2010 Summer Externs

Suhrim Fisher - ASLAP Foundation Summer Fellowship
• Currently a student at The Ohio State University, College of Veterinary Medicine to graduate in 2013
• Involved with clubs such as SCAVMA, Radiology club, VBMA, ASLAP Integrated Medicine club, VOICE, and Community outreach committee for Josh Project

Mentor: Dr Stephanie Lewis, DVM, MS, DACLAM
Director, Experimental Surgery ULAR; Director, Large Animal Clinical Medicine ULAR; Assistant Professor Clinical VPM

Dr. Lewis is Attending Veterinarian for OSU facilities and as the Director of Large Animal Clinical Medicine provides clinical and didactic support for the Laboratory Animal Residency Program. She is a member of ASLAP and AALAS, serves on the IACUC at OSU and at the Veterinary Institute at Bradford.

Resident Mentor: Dr Michelle Creamer, DVM, BS

Dr Creamer is currently a Resident of Laboratory Animal Medicine at The Ohio State University, University Laboratory Animal Resources to graduate in 2012. She received her DVM degree from Virginia-Maryland Regional College of Veterinary Medicine in May 2010. She is a member of AALAS and ASLAP.

Research Project: Prevalence of Infection of *Ehrlichia canis*, *Borrelia burgdorferi* and *Rickettsia rickettsii* in Unconditioned Laboratory Dogs (*Canis familiaris*)
ABSTRACT- Surhim Fisher

Prevalence of Infection of *Ehrlichia canis*, *Borrelia burgdorferi* and *Rickettsia rickettsii* in Unconditioned Laboratory Dogs (*Canis familiaris*). S. Fisher, M. Creamer and S. Lewis. University Laboratory Animal Resources

To obtain optimal research data, it is ideal to use Class A laboratory dogs as defined by the AWA, which are bred specifically for research. Class B dogs are random source, not purposely bred for research and typically unconditioned and therefore more likely to be infected with tick-borne diseases. There are many tick-borne bacterial infections that dogs are susceptible to including *Ehrlichia canis*, *Borrelia burgdorferi* and *Rickettsia rickettsii*. *Ehrlichia canis* is transmitted by brown dog tick and causes Canine Monocytic Ehrlichioses (CME). *Borrelia burgdorferi* is transmitted by deer tick and causes Borreliosis (Lyme disease). *Rickettsia rickettsii* is transmitted by American dog tick and Rocky Mountain wood tick and causes Rocky Mountain spotted fever. These tick-borne diseases may cause clinical signs in dogs such as fever, anorexia, malaise, lameness, rash and bleeding episodes or may be subclinical. Exposure to these pathogens can introduce undesired variables to hematologic, biochemical, and histopathologic parameters in research. These pathogens cause chronic immune stimulation and therefore the dogs exposed to them are suboptimal candidates for immunology or infectious disease research. For our study, we collected blood samples from Class B dogs (n=27) and submitted them for hematologic and biochemistry analysis and tick-borne pathogen serology. No dogs exhibited clinical signs of disease. 17 of 27 dogs (62.96%) had antibodies to one or more tick-borne pathogens. No specific hematologic or biochemical differences were apparent between seronegative and seropositive dogs. Only 1 dog (0.4%) showed abnormal CBC result that may be consistent with chronic infection of tick-borne diseases. These serologic and molecular results indicate prior exposure without active infection of concurrent clinical tick-borne diseases in Class B laboratory dogs at an Ohio State University research facility.

Keywords: Tick-borne Disease, Ehrlichiosis, Lyme disease, Rocky Mountain spotted fever
Prevalence of infection of *Ehrlichia canis*, *Borrelia burgdorferi* and *Rickettsia rickettsii* in Unconditioned Laboratory dogs (*Canis familiaris*)

Suhrim Fisher, Michelle Creamer, and Stephanie Lewis

University Laboratory Animal Resources

**BACKGROUND**

**Class A Laboratory Dogs**

- Bred by USDA licensed Class A dealers for sole purpose of research
- Random-source, not bred for sole purpose of research
- Dealt by USDA licensed Class B dealers
- Predominantly hound dogs previously used as hunting dogs
- Favor over Class A dogs by some researchers due to lower costs, flexibility in the size and age of animals and appropriateness for acute, terminal procedures
- At higher risk of acquiring tick-borne disease prior to their arrival to research facilities due to their occupational exposure to ticks and other ectoparasites

**Tick-Borne Diseases in Dogs**

- *Canine Monocytic Ehrlichioses (CME)*
  - Bacterial disease in dogs caused by *Ehrlichia canis* or *E. chaffeensis*
  - Transmitted by brown dog tick, *Rhipicephalus sanguineus*
  - Clinical signs: fever, depression, weight loss, ocular and nasal discharge, ataxia, heptomegaly, splenomegaly, painful joints, thrombocytopenia and death

- *Borrelia burgdorferi (Lyme disease)*
  - Zoonotic bacterial disease caused by *Borrelia burgdorferi*
  - Transmitted by deer tick, *Ixodes pacificus* and I. scapulari
  - Clinical signs: fever, depression, regional lymphadenopathy with or without painful joints, renal and neurologic abnormalities

- *Rocky Mountain Spotted Fever*
  - Zoonotic bacterial disease caused by *Rickettsia rickettsii*
  - Transmitted by American dog tick, *Dermacentor variabilis* and Rocky Mountain wood tick, *D. andersoni*
  - Clinical signs: fever, depression, polyarthritids, vomiting, diarrhea, focal retinal hemorrhage, thrombocytopenia, altered mental states, paraspinous hyperesthesia, peltchiae and ecchymoses

**MATERIALS AND METHODS**

**Blood Collection**

- Twenty seven, > 6 months of age Class B dogs
  - Large hound breeds, many were retired hunting dogs from diverse geographic location of state of Kentucky
  - Jugular venipuncture with 21 gauge needles and vacutainer
  - One 9ml serum separator tube and one 3ml EDTA tube per dog
  - Blood sent to Anitech for CBC, chemistry and tick serology profile

**RESULTS**

- **6 of 27 dogs (22.22%)** had antibodies to *E. canis*
- **1 of 27 dogs (3.70%)** had antibodies to *B. burgdorferi*
- **16 of 27 dogs (59.26%)** had antibodies to *R. rickettsii*
- Dealt by USDA licensed Class B dealers
- Out of those 17:
  - **5 dogs (29.41%)** were seropositive for *E. canis* and *R. rickettsii*
  - **1 dog (5.88%)** was seropositive only for *R. rickettsii*

**Serology**

- • Seropositive for *E. canis* and *R. rickettsii*
- • Consistent with active infection
  - Most consistent with vaccination and/or natural exposure to *Borrelia burgdorferi*
  - Most consistent with vaccination and/or natural exposure to *Borrelia burgdorferi*
  - Rocky Mountain Spotted Fever (*Rickettsia rickettsii*)
  - Single titer of 1:1042
  - Consistent with active infection
  - Dogs tested during the active phase of disease may have a negative or low positive serology

**RECOMMENDATIONS**

- • These serologic results indicate prior exposure without concurrent active infection of clinical tick-borne diseases in Class B laboratory dogs at an Ohio State University research facility
- • Researchers should be cautious when using unconditioned Class B laboratory dogs for immunology or infectious disease research
- • The use of quantitative real-time PCR to enhance sensitivity and the use histopathology and immunohistochemistry to identify pathogens in tissue sections are recommended for future studies

**CONCLUSIONS**

- • These underlying infections may increase the exposure of tick-borne diseases to the animal care staffs
- • These pathogens cause chronic immune stimulation and therefore the dogs exposed to them will be suboptimal for studying immunology or infectious disease research

**ACKNOWLEDGEMENT**

- • Special thanks to: Mr. Bob Perry for hospitality
- • Ms. Staci Stahlke for assisting in data collection
- • USDA APHIS, Animal Welfare for financial support

**REFERENCES**


Caroline Hilty

- Currently a student at The Ohio State University, College of Veterinary Medicine to graduate in 2013
- Ms. Hilty is a member of ASLAP, OVMA, SCAVMA, AAZV and the shelter medicine club

**Mentor:** Carrie Freed, MLAS, DVM, DACLAM, Director, Rodent Clinical Medicine ULAR; Assistant Professor Clinical VPM

Dr. Freed is Attending Veterinarian for OSU facilities and as the Director of Rodent Clinical Medicine provides clinical and didactic support for the Laboratory Animal Residency Program. She is a member of ASLAP and AALAS, serves on the IACUC, the Institutional Biosafety Committee, ASLAP Faculty Liaison, and is a BSL3 Veterinarian at OSU.

**Resident Mentor:** Dr. Adrienne Dardenne, DVM

Dr. Dardenne is currently a Resident of Laboratory Animal Medicine at The Ohio State University, University Laboratory Animal Resources to graduate in 2012. She received her DVM from Louisiana State University in May 2010. She is a member of AALAS and ASLAP.

**Research Project:** Evaluation of Rodent Anesthesia: Do Strain Specific Responses Exist?
EVALUATION OF RODENT ANESTHESIA: DO STRAIN SPECIFIC RESPONSES EXIST?

C Hilty, A Dardenne DVM, and C Freed MLAS, DVM, DACLAM. University Laboratory Animal Resources.

The mouse is the most frequently used animal model for biomedical research. To meet research needs, surgical anesthesia is commonly induced, yet information is limited regarding strain specific variability in physiologic responses. This study investigated isoflurane, a commonly used inhalant anesthetic and a parenterally administered cocktail consisting of a dissociative agent (ketamine), sedative (acepromazine) and an alpha-2 agent (xylazine) in the C57BL/6 and BALB/c inbred mouse strains. For both groups, a surgical plane of anesthesia was maintained for 20 minutes. For the ketamine-xylazine-acepromazine (KXA) group, a range of drug doses was required as individual responses to the initial dose were not consistent. The alpha-2 agonist, yohimbine, was administered during the recovery period. The induction rate, duration and depth of anesthetic plane, and recovery time were noted for each strain and each anesthetic protocol. Physiologic parameters were monitored during anesthetic events and electrocardiograms (ECG) were evaluated on a subset of animals. In addition, blood samples were collected at baseline and following anesthetic events for analysis. No statistically significant differences between strain were observed based on the evaluated parameters in the two strains that were selected. Not surprisingly, significant differences were noted between the anesthetic protocols for both the C57BL/6 and BALB/c strains. Specifically, the induction period and recovery time was significantly shorter for the isoflurane group when compared with the KXA group, which was consistent across both inbred strains. Overall, based on our study, isoflurane is still the recommended option for achieving a surgical plane of anesthesia. However, if injectable protocols are needed based on research needs, a dose of 150/20/3 mg/kg KXA effectively produces a surgical plane of anesthesia in both the C57BL/6 and BALB/c strains.

Keywords: anesthesia, rodents, isoflurane, ketamine, xylazine, acepromazine
Evaluation of Rodent Anesthesia: Do Strain Specific Responses Exist?
C Hilty, A Dardenne, C Freed
University Laboratory Animal Resources

BACKGROUND

The purpose of this study was to identify strain specific responses of C57BL/6 and BALB/c mice to two anesthetic protocols by evaluating survival reflexes and physiological parameters. This study also aimed to provide guidance to investigators in choosing the most appropriate method of anesthesia for their research needs.

METHODS

Each mouse underwent both anesthetic protocols, allowing for a one week washout period between evaluations. All evaluations occurred between 8 AM and 12 PM in order to minimize impact of the circadian rhythm on the data. For inhalant anesthesia administration, an induction chamber was created by modifying a plastic container with a hole in the lid. Induction of anesthesia was defined by the loss of the righting reflex, at which point the animal could then be placed in a dorsal recumbent position. Animals were maintained on a heating pad and RR was measured every 10 minutes. Depth of anesthesia was determined by checking the pedal withdrawal reflex using a modified hemostat (Figure 1). A lock was placed on the hemostat so that a consistent pressure could be applied while potential trauma to the foot was minimized. Reflexes were checked every 10 minutes (Figure 2). For the ISO group, the duration of surgical anesthesia was clearly defined by the user and therefore not evaluated. Two-tailed t tests were performed to look for statistical significance between mouse strains as well as between anesthetic protocols. Statistical analysis was not performed on the blood parameters, HR, or RR.

RESULTS

Data was included only when the animal reached a surgical plane of anesthesia for a minimum of 20 minutes. 100% of the animals in the ISO group were included while only 80% of the KXA group were included. Of the remaining, 20% of animals in the KXA group, 2 did not reach a surgical plane and 2 died during induction of anesthesia. The initial study design also included a third anesthetic group consisting of only ketamine and xylazine, although animals did not consistently reach a surgical plane so the data was not included. For the ISO group, the duration of surgical anesthesia was clearly defined by the user and therefore not evaluated. Two-tailed t-tests were performed to look for statistical significance between mouse strains as well as between anesthetic protocols. Statistical analysis was not performed on the blood parameters, HR, or RR.

CONCLUSIONS

1. Inhaled isoflurane efficiently induces a easily controlled surgical plane of anesthesia in both B6 and C mice.
2. Inhaled ISO provided a statistically significant shorter induction and recovery rate when compared to KXA.
3. If injectable protocols are desired, a dose of 15/203 mg/kg KXA effectively produces a surgical plane of anesthesia in both the B6 and C strains.
4. Strain-specific differences in physiological parameters and survival reflexes were not observed between ISO and KXA anesthesia for the B6 and C strains of mice.

ACKNOWLEDGMENTS

Special thanks to:
- C Test Labs and Andy Kljakovic, DVM, PhD for assistance with ECG recordings
- Kathy Hoden-Davis, DVM, PhD, DACLAM for providing statistical analysis and research guidance
- Brenda Green Research Fellowship for funding my summer research experience through the College of Veterinary Medicine at the Ohio State University.

REFERENCES

Rebecca Erickson

- Currently a student of The Ohio State University, College of Veterinary Medicine to graduate in 2013
- Member of SCAVMA, SCASLAP, Shelter Medicine Clubs, Behavior Club

**Mentor:** Valerie Bergdall, DVM, DACLAM, Director, University Laboratory Animal Resources (ULAR) Professor Clinical Veterinary Preventative Medicine (VPM)

Dr. Bergdall is the Director of the University Laboratory Animal Resource Department (ULAR) and also serves as the Institutional Attending Veterinarian overseeing all animals used in biomedical research at The Ohio State University. Dr. Bergdall has served on the ACLAM Training Program Oversight Committee, Career Pathways Committee, Standard Setting Study Task Force, Minimal Competency Task Force, and Recertification Oversight committees. She is a member of AALAS and ASLAP and serves on the ASLAP Veterinary Student Liaison Committee. She currently serves on the NCRR/NIH scientific review group and is a co-investigator on an NIH grant investigating wound healing. Dr. Bergdall provides fiscal, clinical and didactic support for the Laboratory Animal Residency Program.

**Resident Mentor:** Dr Dan Domer, DVM, BS
Dr Domer is currently a Resident of Laboratory Animal Medicine at The Ohio State University, University Laboratory Animal Resources to graduate in 2011. He received his DVM from the University of Missouri - Columbia, College of Veterinary Medicine in 2003. He is a member of AALAS and ASLAP.

**Research Project:** Ammonia Levels in Mouse Cages Using Standard Corncob and Novel Corncob Bedding
Abstract - Rebecca Erickson

AMMONIA LEVELS IN MOUSE CAGES USING STANDARD CORNCOB AND A NOVEL CORNCOB BEDDING. R. Erickson, D. Domer, and V. Bergdall. University Laboratory Animal Resources

Cage changing frequency in laboratory mice can have impacts on animal health and stress levels, human health, and operational costs of husbandry. Cage changing frequency is dictated by intracage ammonia levels. Ammonia gas is released from urea by urease, which is produced by fecal bacteria and bacteria present in bedding before use. PureLite is a proprietary method of processing corncob bedding, and it has been shown to have significantly less bacteria than standard corncob bedding. The use of PureLite bedding could lead to a decrease in intracage ammonia levels and thus decrease the frequency of cage changing and sanitation without adverse effects to the animals. For this study, mice were placed 5 per cage with non-sterilized standard corncob, non-sterilized PureLite, sterilized standard corncob, or sterilized PureLite bedding. Intracage ammonia levels were measured daily using an ammonia tube test device, and the number of days to reach 25 ppm ammonia was documented. The study was performed in static cages and will be repeated in ventilated cages. In static housing, cages with non-sterile corncob bedding took significantly longer to reach 25 ppm than sterile corncob. Non-sterile PureLite lasted longer than sterile corncob, and sterile PureLite lasted longer than non-sterile PureLite; however, these differences were not significant. No significant difference was found between non-sterile corncob and non-sterile PureLite, suggesting that in static, non-sterile cages, PureLite bedding may not offer a significant advantage over traditional corncob in controlling ammonia levels. Further experiments will continue to investigate PureLite’s effect on cage ammonia levels as well as other aspects of the rodent microenvironment.

Keywords: rodents, husbandry, ammonia, bedding, corncob, PureLite
AMMONIA LEVELS IN MOUSE CAGES USING STANDARD CORNCOB AND A NOVEL CORNCOB BEDDING

R. Erickson, D. Domer, J. Hickman-Davis, V. Bergdall

University Laboratory Animal Resources

BACKGROUND

- Cage change frequency in laboratory mice can impact the microenvironment and well-being of the animals, the health of the human caretakers, and the operational costs of husbandry.
- Cage change frequency is dictated by intragas ammonia levels. Ammonia gas is released from urine by urease, which is produced by fecal bacteria and bacteria present in bedding before use (Figure 1). Urease activity increases with the size of the microbial population.
- Ammonia levels of 25 ppm have been determined by OSHA to be the human 8-hour exposure limit. This level is considered the "gold standard" for cage change frequency.
- Standard cage change frequencies are twice weekly for static mouse cages and every two weeks for ventilated cages.
- Previous studies have indicated that cage changing is stressful to mice and can cause disruption of the microenvironment, increased corticosterone levels, and decreased ease of handling.
- PureLite is a proprietary method of processing corn cob bedding, and it has been shown to have significantly less bacteria, yeast, and mold than standard corn cob bedding (Figures 2 and 3).
- The cost of PureLite bedding is approximately 30% greater than traditional corn cob bedding.

MATERIALS & METHODS

- C57BL/6J female mice were housed according to the institutional standard using the Lab Products Micro-Isolator caging system.
- For the purposes of sampling ammonia levels with minimal disruption of the microenvironment, a 1/4" hole (approximate diameter of gas detector tubes) was drilled into the side of each cage (Figure 4). The holes were covered with tape between samplings.
- Room temperature, humidity, light, and airflow (if applicable) were recorded daily throughout each study.
- Abstraction means of processing corn cob bedding, and it has been shown to have significantly less bacteria, yeast, and mold than standard corn cob bedding (Figures 2 and 3).
- The cost of PureLite bedding is approximately 30% greater than traditional corn cob bedding.

RESULTS

- Data is presented as number of days for intracage ammonia levels to reach 25 ppm.
- Results from Studies 1 and 2 are shown in Figure 7.
- The results from Studies 1 and 2 (using test paper and gas detector tubes, respectively) did not differ significantly for any of the treatment groups (Figure 8), so the data from both studies were combined for the purpose of statistical analysis.

PURPOSE & HYPOTHESIS

- Cages with PureLite bedding will begin with a decreased initial intracage bacterial load, leading to a delay in the increase of ammonia concentration. This would increase the interval required for cage changing and sanitation.
- Reduction in cage change frequency could result in decreased animal stress from cage changing; increased efficiency of animal husbandry; and decreased exposure of human caretakers to allergens, infectious agents, and rodent pathogens.

REFERENCES


ACKNOWLEDGEMENTS

Funding provided by NIH T35 Research Training Grant.