initial cytokine environment, type of antigen presenting cell/costimulation and genetic background. Th1 cells produce type-1 cytokines (IL-2, IFNγ, TNFα, TNFβ). They activate dendritic cells for intracellular killing of pathogens, mediate cell mediated immunity. Th2 cells produce type-2 cytokines (IL-4, IL-5, IL-10, IL-13). They provide help to B cells in antibody response, mediate allergy and immunity to extracellular pathogens including parasites.

**Webb et al.** [**Differences in Behavior Between Elderly and Nonelderly Captive Chimpanzees and the Effects of the Social Environment**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00007)**,** **pp. 783-789**

Domain 4: Animal Care

Tertiary Species: Other Nonhuman Primates

SUMMARY: Little research exists that addresses the ways in which captive chimpanzee behavior differs as a function of the interaction of age and aspects of the captive environment. The authors examined overall differences in behavior between elderly (35 y and older) and nonelderly (younger thaan 35 y) captive chimpanzees.

Behavioral management techniques are designed to enhance the welfare of NHP in captivity. Specific attempts to enhance the welfare of captive elderly chimpanzees are becoming more common, including the use of acupuncture and laser therapy for arthritis, self-medication paradigms, and systematic programs that use personalized care approaches for geriatric animals.

Behavioral management techniques have focused on methods to enhance the captive environment of elderly chimpanzees in ways that are functionally appropriate,  promote species-typical behaviors, and encourage locomotor activities that are necessary for healthy aging.

At the National Center for Chimpanzee Care, more than 30% of the animals are considered elderly. The authors want to ask the following questions:

1) In what ways does overall behavior differ between elderly and nonelderly chimpanzees?

2) At what age does a chimpanzee begin to behave like an elderly chimpanzee?

3) What are the size and composition characteristics of a geriatric group compared with a nongeriatric group in the program?

4) Among elderly chimpanzees, does behavior differ as a function of living in a geriatric compared with a nongeriatric group?

Results showed that elderly animals show a number of important, welfare-related differences in behavior, including greater rough scratching and inactivity, lower behavioral diversity and affiliation. Compared to nonelderly groups, geriatric groups were characterized by higher average group age, higher mobility impairment scores, smaller group size, and a lower percentage of male chimpanzees.

This findings are consistent with previous reports of decreased aggressive and manipulative behavior and increased inactivity and solitary behavior in older chimpanzees.

Future investigations should focus on the implementation and assessment of preventive measures aimed at maintaining appropriate levels of welfare-related behaviors, such as locomotion and species- typical behaviors, within geriatric groups. For example, structural enhancements to the physical environment and increased voluntary participation and choice within the environment have been used to increase locomotion, activity, and behavioral diversity of aged chimpanzees.

QUESTIONS

1. List some of age-related ailments that elderly chimpanzees have to face in their life?

2. Which one of the following statements is false regarding changes in the behavior of elderly chimpanzees in the wild?

a. Social behavior is characterized by withdrawal from the group and social interactions.

b. Females spend less time with family members.

c. Male dominance rank can drop with old age, but female rank often remains the same.

d. Male chimpanzees seem to prefer to mate with older- even geriatric- females.

ANSWERS

1. For example: hypertension, heart disease, mobility impairment, arthritis, Alzheimer pathology, diabetes, etc.

2. b is false. Females spend more time with exclusively family members.

***Animal Health Surveillance***

**Tosa et al.** [**Multiplex Immunochromatographic Assay for Serologic Diagnosis of Major Infectious Diseases in Laboratory Mice**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00008)**,** **pp. 790-795**

Domain 3: Research

Primary Species: Mouse (Mus musculus)

SUMMARY: This research group developed and analyzed a multiplex immunochromatographic assay (ICA) for mice in order to detect serum antibodies for three pathogens: mouse hepatitis virus (MHV), Sendai virus and C. piliforme. Serum samples, positive and negative, for MHV, Sendai virus and C. piliforme were used as controls. In order to develop a multiplex ICA test strip, four major components were prepared (1) a colloidal gold conjugate where a protein A-conjugated colloidal gold was prepared, (2) a conjugate pad, coated with the prepared colloidal gold conjugate, and (3) a nitrocellulose membrane in which antigens for MHV, Sendai virus, C. piliforme and rabbit IgG mixed with other reagents were immobilized in this membrane, an ICA strip containing the 3 antigens and the control as separate lines was considered a multiplex ICA test strip; (4) absorbent pad. All these components were assembled on a membrane card.  (Figure 1A). An ICA strip was added into a microtube containing PBS-diluted serum samples or whole blood; a sample that showed test and control lines detecting rabbit IgG was considered as positive, and a sample that showed only the control line was considered as negative.

Single ICA tests were performed using different concentrations of positive serum (1:50, 100, 200) and antigens (1:1, 10 and 100); multiplex ICA using all 3 antigens specifically and simultaneously detected antibodies to the corresponding antigens. Regarding sensitivity and specificity of the multiplex ICA assay, the strip detected the antigen-specific antibody in all Sendai virus positive serum samples, as well as in the   MHV-positive serum samples. However, specific antibody to C. piliforme was detected in only 90% of the positive serum samples. In addition, a comparison between ICA for PBS-diluted antibody-positive serum and diluted antibody-positive sera mixed with whole blood was performed; the multiplex ICA test specifically detected antibodies to the MHV, Sendai virus and C. piliforme antigens in the presence of whole blood suggesting that the assay can detect these antibodies in whole blood without the need to remove RBC from samples.

The research group concluded that the multiplex ICA assay will be a useful tool for future diagnostics in laboratory mice; and future studies involving serologic testing of field samples as well as incorporating other infectious agents are needed for achieving practical use of multiplex ICA.



**Figure 1:** Detection method and scheme of the immunochromatographic test (ICA) strip. (A) Structure of the ICA strip. Antigens and rabbit IgG were placed at the test lines and control line, respectively. The ICA strip consisted of 4 membrane pads: sample pad, conjugate pad, nitrocellulose membrane, and absorbent pad. The conjugate pad contained the protein A–colloidal gold conjugate.

QUESTIONS

1.  What is the principle of immunochromatography?

2.  True or False: Which of the following statements about multiplex ICA is true?

a.   Multiplex ICA assay detects antibodies to their corresponding antigens in a small amount of serum

b.   Multiplex ICA assay has high levels of specificity and sensitivity among antigen-positive as well as uninfected serum samples

c.   Multiplex ICA can be used in diluted whole blood

d.   All of them are true

ANSWERS

1.   Immunochromatography is a combination of chromatography and immunoassay, the antigen-antibody reaction which occurs in a membrane is used to determine the target analyte in the sample. It uses a nitrocellulose along with some dyes which produces colored lines depending on the presence or absence of target analyte.

2.  d

**Lee et al.** [**Detection of *Myocoptes musculinus* in Fur Swab and Fecal Samples by Using PCR Analysis**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00009), **pp. 796-801**

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

Primary Species: Mouse (Mus musculus)

SUMMARY: Successful detection of common mites *Mycoptes musculinus, Myobia musculi, Radfordia affinis*, and *Demodex musculi* in a research rodent colony can be challenging in terms of accuracy, time, and cost but are important for animal welfare and research outcomes.  The authors developed a PCR assay to address the common limitations associated with the detection of *Mycoptes musculinus* and presented the findings for accuracy of the assay.  The authors tested both fur swabs and fecal samples to find both mites in the fur and eggs ingested during grooming.  The goal was to develop a PCR-based assay for M. musculinus that could detect mite DNA in fecal samples, given the assumption that mites and mite eggs are ingested during the grooming process.  The authors designed forward primers within the 18S rRNA gene and reverse primers within the 28S rRNA gene that would specifically target the *Psoroptidia* mite species.  The resulting species specific primers were able to identify *M. musculinus*.  The standard curve analysis demonstrated that the limit of detection of the *M. musculinus* PCR assay is 100 copies.  The authors concluded that fecal samples were able to be used in a PCR assay to accurately detect *M. musculinus*in laboratory mice.

QUESTIONS

1. Common fur mites Myobia musculi and Radfordia affinis commonly infest the skin and hair shafts in what regions of the body?
	1. Ventral thoracic and abdominal region
	2. Inguinal and axillary region
	3. Intrascapular and dorsal cervical region
	4. Base of the tail
2. Which common fur mite has claws on the tarsi of the second pair of legs that are paired?
	1. *M. musculi*
	2. *R. affinis*
	3. *M. musculinus*
	4. *D. musculi*
3. Which is NOT a common adverse clinical signs associated with mite infestation?
	1. Ulcerative dermatitis
	2. Decreased life span
	3. Increased belligerence
	4. Altered IgE levels

ANSWERS

1. c
2. b
3. c

***Anesthesia***

**Sarfaty et al.** [**Concentration-dependent Toxicity after Subcutaneous Administration of Meloxicam to C57BL/6N Mice (*Mus musculus*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00010), **pp. 802-809**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: The objective of the current study was to evaluate the  safety of meloxicam (20 mg/kg) given subcutaneously (SC) in C57BL/6N mice. The primary question was whether meloxicam at 20 mg/kg SC results in pathology either at the injection site or systemic NSAID toxicity. The dose regimen of the analgesics that were evaluated included two concentrations: Meloxicam (5 mg/mL; 0.1 mL;  SC; SID) and Meloxicam (1 mg/mL; 0.5 mL;  SC; SID) for 6 days at same injection site (Interscapular Region). Author’s evaluated the injection site pathology via gross physical examination and histopathology exam on tissues obtained on necropsy which was evaluated based on a severity score (7-stage scale; 0 being normal and 6 being most severe). Based on data obtained, the authors concluded that meloxicam (1 mg/mL) can be administered safely at a dosage of 20 mg/kg SC to C56BL/6N mice for as long as 6 d .Whereas SC injection of meloxicam at its standard concentration (5 mg/mL) caused marked dermal necrosis, steatitis, and myositis at the injection site in all mice.  This study underscore the use of at least 20 mg/kg SC of meloxicam is necessary for sufficient analgesia in clinical postoperative contexts and the same dosage at the concentration of 1 mg/ml results in minimal pathology.

QUESTIONS

#### 1.  In the present study, while studying the injection-site pathology, none of the mice in Meloxicam (1mg/mL) group showed pathology based on which parameter as compare to Meloxicam (5 mg/mL)?

a. Cavitary Lesions

b. Subcuticular/fat/muscle necrosis

c. Full-thickness skin necrosis

d. All of the above

2.  In the study, the authors noticed in what group of mice were significantly more likely to develop gastritis?

a. Mice Injected with Meloxicam (5 mg/mL, SC)

b. Mice Injected with Meloxicam (1mg/mL, SC)

c. Mice Injected with Saline

d. None of the above

3.   Base on the findings from the study, what dose regimen for meloxicam (20 mg/kg) administration is recommended by authors for C56BL/6N mice for as long as 6 d ? ( SC: Subcutaneous; ID: Intradermal)

a. Meloxicam (5 mg/mL; 0.1 mL;  SC; SID)

b. Meloxicam (1 mg/mL; 0.5 mL;  SC; SID)

c. Meloxicam Oral (20 mg/kg)

d. Meloxicam (1 mg/mL; 0.5 mL; ID; SID)

4. A meloxicam dose of at least \_\_\_\_\_\_\_\_appears necessary for effective postoperative analgesia in mice?

a. 20 mg/kg

b. 10 mg/kg

c. 40 mg/kg

d)    5mg/kg

5.  True or False: C57BL/6 mice, among popular inbred mouse strains, may be the most sensitive to nociception?

6.  True or False: C57BL/6 have lower baseline MGS scores than C3H/He and CD1 mice, in the absence of a noxious stimulus.

ANSWERS

1. d

2.  c

3.  b

4.  a

5.  True

6.   True

**Bressan et al.** [**Use of Rodent Sedation Tests to Evaluate Midazolam and Flumazenil in Green Iguanas (*Iguana iguana*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00011), **pp. 810-816**

Tertiary Species:  Reptiles

Domain 2: Management of Pain and Distress

SUMMARY: The goal of the study was to evaluate various sedation tests used in rodents models to better understand midazolam and flumazenil effects in the green iguana (iguana iguana) and create a scheme for future sedative evaluation in reptiles. This study used 10 juvenile green iguanas. The subjects were either given 2 mg/kg midazolam, 0.05 mg/kg flumazenil, or 0.4 mL/kg of 0.9% saline IM in the forelimbs. This is a double randomized study with 15 d wash out period and each animal was used 8 times. The animals were assessed at 0, 15, 60, 180, and 300 minutes by an observer that did not know what treatment regimen the animal received.

The sedation tests included the open field test, forced swim test, behavioral test, traction test, and the limb withdrawal latency time (LWLT) was determined when the animal was presented with a noxious stimulus (heat source). A non-validated scale was created to evaluate the animal’s responses.

The results demonstrated that the forced swim test and behavioral scoring were the best tests to verify the sedation effect of drugs in iguanas. The forced swim test was easy to apply and produced quantitative data when comparing treatment groups. Iguanas in this test were most affected at 15-60 minutes where animals head their heads above water and floated. The behavioral scale showed significant differences between treatment groups with the greatest affects at 15-60 minutes and mild sedation at 180 minutes. The open field testing showed no difference between the sedated and non-sedated groups. The traction test was useful only in evaluating deep sedation as the iguanas were able to hold onto the grill under light to mild sedation.

Midazolam is an effective sedation agent in reptiles. The authors used the forced swim and behavioral tests to further explore flumazenil’s reversal effects on midazolam. The reversal effect was observed in the midazolam flumazenil group in which the animal received flumazenil 30 minutes after the midazolam; the sedation was decreased after injection and not detected by 180 minutes. Animals in the swim test receiving midazolam/saline were unable to swim at the 15 minute mark with sedation lasting for 240 minutes. Animals that received flumazenil were able to swim 30 minutes after injection. The LWLT was longer 15-30 minutes after midazolam with effects lasting up to 60 minutes with saline administration. However, when given flumazenil the animals had a lower than baseline LWLT at 60 minutes.

QUESTIONS

1. What is the forced swim test?
2. What is the traction test?
3. What is the open field test?

ANSWERS

1. The test involves placing a mouse in a cylinder of water and then observing how long it swims, its behavior, or its mobility.
2. This test is used to assess a drugs effect in rodents. The test involves placing a mouse’s forepaws on a suspended small diameter wire. Normally rodents grasp the wire with forepaws and hind feet to prevent falling.
3. The test is used to verify the effect of a drug on an animal’s movement and activity/exploration. The animal is placed in an enclosure (circle or rectangular area with walls to prevent escape). The animal is then monitored for distance moved, time spent walking, grooming, and location on the field.

***Experimental Use***

**Personett et al.** [**Hematologic Parameters and Blood Cultures from the Gingival Vein Compared with the Cranial Vena Cava in Guinea Pigs**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00012), **pp. 817-822**

Domain 3: Research

Secondary Species: Guinea Pig (Cavia porcellus)

**SUMMARY:** Due to their unique anatomy (short limbs, neck, and tail), there are limited routes of blood collection in guinea pigs (GPs). This paper compares gingival vein puncture with the more commonly used cranial vena cava puncture and their effects of CBC and blood culture results. To collect from the gingival vein, the GP must be anesthetized, and an assistant needs to occlude the vein in the area of the mandibular symphysis. A 28G insulin needle w/ syringe is used. The success rate for obtaining an unclotted sample for CBC was 38%, even though up to 800uL was obtained from the gingival vein. This is likely due to 1) perceived slower collection times, 2) use of a small gauge needle, both resulting in rapid coagulation. Only MCV differed between blood obtained from the cranial vena cava and gingival veins. The gingival vein can be used for CBC, but not for blood cultures, even with prior disinfection using sterile saline or 0.05% chlorhexidine solution. Both techniques resulted in no/minimal (slight bruising) adverse effects on GP welfare. Since both techniques required general anesthesia, anesthesia may have some effect on CBCs. The gingival vein can be also used in mice, rats, and hamsters. The authors recommend further investigation on the effects of heparinizing the syringe prior to blood collection on CBC/chem values.



**QUESTIONS**

1.  What are the landmarks for cranial vena cava puncture?

2.  Why is blood collection from the gingival vein more difficult in GPs versus rats?

**ANSWERS**

1.   While the GP is in dorsal recumbency, insert needle caudally **just lateral to the manubrium under the right first rib** at a 30 degree angle to the horizontal axis of the body. Insert the needle ~0.5” and pull back on the plunger slowly.

2.  The area where the gingival vein is the widest in a GP is **more caudal and deep** compared to the rat; overall the gingival vein is narrower in the GP (Rodrigues, et al., 2017)

**Thurman et al.** [**Effect of 3 Euthanasia Methods on Serum Yield and Serum Cortisol Concentration in Zebrafish (*Danio rerio*)**](https://www.ingentaconnect.com/contentone/aalas/jaalas/2019/00000058/00000006/art00013)**,** **pp. 823-828**

Domain 3: Research

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY:Zebrafish are a common animal to be used in a wide variety of research models. Their popularity is in part due to their small size, external fertilization and transparent embryos. Techniques for euthanizing zebrafish have been evaluated but a comparison of the potential stress between different techniques has not been evaluated. In this paper the authors compared MS222, Benzocaine, and rapid chilling. The outcomes that were compared were time to loss of operculum movement, volume of serum collected and serum cortisol concentration. All three of these techniques are listed as acceptable in the 2013 AVMA Guidelines  for the Euthanasia of Animals. MS222 and Benzocaine are local anesthetics that block nervous responses by blocking sodium uptake and membrane excitability in neurons. Rapid chilling causes a slowing of nerve conduction which is exaggerated in tropical poikilotherms such as zebrafish. Cortisol concentration is accepted as a physiological stress indicator in fish and a human salivary cortisol ELISA has been used in zebrafish. Rapid chilling had a significantly lower time to loss of operculum movement (40s) compared to MS222 (96s) and Benzocaine (86s). The serum yield in all three techniques was similar. Serum cortisol levels did not differ significantly in all methods. The authors recommend transferring fish from a tank with room temperature water poured into the cold water to minimize the potential stress of netting the fish. It is recommended to pilot this first without fish to ensure that adding room temperature water does not take the cold water out of the 2-4 degree Celsius range. There was a concern that the cortisol level may not reflect the pre-mortem levels if there was time for the levels to rise while the animals were being euthanized because cortisol levels can change rapidly under stressful conditions.

QUESTIONS

1. What are some of the reasons that make zebrafish popular as research animals?
2. How does MS222 and Benzocaine induce euthanasia?
3. True or False:   Rapid chilling is not listed as an acceptable method of zebrafish euthanasia in the AVMA Guidelines on Euthanasia of Animals: 2013
4. Of the three methods of euthanasia evaluated which one had the shortest time to loss of operculum movement?

ANSWERS

1. Their popularity is in part due to their small size, external fertilization and transparent embryos.
2. MS222 and Benzocaine are local anesthetics that block nervous responses by blocking sodium uptake and membrane excitability in neurons.
3. False.  It is acceptable.
4. Rapid chilling had a significantly lower time to loss of operculum movement (40s) compared to MS222 (96s) and Benzocaine (86s).