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43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

IN THE CIRCUIT COURT OF LEE COUNTY, ALABAMA

MADELYN BINGHAM,	*	
	*	
Plaintiff,	*	
	*	
v.	*	**Civil Action No.: _____**
	*	
JAIME S. HAMMER, in her Official Capacity as General Counsel for Auburn University,	*	
	*	
	*	
	*	
Defendant.	*	

COMPLAINT FOR VIOLATION OF OPEN RECORDS ACT

COMES NOW, Plaintiff Madelyn Bingham and makes the following complaint against Jaime S. Hammer in her official capacity as general counsel for Auburn University:

Parties, Jurisdiction, and Venue

1. Plaintiff is an adult resident citizen of the State of Alabama.

2. Non-party Auburn University ("Auburn") is a state-chartered university located in Auburn, Alabama and subject to the provisions of the Open Records Act.

3. Jaime S. Hammer ("Hammer") is the General Counsel for Auburn and, in that capacity, her office is responsible for responding to Open Records Act requests.

Facts

A. Institutional Animal Care and Use Committee and Animal Testing Protocols.

4. Research universities, like Auburn, perform laboratory testing on animals.

5. In 1966, Congress passed and President Johnson signed the Animal Welfare Act of 1966 ("AWA"), which regulates the treatment of animals in research and exhibition, among other things. It has been amended several times since its passage.

6. An Institutional Animal Care and Use Committee ("IACUC") is a federally mandated committee under the AWA. *See* 7 U.S.C. § 2143 & 9 C.F.R. 2.31.

7. The United States Department of Agriculture regulations require IACUCs to ensure that animal testing meets certain criteria related to personnel, housing, procedures, rationale, objectives, minimization of pain and distress, safety factors, and final disposition of the test subjects. *Id.* A researcher seeking to use animals in experiments submits a protocol (also sometimes called an application) containing information related to these criteria to the IACUC for their institution before testing or research can begin. The IACUC then either approves or disapproves the protocol. If the IACUC approves the protocol, the experiments can proceed at the institution.

8. Auburn has an IACUC which "reviews all animal use protocols, reviews the animal care and use program, and monitors university animal facilities to ensure compliance with standards and regulatory requirements." https://research.auburn.edu/research-administration/compliance/iacuc/index.php (accessed 1/15/26).

9. Other state universities have made their IACUC animal use protocols public. Attached as **Exhibit A, B, & C** respectively, are publicly available cat testing protocols from the University of Georgia, University of Louisville, and Kansas State University. Attached as **Exhibit D** is a publicly available dog and cat testing protocol from the University of Missouri-Columbia.

10. In addition, research funded by the National Institute of Health is subject to the Public Health Service Policy on Humane Care and Use of Laboratory Animals, which requires institutions to implement programs for activities involving animals based on the *Guide for the Care and Use of Laboratory Animals*. It also requires a written Animal Welfare Assurance, describing training offered to scientists and laboratory personnel in animal care and in methods that minimize the number of animals used and minimize animal distress. https://olaw.nih.gov/policies-laws/phs-policy.htm (accessed 1/15/2026). These requirements stem from the Health Research Extension Act of 1985, Public Law 99-158, "Animals in Research" (November 20, 1985).

11. Auburn has disclosed in a federal funding application that it is performing at least one set of experiments on cats requiring IACUC approval. A joint application submitted on behalf of Auburn, Northwestern University, Tufts University, and University of Massachusetts Medical School reveals the project abstract, narrative, methods, and goals, and highly detailed descriptions of the experiments that will be conducted at Auburn in Douglas Martin's lab, the facilities where they will be conducted, who will conduct them, the equipment to be used, the cats to be experimented on, the intended animal care, the justification for using them, when they will be euthanized, and the federal funds requested.

12. Auburn also has breeding colonies to supply cats for these and other experiments, which themselves necessitate active protocols for as long as the university maintains them.

B. The Alabama Open Records Act.

13. Alabama has a long history of government transparency. Cases dating as far back as the nineteenth and early twentieth centuries recognized a broad common law right of access to public records. *See Brewer v. Watson*, 71 Ala. 299, 303 (1882); *Excise Com. of Citronelle v. State ex rel. Skinner*, 60 So. 812 (Ala. 1912). One hundred years ago, the Legislature enacted what is now known as Alabama's Open Records Act, Ala. Code § 36-12-40, *et seq.* (the "ORA"). *See* Ala. Code § 2695 (1923). It now states in relevant part, "Every resident has a right to inspect and

take a copy of any public record of this state, except as otherwise expressly provided by applicable law." Ala. Code § 36-12-40.

14. The Alabama Supreme Court has described a public record as any record "reasonably necessary to record the business and activities required to be done or carried on by a public officer so that the status and condition of such business and activities can be known by our citizens." *Stone v. Consol. Pub. Co.*, 404 So. 2d 678, 681 (Ala. 1981). The Alabama Code also defines a public writing as "all written, typed or printed books, papers, letters, documents and maps made or received in pursuance of law by the public officers of the state... other subdivisions of government in the transactions of public business and shall also include... any other public record authorized by law...." Ala. Code § 41-13-1.

15. As the Alabama Supreme Court has explained:

> Citizens are entitled to information regarding the affairs of their government. Alabama's Open Records Act first appeared in the 1923 Code of Alabama and represents a long history of openness. The Open Records Act is remedial and should therefore be construed in favor of the public. The statutory and judicially created exceptions generally protect an individual's privacy, the integrity of a criminal investigation, public safety and security, or privileged information. The exceptions to the Open Records Act should be strictly construed, because the purpose of the Open Records Act is to permit the examination of public writings and records.

Allen v. Barksdale, 32 So. 3d 1264, 1274 (Ala. 2009).

16. There is, as a result, a presumption in favor of the disclosure of public writings and records. *Chambers v. Birmingham News Co.*, 552 So. 2d 854, 856 (Ala.

1989). The burden is on the government to prove the records sought are subject to an exception or exemption to disclosure. *Id.* at 856.

C. Plaintiff's request for Auburn's IACUC protocols involving cat testing is denied.

17. On July 16, 2025, Plaintiff made an open records request to Auburn for "all IACUC-approved protocols that were active as of July 2, 2025 and involve the use of at least one cat." A true and correct copy of her request is attached as **Exhibit E**.

18. On September 18, 2025, Auburn denied the request, stating "we have not been able to locate any records responsive to your request subject to release under the Alabama Open Records Law." A true and correct copy of the initial denial is attached as **Exhibit F**.

19. On October 27, 2025, Madelyn replied to the denial, pointing out that it was both vague and confusing and asking for a clarification.

20. On October 28, 2025, Auburn provided a longer, but not necessarily more enlightening, explanation for its initial denial. It stated:

> State and federal laws, as well as case law, provide protection for scientific research materials, documents, and records, which are not the same as agency policymaking documents, and may qualify as confidential, privileged, or nonpublic in nature. Research materials, documents, and records, including but not limited to, research protocols, drafts, communications, peer review commentary, preliminary results, underlying data, or study methodologies, etc., are not subject to release under the Alabama Open Records Law if the records sought are prepublication preliminary research documents;

> have not been publicly disseminated, published, copyrighted, or patented; contain intellectual property, trade secrets, or other confidential material; are received in confidence by a public officer; or the release of the records sought will unduly interfere or hinder the discharge of a public officer's duties or government business.

A true and correct copy of the October 27 reply and Second Denial is attached as **Exhibit G**.

21. On December 10, 2025, Plaintiff, by and through the undersigned counsel, sent Auburn a letter pointing out the additional deficiencies in Auburn's second denial. These included a lack of specificity, lack of any citation to the authorities relied on to deny the request, and that this category of document is typically in the public domain—including extensive detail related to Auburn's research. Plaintiff requested that Auburn (1) provide the records in full or (2) provide the records with redactions with a privilege log. *See Something Extra Publ'g, Inc. v. Mack*, 350 So. 3d 663, 671 (Ala. 2021) (Stewart, J., concurring). Plaintiff asked that the records be provided by January 5, 2026. A true and correct copy of counsel's letter is attached as **Exhibit H**.

22. As of the date of this filing, Auburn has not provided the requested records or responded in any way.

COUNT I—ENFORCEMENT OF THE OPEN RECORDS ACT

23. Plaintiff adopts and incorporates all prior paragraphs.

24. On July 16, 2025, Plaintiff made a valid request for public records from Auburn for "all IACUC-approved protocols that were active as of July 2, 2025 and involve the use of at least one cat."

25. Plaintiff has complied with the ORA and was entitled to inspect and make copies of the records requested in exchange for a reasonable fee. Ala. Code § 36-12-41.

26. Hammer, as the public officer responsible for responding to open records requests made to Auburn, denied Plaintiff's request for records.

27. Hammer denied Plaintiff's request for reasons that are vague, untethered to the facts, and unsupported by any legal authority. Auburn has failed to meet its burden of demonstrating the subject records are prohibited from disclosure.

WHEREFORE, Plaintiff hereby demands judgment against Hammer in the form of a writ, declaration, and/or injunction which orders Hammer to provide a copy of the subject records to Plaintiff in exchange for a reasonable fee, sets the amount of such fee in accordance with the ORA, and awards any such further relief to which Plaintiff may be entitled including attorney's fees.

Respectfully submitted,

/s/ J. Evans Bailey
J. Evans Bailey (BAI062)
Attorney for Plaintiff

OF COUNSEL
Rushton, Stakely, Johnston & Garrett, P.A.
184 Commerce Street (36104)
P.O. Box 270
Montgomery, Alabama 36101-0270
(334) 206-3170
ebailey@rushtonstakely.com

PLEASE SERVE DEFENDANTS BY CERTIFIED MAIL

JAIME S. HAMMER
GENERAL COUNSEL
182 SOUTH COLLEGE STREET
101 SAMFORD HALL
AUBURN, ALABAMA 36849

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EXHIBIT A

Quick Info

PI:	Andrew Moorhead
Title:	Infection of mammals with Brugia malayi, Brugia pahangi, and Dirofilaria immitis
Form Code:	A2022 04-009-Y2-A11
Workflow Status:	Approved
Final Approval Date:	2022-10-11
Expiration Date (3 yr):	2025-10-11
Attending Veterinarian:	Leanne Alworth

Section 1: Overview

Animal Use Proposal

The Institutional Animal Care and Use Committee (IACUC) is responsible for ensuring that the use of animals at the University of Georgia is performed according to the highest standards and in an ethical manner. This responsibility is shared with university faculty, staff, and students. The use of animals at the University is a privilege, not a right.

Maintaining this privilege requires compliance with the following regulations, policies and guidelines:

Regulatory documents

- Animal Welfare Act Regulations
- The Guide for the Care and Use of Laboratory Animals
- Guide for the Care and Use of Agricultural Animals in Research and Teaching
- Public Health Service Policy on Humane Care and Use of Laboratory Animals
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training.

UGA IACUC general policies and guidelines

- Humane Care and Use of Animals
- Changes to Approved Animal Use Protocols
- Reporting Unanticipated Outcomes
- Reporting and Investigating Animal Welfare Concerns
- Health Records Standards
- Acclimation of Animals Utilized in Research Following Transportation
- Reuse of Animals for Research or Instruction

This form is intended to facilitate review of requests to use animals for research and instruction. The review process has been designed to communicate rationale, justifications, methods, and materials for using animals through the Office of Animal Care and Use and Attending Veterinarians to the IACUC.

In general, questions on the AUP form are 1 of 2 types. Most questions come directly from the regulations, to gather specific information that the IACUC is required to consider during a protocol review. Some questions are more indirectly related to the regulations. They are for descriptions/clarification, to allow the IACUC reviewer to

understand the particular circumstances and procedures in relation to the research goals and impact on the animals.

We have populated the AUP form with helpful hyperlinks:

1. Hyperlinks to "TIPS" for answering specific questions
2. Hyperlinks to related IACUC policies and guidelines

Here is the first hyperlink to general tips for writing an AUP:TIPS

Important Notes regarding privacy and open records requests:

Please avoid using names of people other than in section 3.1. According to UGA Open Records Office, names cannot be redacted from documents subject to an open records request.

Please avoid using the names of private organizations, such as animal shelters or commercial contract organizations that would want to maintain privacy other than when specifically required, such as in section 2.

Section 2: Key Information

AUP Number:	A2022 04-009-Y2-A11
2.1: Project Title: TIPS	*Infection of mammals with Brugia malayi, Brugia pahangi, and Dirofilaria immitis*
2.2: Principal Investigator: TIPS	Andrew Moorhead
2.3: Primary Funding Agency: TIPS If you are receiving federal money (e.g. Public Health Service, NIH, DOD, DOE, NSF, etc.) you MUST answer this section and specify the funding agency.	*National Institutes of Health and various corporations*

2.3.1 Aliases with Funding Agency TIPS
If you have multiple titles for this work related to different funding sources, please list them here.

Protocol Title Alias: *Animal Models of Infectious Disease: Contract No. HHSN272201000030I Part D02-MAINTENANCE, DEVELOPMENT AND PRODUCTION OF FILARIASIS PARASITES, REAGENTS, AND ASSAYS*
Funding Agency: *NIH*

2.4: Location TIPS
Will any live vertebrate use aspect of the study (course) or vertebrate animal husbandry be conducted at another institution?
○ Yes ◉ No

2.5: Expiring Protocol TIPS
Will this AUP replace an expiring protocol or a protocol that has already expired? ◉ Yes ○ No

Select AUP: A2019 04-010-Y3-A18 - Expires

2.6: Purpose TIPS
What is the Purpose of this animal use protocol?
☐ **Instructional**
☑ **Research**

Section 3: Personnel and Qualifications

3.1: Personnel TIPS (US Government Principles, Principle #8 Guide, p 25-26 AW Regs 9 CFR Part 2, sec 2.31, sec 2.32)

Project Roster: Please provide the names of all the individuals who will work with animals on this project to the IACUC. Include yourself and any other investigators, student employees, post-doctoral fellows, staff research associates, post-graduate researchers, laboratory assistants, and others who will actually work with the animals. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OHSP page.

See IACUC policy: Occupational Health

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals" and "Sharps."

See IACUC policy: Training

See IACUC policy: Volunteers in Research and Instructional Protocols

See IACUC policy: AUP Roster Requirements and PI Eligibility

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

CURRENT ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00

Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	

Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00

Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00

Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	
IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-06-24	0.00
Sharps Training(2022)	2024-06-24	
Staying Healthy While Working With Animals (Version 032023)	2024-06-24	
IACUC 101(Version 041123)	2024-06-24	1.00

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2024-02-16	
Staying Healthy While Working With Animals (Version 032023)	2024-02-16	
IACUC 101(Version 041123)	2024-02-16	1.00
Research Occupational Health Enrollment	2024-02-14	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

Section 4: Project Objective, Significance and Animal Use Justification

Section 4 allows you to clearly state the reasons you are doing this research or instruction. This information is specifically required by the regulations because the IACUC is charged with considering the cost to benefit balance of the research. So, the IACUC needs to know what the benefits may be.

These descriptions must be in lay terms. Some IACUC members are not scientists, and the scientist members have a variety of areas of expertise- most of the people reading this are not experts in your field.

If any acronyms are used, they must be defined the first time they are introduced.

4.1: Objective TIPS (US Government Principles, Principle # 2 Guide, p 12, 25)
["Why are you doing this experiment with animals and what do you propose to learn?"]
Please provide a brief statement, limited to 300 words, outlining the objectives of the procedures in this protocol.

- This must include a statement of your **experimental or teaching objectives.**
- This must be in LAY TERMINOLOGY, understandable by someone with a high school education, with no scientific jargon.
- Please define all abbreviations/acronyms the first time they are used and explain medical terms.
- Please do not submit your grant proposal abstract for this section.

The objective of this protocol is to provide parasitic worms, specifically filarial worms, to North American scientists for research experiments regarding the immunology, pathology and transmission of these worms. We are contracted by the National Institutes of Health (NIH) to perform this service. Also, we provide these resources to other commercial and private entities. Since we provide parasites for other researchers, they do not have to grow the worms in animals reducing the overall number of animals used in research (Three R's).

The three worms Brugia pahangi, Brugia malayi, and Dirofilaria immitis in this protocol are known as filarioid worms. They cause diseases such as elephantiasis (Brugia malayi) in humans. Dirofilaria immitis (canine heartworm) is a significant health problem in dogs. Therefore, research on these parasites is very important. However, the life cycle of these parasites require that the adults be maintained in dogs and cats in order to produce the microfilariae larval stages used for feeding mosquitoes. Microfilariae (a larval stage of the worm)

are fed to mosquitoes. The parasites grow further in the mosquitoes and are then infectious to the definitive host (dog or cat). In order to minimize the use of dogs and cats, we can grow the worms from their infectious stage to adults in the Mongolian gerbil or jird. We can then collect the adult worms from the jird.

There are no in vitro systems that allow the levels of production needed in order to meet the demands of researchers. Therefore, we must maintain the life cycle of the parasite in dogs, cats and jirds. If we did not supply these worms, filariasis research in the United States would be severely affected.

Also, another objective of this protocol is to provide different life stages Dirofilaria immitis (canine heartworm) to private companies for scientists for research experiments regarding drug discovery. Dirofilaria immitis (canine heartworm) is a significant health problem in dogs. Therefore, research on these parasites is very important. Since growing and maintaining D. immitis infections in animals is a time-consuming process, independent laboratories have requested that we perform this service for them. The life cycle of D. immitis requires that the adults be maintained in dogs. Microfilariae (a larval stage of the worm) are fed to mosquitoes. The parasites grow further in the mosquitoes and are then infectious to the definitive host (dog). Since we have literally years of experience with this experimental system, the number of animals used will be decreased, as compared to someone with less experience. Because the University of Georgia is an AAALAC-accredited institution, we can assure that the research performed adheres to the strictest of standards.

Another objective involves the examining the effect of the bacterial endosymbiont, Wolbachia, on filarial biology. Wolbachia are bacteria that live inside the worm and are required for filarial worm survival. These bacteria can be eliminated by the administration of the antibiotic, doxycyline, to the vertebrate host containing the worm. By depleting Wolbachia, we can evaluate the effect on worm survival in the host, as well as in the offspring (microfilariae).

4.2: Significance TIPS (US Government Principles, Principle # 2 Guide, p 12, 25)
Please provide a brief statement of the relevance of contributions your work might make to human/animal well-being or the expansion of knowledge. This statement must provide a rationale for your proposed use of animals. This must be written in LAY TERMINOLOGY, understandable by someone with a high school education, with no acronyms or scientific jargon. Please do not submit your grant proposal abstract for this section. Define all abbreviations the first time they are used and explain medical terms. ["How will the proposed use of animals benefit human or animal health or expand knowledge?"]

The parasites that we grow in animals are used for a myriad of NIH-funded and other researchers to address basic questions about the pathogenesis of filarial diseases. Also, these parasites are used for in vivo testing of known and novel anthelmintic compounds. These studies include the immunological response of the host to the parasite, as well as how the vector (mosquito) interacts with the worm. The discoveries made as a result of this research allows eventual advances in control, treatment and diagnosis of filarial diseases, both human and animal. Also, we provide parasites or blood containing parasites to institutions for teaching purposes. In accordance with NIH practices, when animals are donated to another instution from UGA, the ownership of the animals will be transferred to the recipient institution.

4.3: Justification for the Use of Animals TIPS (US Government Principles, Principle #3 Guide, p 12, 25 AW Regs 9 CFR Part 2, sec 2.31)
Please provide a brief statement justifying your use of animals in the proposed project: ["Why must you use animals in this work?"]
Note: The response needs to be an explanation on the use of a live animal instead of a non-animal alternative (not just a justification for the species).

There exists no other means to produce larval and adult stages of these worms at the levels needed by researchers who utilize our services. Worms have been grown in vitro, but the complete life cycle has yet to be maintained outside of the vertebrate host. As a result, it is impossible to provide parasites to researchers without the use of animals.

Section 5: Animal Information

5.1: Animal Information (Species, strain, use category and other parameters to be used on this protocol).
Provide details for each species to be used. Indicate the number of each species for which you wish to be approved for the 3 year lifespan of the AUP. If you wish to increase these numbers after this AUP is approved, an amendment will need to be submitted.

See IACUC policy: Animal Housing Locations
See IACUC policy: Satellite Animal Facilities and Principal Investigator-Provided Husbandry

Animal Use Categorization TIPS
All animal use must be classified according to the anticipated level of perceived pain/stress/distress experienced by the animal(s). Animals must be classified under the highest category involved at any point. Procedures involving more than momentary or slight pain or distress must be discussed with the Attending Veterinarian in the planning of the research project.

Note that these category letters do NOT match the USDA category letters your grant may include.

Category A: Includes the use of animals in experimental procedures that would be expected to produce little or no pain or distress.

Category B: Includes the use of animals in procedures that involve minor pain or distress of short duration, or in procedures where pain and distress are alleviated through the use of anesthetics, analgesics, and/or tranquilizers.
Animals are not expected to show prolonged (days) clinical signs, other than some mild discomfort, during or after Type B procedures. Type B studies place an explicit responsibility on investigators to explore alternatives to the procedures which may cause pain or distress.

Category C: Includes the use of animals in procedures that involve potential for significant but unavoidable pain or distress to the animals. Type C studies place an explicit responsibility on investigators to explore alternatives to the procedures which may cause pain or distress. Convened quorum of the IACUC will review all Category C, multiple major survival surgeries, and any other proposal as requested by any member(s).

IMPORTANT: The reasons for using these procedures must be explained in a statement by you, the Principal Investigator, justifying their use. Provide scientific justification if withholding appropriate sedation, analgesia, or anesthesia.

Category D: Includes the use of invertebrate animals, cell cultures, embryonated eggs, certain biologic products, tissues obtained post-mortem from vertebrate animals (obtained at necropsy, slaughterhouses, meat markets), or observation of non-captive wildlife species where there is no contact with animals.

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Cat
Strain: TIPS	DSH
Highest Use Category:	Category B
Sex: TIPS	Both
Quantity (Numerical Only): TIPS	30
Housing Location: TIPS	VET MED CENTRAL ANIMAL FACILITY (CAF)
Weight Range: TIPS	*3-5 kg*
Age Range: TIPS	*10-12 weeks*
Preferred Vendor/Source: TIPS	Class A Vendor
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	(•) Yes ( ) No

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Dog
Strain: TIPS	Beagle

	Mongrel
Highest Use Category:	Category B
Sex: TIPS	Both
Quantity (Numerical Only): TIPS	50
Housing Location: TIPS	INTERIM CANINE FACILITY (ICF)
Weight Range: TIPS	*7-25 kg*
Age Range: TIPS	*4-8 months*
Preferred Vendor/Source: TIPS	Class A Vendor
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	◉ Yes ○ No

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Gerbil
Strain: TIPS	Mongolian
Highest Use Category:	Category B
Sex: TIPS	Both
Quantity (Numerical Only): TIPS	1500
Housing Location: TIPS	VET MED CENTRAL ANIMAL FACILITY (CAF)
Weight Range: TIPS	*50-110 grams*
Age Range: TIPS	*see weight*
Preferred Vendor/Source: TIPS	Charles River Laboratories
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	◉ Yes ○ No

5.2: Justification of Animal Numbers TIPS (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data)

Group sizes are expected to represent the minimum number of animals that are needed to achieve the scientific or instructional objectives. Please indicate all the methods used to determine these numbers.
NOTE that if you check certain boxes, you MUST also check the final box "Explanations/Other" and provide specific details.

☐ Statistical tools, such as power analysis, were employed to determine appropriate group sizes to ensure statistically valid outcomes. [If you check this box you MUST check the final box "Explanations/Other" and state the name/s of the statistical tests you used, as well as a brief explanation as to how the test provided you with the number of animals.]
☑ Previous experience with this experimental paradigm indicates this is the minimum number of animals needed.
☐ Consultation with a biostatistician. [If you check this box you MUST check the final box "Explanations/Other" and state the name/s of the tests the biostatistician used, as well as a brief explanation as to how the test provided the number of animals.]
☐ This is a pilot study used to determine feasibility before proceeding with larger, more tightly controlled experiments.
☑ This is an instructional activity and this is the minimum number of animals needed based on class size and optimal student to animal ratios.
☑ Explanations/Other
(Explain below):

For jirds, we must have large numbers in order to meet the demands of researchers to accommodate their orders for Brugia filarial parasites. Typically we need 500 jirds a year to produce the needed worms to ship to investigators.

Dogs and cats will be used for both B. malayi and B. Pahangi infections.

For cats and dogs, there are a 10-20% and 50% infection rate, respectively, with Brugia malayi. Cats maintain a patent infection for only one year, where as dogs maintain infection for 2 years. This means that in order for us to obtain a useable number of infected animals, we have to infect 10 cats and 5 dogs approximately every year. Previous attempts to reinfect cats with B. malayi have not yielded patent infections (per M. Dzimianski, DVM, who has over 40 years of experience with the experimental infection of cats with B. malayi), therefore new cats must be used for each infection. Also, there can be unexpected deaths due the hypertrophic cardiomyopathy present in the vendor colony.

For dogs, we typically need 2-6 Brugia pahangi dogs per year and multiple dogs for a variety of heartworm strains. The need for heartworm dogs cannot be predicted as we do not know exactly how many field isolates we may obtain. Dogs maintain infections of B. pahangi and D. immitis for several years, therefore, we need fewer dogs than cats. Also, we occasionally need adult D. immitis. They only way to obtain adult D. immitis is to euthanize infected dogs.

5.3: Justification for species selection TIPS (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data)

The species has been selected because (check all that apply)

☑ Previous work in the biomedical literature validates the use of this species as an animal model for this disease or biological process.
☐ This is the lowest sentient species that provides appropriate size, tissue or anatomy for the proposed work.
☐ There is a large body of existing research that would need to be repeated if another species was used instead.
☐ Availability of reagents or research tools necessary for this research is unique to this species.
☑ Characteristics of the species make it uniquely suited for the proposed research or instruction.
(Explain Below)

Jirds have been used for many years for the production of adult Brugia spp. Brugia malayi can be maintained in cats and dogs. Brugia pahangi can be maintained in dogs. As far as D. immitis, dogs are the natural host and therefore suitable for laboratory infections.

☐ Other

5.4: Will a breeding colony be maintained? TIPS (Guide, p 25 AW Regs 9 CFR Part 2, sec 2.31)
Definition of a breeding colony: animals mated repeatedly to produce multiple groups of offspring for ongoing research projects and to maintain the colony. This does not include a single mating to produce embryos or one group of offspring for a specific research project.
○ Yes ◉ No

Section 6: Animal Use Attributes and Justification

6.1: Will any experimental condition exist, deleterious phenotype be expected, or technique be performed, which would result in loss of sensation or paralysis in conscious animals? TIPS (Guide, p 123 USDA Animal Care Resource Guide Policy #11)
○ Yes ◉ No

6.2: Will any technique be performed which will involve prolonged physical restraint in unanesthetized animals other than routine caging and handling? (Guide, p 29)
UGA's IACUC has chosen to consider prolonged restraint to be 30 minutes or longer in a natural body position, or 10 minutes or longer in an unnatural body position.
"Physical restraint is the use of manual or mechanical means to limit some or all of an animal's normal movement for the purpose of examination, collection of samples, drug administration, therapy, or experimental manipulation."

[Guide, p. 29]

See IACUC policy: Physical Restraint
Yes No

6.3: Will any agents, such as Complete Freund's Adjuvant or other adjuvants be injected which could cause chronic inflammation and/or pain? TIPS (Guide, p 27 USDA Animal Care Resource Guide Policy # 11)
Yes No

6.4: Will animals be subjected to potentially painful procedures for identification, e.g. toe clipping, branding, etc? (Guide, p 25 USDA Animal Care Resource Guide Policy # 11)
NOTE: ear punch, ear tag, and leg banding are not considered painful and do not need to be listed here.
Yes No

6.5: Will one or both of these situations occur with live animals? TIPS (Guide, p 25-26)

1. **Will live animals ever be transported outside of their housing area? Such as transported between housing locations, or moved from a housing location to a non-housing space for a procedure? NOTE: This would include transportation outside of the housing location, even if it is in the same building.**

   See IACUC policy: Transport

   and/or

2. **Will live animals ever be housed (> 12 hours) in a location that is not a dedicated animal facility (e.g., URAR, AHRC, PDRC, PRC, ADS) - such as housed in a satellite facility or lab space ?**

   See IACUC policy: Satellite Animal Facilities and Principal Investigator-Provided Husbandry

Yes No

6.6: Will animals undergo more than one major survival surgical procedure? TIPS (Guide, p 30 AW Regs 9 CFR Part 2, sec 2.31 USDA Animal Care Resource Guide Policy # 14)

Major survival surgery is defined as the penetration of a body cavity with anything larger than a needle or a surgical procedure that results in the permanent impairment of physical or physiological functions.

Yes No

6.7: Will this experiment involve the study of stress, pain, or abnormal behavior in live animals which cannot be alleviated with drugs because their use would interfere with the research goal?
NOTE: This refers only to projects that induce stress, pain, or abnormal behavior specifically to study these conditions. It does not refer to stress/pain/abnormal behavior that is a potential side effect of the procedures.
(US Government Principles, Principle # 4, 5 Guide, p 27 USDA Animal Care Resource Guide Policies # 11, 12)
Yes No

6.8: Do you expect any adverse effects on animals or overt signs of illness as the result of any procedures or activities performed? If YES, explain. TIPS
This refers to adverse effect/illness from any research related cause, such as an experimental agent, procedure, or genetic predisposition.
DO NOT include monitoring intervals or humane endpoints here - monitoring and humane endpoints should only be listed in sections 11.2 and 11.3, respectively to avoid inconsistencies. (Guide, p 105)
Yes No

6.8.1 (a): TIPS

Describe what clinical signs (not the monitoring intervals) will be monitored to assess the presence of pain, discomfort, or other potential adverse effects that may result from the study.
NOTE: This period includes the time from initiation of experiments until the animals are removed from the study; for surgically manipulated animals, this includes the time after anesthesia recovery until animals are removed from the study. (Guide, p 27, 105, 120 AW Regs 9 CFR Part 2, sec 2.31 USDA Animal Care Resource Guide Policy #11)

In gerbils, post-peritoneal infection of Brugia spp. peritonitis may develop with 24-48 hours after infection. This possibly manifests in a rough hair coat, potentially limited movement, ascites, and lethargy.

Post-SQ infection of Brugia spp, animals could potentially develop lymphedema as manifested by limb swelling. This condition does not normally affect the mentation or movement of the animal. On rare occasions, the hind feet will have swelling of the lateral digits and possible loss of the ungual process. They appear comfortable with no lameness and are in otherwise good health.
A result of SQ infection with Brugia malayi or pahangi in gerbils can be swollen testicles due to either presence of the worms or lymphedema. Secondary to the swelling of testicles, ulcerations may occur on the scrotal sac although it is not clear if the ulceration is due directly to the swelling or due to an indirect effect. The ulceration is usually non life threatening and does not require immediate euthanasia of the animal. The gerbils are often bright, alert, and active (BAR) when they have ulcerations and in our experience usually heal on their own without medical treatment. Infections in gerbils are not usable until at least 3 months post-infection, therefore to gain maximum use from the animal, we must keep them alive for this time. Otherwise, it is a non productive infection and we would need to infect another animal. Hence, we would prefer to maintain gerbils with ulcerations as long as the ulceration does not seem to be causing any pain or distress (hunched posture, or lethargic).

6.8.1 (b): TIPS Check all criteria that will be monitored to assess the presence of pain, discomfort, or other potential adverse effects that may result from the study. (Guide, p 105, 120)
☑ Activity ☑ Appearance ☐ Appetite TIPS (Requires method to assess individual animal intake-- not usually selected for group-housed, ad libitum fed animals) ☑ Behavior ☐ Excreta ☑ Grooming ☐ Guarding/Ambulation/Gait TIPS ☐ Heart Rate TIPS
☐ Licking, biting TIPS ☑ Posture ☐ Respiratory rate and/or quality ☐ Temperature TIPS ☐ Vocalizing ☐ Body Weight / Body Condition TIPS ☑ Wound site
☐ Laboratory tests or other evaluation TIPS (please specify)

6.9: Special Husbandry Requirements/Exceptions with Scientific Justification TIPS:
Please specify any exceptions, such as single housing of social animals, husbandry provided by PI or PI staff, or other special husbandry requirements.
If you need to have approval for any of the following exceptions or unusual husbandry situations, you need to choose the appropriate items offered in the drop down list.
NOTE: "Other" is an option for those situations not named. It is a good idea to scan the drop down list as a reminder, to see if there is anything you should include. If there is something not listed that you think would be good to add, please let us know (iacuc@uga.edu).

See IACUC policy: Satellite Animal Facilities and Principal Investigator-Provided Husbandry
See IACUC policy: Space Requirements
See IACUC policy: Social Housing of Social Animals
See IACUC policy: Reuse of Animals for Research or Instruction
See IACUC Guidelines: Acclimation

Provide a brief justification for each item: (US Government Principles, Principle # 7 Guide, p 25-26, Chapter 3 AW Regs 9 CFR Part 2, sec 2.31)

- ABSL2 requirements
- ABSL3 requirements
- ABSL3-Ag

- Animals not observed daily
- Aversive conditioning
- Barrier housing
- Husbandry performed by PI staff
- Immunocompromised housing
- Metabolic caging
- Non-standard bedding
- Non-standard cage sanitation intervals
- Non-standard space provided
- Non-standard Acclimation
- Other, please describe
- Reverse Light Cycle
- Single housing of social species
- Special diets
- Special medical requirements
- Using animals in multiple projects (this AUP)
- Water treatments
- Wire floor/ wire bottom cages for rodents

Husbandry Requirement / Exception: Special medical requirements
Justification:

Due to the fact that the dogs and cats are infected with filarids, they should not be administered macrocyclic lactone heartworm preventatives. Also, based on recent literature that doxycycline can damage the endosymbiotic bacteria in filarids, no doxycycline or its derivatives, such as tetracycline antibiotics ie minocycline, oxytetracycline, chlortetracycline, should be administered to dogs, cats, or gerbils for any clinical reasons.

Husbandry Requirement / Exception: Single housing of social species
Justification:

Animals can normally be SOCIALLY housed (cats/dogs), or multiply-housed (jirds). However, if animals engage in repeated agression towards one another, they will need to be singly-housed. This exemption will be noted by a laboratory animal veterinarian.

6.10: Environmental Enrichment: Environmental enrichment is provided to all confined research animals in accord with the UGA Environmental Enrichment Policy. Exemption from this enrichment plan requires scientific justification.
See IACUC Policy: UGA Environmental Enrichment Policy

Does this project require exemption from the UGA Environmental Enrichment Policy? (Guide p 25-26, 52)
○ Yes ◉ No

6.11: Food/Water Restriction TIPS
Will food or fluid be restricted? (Guide, p 30 USDA Animal Care Resource Guide Policy # 11)

Note: This does NOT include fasting before anesthesia -- please describe fasting before anesthesia in section 9 (if surgery is being performed) or in 8.1/8.2 (if surgery is not being performed). For species that are not fed daily, do NOT include withholding food if the period of withholding is known to fall within that species' normal feeding intervals (e.g., a couple days for reptiles).

See IACUC policy: Food or Fluid Regulation
○ Yes ◉ No

6.12: Numbers and Use Category Summary: TIPS

The following is an outline of the species selected in section 5.1 grouped by species/highest use category. This represents the number of animals available at the specified use category for animal orders over the 3 year AUP lifespan. If modifications are necessary please make appropriate changes to the species information in section 5.1.

If you wish to increase these numbers after this AUP is approved an amendment will need to be submitted.

HIGHEST USE CATEGORY: B

Species: CAT	**Use Category: B**	**Number of Animals: 30**
Species: DOG	**Use Category: B**	**Number of Animals: 50**
Species: GERBIL	**Use Category: B**	**Number of Animals: 1500**

6.12.1 (a): TIPS

Please provide scientific justification for withholding appropriate sedation, analgesia, anesthesia, or euthanasia.

1. Explain why it is necessary to keep an animal showing signs of pain/distress alive until the humane endpoints are met.
2. Explain why alleviation of the pain/distress through sedation, analgesia, or anesthesia is not possible. You must be specific and explain exactly what outcomes/data may be affected by the use of these agents.

NOTE: For USDA covered species, USDA requires us to report this information on our USDA Annual Report. (US Government Principles, Principle # 4, 5, 6 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31)

Section 7: All 3 Rs: Refinement, Reduction, and Replacement

The Public Health Service Policy and the Animal Welfare Act require assurance that there is appropriate consideration of alternatives refinements to procedures or research-induced conditions, that can cause more than slight or momentary pain or distress in anir (category B and C procedures), consistent with sound research design. Note that this focuses on Refinement, by improving a proced or condition, rather than simply a Replacement of animals, or a Reduction of animals.

7.1: Search for Alternatives Required for USDA-covered species
For projects using USDA covered species, a literature search (7.1) and narrative about the search results (7.3) are required.
For projects which do not use USDA covered species , a literature search is not required, however, a narrative about the consideratio alternatives must still be documented in 7.3
Because the literature search is intended to find alternatives to painful and distressing procedures and disease conditions, the keywo you choose should reflect a search for alternatives to potentially painful or distressful procedures. Suggested terms would be: alternat pain, discomfort, distress, etc along with additional keywords relevant to your proposal. More information regarding alternatives animal use in research can be found at https://research.uga.edu/oacu/alternatives/. (Guide, p 25 US Government Principles, Principle AW Regs 9 CFR Part 2, sec 2.31 USDA Animal Care Resource Guide Policies # 11, 12)

Alternatives include methods that

1. **Refine** existing tests by minimizing animal distress,
2. **Reduce** the number of animals necessary for an experiment,
3. **Replace** whole-animal use with in-vitro or other tests.

Again, note that only projects using USDA covered species require a literature search. **If a search is required, it must include at lea 2 databases.**

Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*

Keywords Used or Search Strategy: TIPS	*in vitro and Brugia*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*in vitro and Dirofilaria*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*anesthesia and cats*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*cats and blood collection*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo filarial infections*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo Brugia infections*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo Dirofilaria infections*
Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*in vitro and filarial worms*
Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*in vitro and Brugia*
Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*in vitro and Dirofilaria*
Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*anesthesia and cats*
Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*cats and blood collection*
Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*in vitro and filarial worms*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo filarial infections*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo Brugia infections*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*alternatives in vivo Dirofilaria infections*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*gerbils and blood collection*

Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1970-2022*
Keywords Used or Search Strategy: TIPS	*gerbils and blood collection*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*doxycycline and heartworm*

Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*doxycycline and heartworm*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*doxycycline and heartworm*

Source: TIPS	Agricola
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*doxycycline and Dirofilaria immitis*

Source: TIPS	Pubmed
Date of Search: TIPS	*8/2/22*
Years Searched: TIPS	*1960-2022*
Keywords Used or Search Strategy: TIPS	*doxycycline and Dirofilaria immitis*

7.2: Other Sources of Information on Alternatives to Painful and/or Distressful Procedures and Research-Induced Conditions
TIPS (US Government Principles, Principle # 4 AW Regs 9 CFR Part 2, sec 2.31)

1. Consultation with experts (please include credentials and dates; please do not include names, for privacy reasons)
2. Scientific meetings (please specify)
3. Other databases not available for selection in Section 7.1 (Name only. Please complete Section 7.1 with “Other Search” selected

7.3: Narrative on Alternatives to Painful and/or Distressful Procedures and Research-Induced Conditions TIPS (US Governmen Principles, Principle # 4 AW Regs 9 CFR Part 2, sec 2.31)
Do bona fide alternative methods exist that could reduce or avoid pain and distress in the animals in this study? Please check at least of the following options, and, in the text box below it, provide a narrative regarding the search findings and/or your considerations of alternatives.

- ☐ **YES, but they will not be used in this study.**
- ☐ **YES, alternative methods exist and will be employed in this study.**
- ☑ **NO, bona fide alternative methods do not exist that could reduce or avoid pain and distress.**

Provide a brief narrative summarizing your consideration of appropriate alternatives. If literature searches were used (e.g., USDA covered species), please include commentary on the searches.

In order to withdraw blood from the cats (and minimize the chance of our employees being injured) we must sedate the cats with drug (i.e. ketamine-acepromazine) that does not result in the animal becoming hypotensive.

Furthermore, as stated previously, the filarial worms must be grown and maintained in animals, since in vitro methods do nc produce the amount of parasites needed. The parasitemias in the species listed normally do not cause distress.

7.4 Narrative on Duplication: TIPS Please provide a written assurance that the proposed work is not unnecessarily duplicative. The assurance should include a brief narrative on why this work is not unnecessarily duplicative; what makes it novel research or a necessary duplication? (Guide, p 25-26 AW Regs 9 CFR Part 2, sec 2.31)

Each investigator needs new parasites for each experiment, therefore, since they require new parasites, our work is not duplicative.

Section 8: Experimental Design and Procedures

8.1: Brief Descriptions of Experimental/Instructional Procedures: TIPS (Guide, p 25 AW Regs 9 CFR Part 2, sec 2.31)
Identify and briefly describe each procedure that will be used in this project.

Please DO NOT describe the entire experiment in 8.1. Section 8.2 is intended for that.

Procedure outlines for inclusion in your Animal Use Protocol are available for several common procedures at https://research.uga.edu/oacu/procedures/. These procedures (denoted by an asterisk in the dropdown list below) are endorsed by the IACUC and may be cut and pasted directly into the protocol. Ensure you copy the entire procedure including the title. Be sure to use the procedures as described under "Artemis Briefs: Animal Use Protocol Descriptions," because they have the correct wording (verb tense) for an AUP.

Please consult the URAR veterinarians regarding any changes made to the procedures.

In-depth detailed descriptions for each procedure are also available at https://research.uga.edu/oacu/procedures/ Note that these descriptions should not be copy/pasted into your AUP because they do not have the correct wording; they are instructional.

See IACUC policy: Anesthesia and Post-Anesthetic/Post-Operative Monitoring
See IACUC policy: Surgery
See IACUC policy: Analgesia
See IACUC policy: Physical Restraint
See IACUC policy: Food or Fluid Regulation
See IACUC guideline: MS-222 Use
See IACUC guideline: Isoflurane Guidelines
See IACUC guideline: Tribromoethanol Use

Below is a list of common procedures. If you are performing any of these procedures, you are expected to include them in 8.1. The option "Other" is intended for other procedures not listed here.

- Anesthesia/Sedation TIPS
- Behavioral Modification/testing TIPS
- Blood Collection TIPS
- Cannulas (done in-house) TIPS
- Imaging TIPS
- Infectious disease challenge TIPS
- Injections TIPS
- Lavage
- Monoclonal antibody production
- Oral gavage TIPS
- Other
- Pharmacokinetics/Pharmacodynamics TIPS
- Polyclonal antibody production
- Prolonged restraint TIPS
- Surgery TIPS
- Tissue collection (post-mortem) TIPS
- Tissue collection (biopsy of live animal) TIPS
- Vaccination/Immunization TIPS
- Water Restriction TIPS

Procedure: Blood Collection*
Description:

IV from jugular vein (cats). Cats are sedated to facilitate human safety and restraint of cats by handlers. Cats will be bled as needed (prn), not to exceed 10% total blood volume in a two week period

Dogs are bled (from the jugular veins) prn not to exceed 10% total blood volume in any given two week period.

For smaller blood volumes, it may be necessary to use either cephalic or saphenous veins.

Procedure: Blood Collection*
Description:

Lateral Saphenous

Gerbils are bled from the lateral saphenous vein without anesthesia. This amount will not exceed 10% of the total blood volume in a two week period. If two samples are needed within a 1-week period alternate legs will

be used.

Procedure: Anesthesia/Sedation
Description:

See section 10. In summary, cats are administered ketamine/acepromazine either IM or IV.

Procedure: Other
Description:

Gerbils are injected IP or SQ with the L3 larval stage of B. pahangi, B. malayi, and D. immitis.

Cats are injected SQ with the L3 larval stage of B. malayi.

Dogs are injected SQ with the L3 larval stage of B. pahangi, B. malayi, and D. immitis.

8.2: Hypothesis, Experimental Design Details, and Description of the Full Experiment: TIPS (Guide, p 25 AW Regs 9 CFR Part 2, sec 2.31)

Describe the use of animals in your project in detail. Use terminology that will be understood by individuals outside your field of expertise.

NOTE: If you have any related charts, tables, or schematics that help explain the experiments, please email them to the Office of Animal Care and Use (iacuc@uga.edu). There is not a method for attaching them to the AUP form; however, we can add it to the file.

If you find yourself listing multiple experiments and section 8.2 is getting pretty long and complicated, it is best to separate the work into more than 1 AUP.

Each experiment/study described in this section must be answered in 3 parts:
Part A: Hypothesis, Part B: Experimental Design and Part C: Experimental Procedures.
Notes: For instructional protocols, you can answer Parts A and B as Not Applicable.
If multiple studies/objectives are described, address Parts A-C for each study before describing the next.

Part A: Hypothesis TIPS
Please clearly state the experimental hypothesis and specifically explain how your experiment is designed to test the hypothesis:

- How will you capture the variance?
- What data are you collecting to analyze?
- What conclusions could be drawn from the data if generated as planned?

Part B: Experimental Design TIPS

- Clearly define the experimental design, listing each experimental/control group, and the number of animals per group
- You must address specifically how the sample size was determined to be appropriate to capture the variance and test the hypothesis. Please include any assumptions regarding variability, distribution, etc.

Part C: Experimental Procedures TIPS

- Provide a detailed description of all animal procedures in a logical, chronological progression, beginning with receipt of the animals and ending with the study endpoint.
- Specific procedures, test, analyses, treatments, assays, drugs/compounds, duration of the study/timeline, and point at which animals are removed from the study (e.g., euthanized) should be clearly delineated for treatment and control groups.
- You must address specifically what data are being collected
- **Surgical procedures and anesthesia details should be entered into sections 9 and 10, respectively. Surgery and anesthesia details should not be entered here in 8.2, to avoid inconsistencies.**

- **Note that you should <u>not</u> include doses/volumes here for drugs listed in section 10 -you should only list the doses in section 10, to avoid inconsistencies**

The primary purpose of this project is to cyclically maintain and produce the filarial parasites Brugia malayi, B. pahangi, and Dirofilaria immitis by alternate passage through vertebrate and invertebrate hosts and to supply limited amounts of these parasites and parasite-infected hosts to NIH approved investigators.

Brugia spp.

Brugia malayi is one of the filarial parasites responsible for human lymphatic filaiasis and elephantiasis and is endemic to tropical Asia. Besides humans, the domestic cat and dog are natural definitive host for this parasite. The adult parasites dwell within lymphatic vessels and produce microfilariae, the immature first larval stage, which circulates in the blood. Microfilariae when ingested by a susceptible mosquito further develop through two larval molts into an infective third stage larva. When the infective third stage larvae are injected into a susceptible mammalian host, they migrate and develop into the adult stages.

For this project, cats and dogs infected with B. malayi are maintained as blood donors of microfilaremic blood for feeding Aedes aegypti mosquitoes through membrane feeders, as well as providing blood to other researchers. Cats and dogs infected with B. malayi are usually asymptomatic, but may present with a mild lymphedema, with only circulating micro filariae in the blood as evidence of infection. Cats and dogs are injected SC with larvae then these larvae migrate to and subclinically develop in the lymphatics. They are maintained as long as they have a microfilaremia suitable for providing a blood specimen which can be administered to mosquitoes via an artificial mosquito feeder. [Note: The mosquitoes do not feed on live animals in our system. Instead, as stated above, mosquitoes feed on blood from membrane feeders (parafilm.) This artificial system is the commonly used as an alternative to the feeding of mosquitoes on live animals.]

Mongolian gerbils (jirds) are infected with B. malayi or B. Pahangi by subcutaneous injection to produce lymphatic infections. The development and migration of the parasite within the jird is similar to that in the definitive hosts (cat, dog, and human).

To produce adult worms, immature mammalian stages, and microfilariae of B. malayi or B. Pahangi, jirds are infected by intraperitoneal injection with infective third stage larvae. In contrast to infections established by subcutaneous injection, the developing stages do not migrate. The parasite material is harvested from the abdominal cavity of a euthanatized jird.

Brugia pahangi is closely related to B. malayi and is a lymphatic filarid infecting dogs and jirds. The life cycle is similar to that of B. malayi. Dogs infected with B. pahangi are maintained as blood donors of microfilaremic blood for feeding mosquitoes.

Of the 1500 gerbils requested in 5.1, approximately 1200 will be used for Brugia studies.

Dirofilaria immitis
Dirofilaria immitis, the canine heartworm, is one of the most important nematode parasites of dogs. The life cycle with an intermediate mosquito host is similar to that of B. malayi. The adult parasites inhabit the right heart chambers and pulmonary arterial circulation. Infection with heartworm can produce interstitial lung disease, arteritis, right ventricular hypertrophy, right heart failure, and occasionally vena cava syndrome. To negate the risk for these complications, the number of infective larvae given to a dog is minimized (less than 50 larvae injected SC in 1 ml volume). In our experience, a one-time injection of <50 L3 larvae SC into laboratory-maintained dogs can successfully produce subclinical, rather than clinical, heartworm disease. On occassion, it may be necessary to re-infect if the previous infection attempt did not produce a patent infection. Our laboratory has successfully maintained subclinically-infected dogs for several years, with no untoward effects. Dogs infected with D. immitis are maintained as blood donors of microfilaremic blood for feeding mosquitoes and providing microfilaremic blood for teaching purposes.

Occasionally, in order to produce adult heartworms for in vitro procedures or transplantation into dogs (which would be on a separate AUP), we will need to inject dogs with 100 D. immitis L3s. This number of larvae may cause clinical disease. However, as compared to the dogs infected with 50 L3s, these dogs will be euthanized approximately 8 months post-infection, in order to collect adult worms.

No rodent models exist for D. immitis. In order to define a rodent model and investigate the life cycle, we wish to use the jird (which as I stated above) can be used as a filarial model. Jirds will periodically be infected with D. immitis. Jirds are not a natural model of heartworm, which allows us to investigate in vivo potential reasons for this negative host-specificity. These studies will allow us to potentially, in future studies, identify novel biologic or pharmaceutical intervention for heartworms in permissive host species. In order to investigate this important aspect of filarial parasites, we will infect groups

of gerbils (from 1 for pilot experiments to 8 for experiments) either subcutaneously or intraperitoneally. We will then euthanize the animals at different time points, and evaluate the in vivo response to the worms. Previous data indicates that this response happens quickly and the parasite is eliminated quickly (i.e. within hours) from the peritoneal cavity.
Also experiments will be performed where D. immitis and Brugia spp. are co-infected. We do not know whether Brugia will aid in the survival of D. immitis. As Brugia spp. survive in the peritoneal cavity and D. immitis does not, we hypothesize that a co-infection will allow survival of D. immitis. These experiments will use use up to 8 animals for co-infections, and 8 animals each for Brugia and D. immitis (to serve as controls). These experiments will also involve different time points.

The effect of Wolbachia on filarial worms:
Doxycycline (orally at 10 mg/kg BID for 28 days) in Dirofilaria immitis-infected dogs. The purpose of the use of doxycycline is to deplete the bacterial endosymbiont Wolbachia, which is present inside D. immitis. The hypothesis being that Wolbachia levels will be decreased as compared to heartworms in dogs that have not received doxycycline. Doxycycline is a standard part of the American Heartworm Society recommended gold standard heartworm treatment, however, there is only circumstantial evidence of Wolbachia depletion in adult worms (due to the complexity and expense of the model). Also, while this dose is the gold standard, it was determined empirically based on data from other rickettsial organisms.
There should be no adverse effects. Humane endpoints are as previously outlined in this protocol. There would be a maximum of 2 dogs dosed at 1 time. A total of eight to 10 dogs will be dosed.

Dogs may be euthanized after the course of doxycycline and adult worms examined by molecular methods for the presence of Wolbachia. Controls are previously collected frozen worms. After dosing, blood will be drawn and fed to mosquitos as previously described in the protocol. As the comparison will be based on the amount of Wolbachia in both the microfilariae and the third-stage larvae vs. number of dogs, two dogs (1 experimental/1 control) at a time should be enough.

Of the 1500 gerbils requested in 5.1, approximately 300 will be used for D immitis studies.
The typical timeline (infection to end of study) for experiments/infections is as follows:

Gerbils (Brugia SQ-infected): 2 years
Gerbils (Brugia IP-infected): 6 months
Cats (patent Brugia infection): 1 years
Cats (non-patent infection): 6 months
Dogs (Dirofilaria immitis-50 L3s): 5-7 years
Dogs (Dirofilaria immitis-100 L3s): 8 months
Dogs (Brugia spp.)-8 years

8.2.1: Please describe the proposed procedures' (manipulations, infections, surgeries, toxin dosing, etc.) impact on the animals' well being. TIPS (Guide, p 25) Note that this question **must** be answered, even if no impact is expected.

Please state the expected level of morbidity/mortality with this model.
Notes regarding expected morbidity/mortality vs unexpected outcomes: Most procedures, even when performed appropriately and according to the IACUC protocol and veterinary standards, are expected to have some level of morbidity, and possibly mortality, due to expected complications (e.g., anesthesia sensitivities, post-operative infection, or failure to fully recover). The expected level of morbidity/mortality should be based on historical data within the lab and/or the model's known success rate. See the IACUC Policy "Policy on Reporting Unanticipated Outcomes Affecting Animal Well-Being" for further explanation.

SQ infection with Brugia spp. can result in transient lymphedema. However, this has rarely been shown to impact the animal's ability to eat, drink or be ambulatory.

IP injection of gerbils rarely results in signs. Animals may develop peritonitis.

Dirofilaria infection can result in potentially fatal complications. However, with the dose of L3s administered 30-50, most animals will remain subclinical (i.e. class I heartworm disease).

In the case of when 100 L3s are administered, clinical signs may be more severe than those mentioned above. When animals are administered this number of L3s, they will be euthanized by 8 months post-infection, which will be prior to the development of severe clinical signs.

8.3: Will you be using potentially hazardous substances, or recombinant materials that require IBC approval, in live animals? These potentially hazardous substances, include chemicals, biohazards (including human cells/tissues or animal cell lines), recombinant materials, or radiation. TIPS

NOTE: It is the PI's responsibility to identify if any of the substances used fit in any of these categories and obtain the safety information from the responsible UGA department [Environmental Safety Division (ESD), Office of Research Safety (ORS), Radiation Safety (RS), Office of Biosafety (OBS)].
The IACUC review includes verification that any work with animals that involves hazards is done safely and according to recommendations from ESD, ORS, RS, and OBS.
(Guide p 18, 25-26 Occ Health and Safety, p13)

(●) Yes ( ) No

8.3.1 (a): TIPS

Select the category of hazard/material that will be used and then provide the name, the species in which it will be used, a short description of its use and what methods will be employed to ensure it is used safely, and a description of all Personal Protective Equipment (PPE).
Note that if more than one hazardous agent is used simultaneously, the safety guidance for the more significant hazard must be used.
Note that the safety methods described should focus on the use of the hazard in the live animal, not the agent preparation in your lab. You must explain how soiled bedding is dumped when cages are changed and what disposal method will be used for the soiled bedding and carcasses. Note that you must notify URAR in writing before a hazard is administered to animals and that all cages in which such animals are housed must be labeled with a URAR approved label.

☐ Hazardous Chemicals:

☑ Biohazards and recombinant materials:

Note that recombinant materials here includes the use of genetically modified animals ("transgenics"). Description of use in animals and procedures to ensure safety of personnel including PPE as recommended by the Office of Biosafety:

Biohazard/Recombinant Material Name:	*Brugia malayi*
Species Used:	Cat
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*
Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:	
Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.	

Biohazard/Recombinant Material Name:	*Brugia pahangi*
Species Used:	Dog
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*
Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:	
Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.	

Biohazard/Recombinant Material Name:	*Dirofilaria immitis*
Species Used:	Dog
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*
Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:	
Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.	

Biohazard/Recombinant Material Name:	*Brugia Malayi*
Species Used:	Dog
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*

Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:

Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.

Biohazard/Recombinant Material Name:	*Brugia malayi*
Species Used:	Gerbil
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*

Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:

Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.

Biohazard/Recombinant Material Name:	*Brugia pahangi*
Species Used:	Gerbil
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*

Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:

Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.

Biohazard/Recombinant Material Name:	*Dirofilaria immitis*
Species Used:	Gerbil
IBC Approval Number:	(If you're awaiting approval, enter "pending") *2021-0016*

Description of use and procedures to ensure safety of personnel recommended by the Office of Biosafety:

Personnel wear lab coats and gloves when handling infected animals. No other PPE is needed.

☐ Radiation or Lasers:

Section 9: Surgical Information

9.1.1: Surgical Information (AW Regs 9 CFR Part 2, sec 2.31 Guide p 25-26, 105, 115)
Will you be doing surgery?

NOTE: Anesthetizing an animal for terminal tissue collection before the animal is euthanized is considered Non Survival surgery and should be included in section 9.

○ Yes ◉ No

Section 10: Administration of Anesthesia, Analgesia, Other Substances and Biologicals

10.1: Will you be using anesthesia, anesthetic antagonists, analgesics, tranquilizers, etc.? (Guide, p 12, 25, 105, 121 AW Regs 9 CFR Part 2, sec 3.21 USDA Animal Care Resource Guide Policy # 3)
NOTE: All use of anesthesia must comply with the UGA Policy: Anesthesia and Post-Anesthetic/Post-Operative Monitoring
If you are performing anesthesia, it is very important that you are familiar with the policy named above. The PI is responsible for ensuring that the policy requirements are followed by all lab personnel. This includes appropriate records for anesthesia and recovery.

See IACUC guideline: Labeling of Secondary Containers
See IACUC policy: Outdated Drugs and Materials
See IACUC guideline: MS-222 Use
See IACUC guideline: Isoflurane Guidelines
See IACUC guideline: Tribomoethanol Use

◉ Yes ○ No

10.1.1 (a): Anesthesia and agents used in conjuction with anesthesia

List all **pre-anesthetic agents** (e.g., tranquilizers, narcotics), **anesthetic agents, antagonists** and **analgesics** as well as agents used in conjunction with anesthesia such as local anesthetics and anti-cholinergics.

Agent: TIPS	Ketamine
Frequency of Anesthesia: TIPS	*PRN prior to blood withdrawl*
Species	Cat
Dose: TIPS	*10-12 mg/kg*
Route: TIPS	Intramuscular
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Ketamine
Frequency of Anesthesia: TIPS	*PRN prior to blood withdrawl*
Species	Cat
Dose: TIPS	*2.2-4.4 mg/kg*
Route: TIPS	Intravenous
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Acepromazine
Frequency of Anesthesia: TIPS	*PRN prior to blood withdrawl*
Species	Cat
Dose: TIPS	*0.1 mg/kg*
Route: TIPS	Intramuscular
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Acepromazine
Frequency of Anesthesia: TIPS	*PRN prior to blood withdrawl*
Species	Cat
Dose: TIPS	*0.02-0.04 mg/kg*
Route: TIPS	Intravenous
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Ketamine

Frequency of Anesthesia: TIPS	*PRN for sedation prior to euthanasia*
Species	Cat
Dose: TIPS	*10-12 mg/kg*
Route: TIPS	Intramuscular
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Acepromazine
Frequency of Anesthesia: TIPS	*PRN for sedation prior to euthanasia*
Species	Cat
Dose: TIPS	*0.1 mg/kg*
Route: TIPS	Intramuscular
Volume: TIPS	*injectable volumes depend on cat weight*
Agent: TIPS	Xylazine
Frequency of Anesthesia: TIPS	*PRN for sedation prior to euthanasia*
Species	Dog
Dose: TIPS	*0.6-1.1mg/kg*
Route: TIPS	Intravenous
Volume: TIPS	*injectable volumes depend on dog weight*

10.1.1 (b): TIPS

Monitoring of Anesthesia (Guide, p 12, 119 USDA Animal Care Resource Guide Policy # 3)
Check all items that you will be monitoring and provide a brief description of the monitoring, depth assessment, thermal support, and recovery, in the text box below.

See IACUC Policy: Anesthesia, Survival Surgery and Post-Anesthetic/Post-Operative Monitoring

Include:

- How frequently each of these variables will be monitored.
  - Note that some variables, such as respiration, should be monitored (observed) continuously, so do not say you will monitor respiration at specific intervals. You can say you will count and record the respiratory rate at specific intervals.
- How you determine that the depth of anesthesia is adequate before a painful procedure is performed (e.g., lack of response to a firm toe pinch).
- The method of thermal support that will be provided.
  - Unless you are using a warm water circulating pad, or a warm air blower, you must include a comment that you will have a barrier between the animal and the pad. With the exception of water circulating, or warm air thermal support, the use of a heating pad requires a barrier between the animal and the pad (e.g., a folded towel underneath the recovery cage).

NOTE: An animal must be monitored continuously during the immediate recovery from anesthesia until it is able to hold itself in a normal, upright position.

☐ Body Temp: TIPS ☐ Pulse Oximetry ☐ Blood Gas ☐ Doppler Blood Flow ☐ Mucus Membrane Color ☐ Capillary Refill Time ☐ ECG ☐ Blood Pressure ☑ Respiration Rate ☐ Capnography (Measuring CO2 levels) ☐ Muscle Tone

Cats will be sedated for PRN blood draw. Cats will be kept warm by wrapping them in a towel. The animals will be monitored constantly by lab personnel until sternally recumbant.

10.1.1 (c): TIPS

Provide a plan for immediate (<48 hours) and extended (>48 hours) analgesia after a potentially painful procedure: (Guide, p 12, 25, 105, 120 AW Regs 9 CFR Part 2, sec 2.31 USDA Animal Care Resource Guide Policy # 3)

See IACUC Policy: Anesthesia, Survival Surgery and Post-Anesthetic/Post-Operative Monitoring

Include:

- The drug/s to be administered, and the frequency at which they are administered.
- Be sure to distinguish the standard analgesia plan from additional analgesia that will be provided "if needed." In other words, what is the standard plan, that all animals will receive, and what will be done if an animal is still showing signs of pain after the standard plan has been completed?

No surgery is being performed.

10.1.1 (d): TIPS

Describe how animals will be assessed to determine the need for analgesia after a potentially painful procedure: (Guide, p 120)

See IACUC Policy: Anesthesia, Survival Surgery and Post-Anesthetic/Post-Operative Monitoring

Include:

- What signs will be monitored?
- How often will animals be monitored? Note that animals should be assessed for the need for additional analgesia at time intervals appropriate to the analgesic being used. I.e., if the analgesic lasts for 6-8 hours, the animals should be reassessed 6-8 hours after the previous dose.

No surgery is being performed.

10.2: Will you be administering substances to live animals other than anesthetics, analgesics, etc. listed in 10.1? (This would include any therapeutic drugs; cells or tissue extracts and experimental or study materials such as infectious agents, antigens, adjuvants, or other reagents) (Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31)

See IACUC guideline: Labeling of Secondary Containers
See IACUC policy: Outdated Drugs and Materials

◉ Yes ○ No

10.2.1 (a): Therapeutics:

List all therapeutic drugs such as anti-infectives (antibiotics, antifungals, antivirals, and parasiticides), steroids, chemotherapeutics, etc.:

Agent: TIPS	Doxycycline
Frequency and length of time: TIPS	*BID x 28 days*
Species	Dog
Dose: TIPS	*10 mg/kg*
Route: TIPS	Oral
Volume: TIPS	

10.2.1 (b):

Cells or Cell Tissue Extracts: List all isolated cells, cell lines and cell or tissue/cell extracts. Rodent cell lines and cell/tissue products can only be used in animals after confirming the absence of rodent infectious agents. (Guide, p 25-26 AW Regs 9 CFR Part 2, sec 2.31)

See IACUC Policy: Testing Biological Materials

Cell(s)/Tissue(s): TIPS	
Species	Choose a Species...
Dose: TIPS	
Route: TIPS	Choose a Drug Route...
Volume: TIPS	

10.2.1 (c):

Experimental / Study Agents: List all other substances administered to live animals not listed in 10.1.1, 10.2.1(a), or 10.2.1 (b): experimental drugs, infectious agents, vaccines, nanoparticles, imaging contrast agents, or other substances/reagents/compounds.
For drugs under study in the experimental component of your protocol, drug type or group (e.g., non-steroidal anti-inflammatory agents, alpha2-adrenergic receptor blockers) will suffice; however specific drugs should be indicated if known. (Guide, p 25 AW Regs 9 CFR Part 2, sec 2.31)

Agent: TIPS	*Brugia malayi*
Frequency and length of time: TIPS	*once*
Species	Cat
Dose: TIPS	*200-500 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Brugia pahangi*
Frequency and length of time: TIPS	*1-3 times*
Species	Dog
Dose: TIPS	*200-500 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Dirofilaria immitis*
Frequency and length of time: TIPS	*1-3 times*
Species	Dog
Dose: TIPS	*20-100 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Brugia Malayi*
Frequency and length of time: TIPS	*1-3 times*
Species	Dog
Dose: TIPS	*200-500 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*<1ml L3's*
Agent: TIPS	*Brugia malayi*
Frequency and length of time: TIPS	*1-3 times*
Species	Gerbil
Dose: TIPS	*100-1000 L3s*
Route: TIPS	Intraperitoneal
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Brugia malayi*
Frequency and length of time: TIPS	*1-3 times*

Species	Gerbil
Dose: TIPS	*100-400 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Brugia pahangi*
Frequency and length of time: TIPS	*1-3 times*
Species	Gerbil
Dose: TIPS	*100-400 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Brugia pahangi*
Frequency and length of time: TIPS	*1-3 times*
Species	Gerbil
Dose: TIPS	*100-1000 L3s*
Route: TIPS	Intraperitoneal
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Dirofilaria immitis*
Frequency and length of time: TIPS	*1-3 times*
Species	Gerbil
Dose: TIPS	*100-500 L3s*
Route: TIPS	Subcutaneous Injection
Volume: TIPS	*< 1 ml*
Agent: TIPS	*Dirofilaria immitis*
Frequency and length of time: TIPS	*1-3 times*
Species	Gerbil
Dose: TIPS	*20-500 L3s*
Route: TIPS	Intraperitoneal
Volume: TIPS	*< 1 ml*

10.3: Are non-pharmaceutical-grade chemicals or other substances used? (Does not apply to test articles) (Guide, p 12, 31 USDA Animal Care Resource Guide Policy # 3) TIPS

See IACUC guideline: MS-222 Use
See IACUC guideline: Tribromoethanol Use

○ Yes ◉ No

Section 11: Monitoring

11.1: Monitoring Interval: TIPS (Guide, p 12, 25-26)

Indicate, by checking one or more of the appropriate boxes below, the minimum frequency with which your lab personnel will monitor your animals during and after all procedures. If more than one frequency will be followed (i.e., different studies, different time periods during the study), please check all of the appropriate boxes.

- This question does not refer to the standard daily observations made by University Research Animal Resources staff.
- Note that for USDA covered species, this monitoring by lab personnel for signs of pain/distress related to the research procedures must be documented in the clinical health record.

"Approximately" below indicates within the range of 1 before to 1 hour after the time stated.

☐ Daily

☐ Approximately every 6 Hours ☐ Approximately every 8 Hours ☐ Approximately every 12 Hours ☐ Approximately every 24 Hours

☑ Other

11.2: Provide a brief description of the monitoring interval checked above. TIPS (Guide, p 12, 26)
NOTE: THE MONITORING DESCRIBED IN 11.2 SUPERCEDES DESCRIPTIONS IN OTHER SECTIONS IF OTHER SECTIONS ARE NOT CONSISTENT WITH 11.2. THE IACUC'S EXPECTATION IS THAT THE MONITORING SCHEDULE IN 11.2 WILL BE FOLLOWED. Therefore, we strongly recommend that you ONLY list monitoring schedules in 11.2, with the exception of questions that ask about monitoring related specifically to anesthesia (10.1.1(b), 10.1.1(d)) and surgery (9.1.1(f)).

- If the frequency will vary during the experiment (e.g., the time from injection to tumor visible to the time of euthanasia) describe all periods of time.
- Note that the interval between checks should include a specific hour range (i.e., "every 10-12 hours" or "once between 7 to 9 am and a second check between 6-8pm" not "twice a day")
- If the frequency will vary by experimental group (e.g., animals infected with different strain of virus) describe the frequency to be used for each group.
- If parameters are being measured to determine the humane endpoints (e.g., weight, tumor size) include the frequency at which the measurements will occur.

After injection of L3s, animals will be monitored for lymphedema in testicles and limbs weekly for 4 weeks post injection. If lymphedema is noted, it will be documented and monitored weekly until resolution.

Gerbils that present with swollen testes or digits as a result of the parasitic infection will be monitored weekly until resolved. If the scrotum ulcerates due to the swelling, the animal will be monitored every 3-4 days until resolved. In our experience, these ulcerations develop slowly, so the interval of monitoring has been adequate.

Gerbils infected IP will be monitored daily for 48 hours for signs(lethargy, hunched posture, rough haircoat, ascities, and obtundation) of peritonitis.

If dogs infected with D. immitis develop clinical signs once patent, which is approximately 6 months post infection, they will be monitored at a frequency decided upon in consultation with the URAR vets.

11.3: Humane Endpoints TIPS (Guide, p 12, 25-26 AW Regs 9 CFR Part 2, sec 2.31)
NOTE: THE HUMANE ENDPOINTS DESCRIBED IN 11.3 SUPERCEDE DESCRIPTIONS IN OTHER SECTIONS IF OTHER SECTIONS ARE NOT CONSISTENT WITH 11.3. THE IACUC'S EXPECTATION IS THAT THE HUMANE ENDPOINTS IN 11.3 WILL BE FOLLOWED. Therefore we strongly recommend that you ONLY list humane endpoints in 11.3

If your protocol is classified as a Category B or C, please provide the humane endpoints that will be employed in this study w meeting the scientific objectives. These endpoints will be used to determine the earliest opportunity when animals are to be remo from the study, treated or euthanized. Please provide the criteria that are used to determine humane endpoint conditions, being objective and specific as possible (e.g., "severely ill" and "significant weight loss" are not adequately specific and objective). Note tha a situation in which the need for euthanasia of an individual animal is in question, the University Attending Veterinarian, or her designee, has the authority, and responsibility, to make the final decision regarding euthanasia of an animal.

Tips for common humane endpoints used:

- Weight loss: indicate the comparison- a baseline weight, comparison to controls, comparison to growth curves
- Lethargy: any decrease of activity, even very mild, is considered lethargy- if you incorporate lethargy in your endpoints and mea something other than a very mild decrease in activity, you need to describe what level of lethargy you mean.

- Body Condition Scores: indicate the scale (e.g., 1-5, 1-10) and provide a reference for a published BCS, or a description of an animal at each score

If dogs or cats exhibit prolonged (greater than 2 months) lymphedema (vs. the transient 3-week lymphedema after initial infection associated with infection with Brugia spp. A URAR veterinarian will be consulted on the case to determine a course of action.

Post SQ infection of Brugia spp, gerbils that present with swollen testes and/or ulcerated scrotum rarely show signs of pain o discomfort. Clinical signs might include lethargy, hunched posture, rough haircoat, and obtundation. If clinical signs were to occu a URAR veterinarian would be consulted on the case for an appropriate treatment plan or euthansia. The URAR veterinarian wi be consulted if the ulceration is larger than one centimeter in size for an appropriate treatment plan or euthansia. If at anytime a ulceration were to have a purulent discharge, the animal would be euthanized. On rare occasions, the hind feet will have swellin of the lateral digits and possible loss of the ungual process. They appear comfortable with no lameness and are in otherwise goo health. A URAR veterinarian would be consulted on the case for an appropriate treatment plan.

In gerbils that have been administered L3s intraperitoneally, peritonitis can develop. Clinical signs are described in 11.2. If thi occurs, gerbils will be euthanized promptly.

Occasionally, dogs infected with D. immitis will display clinical signs, such as dyspnea and coughing. Regarding these signs, if a occasional cough is detected, the animal would be examined, the exam would be noted in the record, and the dog would b observed more closely. If dyspneic (trouble breathing), the dog would be examined, the URAR veterinarian would be notified and treatment plan would be discussed or the dog will be euthanized. Either Andrew Moorhead, Michael Dzimianski, or Tanya Coope can make the decision the euthanize during consultation with the URAR veterinarian. During the weekend, if a problem is noted b the animal care staff, one of the above individuals will be notified and in conjunction with the URAR veterinarian will make th decision as whether to euthanize the animal. If euthanasia is to be performed, it will be done so by one of the named individuals o the URAR veterinarian.

It may become necessary, for medical reasons, to image these animals using radiography or echocardiogram. For cases showin clinical signs, the URAR veterinarian will be consulted on the case to determine the appropriate course of treatment and/o imaging.

Section 12: Animal Disposition

12.1: Final Disposition TIPS (Guide, 26, 105, 123)

For each species listed on this AUP, describe what will happen to these animals at the end of this project:
By default, PI retains the right to transfer animals to other protocols, or use the option of adoption or resale, at the discretion of the attending veterinarian and in accordance with UGA policy.
Please note that while euthanasia may not be a planned outcome of the study, and is not chosen as a method of planned disposition in 12.1, a method of euthanasia must be described (in question 12.2) in case an animal experiences unanticipated pain or distress (e.g. untreatable injury or illness).

Cat

☑ Planned transfer to other projects (Transfer of animals to a different PI or to a different AUP must be handled through URAR, via AnOps) (Explain in the text box):

If other investigators need cats, and the cats have a low microfilaremia or are amicrofilaremic they will be transferred. Transferees will be notified of potential transmission of the disease by the mosquito vector, so that they may exercise all appropriate precautions. These animals cannot be adopted out, because they have been infected with a human pathogen.

☐ Adoption or resale* (Explain in the text box):

☐ Return to the wild* (Explain in the text box):

☐ Return to the herd or flock* (Explain in the text box):

☑ Euthanasia (Please DO NOT describe the method here. Describe the methods in 12.2)

Euthanasia Solution IV 100mg/kg.

Dog

☑ Planned transfer to other projects (Transfer of animals to a different PI or to a different AUP must be handled through URAR, via AnOps) (Explain in the text box):

If other investigators need dogs, and the dogs have a low microfilaremia or are amicrofilaremic they will be transferred. Transferees will be notified of potential transmission of the disease by the mosquito vector, so that they may exercise all appropriate precautions.

☑ Adoption or resale* (Explain in the text box):

Dogs that are no longer necessary to the research and are deemed adoptable by a URAR veterinarian will be available to be adopted.

☐ Return to the wild* (Explain in the text box):

☐ Return to the herd or flock* (Explain in the text box):

☑ Euthanasia (Please DO NOT describe the method here. Describe the methods in 12.2)

Euthanasia Solution IV 100mg/kg.

Gerbil

☐ Planned transfer to other projects (Transfer of animals to a different PI or to a different AUP must be handled through URAR, via AnOps) (Explain in the text box):

☐ Adoption or resale* (Explain in the text box):

☐ Return to the wild* (Explain in the text box):

☐ Return to the herd or flock* (Explain in the text box):

☑ Euthanasia (Please DO NOT describe the method here. Describe the methods in 12.2)

Gerbils will be euthanized by CO2.

12.2: Euthanasia Method TIPS (Guide, p 126, 105, 123 AW Regs 9 CFR Part 2, sec 2.31 USDA Animal Care Resource Guide Policy # 3)
Describe the method of euthanasia for each species listed in this AUP:
**Please note that while euthanasia may not be a planned outcome of the study, a method of euthanasia must be described in case an animal experiences unanticipated pain or distress (e.g. untreatable injury or illness).*
**Please note that if the method you are using is not approved by the AVMA Guidelines on Euthanasia, you need to justify the method (Check the "Other" box at the end of 12.2 to provide a text box for the justification).*

See IACUC policy: CO2 Euthanasia
See IACUC guideline: Guillotine Maintenance

Cat

☑ Injectable Drugs: If you are using a drug, you must indicate the name, dose and route of administration of the drug.(Check the "Other" box at the end of 12.2 to provide a text box for this information). NOTE: For wildlife studies, it is the responsibility of the researcher to prevent scavenging animals from consuming bodies contaminated with drugs that are potentially dangerous.

☐ Carbon Dioxide: CO2 euthanasia requires a second, physical method of euthanasia to ensure death (**Check the "Other/Secondary method after CO2" box at the end of 12.2 to provide a text box for the description of the secondary physical method**).

☐ Euthanasia by Physical Methods: This refers to a physical method as the primary method of killing the animal (i.e., not as a verification of death after the animal has been killed with CO2 or anesthetic overdose). Examples are cervical dislocation, decapitation, and captive bolt. If animals are conscious at the time of the physical method, you are required to describe the training and/or experience for the persons using this method. Please provide a description of the method/s and information on personnel training and experience in the following box:

☑ Please use this box to describe:

1. Any methods other than Injectable Drugs, CO2, or Physical Methods
   And/Or
2. Details about any injectable drugs used to euthanize
   And/Or
3. A secondary physical method after CO2

Euthanasia Solution IV 100mg/kg.

Dog

☑ Injectable Drugs: If you are using a drug, you must indicate the name, dose and route of administration of the drug.(Check the "Other" box at the end of 12.2 to provide a text box for this information). NOTE: For wildlife studies, it is the responsibility of the researcher to prevent scavenging animals from consuming bodies contaminated with drugs that are potentially dangerous.

☐ Carbon Dioxide: CO2 euthanasia requires a second, physical method of euthanasia to ensure death (**Check the "Other/Secondary method after CO2" box at the end of 12.2 to provide a text box for the description of the secondary physical method**).

☐ Euthanasia by Physical Methods: This refers to a physical method as the primary method of killing the animal (i.e., not as a verification of death after the animal has been killed with CO2 or anesthetic overdose). Examples are cervical dislocation, decapitation, and captive bolt. If animals are conscious at the time of the physical method, you are required to describe the training and/or experience for the persons using this method. Please provide a description of the method/s and information on personnel training and experience in the following box:

☑ Please use this box to describe:

1. Any methods other than Injectable Drugs, CO2, or Physical Methods
   And/Or
2. Details about any injectable drugs used to euthanize
   And/Or
3. A secondary physical method after CO2

Euthanasia Solution IV 100mg/kg.

Gerbil

☐ Injectable Drugs: If you are using a drug, you must indicate the name, dose and route of administration of the drug.(Check the "Other" box at the end of 12.2 to provide a text box for this information). NOTE: For wildlife studies, it is the responsibility of the researcher to prevent scavenging animals from consuming bodies contaminated with drugs that are potentially dangerous.

☑ Carbon Dioxide: CO2 euthanasia requires a second, physical method of euthanasia to ensure death (**Check the "Other/Secondary method after CO2" box at the end of 12.2 to provide a text box for the description of the secondary physical method**).

☐ Euthanasia by Physical Methods: This refers to a physical method as the primary method of killing the animal (i.e., not as a verification of death after the animal has been killed with CO2 or anesthetic overdose). Examples are cervical dislocation, decapitation, and captive bolt. If animals are conscious at the time of the physical method, you are required to describe the training and/or experience for the persons using this method. Please provide a description of the method/s and information on personnel training and experience in the following box:

Gerbils will be euthanized by CO2 followed by thoracotomy or cervical dislocation.

Please use this box to describe:

1. Any methods other than Injectable Drugs, CO2, or Physical Methods
   And/Or
2. Details about any injectable drugs used to euthanize
   And/Or
3. A secondary physical method after CO2

Gerbils will be euthanized by CO2 followed by thoracotomy or cervical dislocation.

Section 13: Permits

13.1: Permits TIPS (Guide, p 106 State and federal laws that require permits for collection of certain wild animals)
Do you have any permits that may be required for handling, importation, collection or maintenance of animals used in this proposed study?
NOTE: If a state or federal permit is required for this work, the permit must be obtained before the handling of wild animals for this study can begin.

○ Yes ◉ No

Section 14: Certifications and Submission

I, Andrew Moorhead, certify that:

This Protocol provides a complete and accurate description of all proposed uses of live vertebrate animals in this research activity. Any proposed revisions to animal care and use procedures will be promptly forwarded in writing to the IACUC for review and approval prior to implementation.

I agree to abide by all applicable laws, policies and regulations, including the U.S. Animal Welfare Act, the National Research Council Guide for the Care and Use of Laboratory Animals, the Public Health Service Policy on the Humane Care and Use of Laboratory Animals, the FASS Guide for the Care and Use of Agricultural Animals in Research and Teaching, and all UGA policies and procedures regulating the humane use of vertebrate animals in instruction and research.

I will comply with all regulations governing the importation, collection and/or maintenance of wild species, including obtaining permits from all applicable regulatory agencies prior to the acquisition of animals.

I have completed (or agree to complete) training (Training Page) offered by the Office of Research Integrity and Safety. I agree to participate in required training at least once every 3 years to assure that I remain current on laws, regulations, guidelines and policies pertaining to the use of animals.

The information in this form agrees with the animal use section of the grant application. By checking this box, I provide assurance to the institution that my IACUC protocol is congruent with my grant application. I am responsible for amending my grant as necessary, to remain congruent with any IACUC protocol amendments, and vice versa.

All procedures involving live animals will be performed under my supervision.

- I accept responsibility for ensuring that all personnel working with live vertebrate animals are aware of, and will not deviate from, the IACUC approved procedures outlined in this protocol; that they will adhere to the regulations regarding the humane treatment of animals and that they will receive proper training as required by the IACUC.
- All personnel having direct contact with live vertebrate animals, including myself, have been or will be trained in humane and scientifically acceptable procedures for animal handling, administration of therapeutic drugs and euthanasia to be used in this project.
- Personnel will be allowed adequate time to obtain necessary training for this project and will not begin any procedures with live animals until they have been successfully trained.
- Listed participants will perform only those procedures for which they have received adequate training.

☑ Extramural or intramural peer review for scientific merit will be conducted before this proposed work is initiated.

☑ I understand that personnel with animal contact will be required to enroll in the occupational health and safety program. (OHSP Policy) I agree to inform all persons working with animals under my supervision regarding the availability of this program. (Contact the IACUC office if you need additional information.)

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2022-10-03

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Electronically Signed by Leanne Alworth -->

Date: 2022-10-03

IACUC Member Approval

Signature of IACUC Member: <-- Electronically Signed by Second Member Reviewer -->

Date: 2022-10-03

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Yet Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2022-10-11

Revision Comments:

2022-09-02 11:17:18 Leanne Alworth wrote:
Hi Andy,
Thank you for submitting this new AUP. In order to fulfill the IACUC's regulatory requirements, some additional information is needed. Please revise the AUP form (edit the text of the form itself) to respond to the questions.
Instructions for revising this AUP:
1. Go to the PI tab, and choose 'Submissions'
2. The AUP to be modified should be in the section labeled "Under Revision"
3. Click 'modify' on the AUP you wish to revise
4. Directly edit the text in the AUP form to answer the questions
5. At the bottom of the form, click on "save and submit" if you are ready to submit; or click on "save" if you are not ready to submit yet, but are logging off Artemis and want to save your changes.
6. Please do not simply answer the questions in the Revision Comments section—we would need to send it back to you to edit the actual AUP form.
Questions: Please revise the AUP to clarify the following:
Notes for Section 3. This AUP will be reviewed to ensure that all personnel are up to date on training requirements and OHSP requirements needed before the AUP can be approved. If any training or OHSP requirements are not up to date, you will receive an official email from the Research Support Services Training Coordinator, Shelby Robinson, about the specific items that are due.
Meanwhile, to speed the review process, I am giving you a heads up that some training/OHSP requirements appear to be due, or will be due soon, so you can start addressing any needs now.

Moorhead: needs to complete LAT14 in PEP (rodent anesthesia)
[redacted] *needs to complete LAT14*
[redacted] *needs to complete LAT14*
Cooper: needs to complete LAT14
Dzimianski: R-OHSP enrollment will be due 10/7
Evans: needs to complete LAT14
Greenway: needs to complete LAT14
Herrera: needs to complete LAT14
Miller: needs to complete LAT14; R-OHSP enrollment will be due 9/20
Moss: needs to complete LAT14
[redacted]*: needs to complete LAT14*
Reece: needs to complete LAT14; 1 hour of animal related CE needs to be submitted to Ms Robinson (due 10/23)
Sakamoto: 1 hour of animal related CE needs to be submitted to Ms Robinson; R-OHSP enrollment due
[redacted] *needs to complete LAT14*
[redacted] *needs to complete LAT14*

If you have any questions, please contact Shelby at Shelby.Robinson@uga.edu.

1. 3.1: [redacted] *please remove the reference to working in the lab for 1 month (not up to date with experience)*
2. 3.1: [redacted] *Just making sure the comment about being under the supervision of techs and vets is still accurate*
3. 3.1: C. Pulaski: please remove the reference to the surgeries, because no surgeries are listed on the AUP.
4. 3.1: [redacted]*: please remove the reference to working in the lab for 1 month (not up to date with experience)*
5. In general, please make sure any information added to the previous AUP via amendments that is still needed is included on this new AUP:
**A2: Gravity-assisted abdominal lavage in gerbils, instead of euthanasia (8.1, 8.2)*
**A8: Microfilariae suppression test in dogs (8.1, 8.2)*
**A9: Singly housing dogs for a workday to collect urine sample (6.9, 8.1, 8.2)*
**A9: Ace for dog blood collection (8.1, 10.1.1(a/b))*
**A13: Fasting cats before sedation, lab then feeds (8.1, 6.9 (Husbandry performed by PI staff))*
**A17: Cerenia for nausea in cats (10.2.1(a))*

6. 5.2: Thank you for the clear explanation of the animal numbers. One box that was checked is 'Consultation with a biostatistician". USDA has told us that if statistics are listed as a rationale (i.e., that box is checked), that a discussion of the actual statistical tests needs to be included in the explanation. If you have specific statistical tests that were used to determine the numbers, please include them in the text box explanation. If you do not have any specific tests to describe, please uncheck that box.
7. 5.3: We recommend that you do not list the names of colleagues who are not on the AUP roster, in case of Open Records requests, to protect their privacy.
8. 6.8.1a: Recommendation: We try to keep information about interventions only in 11.3, to avoid inconsistencies. So, we recommend that you do not include the final sentence in paragraph 2 and the final sentence in paragraph 3 (If this happens, we will contact URAR) and just keep that information in 11.3.
9. 6.8.1a/6.8.1b: In part 6.8.1a, inappetance is mentioned as a possible sign of peritonitis in gerbils injected IP. And in 6.8.1b, the box for 'Appetite' is checked. If you will be assessing appetite in gerbils, in the text box under 6.8.b, please explain how you assess appetite in these socially housed ad lib fed animals. Or, does the checking of the box 'Appetite' refer to dogs and/or cats? If so, please clarify that in the 6.8.1b text box.
10. 6.9/Special medical requirements: Please clarify that no doxy should be provided for clinical reasons (since animals will be treated with it for research purposes as per 8.2)
11. 7.1: In 2 searches, neuter is mentioned. Please remove those searches if animals will not be neutered on this AUP.
12. 8.1/Blood collection: Recommendation: Please move the final sentence (If damage occurs, the URAR veterinarian…with our research) from this location to 11.3, where interventions are described.
13. 8.2: Under Brugia (paragraphs 2 and 5 of the Brugia section) it is stated that cats and dogs (P2-malayi) and dogs (P5 pahangi) are maintained as blood donors to feed mosquitoes. Which suggests that is their only purpose for Brugia infections. Just making sure that these animals are not also used to provide blood to other researchers.
14. 8.2/Brugia: Recommendation: We recommend you remove the information about transient lymphedema from this section, because it is described elsewhere, such as 6.8.1 and 8.2.1, and does not need to be included in the description of the experiments. (Occasionally, an infected cat or dog will have…consultation with the URAR veterinarian).
15. 8.2: D immitis: Recommendation: In paragraph 1, we recommend you remove the sentence about possible interventions if clinical signs are noted (If any clinical signs are noted,…with the URAR veterinarian)
16. 8.2: D immitis: Recommendation: In paragraph 2, we recommend you remove the sentence about possible interventions if clinical signs are noted (Any clinical signs that are noted…with the URAR veterinarian)

17. 8.2: D immitis: Recommendation: We recommend you remove paragraph 3, about imaging, and include that information only in 11.3, with the interventions.
18. 8.2: D immitis: regarding the experiments with coinfection with Brugia, can you provide a bit more detail —for example would you be comparing gerbils with single infections with gerbils with coinfections? And/or would these studies be more like the gerbil negative host-specificity, where you infect and then assess at various time points.
19. 8.2: D immitis: regarding the experiments with coinfection with Brugia, would these studies also use approximately 8 gerbils per study?
20. 8.2: Wolbachia: In paragraph 1, the sentence about the hypothesis and the AHS gold standard is not quite clear.
Does this indicate that the hypothesis is already part of the gold standard treatment? (Seems like it wouldn't still be a hypothesis in need of testing in that case)
Or that it could become part of the standard treatment if the hypothesis is proven correct?
Finally, "Wolbachia levels will be decreased as compared to Doxycycline" is not clear.
21. 8.2: D immitis: please clarify the data collected for the dog doxy studies. In the second to last paragraph, initially dogs are euthanized to collect adult worms. However, at the end of that paragraph, it is suggested that L3s are being compared. Please clarify.
22. 8.2: Timelines: Because in 12.1, some animals may be transferred or adopted, it would be better to list the timeline as 'infection to end of study' rather than 'infection to euthanasia'
23. 8.2: Timelines: This lists a cat Brugia malayi infection as lasting 2 years. However, in 5.2, the length is described as having a patent infection only 1 year, as part of the rationale for needing to infect more cats. Please make sure these 2 sections are not inconsistent, so as to ensure the rationale for cat numbers is supported throughout the AUP.
24. 8.2.1: Because in 6.8.1a you listed slightly different signs of peritonitis, they are not consistent. But, the specific signs are not needed in this section, so please remove them from this section, and simply say 'signs of peritonitis'.
25. 8.2.1: Recommendation: We recommend you do not list interventions in this section, only the expected impact on the animals. So we recommend you remove:
paragraph 1: Remove 'If this were the case…would be implemented'; paragraph 2: revise to state ' IP Injection of gerbils rarely result in signs. Animals may develop signs of peritonitis.'
paragraph 3: Remove ' If the animal displays clinical signs…will be performed'

26. 8.2.1: For D immitis, the 100 L3 dose, and possible clinical signs, is not mentioned. Please add that also.
27. 10.1.1a: In general, we do not require the AUP to list the euthanasia drug (pentobarbital) under anesthesia. Also, is the drug truly pentobarbital, or is it a commercial euthanasia solution containing pentobarbital?
28. 10.1.1a: Sorry, but the doxy should really be in 10.2.1a (therapeutics).
29. 10.2.1c: For Cats, in 5.2 it is explained that cats cannot be reinfected, however, here the Frequency is listed as 1-3 times. Please make sure these 2 sections are consistent. If cats are not usually reinfected, for the reasons stated in 5.2, but may sometimes be reinfected, you can keep 5.2 and 10.2.1 c as they are, but then explain in 8.2 why cats might sometimes be reinfected.
30. 11.2: A lot of this section is about the interventions/humane endpoints, not the monitoring schedule. In order to keep the interventions clear, it is best to keep them in 11.3.
Please remove and move to 11.3 (if it is not already in 11.3):
P1: 'If lymphedema is greater than the mild…' to the end of the paragraph
P2: the whole paragraph
P3: 'If at any time the case worsens...' to the end of the paragraph
P4: 'If this occurs…euthanized promptly'
P8: the whole paragraph. Instead, describe how often dogs with D immitis are checked by the lab for signs of heartworm clinical signs.
P9: the whole paragraph
31. 11.2: Recommendation: You do not need to describe monitoring after anesthesia, because it is already in section 10.1.1b
32. 11.2: For the gerbil peritonitis, please make sure the signs are consistent with 6.8.1a. For example, do ascites and/or inappetance need to be added here?
33. 11.3: For the gerbil peritonitis, please make sure the signs are consistent with 6.8.1a. and 11.2, or simply state 'Clinical signs are described in 11.2'
34. 12.1: For cat, dog, and gerbil, under the 'Euthanasia' box, you do not need to describe the method (sorry, we cannot get the form to not include that box). 12.1: Dog/Adoption, please refer to the IACUC Adoption policy, or do not refer to any specific policy.
35. 12.1: Dog/Adoption, also the IACUC adoption policy allows for a mechanism for an animal to be adopted by people who are not CVM faculty, staff, or student, so if you prefer the flexibility to adopt to others, please remove those limitations.
36. 12.2: Cat: Please check the final box ('Please use this box to describe:') and list the drug name, dose, and route. Please be sure to refer to it as euthanasia solution if it is not truly pentobarbital.
37. 12.2: Dog: Please check the final box ('Please use this box to describe:') and list the drug name, dose, and route. Please be sure to refer to it as euthanasia solution if it is not truly pentobarbital.

Thanks,
Leanne

2022-10-03 14:01:12 Leanne Alworth wrote:
Hi Andy

The second IACUC member had only 1 comment to address:

6.8.1: Minor point of clarification - It is stated there is the possibility of "swelling of the testicles, ulcerations may occur on the testicle...". I assume the swelling is in the scrotal sac and the ulcerations are on the scrotal skin, rather than the testicles themselves? May be good to clarify, as an ulceration of the scrotal skin is easy to visualize but an ulceration of the testicle is not.
----

thanks
L

2022-10-03 15:50:45 Leanne Alworth wrote:
Hi Andy

I don't see that 6.8.1 was updated. Perhaps the person who made the changes did not "save"?

thanks
L

Renewals

Renewal Form Number: A2022 04-009-R1
Species: Cat Use Category: Category A Number Used in the Past Year:

Indicate briefly what you have accomplished in the last year with this study.
If your study is a Category C, please consider the following questions and provide responses below:

1. What is the percentage of experimental animals being assessed by humane endpoints that were found dead before reaching an established humane endpoint?
2. Does the monitoring interval established by your lab allow you to collect appropriate data yet identify moribund animals and remove them from the study to minimize suffering?

Auto Generated Submission

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-08-12

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

Final Approval

Received Final Approval on: 2023-08-16

Revision Comments:

No Revision Comments made for this Renewal.

Amendments

A2022 04-009-A2

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

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2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00

Occupational Health and Safety Enrollment	2014-01-06	0.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	

IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	

UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2022-10-27

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2022-11-10

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A1

1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.

Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00

Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00

Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00

Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2023-05-14	0.00

Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-02-16

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-02-17

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A3

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

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2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	

Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50

Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00

Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00

IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00

Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50

Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00

IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-02-17

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-02-21

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A4

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

This amendment is to request the use Dexmedetomidine 3mcg/kg-6mcg/kg, buprenorphine 0.03mg/kg, and Ketamine 10mg/kg as a single injection IM as a pre-medication for euthanasia in dogs.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...

Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	Yes No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00

UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	

UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00

Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00

UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

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Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-02-22

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Electronically Signed by Leanne Alworth -->

Date: 2023-02-23

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-02-27

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A6

1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00

IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00

Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00

LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00
UGA IACUC 101	2019-03-22	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-03-16

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-03-22

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A9

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

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2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs

Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00

Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00

Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00
UGA IACUC 101	2019-03-22	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-04-17

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-04-25

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A10

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

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2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health

Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

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Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00

Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	
IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00
UGA IACUC 101	2019-03-22	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00

IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-05-02

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-05-09

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A11

1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	Yes No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	

Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00

IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00

Occupational Health and Safety Enrollment	2008-01-31	0.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-06-13	
Staying Healthy While Working With Animals (Version 032023)	2023-06-13	
IACUC 101(Version 041123)	2023-06-13	1.00
Research Occupational Health Enrollment	2023-06-12	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50

Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	

IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training - Old Version 3	2021-05-17	0.50
Research Occupational Health Enrollment	2021-05-17	0.00
Staying Healthy While Working With Animals (ver2021)	2021-05-17	1.00
IACUC 101 (2021)	2021-05-17	1.00
Sharps Training (2021)	2021-05-17	
IACUC 101(Version 041123)	2021-05-17	1.00
Sharps Training - Old Version 3	2021-05-14	0.50
Staying Healthy While Working With Animals (ver2021)	2021-05-14	1.00
IACUC 101 (2021)	2021-05-14	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted] *el.*

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2021-12-14	0.00
Research Occupational Health Enrollment	2021-03-26	0.00
UGA IACUC 101	2021-03-01	1.00
Sharps Training (2021)	2021-02-06	
Staying Healthy While Working With Animals (ver2021)	2021-02-06	1.00
Sharps Training - Old Version 3	2021-02-06	0.50
Staying Healthy While Working With Animals (ver2020)	2021-02-06	1.00
Occupational Health and Safety Enrollment	2018-03-05	0.00
Research Occupational Health Enrollment	2018-03-05	0.00
Staying Healthy while Working with Laboratory Animals	2018-03-01	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-28	
IACUC 101 (2021)	2018-02-28	1.00
UGA IACUC 101	2018-02-28	1.00

Cassan Pulaski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

DVM with 6 years clinical experience and 7 years animal research experience. Under supervision of PI or listed experienced personnel, responsibilities include animal restraint, venipuncture, euthanasia, assistance with infection of animals with filarial parasites, and can administer medications when required. Can also manage medical cases.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-05-30	
Research Occupational Health Enrollment	2023-01-30	0.00
Research Occupational Health Enrollment	2022-12-20	0.00
IACUC 101 (2021)	2022-10-26	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2020-01-06	
Research Occupational Health Enrollment	2019-12-10	0.00
Staying Healthy while Working with Laboratory Animals	2019-11-26	1.00
UGA IACUC 101	2019-11-26	1.00
UGA IACUC 101 (Retired June 2020)	2019-11-26	
IACUC 101 (2021)	2019-11-26	1.00
Sharps Training - Old Version 3	2019-11-26	0.50
Sharps Training (2021)	2019-11-26	

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	

UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-14	0.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-20	0.00
Staying Healthy While Working With Animals (ver2021)	2022-09-13	1.00
IACUC 101 (2021)	2022-09-13	1.00
Sharps Training(2022)	2022-09-10	

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00
UGA IACUC 101	2019-03-22	1.00

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Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-04-11	0.00
Staying Healthy While Working With Animals (ver2021)	2022-04-11	1.00
IACUC 101 (2021)	2022-04-11	1.00
Sharps Training (2021)	2022-04-11	

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2023-07-06

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2023-07-12

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A12

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

The purpose of this amendment is to add saliva collection from dogs to test a diagnostic method for heartworm to the AUP. Saliva will be collected from under the tongue and from the surface of the mucosal lining of the lips and cheek via a Salimetrics SalivaBio's Children's Swab (SCS) System (https://salimetrics.com/product/swab-method-animals-50pk/). Originally designed for Children, the SCS system also features characteristics that make it viable for animal saliva collections. Saliva testing for heartworm determination will be performed at the same time as Knott's and DiroCHEK test for comparison. Nine dogs will be used (6 infected and 3 control) for proof of concept. Not all dogs on this AUP will have the procedure. There will be no more than 3 collections per dog.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...

Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

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Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

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Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

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Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00

Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00

Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	
IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	

UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00

UGA IACUC 101	2019-03-22	1.00

REMOVED FROM AUP:

[redacted]

Cassan Pulaski

[redacted]

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2024-01-03

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Electronically Signed by Leanne Alworth -->

Date: 2024-01-04

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2024-01-11

Revision Comments:

2024-01-03 09:40:11 Leanne Alworth wrote:
Hi Andy,
Thank you for submitting this amendment to your base AUP. In order to fulfill the IACUC's regulatory requirements, some additional information is needed. Please revise the amendment (edit the text of the amendment itself) to respond to the questions.
Instructions for revising this amendment:
1. Go to the PI tab, and choose 'amendments'
2. The amendment to be modified should be in the section labeled "Under Revision"
3. Click 'modify' on the amendment you wish to revise
4. Directly edit the text in section 1 to answer the questions
5. At the bottom of the form, click on "save and submit" if you are ready to submit; or click on "save" if you are not ready to submit yet, but are logging off Artemis and want to save your changes.
6. Please do not simply answer the questions in the Revision Comments section—we would need to send it back to you to edit the actual amendment section
Questions: Please revise the amendment to clarify the following:

1. What is the minimum number of dogs that will have this procedure, and how did you determine that number is adequate for the data analysis? If statistical methods were used, please be specific about the tests.
2. Will all dogs on this AUP potentially have the saliva collection procedure?
3. Will individual dogs have the procedure more than once? If so, approximately how many times will saliva be collected?

Thanks,
Leanne

A2022 04-009-A13

1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

This amendment is to add personnel.

2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...
Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	○ Yes ◉ No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00

Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	
IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	

UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00

Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00
UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00

UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	
IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs

Sharps Training(2022)	2024-02-16	
Staying Healthy While Working With Animals (Version 032023)	2024-02-16	
IACUC 101(Version 041123)	2024-02-16	1.00
Research Occupational Health Enrollment	2024-02-14	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101 (2021)	2023-02-06	1.00
Staying Healthy While Working With Animals (ver2021)	2023-02-06	1.00
Research Occupational Health Enrollment	2022-02-02	0.00
Sharps Training - Old Version 3	2019-04-18	0.50
Research Occupational Health Enrollment	2019-03-27	0.00
Staying Healthy while Working with Laboratory Animals	2019-03-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-03-22	
IACUC 101 (2021)	2019-03-22	1.00
UGA IACUC 101	2019-03-22	1.00

REMOVED FROM AUP:
None

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2024-02-21

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2024-02-29

Revision Comments:

No Revision Comments made for this Amendment.

A2022 04-009-A14

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1) Requested Modification

Provide details of the amendment to the approved AUP listed above. If more than one modification is requested, please number them.
Note: To request additional animals, please see section 2 of this form. For personnel modifications, please see section 3.

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2) Additional Animal Information

Species: (US Government Principles, Principle #3 Guide, p 12 AW Regs 9 CFR Part 2, sec 2.31 Internal record keeping/reporting data) TIPS	Choose a Species...

Strain: TIPS	No Strains Available
Highest Use Category:	Category A
Sex: TIPS	Male
Quantity (Numerical Only): TIPS	
Housing Location: TIPS	Choose a Facility...
Weight Range: TIPS	
Age Range: TIPS	
Preferred Vendor/Source: TIPS	Choose a Vendor...
Is the use of this species covered by the USDA Animal Welfare Act? TIPS	Yes No

3) Additional Personnel

Project Roster: Please provide the names of all additional individuals who will work with animals on this project to the IACUC. You do not need to include the staff of the facility in which your animals will be housed.

Occupation Health Program: Supervisors must enroll their employees in the OVPR Occupational Health and Safety Program. Please enroll personnel by having them complete a "Risk Assessment/Animal Contact Health Surveillance Questionnaire", available at the OACU OHS Page.

Training: Supervisors are responsible for insuring that their employees are adequately trained both in the specifics of their job and in the requirements of the Federal Animal Welfare Act.

All individuals working with live vertebrate animals, including the protocol Principal Investigator (PI), must complete federally required training on the pertinent laws and regulations covered in the "IACUC 101" course and health and safety covered in "Staying Healthy While Working with Animals."

The PI is responsible for keeping this roster for these individuals current. If staff is added or removed from this project, please modify the protocol to reflect this change; this is an administrative change and does not requires full IACUC review.

NEW INFORMATION:

Please be advised, for new personnel you must click the plus sign (+) by each individual's name to open a textbox in which you must provide the responsibilities as related to the work described in this animal use protocol and any relevant training/experience with these activities/these species.

PERSONNEL ROSTER:
Andrew Moorhead *(PI)* [-]

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

PI-final decision on animals. Dr. Moorhead, DVM, MS, Ph.D, 20+ years of experience with laboratory animals and has completed a laboratory animal medicine residency. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-27	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
IACUC 101 (2021)	2022-03-02	1.00
Sharps Training - Old Version 3	2021-06-16	0.50
Sharps Training (2021)	2021-06-16	
Research Occupational Health Enrollment	2021-01-28	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
UGA IACUC 101	2019-04-23	1.00

UGA IACUC 101 (Retired June 2020)	2019-04-23	
IACUC 101 (2021)	2019-04-23	1.00
Research Occupational Health Enrollment	2019-04-03	0.00
Sharps Training - Old Version 3	2018-08-10	0.50
Research Occupational Health Enrollment	2018-08-02	0.00
Continuing Education for Animal Research Credit	2017-07-26	1.00
Occupational Health and Safety Enrollment	2015-05-28	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-05-25	0.00
Research Occupational Health Enrollment	2022-02-14	0.00
Sharps Training - Old Version 3	2021-01-21	0.50
Staying Healthy While Working With Animals (ver2020)	2021-01-21	1.00
Staying Healthy While Working With Animals (ver2021)	2021-01-21	1.00
Sharps Training (2021)	2021-01-21	
UGA IACUC 101	2021-01-20	1.00
IACUC 101 (2021)	2021-01-20	1.00
IACUC 101(Version 041123)	2021-01-20	1.00

Elizabeth Boudreau [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Elizabeth will assist in animal handling/restraint, gerbil euthanasia and medication administration. She has previously worked in our laboratory for a period of a year where she learned the relevant skills.

Training Courses Completed:

Course Name	Completion Date	CEUs
Staying Healthy While Working With Animals (Version 032023)	2024-06-25	
Research Occupational Health Enrollment	2024-01-08	0.00
IACUC 101(Version 041123)	2024-01-02	1.00
Research Occupational Health Enrollment	2022-01-06	0.00
Research Occupational Health Enrollment	2021-02-12	0.00
IACUC 101(Version 041123)	2021-02-09	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-02-09	
Sharps Training (2021)	2021-02-09	

IACUC 101 (2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2021)	2021-02-09	1.00
Staying Healthy While Working With Animals (ver2020)	2021-02-09	1.00
UGA IACUC 101	2021-02-09	1.00
Sharps Training - Old Version 3	2021-02-09	0.50

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-03-21	0.00
IACUC 101 (2021)	2022-08-29	1.00
Staying Healthy While Working With Animals (ver2021)	2021-12-08	1.00
Sharps Training (2021)	2021-07-14	
Sharps Training - Old Version 3	2021-07-14	0.50
Research Occupational Health Enrollment	2021-01-28	0.00
IACUC 101 (2021)	2019-10-22	1.00
UGA IACUC 101 (Retired June 2020)	2019-10-22	
UGA IACUC 101	2019-10-22	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-04-10	
Research Occupational Health Enrollment	2019-03-13	0.00
Continuing Education for Animal Research Credit	2018-12-13	1.00
Sharps Training - Old Version 3	2018-08-10	0.50
Occupational Health and Safety Enrollment	2018-05-03	0.00
Research Occupational Health Enrollment	2018-05-03	0.00
Occ Health Update	2016-12-12	0.00
Staying Healthy while Working with Laboratory Animals	2016-10-24	1.00
UGA IACUC 101 (Retired June 2020)	2016-10-24	
UGA IACUC 101	2016-10-24	1.00
Occupational Health and Safety Enrollment	2014-01-06	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs

Continuing Education for Animal Research Credit	2023-04-21	1.00
IACUC 101 (2021)	2022-03-22	1.00
Staying Healthy While Working With Animals (Version 032023)	2021-07-14	
Staying Healthy While Working With Animals (ver2021)	2021-07-14	1.00
Sharps Training (2021)	2021-02-18	
Sharps Training - Old Version 3	2021-02-18	0.50
Research Occupational Health Enrollment	2021-02-16	0.00
UGA IACUC 101	2021-01-23	1.00
Occupational Health and Safety Enrollment	2018-02-27	0.00
Research Occupational Health Enrollment	2018-02-27	0.00
Staying Healthy while Working with Laboratory Animals	2018-02-26	1.00
UGA IACUC 101 (Retired June 2020)	2018-02-26	
IACUC 101 (2021)	2018-02-26	1.00
Sharps Training - Old Version 3	2018-02-26	0.50
UGA IACUC 101	2018-02-26	1.00

Tanya Cooper [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Tanya is a RVT with 30+ years experience (12 years with laboratory animal species, specifically in a medical role). Tanya will be responsible for animal records, medical management (with consultation of veterinarians), and technical assistance (e.g. venipuncture, anesthesia).

Training Courses Completed:

Course Name	**Completion Date**	**CEUs**
Research Occupational Health Enrollment	2024-01-29	0.00
Continuing Education for Animal Research Credit	2022-04-05	1.00
IACUC 101 (2021)	2022-02-08	1.00
Sharps Training (2021)	2021-07-26	
Sharps Training - Old Version 3	2021-07-26	0.50
Research Occupational Health Enrollment	2021-02-23	0.00
Research Occupational Health Enrollment	2019-04-03	0.00
Staying Healthy while Working with Laboratory Animals	2019-04-02	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-02	
IACUC 101 (2021)	2019-04-02	1.00
UGA IACUC 101	2019-04-02	1.00
Sharps Training - Old Version 3	2018-09-06	0.50
Research Occupational Health Enrollment	2018-07-11	0.00
Continuing Education for Animal Research Credit	2017-10-21	1.00
Occupational Health and Safety Enrollment	2017-09-25	0.00
Research Occupational Health Enrollment	2017-09-25	0.00
Occ Health Update	2016-10-11	0.00
Staying Healthy while Working with Laboratory Animals	2016-01-04	1.00
Occ Health Update	2014-11-25	0.00
Staying Healthy while Working with Laboratory Animals	2013-01-07	1.00
Occupational Health and Safety Enrollment	2008-01-31	0.00

Michael Dzimianski [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Scientist-Parasite Resource Coordinator. Bleeding of animals. Dr. Dzimianski, DVM, has over 40 years experience with filarial-infected animals. Animal handling, venipuncture, making medical decisions, euthanasia.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-13	0.00
Continuing Education for Animal Research Credit	2022-07-06	1.00
Sharps Training (2021)	2021-07-13	
Sharps Training - Old Version 3	2021-07-13	0.50
IACUC 101(Version 041123)	2021-06-15	1.00
IACUC 101 (2021)	2021-06-15	1.00
Research Occupational Health Enrollment	2019-10-07	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-14	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-07-05	
Sharps Training - Old Version 3	2018-09-12	0.50
IACUC 101 (2021)	2018-08-14	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-14	
UGA IACUC 101	2018-08-14	1.00
Continuing Education for Animal Research Credit	2016-07-05	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2008-02-21	1.00

Christopher Evans [-]

Can edit this submission form and draft amendments/renewals for this protocol: Yes No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Research Professional. Has over 9 years experience with filarial-infected animals and has been trained in procedures by Dr. Dzimianski. Chris participates in animal restraint and bleeding.

Training Courses Completed:

Course Name	Completion Date	CEUs
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-08-07	0.00
Staying Healthy While Working With Animals (ver2021)	2022-03-22	1.00
Sharps Training (2021)	2022-03-18	
IACUC 101 (2021)	2022-03-16	1.00
Sharps Training - Old Version 3	2021-07-21	0.50
Sharps Training (2021)	2021-07-21	
Research Occupational Health Enrollment	2019-09-04	0.00
Continuing Education for Animal Research Credit	2019-08-20	1.00
IACUC 101 (2021)	2019-05-08	1.00

UGA IACUC 101 (Retired June 2020)	2019-05-08	
UGA IACUC 101	2019-05-08	1.00
Sharps Training - Old Version 3	2018-09-12	0.50
Continuing Education for Animal Research Credit	2016-07-07	1.00
Research Occupational Health Enrollment	2016-06-06	0.00
Occupational Health and Safety Enrollment	2016-06-06	0.00
Staying Healthy (Non-ALL)	2007-09-18	1.00

Katelin Greenway [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Animal restraint, medical treatments, and monitoring. Will be trained and supervised by senior personnel. She has 6+ years experience working with animals.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2023-08-28	0.00
Continuing Education for Animal Research Credit	2022-03-31	1.00
IACUC 101 (2021)	2022-03-22	1.00
Sharps Training - Old Version 3	2021-10-15	0.50
Research Occupational Health Enrollment	2021-09-30	0.00
UGA IACUC 101	2019-04-09	1.00
IACUC 101 (2021)	2019-04-09	1.00
UGA IACUC 101 (Retired June 2020)	2019-04-09	
Staying Healthy while Working with Laboratory Animals	2019-03-20	1.00
Research Occupational Health Enrollment	2018-09-17	0.00
Sharps Training - Old Version 3	2018-09-06	0.50
Sharps Training (2021)	2018-09-06	
Occ Health Update	2018-06-18	0.00
Research Occupational Health Program Declined	2018-06-18	0.00
OHSP Decline to Participate	2017-09-25	0.00
UGA IACUC 101 (Retired June 2020)	2016-04-08	
UGA IACUC 101	2016-04-08	1.00
Staying Healthy while Working with Laboratory Animals	2016-04-08	1.00

Courtney Herrera [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Courtney is a SAMS departmental veterinary assistant with 6 years of veterinary experience. She will assist with venipuncture, sedation, restraint and anesthesia during this research

Training Courses Completed:

Course Name	Completion Date	CEUs

Staying Healthy While Working With Animals (Version 032023)	2023-12-19	
IACUC 101(Version 041123)	2023-07-26	1.00
Staying Healthy While Working With Animals (ver2020)	2020-09-29	1.00
Staying Healthy While Working With Animals (ver2021)	2020-09-29	1.00
Staying Healthy While Working With Animals (Version 032023)	2020-09-29	
Sharps Training - Old Version 3	2020-07-20	0.50
UGA IACUC 101	2020-07-20	1.00
Sharps Training (2021)	2020-07-20	
IACUC 101(Version 041123)	2017-09-18	1.00
IACUC 101 (2021)	2017-09-18	1.00
UGA IACUC 101 (Retired June 2020)	2017-09-18	
UGA IACUC 101	2017-09-18	1.00
Staying Healthy while Working with Laboratory Animals	2017-09-18	1.00
Sharps Training - Old Version 3	2017-09-18	0.50
Research Occupational Health Program Declined	2017-09-08	0.00
OHSP Decline to Participate	2017-09-08	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2023-04-29	
Staying Healthy While Working With Animals (Version 032023)	2023-04-29	
IACUC 101(Version 041123)	2023-04-29	1.00
Research Occupational Health Enrollment	2023-04-26	0.00

[redacted] *(Added to AUP)* [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2024-06-24	0.00
Sharps Training(2022)	2024-06-24	
Staying Healthy While Working With Animals (Version 032023)	2024-06-24	
IACUC 101(Version 041123)	2024-06-24	1.00

Kaori Sakamoto [-]

Can edit this submission form and draft amendments/renewals for this protocol: ◉ Yes ○ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

Dr. Sakamoto is a DVM, Ph.D. Dip. ACVP with 20 years experience as a veterinary pathologist. She is the designated pathologist for this protocol, and the FR3.

Training Courses Completed:

Course Name	Completion Date	CEUs
Research Occupational Health Enrollment	2022-09-20	0.00
Continuing Education for Animal Research Credit	2022-09-15	1.00
Sharps Training - Old Version 3	2022-04-25	0.50
IACUC 101(Version 041123)	2021-07-28	1.00
IACUC 101 (2021)	2021-07-28	1.00
LAT 13: Aseptic Technique and Surgical Support and Anesthesia	2019-12-05	
Research Occupational Health Enrollment	2019-09-06	0.00
Staying Healthy while Working with Laboratory Animals	2019-08-24	1.00
Sharps Training - Old Version 3	2018-09-10	0.50
Sharps Training (2021)	2018-09-10	
IACUC 101 (2021)	2018-08-24	1.00
UGA IACUC 101 (Retired June 2020)	2018-08-24	
UGA IACUC 101	2018-08-24	1.00
Continuing Education for Animal Research Credit	2018-08-21	1.00
Occ Health Update	2016-07-20	0.00
Research Occupational Health Enrollment	2016-07-20	0.00
Occupational Health and Safety Enrollment	2015-01-16	0.00
Staying Healthy (Non-ALL)	2008-09-17	1.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
Sharps Training(2022)	2024-02-16	
Staying Healthy While Working With Animals (Version 032023)	2024-02-16	
IACUC 101(Version 041123)	2024-02-16	1.00
Research Occupational Health Enrollment	2024-02-14	0.00

[redacted] [-]

Can edit this submission form and draft amendments/renewals for this protocol: ○ Yes ◉ No

Describe this person's 1) responsibilities as related to the work described in this animal use protocol and 2) any relevant training/experience with these activities/these species.

[redacted]

Training Courses Completed:

Course Name	Completion Date	CEUs
IACUC 101(Version 041123)	2023-08-22	1.00
Continuing Education for Animal Research Credit	2023-04-21	1.00
Research Occupational Health Enrollment	2022-09-07	0.00
Research Occupational Health Enrollment	2020-08-24	0.00
Sharps Training - Old Version 3	2020-08-20	0.50
Staying Healthy While Working With Animals (ver2021)	2020-08-20	1.00
Sharps Training (2021)	2020-08-20	
Staying Healthy While Working With Animals (ver2020)	2020-08-20	1.00
UGA IACUC 101	2020-08-18	1.00
IACUC 101 (2021)	2020-08-18	1.00
IACUC 101(Version 041123)	2020-08-18	1.00

REMOVED FROM AUP:
[redacted]

PI Approval

Signature of Principal Investigator: <-- Electronically Signed by Andrew Moorhead -->

Date: 2024-07-03

The Attending Veterinarian verifies that the elements of this proposal have been assessed regarding the use of appropriate techniques in utilization of animals and that consultation with the PI will occur as necessary to resolve issue to minimize pain and distress.

Signature of Attending Veterinarian: <-- Not Signed by Attending Veterinarian -->

IACUC Member Approval

Signature of IACUC Member: <-- Not Signed by IACUC Member -->

IACUC Chair Approval

Signature of IACUC Chair: <-- Not Signed by IACUC Chair -->

Final Approval

Received Final Approval on: 2024-07-15

Revision Comments:

No Revision Comments made for this Amendment.

ELECTRONICALLY FILED
3/26/2026 10:17 AM
43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

EXHIBIT B

[redacted]
IACUC Proposal #21889
Approval Date: 5/17/21
Administratively Closed: 4/30/23

IACUC Application (Version 1.15)

1.0 General Information

***Please enter the full title of your study:**

1) Effects of cervical spinal cord injury on swallow and 2) Regulation of swallow and the effects of high cervical spinal cord injury 3) Influence of Opioids on the Brainstem Respiratory Network Central and Peripheral Regulation of Laryngeal Adduction

***Please enter the Short Study Title you would like to use to reference the study:**

Airway Protection
* This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.

2.0 Add Department(s)

2.1 List the departments associated with this study. Add the Principal Investigator's department as the PRIMARY DEPARTMENT. For research conducted at a Norton facility add: Norton Healthcare. For research conducted at a Jewish Hospital/KyOne facility (e.g. Frazier, St Mary's, etc) add: Ky One Health. For research conducted at University Hospital /James Graham Brown Cancer Center add: University Hospital:

Primary Dept?	Department Name
◉	**U of L** - *42* - Neurological Surgery

3.0 Assign key study personnel (KSP) access to the project

3.1 *Please add a Principal Investigator for the study:

[redacted]

3.2 In this section, please add any project personnel that needs to have access to the submission or will need to approve the submission. In the case of the Undergraduate Research Symposium, your mentor and co-authors would be included in this section.

A) Additional Investigators

[redacted]

B) Research Support Staff

[redacted]

[redacted]

3.3 *Please add a Study Contact. The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact. Adding someone here does not add them as study personnel.

[redacted]

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. If applicable, please add Kentucky One Health, Norton Healthcare or UMC Research as a study Contact.

3.4 Please select the Designated Approvers:

Add the name of the individuals authorized to approve and sign off on this protocol.

4.0 IACUC Form Type

4.1 Please indicate the type of form you are submitting

- (●) Live Animals (including non-observational field studies)
- ( ) Tissue Only
- ( ) Field Investigations – Observational Only

- **Live Animals** - Use this form if you will be using any live animals at UofL, including description of Core Animal Laboratories. Field studies that involve capture, an invasive procedure, harms or materially alters/influences the behavior or activities of a wild animal should use this form.
- **Tissue Only** - Use this form if you will be using ***fresh*** or ***frozen*** (not fixed) tissues, organs, or carcasses obtained from animals outside of UofL or from animals assigned to, and euthanized by, PIs with other IACUC *Proposals*. If you plan to handle live animals in any way, a *Live Animal* form must be used.
- **Field Investigations – Observational Only** – Use this form if you will be conducting a field investigation limited to observation of free-living, wild ***vertebrate*** animals in their natural habitat and there will be no manipulation of the animal or its environment. **Note:** Field studies that involve capture, an invasive procedure, harms, or materially alters or influences the behavior or activities of a wild animal **must use the Live Animals form.**

5.0 Emergency Contacts

5.1 Indicate the Key Study Personnel who will act as emergency contacts

Study Personnel	Phone Numbers
[redacted]	During Work Hours 502-852-5794 After Hours [redacted]

6.0 Welcome Page

6.1

Welcome to **IACUC** @ Louisville **Replacement Reduction Refinement Responsibility Do I need an IACUC protocol?** For best viewing of form materials, it is recommended that you expand your window as much as possible.

7.0 Proposal Purpose / 3 Year Renewal

7.1 Proposal Purpose (Check all that apply)

- [x] Research using live animals
- [ ] Teaching and Training
- [ ] Core Animal Laboratory Proposal

Is this a 3-Year Renewal?

(•) Yes ( ) No

7.2 Previous Proposal number:

17164

Do you currently have animals in-house in ongoing studies under your expiring Proposal that will be transferred to this Proposal?

(•) Yes ( ) No

All ongoing experiments and animals **must** be described and accounted for on this "renewal" Proposal (in Section 13 Experimental Groups). You should describe what procedures/studies were done to the animals under the expiring Proposal and what procedures/studies will be done under this renewal. **Note:** The number of animals transferred from the expiring Proposal to this renewal will contribute to the total number of animals approved.

Have you described all ongoing experiments and accounted for animals that will be transferred onto this Proposal in Section 13.1?

(•) Yes ( ) No

7.3 Scientific Review and Funding Source(s)

Scientific review has been, or will be, performed by an internal or external review panel before experimentation begins. Select all that apply:

- [x] Federal Agency
- [ ] State Agency
- [x] Private Foundation
- [ ] U of L Review Panel
- [ ] Industry Sponsor
- [ ] Department Chair or Designee
- [ ] Other

List any funding sources applicable to this Proposal

Grant No.	Sponsor	Title
NS 110169	NIH	Regulation of swallow and the effects of high cervical spinal cord injury
CNF546714	Craig H. Neilsen Foundation	Effects of cervical spinal cord njury on swallow

8.0 Species to be used in this Proposal (Only ONE Allowed)

8.1 Select species in drop down list below. Note: If the species you would like to use is not listed, please contact the IACUC Office (IACUC@Louisville.edu, 852-7307) so that it may be added to the form.

Select Species to be used in the Proposal (for Field Study, select "Wild Caught Species")

Cat

8.2 Justification of Selected Species

Give rationale for the selection of this species. In all cases, a "lower" species should be given primary consideration.

Adult cats are used for three reasons: (a) While swallow has been assessed using videofluoroscopy in various rodent models of disease, aspiration has never been seen. Russell, et al. (Dysphagia, 28; 95-104; 2013) thoroughly discussed how the anatomical specifics of the rodent do not make aspiration or its use as an outcome measure possible. (b) The cat has been used extensively as a model system for studying vertebrate motor control, including cough, which is a critical respiratory-related defense with similarities to swallow. The extensive literature on feline motor control during breathing and cough, including our own studies, supports the importance of this model for translation. Relative to other species, a large amount of information exists on the physiology and pharmacology of airway protection in the cat. Further, because the rat lacks airway protective behaviors such as cough and/or vomiting, use of cats affords greater translational potential for the current study. (c) The complex instrumentation during the terminal electrophysiology procedures requires that animals of this size be used.

8.3 List strains, lines, stocks, breeds, *etc.* in the table below. - NOTE: it is acceptable to group multiple strains/lines on a single row AS LONG AS the description of potential adverse phenotypes is the same for all strains/lines included in that row. For example, you may be using a number of transgenic lines. You are not required to list every line as long as the expected phenotypes are the same.

General Information	Strain Origin	Decription of Strain and Adverse Phenotypes (if any)
Strain or Lab Name [redacted] Is strain irreplaceable? ○ Yes ◉ No	◉ Commercial Vendor ○ Import from Colleague ○ Existing colonies bred at UofL ○ New genetically-modified line created at UofL ○ Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) None
Strain or Lab Name [redacted] Is strain irreplaceable? ○ Yes ○ No	◉ Commercial Vendor ○ Import from Colleague ○ Existing colonies bred at UofL ○ New genetically-modified line created at UofL ○ Wild Caught	Describe any adverse phenotypes (diabetes, tumor growth, seizures etc.) None

8.4 Are any of the strains/lines above genetically-modified (transgenic, knock-out, knock-in, etc.)?

○ Yes ◉ No

8.7 Are you willing to share tissues from animals or transfer live animals to other colleagues at U o f L?

◉ Yes ○ No

8.8 Related Proposals Are the experiments similar or related to those described in another proposal for a different species? For example, multiple species may be needed to satisfy regulatory requirements.

○ Yes ◉ No

9.0 Lay Project Summary (250 words max)

9.1 Provide a non-technical summary of the proposed research, using language that a person not trained in biomedical sciences can understand. Describe the significance of the project and the reasons for which it has been proposed. This description should allow the reader to weigh the potential human or other animal health benefits against animal welfare concerns.

Lay Summary (250 words maximum):

A variety of neuromuscular/ neurotraumatic diseases result in impaired airway protection including breathing, swallow and/or swallow function.
Impairment of these airway protective behaviors results in an increase in pulmonary infections due to aspiration. Pulmonary complications related to inadequate airway defense are the leading cause of death in patients with spinal cord injuries and Parkinson's disease.

The long-term goal of this project is to determine brainstem and spinal mechanisms that control the regulation of airway protection. Our central hypothesis is the brainstem is reliant on feedback from the spinal cord to regulate airway protective behaviors. Loss of this information creates mis-timed swallows, laryngeal dysregulation and increases aspiration risk. The cat model is essential to the study. These animals exhibit the same cough and swallow reflex as humans, even when they are anesthetized.

Currently there are no effective treatments for swallow disorders beyond the limited effects of exercise. This work provides the foundation to translate experimental models into clinical effects.

10.0 Technical Summary (800 words max)

10.1 Technical Summary Describe specific aim(s) and the long-term goals of the project.

The long-term goal of this project is to determine the role of tracheal, pharyngeal, and spinal sensory feedback in the regulation of cough and swallow.

Our central hypothesis is that an aspiration event produces a series of coughs and swallows which are expressed in various behavioral interaction patterns, and that there are decipherable rules that regulate the various patterns of expression. Cough and swallow are airway protective behaviors. The pharyngeal phase of swallow prevents aspiration of oral material (saliva, food and liquid), by epiglottal movement, laryngeal adduction, and clearing the mouth and pharynx. Cough is an aspiration-response behavior that clears material from the airway. Coordination of these behaviors is vital to protect the airway from further aspiration-promoting events, such as a swallow occurring during the inspiratory phase of cough. The peripheral inputs, operational characteristics, and primary strategies that coordinate cough and swallow are unknown. This lack of information impedes understanding of the deficits in airway protection, with co-occurrence of dystussia and dysphagia, which occurs with diseases such as Parkinson's disease and Alzheimer's disease.

The Specific Aims of the project are: 1) Identify the operational principles that govern the coordination of cough and swallow motor patterns following activation of tracheal and pharyngeal afferents during an aspiration event; 2) Develop a predictive computational distributed network model of the central influence of tracheal sensory pathways on the expression of cough and swallow; 3) Identify the role of neurons in the reticular formation in processing tracheal and pharyngeal afferent feedback on cough and swallow;

5) Identify the effects of cervical spinal cord disruption on the swallow motor pattern; and 6) Determine the therapeutic efficacy of 5-HT_{1A} agonists on recovery of swallow function after spinal cord injury.

11.0 Justification for Animal Use

11.1 Justification for Animal Use

Describe why the Proposal requires the use of animals, as opposed to *in vitro* or *in silico* approaches. (250 words or less)

Adult cats are used for three reasons: (a) While swallow has been assessed using videofluoroscopy in various rodent models of disease, aspiration has never been seen. Russell, et al. (Dysphagia, 28; 95-104; 2013) thoroughly discussed how the anatomical specifics of the rodent do not make aspiration or its use as an outcome measure possible. (b) The cat has been used extensively as a model system for studying vertebrate motor control, including cough, which is a critical respiratory-related defense with similarities to swallow. The extensive literature on feline motor control during breathing and cough, including our own studies, supports the importance of this model for translation. Relative to other species, a large amount of information exists on the physiology and pharmacology of airway protection in the cat. Further, because the rat lacks airway protective behaviors such as cough and/or vomiting, use of cats affords greater translational potential for the current study. (c) The complex instrumentation during the terminal electrophysiology procedures requires that animals of this size be used. Specific lesions are being used to for 2 reasons: 1) increase our understanding of specific tracks/areas which lead to disordered airway protection; and 2) reduce variance in outcomes related to injuries to reduce overall animal usage.

No suitable alternatives have been identified to replace the use of live animals for the studies associated with this protocol. The lack of suitable alternatives was determined by a continual review of the literature on a weekly bases.

12.0 Assurance of Non-Duplication

12.1 Assurance of Non-Duplication

Provide a *written assurance* that the proposed activities do not unnecessarily duplicate previous or ongoing experiments. Describe methods and sources (journals, abstracts, *etc.*) to support this assurance. Include the date the search was performed, years included in the search, and keywords used. [Examples can be found in the help button to the right. Links to Databases: PubMed; Google Scholar]

Database Info	Keywords and Description of Results
PubMed 03/18/2021 Years Searched ALL years in database	List Keywords Cough, swallow, reflex, cat model, spinal cord njury, dysphagia, buspirone Describe Results There is limited research ongoing in cough and swallow, there are < 20 relevant papers. The other groups currently using this model are fellow collaborators and no duplication of effort and work is a significant goal of the international collaborative consortium. The consortium meets every 2 years in Martin Slovakia, which is the birthplace of modern cough research. Additionally, Skype calls take place every 2-3 months with members of the group.
Google Scholar 03/18/2021 Years Searched All in database	List Keywords Cough, swallow, reflex, cat model, spinal cord njury, dysphagia, buspirone Describe Results < 20 relevant papers (same as above).

13.0 Experimental Groups

13.1 Describe Experimental Groups in Table Below

Provide a description of each experimental group in enough detail that reviewers can understand what happens to each animal assigned to that group. - Group Name or Number: May be a user defined number or brief descriptive name. *Example*: heart transplant; dietary restriction. - Pain Class: Class 0 - Animals will be acquired/held, but not used or manipulated in any way. - Class I - Animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. - Class II - There is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include induction of cancer/tumors, biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic catheters, as well as survival and non-survival surgery. - Class III - Animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section. - Treatment/Description: Devise a brief descriptive title for each procedure and describe the treatments each animal in this group will receive, including the time period between procedures. For studies in which the exact sequence or number of procedures cannot be determined, include a range of potential time periods and note the maximum potential procedures to be performed on the animals in that group. *Example:* This group will receive heart transplant followed by stem cell treatments. The stem cells will be given IV by tail vein injection 10 days after heart transplant surgery. In later sections you will describe "heart transplant" (Survival Surgery), "stem cell (IV) injection," etc. in the "*Procedures Table*" below. Note: You may also include in each group a small number of variables as long as it is very clear from the description what will happen to the animals in these groups and the sample size used. Example: Following an acclimation period of 14 days, animals will receive treatment with XJ-47 in the drinking water at 3 levels (0, 15, and 30 mg/ml) for 30 days. At this point, we will perform intrasplenic implantation of WKW-95 (or control group) and follow groups of animals for an additional 30, 60, or 90 days until euthanasia and tissue collection. 10 animals /group x 3 dose groups x 2 implantation groups x 3 time points = 180. - Number of Animals in This Group: The number of animals needed in the group is generally the sample size ("n"). If multiple variables were included in the "Treatment/Description," then this number may be a multiple of the sample size.

Group Name or Number and Pain Classification	Treatment/Description	Number of Animals in This Group
	Adult cats may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats will be brought in to the laboratory and anesthetized to a surgical level of anesthesia. The an artery (femoral and/or vertebral) and a vein (forelimb and/or femoral) will then be cannulated. Arterial blood (0.2-0.3 ccs) may be removed at least 1x an hour for blood gas measurements and supplemental anesthetic or therapeutic agents will be applied through the vein as necessary. Next the trachea will be cannulated and EMG wires:Parasternal intercostal, diaphragm (costal and crural), laryngeal, abdominal, and pharyngeal muscles will be exposed and electrodes placed for EMG recordings of muscle activity during cough and swallow. This should take place over the next two hours. To optimize the EMG placement, the animal will be rotated, ear bars will be placed, and fixed to the	

Group Name or Number Modulation of airway reflexes- terminal electrophysiology Pain Class (See definition above) Class II		stereotaxic frame. Crainiotomy and/or laminectomy will be performed within the next two hours. Stimulations will be performed again for baseline measures taken. Microinjection and/or infusion of experimental compounds will be administered (Doxapram, Baclofen, Codeine,Cobalt Chloride, Insogubacine, Capsaicin, Citric Acid, Substance P, Glutamate, Allostatin, Saporin, Kynurenic Acid, 8-OH-DPAT, and/or WAY-100635), then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e. g. 1 hour, 2 hours, 3 hours and 4 hours). In some animals we may elect to stimulation the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. A bilateral electrode will be placed around 1 or both of the SLN or on the skin without cutting/damaging the nerve. Stimulation will be at <4V and at a range from 5-500 Hz. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. In some animals an acute spinal cord injury will be performed (in procedures designated as a non-survival procedure) and then the stimulations will be repeated (as described above) and this may include microinjection and/or infusion of experimental compounds will be administered (Doxapram, Baclofen, Codeine,Cobalt Chloride, Insogubacine, Capsaicin, Citric Acid, Substance P, Glutamate, Allostatin, Saporin, Kynurenic Acid, 8-OH-DPAT, and/or WAY-100635), then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e.	69	

	g. 1 hour, 2 hours, 3 hours and 4 hours). At the end of Anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo perfusion. A small pilot project will be performed on 8 animals prior to the terminal electrophysiology procedure. Cats will be given buprenorphine IM at a dose of 0.02 mg/kg or 0.03-0.04 mg/kg ~q12 hours for ~48 hours. Videofluorocopic swallow evaluations will be performed during a control period (~24-48 hours before first dose of buprenorphine), at least ~1-hour post the first dose of buprenorphine, depending on change in swallow function swallow studies may be repeated with no more than 2 every 24 hours and a total of 7 per week. Following the 48 hours on buprenorphine swallow function may then be evaluated once per day for up to seven days. Cats may be evaluated at both dosages (0.02 mg/kg and 0.03-0.04 mg/kg), with a period of at least 1-week between evaluations.	
	Adult cats may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats will be brought in to the laboratory and anesthetized to a surgical level of anesthesia. An artery (femoral and/or vertebral) and a vein (forelimb and/or femoral) will be cannulated. Arterial blood (0.2-0.3 ccs) may be removed at least 1x an hour for blood gas measurements and supplemental anesthetic or therapeutic agents will be applied through the vein as necessary. Next the trachea will be cannulated and EMG wires placed. This should take place over the next two hours. To optimize the EMG placement stimulations including: cough, swallow,	

Group Name or Number Neuron Recording- terminal electrophysiology Pain Class (See definition above) Class II	alteration in respiratory state, or laryngeal cooling will be assessed. The animal will be rotated, ear bars will be placed, and fixed to the stereotaxic frame. Crainiotomy and/or laminectomy will be performed within the next two hours. Recording electrode/s will then be placed in targeted areas of the brainstem and/or spinal cord to extracellularly record from neurons of interest. Then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed. In some animals we may elect to stimulation the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. A bilateral electrode will be placed around 1 or both of the SLN or on the skin without cutting /damaging the nerve. Stimulation will be at <4V and at a range from 5-500 Hz. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. At the end of the experiment anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo perfusion.	46
	Adult female cats will be spayed (by vendor or by the lab) prior to or during the training period. Dependent upon cat's personality, will take 1-12 weeks. During training, cats will receive their feed in the RRC housing room, MDR 517/520 and/or the CII-009 fluoroscopy room. Intake will be monitored to assure resting energy requirements (RERs) are met, and a body condition score (BCS) of 5 targeted. Following training, baseline videofluroscopic examination of swallow, aryngeal imaging or behavioral testing will be performed. Following	

Group Name or Number		baseline data collection, animals will receive spinal cord injuries using appropriate anesthesia. Pain medication is provided for 48 post-op. Bladder and bowel care will be provided as needed until voluntary control returns. A high calorie, high protein diet may be used in combination with other feed during the acute recovery period. Cats may be housed separately after surgery to monitor food intake, bowel and urine output as well as prevent cats from grooming incision lines of other cats. A thick bedding of shredded newspaper or thick foam must cover the entire cage floor after injury. It must be maintained for the rest of the study to prevent skin ulcers and peripheral nerve compression. Resting boards will be removed permanently from the cages of spinal cord injured cats. This must be done to prevent cats from 1) rubbing their incision sites which could further damage their spinal cords; 2) from jumping or rolling off of them and potentially hurting themselves; and 3) lying /sitting for extended periods on a the hard board surface on a sensory compromised limb/hindquarters which is likely to cause skin breakdown and/or nerve compression. Small litter pans will be added in the cages of spinal cord injured animals. These pans must be small enough to prevent the animal from laying in the pan. Like resting boards (above), the hard pan and litter can cause potential peripheral nerve compression and/or skin breakdown. Data collection may begin as early as 1-2 wks post-injury, may be collected 1-2 times a week and may continue for as long as ~22 wks. Administration of buspirone may begin 24 hours post-injury and continue for ~22 weeks. After final behavioral data collection, cats will then undergo a above described terminal procedure: tract tracing group, neuron recording group or modulation of airway protective behaviors group	
Survival Spinal Cord Injury			74
Pain Class (See definition above)			
Class II			

	(explained above). Tract tracing groups will undergo a 2nd spinal surgery for tracer placement followed by transcardial perfusion 3-13 days later. Tracers may include lipophilic dyes (e.g. 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI), 3,3'-Dioctadecyloxacarbocyanine Perchlorate (DiO), DiY etc - these are Carbocyanine and aminostyryl dyes with saturated alkyl substituent that come in different colors), biotinylated dextrose axmine (BDA), horseradish peroxidase (HRP), Fast Blue, Cholera Toxin B, and/or FluoroGold (FG). A total of 20 animals are requested for each group including a 10% attrition. Additionally, 10 animals are needed for control tract tracing that did not undergo a spinal cord injury. In a subset of animals opioid (buprenorphine) will be administered ~1 hour before videofluoroscopy and/or laryngeal imaging before and after the spinal cord injury.	

13.2 Will You Maintain a Breeding Colony?

○ Yes ◉ No

13.4 Total Number of Animals Requested **Remember to include those from Breeding colony if relevant.**

189

13.5 Animal Number Justification *Provide specific justification for the number of animals to be used in each group i.e., the sample size.* **This must address statistical significance as it relates to experimental design. Please be as detailed as possible. Statistical power analysis or calculation given a known or expected error/failure rate and difference between groups is preferred, but experience with the model may be acceptable. [IACUC Fact Sheet] For teaching or training, the number of "students" per animal and expected number of training exercises may suffice. Note: Details of treatments, procedures, and other experimental variables should be included in the "Treatment/Description" column in the table above.**

Modulation of airway reflexes - terminal electrophysiology:
Three conditions will be needed to complete this specific aim. Condition A: 40 animals will undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition B: 40 animals will undergo an acute spinal cord injury and undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition C: 30 animals will undergo airway reflex testing and modulstion with adminstration of compounds via infusion or microinjection as the terminal procedure after a survival spinal cord injury (Described below). Condition A (40) + Condition B (30) + Condition C (30) = 100 animals.

We have completed 15 animals from Condition A; 10 animals from condition B; and 6 from Condition C. These have been removed removed from the sample size calculation. For a total of 69 animals. These sample size calculations are based on % change in our previous studies in EMG, with an alpha level of $p< 0.05$ and a power of .9.

For the small pilot project- at ~48 hours post-spay surgery (~1hour post last buprenorphine administration) aspiration was noted on a few animals. This small pilot project on 8 animals (4 males & 4 females) will allow for us to understand the effect of the opioid along on swallow function, as this will be a factor in all studies performed after cervical spinal cord injury.

A modification to this IACUC Proposal will be made to include all required information for the unnamed agent when the agent has been provided for use. No animal will be administered an unnamed test agent without an approved modification so that the IACUC veterinarians may determine the effect of the agent on the health of the animal(s) to which the agent is administered and so that the Institutional Biosafety Committee, Department of Environmental Health and Safety, and/or Radiation Safety may determine the effect of the agent on the laboratory and RRF staff members who will or may come into contact with the animal(s) administered the agent

Neural Recording - terminal electrophysiology:
The historical yield for these types of experiments is that 5% of total neuron pairs produce features that suggest functional interactions between the neurons. Given the complex nature of this network, as well as the fact that we will study a population of neurons about which there is little published information, we anticipate approximately 1700 pairs with evidence of functional interactions. Therefore, we estimate that the minimum number of neuron phenotypes that we will encounter in these experiments is 19, representing (19)(18)/2=171 possible combinations. Allowing for at least ten examples of each yields a total of 3,400 pairs with significant functional interactions. This number represents 5% of the total pairs required, which is 3,400/0.05=68,000 pairs. We anticipate a yield of approximately 350 pairs per animal or 68,000 pairs/350 pairs per animal=194 total animals for the project. Fifty animals will be used to complete this protocol. Should our yields per animal be higher than anticipated, fewer than 50 animals will be used.

We have completed 4 animals from this experiment, for a total of 46 animals needed.

Survival Spinal Cord Injury:
Four conditions will be needed to complete this aim (A: tract tracing, B: modulation of airway reflexes - terminal electrophysiology and C: neural recording) and D: control tract tracing (w/out sci). Based on the variability of the measures from the videofluroscopic evaluation and % change in our previous studies in EMG, with an alpha level of $p< 0.05$ and a power of .9 the following animals are needed: Condition A (20) + Condition B (30) + Condition C (20) and Condition D (without SCI) (10) = 80 animals.

We have completed 2 animals from A, 2 animals from 4, which reduces the animals numbers to 74.

14.0 Field Studies

14.1 Does proposal involve field studies?

○ Yes ◉ No

15.0 Procedures

15.1 Procedures Checklist **Indicate the types of procedures used in this proposal (check all that apply). Definitions: *Minor*: Any surgical intervention that does not expose a body cavity and causes little or no physical impairment. Example: laparoscopy; wound suturing; peripheral vessel cannulation; percutaneous biopsy; routine farm-animal procedures such as dehorning, castration; prolapse repair; and most procedures done on an "outpatient" basis in veterinary clinical practice. *Major:* Any surgical intervention that penetrates and exposes a body cavity; any procedure that has the potential for inducing permanent physical or physiologic impairment; and/or any procedure associated with orthopedics or extensive tissue dissection or transection. - For *Multiple Survival Surgical Procedures, i.e.,* surgical procedures that will be performed under separate anesthetic periods from which the animal will recover from each anesthetic period, describe each surgical procedure separately (there must be two separate procedures in the "Procedures Table"). - Be sure to save often!**

- ☐ None: Animals will be acquired/held and/or bred, but not used or manipulated in any way (exceptionally rare)
- ☐ Genotyping
- ☐ Individual Animal Identification
- ☑ Non-Surgical: may require anesthesia, but do not involve a surgical incision. Examples include test article administration, behavioral assessment, tissue collection (prior to euthanasia), imaging,

irradiation, etc.

- ☑ Surgery - Non-Survival: a surgical procedure, performed under anesthesia, from which the animal does not recover from the anesthetic (also known as terminal or acute surgery)
- ☑ Surgery - Survival: a surgical intervention from which the animal is expected to recover from the anesthesia
- ☑ Surgery - Multiple: TWO or more survival surgical procedures (major or minor) between which the animal recovers from anesthesia

15.4 **Procedures** Note **when anesthetics or analgesics will be used, DO NOT provide dose, route, frequency, *etc.*, as these must be included in a later section.**

Procedure Type and Name (descriptive title used in EXPERIMENTAL GROUP section above)	**Description. Provide sufficient detail such that the reviewer can understand exactly what will occur and the potential impact of the procedure on the health and well-being of the animal. For surgical procedures include incision site, all tissue manipulations, temporary wound closures, etc. Indcate when anesthetics or analgesics will be used, but DO NOT include doses - these will be described in a later section.**
Non-Surgical Procedure Name Videofluoroscopic evaluation of swallow function	Using a fluoroscopy x-ray which allow for movie-like imaging during procedures, the cats will be presented with barium laced liquids that allow swallow will be tracked as the bolus moves from the mouth to the stomach. In some animals we may give buprenorphine IM ~ 1hour before beginning the evaluation. Animals may be fasted for 4-6 hours prior to this procedure. In some animals we will choose to evaluate their swallow unanethesized . Animals will be acclimated to the procedure in [redacted] prior to initial testing. If the acclimation period is unsuccessful then the animal will be transferred to another group, which does not require behavioral assessments. The animals will be taken to [redacted] to use the GE OEC 6800 Miniview C-Arm or the [redacted] to use the the GE Innova 2100 fluoroscopy. Both at at 30 frames per second and recording from the head to below the diaphragm swallow will be evaluated. The animals will lick a liquid/food laced with barium. The swallowed material will be tracked from the mouth to the stomach. The procedure should take less than <20 minutes. This is a commonly used clinical procedure. Barium may lead to constipation, however usually resolved within 24 hours. If the animal has not consumed their daily required calories during this procedure the remaining food will be provided in the home cage. This testing may be done up to 2 times in a 24 hour period and no more than 7 times a week.
	Animals will be acclimated to the training environment and testing procedures. Animals may be transported to [redacted] for behavioral training. Cats are acclimated to the training room, runways and tasks over 1-3 weeks. If the acclimation period is unsuccessful then the animal will be transferred to another group, which does not require behavioral assessments. The length of this period is dependent upon the animal's demeanor (timid cats take longer). Initially they are brought

Non-Surgical Procedure Name Training	into the room and allowed to explore the equipment and eat on the floor or at the platforms at the ends of the runways. When they appear comfortable eating in the training room, they are encouraged to cross the flat simple runway for food placed along the runway and/or at either end on the platforms. When they appear comfortable crossing the simple runway, more difficult runways are introduced. Initially, cats may be assisted across the runways by holding near the base of their tail or on their torso to help with balance, weight support, and encourage forward progression. While cats are initially learning the tasks, crossing will be attempted for 5-10 minute bouts. Once cats are readily performing the task independently, they may continually cross for 15-30 minutes (to achieve 20+ crossings on a single runway), pausing briefly at each end to receive a treat, prior to a rest period off the runway (typically 15-30 minutes while another cat crosses). Most cats cross all runways by 3 weeks. After 1-3 weeks, prior to SCI, training focuses on consistency of crossing speed. When cats are well trained, they typically will perform 2-3 tasks/day which represents <1 hour of total training (crossing) time/day/cat. Additionally, animals may be exposed to [redacted] and/or the [redacted] to increase compliance during swallow testing. This may also be done in the housing room, using food/liquid they will be exposed to during the swallow testing.
Non-Surgical Procedure Name Behavioral Testing	Animals may be transported to MDR-15 or behavioral testing and using a food reward walk across different platforms/structures to evaluate locomotion/standing. Cats are acclimated to the training room, runways and tasks over 1-3 weeks. If the acclimation period is unsuccessful then the animal will be transferred to another group, which does not require behavioral assessments. The length of this period is dependent upon the animal's demeanor (timid cats take longer). Initially they are brought into the room and allowed to explore the equipment and eat on the floor or at the platforms at the ends of the runways. When they appear comfortable eating in the training room, they are encouraged to cross the flat simple runway for food placed along the runway and/or at either end on the platforms. When they appear comfortable crossing the simple runway, more difficult runways are introduced. Initially, cats may be assisted across the runways by holding near the base of their tail or on their torso to help with balance, weight support, and encourage forward progression. While cats are initially learning the tasks, crossing will be attempted for 5-10 minute bouts. Once cats are readily performing the task independently, they may continually cross for 15-30 minutes (to achieve 20+ crossings on a single runway), pausing briefly at each end to receive a treat, prior to a rest period off the runway (typically 15-30 minutes while another cat crosses). Most cats cross all runways by 3 weeks. After 1-3 weeks, prior to SCI, training focuses on consistency of

	crossing speed. When cats are well trained, they typically will perform 2-3 tasks/day which represents <1 hour of total training (crossing) time/day/cat.
Surgical - Non-Survival Procedure Name Alteration in Respiratory State	While under anesthesia, in some animals we will choose to observe how changes in the breathing pattern will effect the EMGs or neurons of interest. We will hyperventilate, (hyperventilation is the method in which we create a hypocapnic condition in the animal) the animal by putting them on a ventilator and increasing the respiratory rate (we will then run an arterial blood gas to insure the hypercapnia has been reached then conduct the cough protocol. We will also be subjecting these animals to a hypercapnic or hypoxic condition to observe this modified physiological state . This is done by supplementing the spontaneously breathing animal with
Surgical - Non-Survival Procedure Name Blood Collection	Every 60-90 minutes 1cc of blood will be collected from the femoral artery cannula and run for blood-gas measurement. The line will be flushed with heparinized saline. A total of 8-22cc's of blood. While the animal is under general anesthesia
Surgical - Non-Survival Procedure Name Cough stimulation	Cough will be stimulated by placement of a 4-5 inch piece of small tubing caudal into the trachea to the level of the carina or rostral into the larynx and slowly rotating. While the animal is under general anesthesia
Surgical - Non-Survival Procedure Name Cough-swallow stimulation	The described cough-swallow stimulation will be overlapped to produce cough and swaiiow in sequence. While the animal is under general anesthesia
Surgical - Non-Survival Procedure Name Craniotomy	An occipital craniotomy will be performed and the caudal portion of the cerebellum will be removed to allow access to the brainstem. While the animal is under general anesthesia
Surgical - Non-Survival Procedure Name Electrical Simulation of the superior laryngeal nerve	In some animals we may elect to stimulation the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. A bilateral electrode will be placed around 1 or both of the SLN or on the skin without cutting/damaging the nerve. Stimulation will be at <4V and at a range from 5-500 Hz. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Electromyography placement (EMG)	Parasternal intercostal, diaphragm (costal and crural), laryngeal, abdominal, and pharyngeal muscles will be exposed and electrodes placed for EMG recordings of muscle activity during cough and swallow. While the animal is under general anesthesia.

Surgical - Non-Survival Procedure Name Microinjection into the brainstem	In some animals we will microinject compounds that are known antitussives or alter areas known to participate in the control of cough and swallow into the areas of the brainstem of interest. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Multi-electrode Array	Up to Three multi--electrode arrays will be placed in the brainstem or spinal cord. Electrodes will be advanced until the number of simultaneously recorded neurons is maximized for each array. Once the number of well--isolated neurons is maximized, recordings will be obtained during breathing for approximately 30 minutes. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Placement of catheters	An incision will be made in the medial aspect of the right hindlimb and catheters (made of polyethylene tubing) will be placed in the artery and vein for additional venous access for fluid maintenance, supplemental anesthesia and arterial pressure observation. An incision will be made through the left armpit, the axillary artery is exposed and cannulated to allow for infusion of compounds into the vertebral artery. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Placement of ear bars	In only non-survival experiments, while the animal is under general anesthesia, a local anesthetic will be infiltrated into the skin around the ear canal for analgesia. Ear bars will be placed bilaterally and the head affixed to the stereotaxic apparatus.
Surgical - Non-Survival Procedure Name Superior laryngeal Nerve Cooling	In some animals the SNL will be placed over an aluminum hook which is attached to a small reservoir which can hold a small amount of dry ice. The nerve will be cooled to approximately 5C for a short duration and cough/swallow stimulation will be conducted. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Swallow simulation	A 3 cc syringe will be attached to a 1 inch piece of PE-90. 3 cc's of a water bolus will be injected into the mouth. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Acute Spinal Cord Injury	PROCEDURE FOR NON-SURVIVAL TERMINAL ELECTROPHYSIOLOGY EXPERIMENTS: The spinal column will be exposed by making an incision through the skin and then the muscle overlying the vertebra. A bilateral laminectomy will be performed to expose the underlying spinal level. The dura will be slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. The spinal cord will either be: completely severed, over severed, or under severed, using iridectomy scissors or a midline incision will be made. More than one cut may be made. Any fibers left adhering to the ventral or lateral

	dura may be gently lifted with suction and cut with iridectomy scissors. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Distension	In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended by a balloon to test the effects of mechanoreceptors activity on cough and swallow. This may be combined with the above described cough and swallow protocols. While the animal is under general anesthesia.
Surgical - Non-Survival Procedure Name Swallow/cough Suppression	In some animals we may bath the trachea, esophagus, larynx, and/or pharynx in capsaicin, citric acid, and/or acetic. This may be combined with the above described cough and swallow protocols. While the animal is under general anesthesia.
Surgical - Survival Major	Spays may be done by the vendor (prior to purchase) or after the cats arrive at U of L. If feasible, the choice will be made to have the vendor perform the procedure. If performed by the researcher at U OF L (who has performed many of these across the last 20 years), the basic procedure is as described below Cats will undergo a spay procedure (ovariohysterectomy) under deep anesthesia in which the entire uterus, with ovaries, is removed. For spays, animals typically are fasted the evening prior to surgery (4- 6 hrs). Acetylpromazine may be given as a pre-sedative prior to anesthetic induction with isoflurane on the day of surgery (30 minute minimum). Alternatively, buprenorphine may be used as a pre-sedative instead of acetylpromazine, as well as for post-surgical analgesia. Atropine and buprenorphine will be given before or after anesthetic induction. Eye ointment will be applied, the animal intubated and an IV line placed. The surgical site will be shaved and prepped with approved cleaning solutions for sterile surgery (e.g. alcohol and chlorhexidine solution 3x) and the animal then moved to the surgery area. Vitals will be continuously monitored and charted every ~15 minutes (e.g. body temperature, respiration, EtCO2, heart rate). A skin incision, ~3", between the umbilicus and the pubic symphysis will be made. This will be followed by muscle incision along the linea alba. The uterine horns will be located as they extend from behind the bladder. The horns will be followed rostrally to identify the ovaries. The horns and ovaries will then be gently separated from fascia. Hemostats will be used to clamp off the blood flow at the level of each ovary as well as at the level of the uterine cervix. Absorbable sterile suture (2-0 or 3-0) will be used to tightly tie off the vessels at each level. The entire uterus will then be removed and the abdominal cavity closed in layers. The muscle will be closed using a 2-0 or 3-0 absorbable monofilament. Subcutaneous tissues will be closed using a 2-0 or 3-0 absorbable monofilament. The skin also will be sutured with a 2-0 or 3-0 non-absorbable

Procedure Name	
Spay/Ovariohysterectomy	monofilament sterile suture using an inverted, interrupted stitch (subcuticular). The cat will be allowed to recover for a minimum of 2 weeks prior to any other surgical procedure. A strong pain medication (buprenorphine) is used to alleviate post-operative pain for at least 48 hrs post-op. General comments: Surgery is performed in a designated surgical room using strict sterile technique. If needed, due to problems with an IV line or oral intake, fluid (sterile saline, ringers, or like solution) may be given SQ up to 2 weeks after surgery. Body temperature is supported during surgery using recirculating water heating blanket/pads, warm water bottles or packs, warmed blankets and /or IV line warmer, and monitored using either a rectal or esophageal probe/thermometer. Animals typically are recovered in a temperature controlled recovery unit. If one of these is not available, cats are recovered on temperature controlled recirculating water heating pads/Bair hugger or other similar warming system. If body temperature is slow to recover to normal levels, warm blankets /towels are placed in the recovery unit. Animals are checked a minimum of 3x/day for the first 48 hours post-op and then at least 1x daily during the first postoperative week. If the incision line should become compromised due to suture break/exposure, over grooming, etc., a topical antibiotic ointment (typically a triple antibiotic ointment such as Neosporin) may be used on the site, an E collar used to limit the animal's access to the site and/or a topical deterrent spray or gel with a foul taste (such as Grannick's Bitter Apple) used to reduce grooming of the site. If the compromise is significant, warranting re-closure, the edges may be closed using an appropriate medical adhesive (as above) or restitched. The animal may be re-anesthetized with isoflurane (box and then face mask) to use adhesive or to stitch. If more than a week out from surgery, the general antibiotic may be re-administered. ***Although our lab is fully capable of this procedure and has performed it >100 times, in recent years, sometimes we opted to have it performed by the vendor as it is more cost efficient and decreases research time.
	Cats may undergo a surgery ~2 to 150 days prior to their sacrifice which first includes anesthesia, as described below. Cats will undergo the procedure under deep anesthesia, as monitored by the procedure below. Spinal Cord. A laminectomy will be performed to remove the reformed bone, and the sutured dura re-slit using an 11-scalpel blade. A few microliters (0.1-3) of a neuronal tracer (such as Diamidino yellow, FluoroGold, Lipophilic Tracer DiI, Lipophilic Tracer DiO, or Horseradish peroxidase) will be introduced into the injured/uninjured spinal cord and/or dorsal root ganglia. Dependent upon the tracer used, it will be placed adjacent to the lesion or up to ~14 mm away (or in uninjured animals in control areas). The dura (optional), muscle, subcutaneous layer and skin will be closed in layers using 2-0 and 3-0 absorbable

Surgical - Survival Major Procedure Name Neuronal Tracer Injection	monofilament suture as indicated above Procedure length is ~1-2 hours. Muscle. A) Diaphragm. There are multiple accepted methods, we will use one of the following: 1) a laparotomy is performed and the tracer is painted onto the surface of the diaphragm; 2) An injection is made into the costal diaphragm through the skin or following a laparotomy; or 3) an injection is made into the plural space with no incision. B) Tongue, no incision is necessary. C) Pharynx. An incision will be made lateral to the thyroid notch and careful blunt dissection will separate the larynx from the strap muscle complex. The larynx will be rotated and the middle/lower pharynx will be visualized. OR the pharynx will be assessed through the oral cavity. 4) Larynx, no incision necessary and will be assessed through the oral cavity. In all cases a few microliters (0.1-10) of a neuronal tracer (such as Diamidino yellow, FluoroGold, Lipophilic Tracer DiI, Lipophilic Tracer DiO, and/or Horseradish peroxidase, cholera toxin B) will be injected /painted onto a local muscle area. If incisions are made the muscle, subcutaneous layer and skin will be closed in layers using 2-0 and 3-0 absorbable monofilament. Procedure length is ~1-2 hours. The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.
Non-Surgical Procedure Name Laryngeal Imaging	In some animals we will choose to image their vocal folds (larynx). Animals will be anesthetized and a laryngoscope will be placed to lower the epiglottis to view the vocal folds. Then a flexible endoscope with video capabilities will be run along the laryngoscope. Video will be taken on the larynx during breathing, and some animals will alter respiratory state (as described above). Video will be recorded through out the procedure. In some animals we may give buprenorphine IM ~ 1hour before beginning the evaluation.
	PROCEDURE FOR SURVIVAL ANIMALS: Animals typically are fasted the evening prior to surgery (4- 6 hrs). Acetylpromazine will be given prior to anesthetic induction with isoflurane on the day of surgery (30 minute minimum). Atropine and an antibiotic will be given before or after anesthetic induction. Eye ointment will be applied, the animal intubated and an IV line placed. The surgical site will be shaved and prepped with approved cleaning solutions for sterile surgery (e.g. alcohol and chlorhexidine solution 3x) and the animal then moved to the surgery area. Vitals will be monitored every 10-15 minutes (e.g. body temperature, respiration, EtCO2, heart rate). The animal may receive local lidocaine or

Surgical - Survival Major Procedure Name Survival Spinal Cord Injury	bupivacaine injection/s into the neck muscle prior to incision or during closing or into the epidural space following the injury. The spinal column will be exposed by making an incision through the skin and then the muscle overlying the targeted vertebra from C2-T10. A bilateral laminectomy will be performed to expose the underlying spinal level. The dura will be slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. For the hemisection: one half of the spinal cord will severed using iridectomy scissors. For dorsal rhizotomy the dorsal quadrant will be severed with iridectomy scissors. For the medial myelotomy a midline incision will be made to separate the left and right sides of the spinal cord. For ventral quadrant half oc the ventral spinal cord (quadrant) will be disrupted using iridectomy scissors or a small blade. Any fibers left adhering to the ventral or lateral dura may be gently lifted with suction and cut with iridectomy scissors. Durafilm and gelfoam will be placed on top of the dura and the back closed in layers (muscle, subcutaneous (as needed) and skin) using 2-0 and 3-0 absorbable monofilament suture as indicated above. Typical procedure length: 2-3 hours. The animal may be placed on a ventilator to help with respiratory support. Although we have now performed more than 10 cervical SCI in non-survival experiments and no animals required ventilator support. The animal may also get a neuronal tracer injection following the spinal injury (within the same surgical procedure). A strong pain medication (buprenorphine) is used to alleviate any post-operative pain and will be be given close to the end of surgery (after the injury is made as it may affect injury magnitude). Surgery will be performed in a designated surgical room using strict sterile technique. If needed following surgery, due to problems with an IV line or oral intake, fluid (sterile saline, ringers, or like solution) may be given immediately or out to 2 weeks. Body temperature is supported during surgery using recirculating water heating blanket/pads, warm water bottles or packs, warmed blankets and/or IV line warmer, and monitored using either a rectal or esophageal probe/thermometer. Animals typically are recovered in a temperature controlled recovery unit. If one of these is not available, animals are recovered on temperature controlled recirculating water heating pads/Bair Huggers or similar heating system. If body temperature is slow to recover to normal levels, warm blankets/towels are placed in the recovery unit. Animals receive pain medication (buprenorphine) for 48 hrs. If any pain-related behaviors are seen after 48 hrs, buprenorphine may be extended for an additional 24 hrs. Animals are checked a minimum of 3x/day for the first 48 hours post-op and then checked at least 1x daily during the first post-operative week. If the incision line should become compromised due to suture break, over grooming, etc., an antibiotic ointment may be used on the site and an e collar may be used to limit the animal's access to the site. If the

	compromise is significant, warranting re-closure, the edges may be closed using an appropriate medical adhesive (as above) or restitch. The animal may be re-anesthetized with isoflurane (box and then face mask) to use adhesive or re-stitch. If more than a week out from surgery, a general antibiotic may be re-administered. If an exposed suture tail (cut end) is seen at the incision line, it may be clipped level with the skin.
Surgical - Non-Survival Procedure Name Laminectomy	Also for Survival Procedure Removal of bone covering spinal segment of interest.
Surgical - Non-Survival Procedure Name Perfusion	Trans or intra-cardial perfusion may be performed. Initially, animals will be anesthetized with isoflurane or sevoflurane. Once anesthetized, Sodium Pentobarbital (IP, IV or IA) will be given. As needed, animals will be given one or more supplemental doses of sodium pentobarbital (typically 25% of initial dose) to achieve or maintain a deep plane of anesthesia (IA, IP or IV). They will then be given 1 cc of a blood thinner (e.g. 10% heparin, IV) followed ~20 minutes later by a vasodilator (e.g. ~1 cc of 1% sodium nitrite). Immediately following administration of sodium nitrite, they may be perfused transcardially with 0.9% saline typically followed by a fixative (eg. 4% paraformaldehyde and/or 1.25%-4% glutaraldehyde in a buffer solution (pH 7.4)). It is critical that the heart continue beating until the perfusion of fluids has begun in order to prevent collection of blood in the vascular system which interferes with post-mortem histological procedures. This is why Nembutal is the drug of choice and other euthanasia options are not good options as they rapidly stop the heart. By providing a bolus of Nembutal IP after the animal is initially anesthetized, a deep plane of anesthesia is easily maintained and the heart continues beating for a long time which allows completion of the transcardial perfusion steps required. The tissues of interest (including spinal cord, brain, leg bones, muscles, etc) will be removed after perfusion. If the heart is not beating strongly enough, an option may be exercised to introduce the blood thinner and/or vasodilator directly into the left ventricle immediately prior to starting the intracardial perfusion of saline.
Non-Surgical Procedure Name Bladder and Bowel care	The Crede method along with peri-anal stimulation will be used to assist with bladder and bowel emptying as needed. Mineral oil may be given in the food to facilitate bowel emptying. Typically this assistance is required for only 1-5 days post-op. If a animal does not have a sufficient bowel movement within the first 5-10 days post-injury, subcutaneous fluids may be given (typically 100-200cc) or a warm sudsy enema using a Tom cat catheter and large syringe or suppository may be used. Prior

	to enema, up to 200 mls/day of subcutaneous fluids (saline, lactated ringers or like solution) may be given to encourage bowel activity.

Project Participants

Provide name(s) (in order of greatest involvement) of individual(s) participating in or involved with this *Proposal* (experimental procedures, animal observation or care, *etc.*) and describe their role in the proposed study. PI and Co-PIs should also be included. The experience described for each person should match their role in the studies described in this *Proposal*. If not, please indicate how they will obtain sufficient training.

Name	Role (List Specific Procedures to be performed)	Experience
[redacted]	[redacted] will perform all outlined procedures.	see training log
[redacted]	[redacted] my lead and/or assist with spinal cord injuries and survival procedures.	see training log
[redacted]	[redacted] may perform and /or assist will all procedures.	see training log
[redacted]	[redacted] will have a support role and may assist and/or perform all procedures.	see training log
[redacted]	[redacted] may perform and /or assist will all procedures.	see training log
[redacted]	[redacted] may perform and /or assist will all procedures.	see training log
[redacted]	[redacted] may perform and/or assist will all procedures.	see training log

If there are any non-UofL personnel who will be handling animals or performing any procedures as part of this Proposal, please provide their names, the functions they will perform, and the relevant experience /training they have in performing those functions.

Name	Procedures Performed and Experience
No records have been added	

☑ As Principal Investigator (or designee), I attest that the Key Study Personnel selected for this study have, or will obtain, the necessary experience, training, and are proficient, or will be proficient, in performing all of the procedures listed above.

15.5 Multiple Survival Surgery

Definition: Two or more Survival Surgical procedures (major or minor) between which the animal recovers from anesthesia.
No animal may be used in more than one MAJOR operative procedure from which it is allowed to recover, unless: 1) justified for scientific reasons, 2) required as routine veterinary procedure or to protect the

health or well-being of the animal, or 3) other special circumstances as determined by the Administrator, APHIS, USDA.

15.6 The details of each independent surgical procedure should be described as Survival Surgical Procedures in the Procedures table. Provide a scientific justification for performing multiple survival surgical procedures.

Justification should include why the procedures could not be done during the same anesthetic period and any special methods of monitoring the animals for recovery from each procedure, and the minimum time allowed between each procedure.

Procedure Names (use terms used in Procedures and Experimental Groups sections)	Justification for Multiple Procedures	Minimum time allowed between each procedure.
Spay/Ovariohysterectomy Spinal Cord Injury of cervical or thoracic region from C2-T10 Neuronal Tracer Injection	1. All animals will be spayed at UofL or at the animal vendor facility. The cat will be allowed to recover for several weeks prior to any other surgical procedure. This cannot be performed at the same time as other surgeries because of the confounding nature of the spay surgery. 2. Animals will be allowed to recover for several weeks following the hemisection prior to any other surgical procedure. 3. Cats may undergo a surgery ~2 to 150 days prior to their sacrifice to place a neuronal tracer for histological analysis which first includes anesthesia. This may be performed at the same time as the spinal injury in some animals	At least 2 weeks between any surgical procedures
Spay/Ovariohysterectomy Neuronal Tracer Injection	1. All animals will be spayed either at UofL or at the animal vendor facility. The cat will be allowed to recover for several weeks prior to any other surgical procedure. This cannot be performed at the same time as other surgeries because of the confounding nature of the spay surgery. 2. Cats may undergo a surgery to place a neuronal tracer for histological analysis ~2 to 150 days prior to their sacrifice which first includes anesthesia. This cannot be performed at the same time as any other procedure because the neurons which transport the tracer are susceptible to death and thus confound the training component.	At least 2 weeks between any surgical procedures

16.0 Anesthetics, Analgesics, and Other Therapeutic Agents

16.1 List ALL pre-anesthetic, anesthetic, analgesic, tranquilizing agents, surgical support fluids, antibiotics, and other veterinary medical therapeutics to be used (even if their use has been described elsewhere in this *Proposal*). Examples include not only peri-operative drugs, but also such drugs as insulin for diabetic animals, or hot/cold packs.

Analgesics "PRN" or "as needed"

The USDA Research Facility Inspection Guide states that "PRN" or "as needed" frequency of administration is not acceptable unless there are detailed instructions and criteria for determining administration of the drug. Non-pharmacological methods, such as hydrotherapy and hot/cold packs, should also be described. Availability of experienced personnel, especially at night and on weekends, should also be assured in protocol review.

IM = intramuscular, IP = intraperitoneal, IV = intravenous, PO = per os (by mouth), SC = subcutanous

Be sure to save often!

Click "Save and Continue" button at top right of screen. (You will move to the next section. You can return to "Anesthetics, Analgesics, and Other Therepeutics" by clicking the appropriate section on the left. This annoying feature will be fixed in future releases!)

Name and Purpose (check labels)	Dose (mg/kg) and Route (check labels).	Frequency (e.g., twice daily) and Duration (e.g., once, three weeks) - check labels
Name: Sodium Bicarbonate Purpose: Other (describe in box below) Other Use, Explain: Acid-base imbalance	Dose: .2 x kg x base deficit Route: IV Other Route:	Frequency: As need based on base deficit from blood gas measurements. Duration: 1-18 hours (duration of experiment)
Name: Atropine Sulfate Purpose: Other (describe in box below) Other Use, Explain: Suppress mucous secretions and reverse hypotension	Dose: .01-10 mg/kg Route: IV Other Route: IM	Frequency: Before trachestomy and as needed based on blood pressure measurement. Duration: 1-18 hours (duration of experiment); for survival surgeries, this will be given once prior to surgery.
Name: Doxapram HCL Purpose: Other (describe in box below) Other Use, Explain: Cough/swallow suppression	Dose: .001-30mg/Kg Route: IV Other Route: IA, Microinjection, Intravetebral	Frequency: 1-10x per hour Duration: 1-3 hours
Name: Baclofen	Dose: .03-1,000ug/Kg	Frequency

Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Route IV Other Route: IA, Microinjection, Intravetebral	1-10x per hour Duration 1-3 hours
Name Codeine Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Dose .001-30mg/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour Duration 1-3 hours
Name Cobalt Chloride Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour Duration 1-3 hours
Name Insogubacine Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour Duration 1-3 hours
Name Capsaicin Purpose Other (describe in box below) Other Use, Explain Cough/swallow expression	Dose 0.016-50 µM Route IV Other Route: IA, Microinjection, Intravetebral, aerosolized in ethanol and saline and placed in the upper and or lower airways.	Frequency 1-10x per hour Duration 1-3 hours

Name: Citric Acid Purpose: Other (describe in box below) Other Use, Explain: Cough/swallow expression	Dose: 0.125-32% Route: IV Other Route: IA, Microinjection, Intravetebral, aerosolized in ethanol and saline and placed in the upper and or lower airways.	Frequency: 1-10x per hour Duration: 1-3 hours
Name: Substance P Purpose: Other (describe in box below) Other Use, Explain: Cough/swallow modulation	Dose: .03-300ug/Kg Route: IV Other Route: IA, Microinjection, Intravetebral	Frequency: 1-10x per hour Duration: 1-3 hours
Name: Glutamate Purpose: Other (describe in box below) Other Use, Explain: Cough/swallow modulation	Dose: .03-300ug/Kg Route: IV Other Route: IA, Microinjection, Intravetebral	Frequency: 1-10x per hour Duration: 1-3 hours
Name: Allostatin Purpose: Other (describe in box below) Other Use, Explain: Cough/swallow modulation	Dose: .03-300ug/Kg Route: IV Other Route: IA, Microinjection, Intravetebral	Frequency: 1-10x per hour Duration: 1-3 hours
Name: Saporin Purpose: Other (describe in box below)	Dose: .03-300ug/Kg Route: IV	Frequency: 1-10x per hour Duration

Other Use, Explain Cough/swallow modulation	Other Route: IA, Microinjection, Intravetebral	1-3 hours
Name Lactated Ringers Solution +/- 5% sucrose Purpose Physiological Fluid Other Use, Explain	Dose 5-10ml/kg/hr Route IV Other Route: IA	Frequency As needed based on the animals physiology Duration 1-18 hours (duration of experiment)
Name Normal Saline Purpose Physiological Fluid Other Use, Explain	Dose 5-10ml/kg/hr Route IV Other Route: IA	Frequency As needed based on the animals physiology for the non-survival surgery. For survival surgery this will be delivered through IV and post-surgery if line is unaffected. Duration 1-18 hours (duration of experiment)
Name Heparin Pork Purpose Physiological Fluid Other Use, Explain	Dose 1MU/ml Route IV Other Route: IA	Frequency As needed based on the animals physiology Duration 1-18 hours (duration of experiment)
Name Artificial Cerebrospinal Fluid Purpose Physiological Fluid Other Use, Explain used mainly for CNS microinjection solutions	Dose .01-1ml Route Other Other Route: CNS microinjection, intra-vetebral artery	Frequency 1-10x per hour Duration 1-3 hours
Name	Dose	Frequency As needed to maintain stable anesthetic depth Duration

Sodium Pentobarbital Purpose Anesthetic Other Use, Explain	35mg/kg Route IV Other Route:	1-18 hours (duration of experiment) for survival surgeries. Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose will always be given.
Name Sevoflurane Purpose Anesthetic Other Use, Explain	Dose .1-5% Route Inhalation Other Route:	Frequency As needed to maintain stable anesthetic depth Duration 1-18 hours (duration of experiment)
Name Isoflurane Purpose Anesthetic Other Use, Explain	Dose .1-5% Route Inhalation Other Route:	Frequency As needed to maintain stable anesthetic depth Duration 1-18 hours (duration of experiment); or until another anesthetic is introduced or euthanasia
Name Beuthanasia aka Euthanasia III Solution Purpose Other (describe in box below) Other Use, Explain Euthanasia	Dose 1 ml / 4.5 Kg Route IV Other Route:	Frequency 1 dose Duration 1x per procedure
Name Saturated KCL Purpose Other (describe in box below) Other Use, Explain Euthanasia	Dose 3 ccs Route IV Other Route:	Frequency 1 dose Duration 1x per procedure

Name: Lidocaine Purpose: Analgesic Other Use, Explain:	Dose: 2-4 mg/kg .03-300ug/Kg (microinjection) Route: IM Other Route: Topical, subcutaneous, CNS microinjection, into the epidural space	Frequency: When needed to maintain noxious stimulus response while under anesthesia Duration: till reduction of stimuli and/or pain
Name: Cefazolin Sodium Purpose: Antibiotic Other Use, Explain:	Dose: 22 mg/kg Route: IM Other Route:	Frequency: 1 dose Duration: n/a
Name: Amoxicilin combined with clavulanate acid (i.e. Clavamox) Purpose: Antibiotic Other Use, Explain:	Dose: 12.5-25 mg/kg Route: PO Other Route:	Frequency: BID Duration: 7 days
Name: Laxatone Purpose: Other (describe in box below) Other Use, Explain: stool softener	Dose: 1/2-1 tsp Route: PO Other Route:	Frequency: during meal time Duration: until evidence of constipation resolves
Name: Mineral Oil Purpose: Other (describe in box below) Other Use, Explain: stool softener	Dose: 1ts-1tbsp Route: PO Other Route:	Frequency: during meal time Duration: until evidence of constipation resolves

Name Acetylpromazine acepromazine) Purpose Pre-anesthetic Other Use, Explain	Dose 0.01 - 0.05 mg/kg Route IM Other Route:	Frequency 1x Duration at least 30 minutes prior to survival surgery
Name Acetic Acid (vinegar) Purpose Other (describe in box below) Other Use, Explain cough/swallow regulation	Dose 1-5% Route Topical Other Route: Topical on mucosa in the upper and/or lower airway	Frequency 1-10x an hour Duration 1-2 hours
Name Insogubacine Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour Duration 1-3 hours
Name Kynurenic Acid Purpose Other (describe in box below) Other Use, Explain Cough/swallow suppression	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour Duration 1-3 hours
Name 8-OH-DPAT Purpose Other (describe in box below) Other Use, Explain Cough/swallow modulation	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour; or via infusion to maintain a steady concentration Duration 1-18 hours (e.g. duration of experiment)

Name WAY-100635 Purpose Other (describe in box below) Other Use, Explain Cough/swallow modulation	Dose .03-300ug/Kg Route IV Other Route: IA, Microinjection, Intravetebral	Frequency 1-10x per hour; or via infusion to maintain a steady concentration Duration 1-18 hours (duration of experiment)
Name Thrombin (bovine) Purpose Other (describe in box below) Other Use, Explain Coagulate blood	Dose 10-1000 units/mL Route Other Other Route: Applied to surface of skin, muscle or bone	Frequency To control bleeding when cauterization is not an option. Duration Applied until bleeding is controlled.
Name Buprenorphine Purpose Analgesic Other Use, Explain Also may be combined with surgical pre-anesthetics	Dose 0.01-0.05 mg/kg Route IM Other Route: SC	Frequency 2-4x/day for at least 48 hours post-surgical procedures. Dosing may be more frequent during first 24 hrs, dependent upon time of surgery, if needed to assure adequate overnight pain relief coverage. Dosing also may be more frequent across the entire 48 hours or dose increased if any pain behaviors are noted. These can include, but are not limited to, panting, freezing posture, hiding, flinching when approached by a known handler, lethargy, vocalizations or growling and hissing from an animal that typically does not show these types of sounds/behaviors. Attention is always given to timing of doses to assure drugs will not wear off during the night. With respect to the SCI surgeries, the first dose is timed to occur after the experimental injury is made but at least 30 minutes prior to discontinuation of anesthesia. With respect to spays and and neuters, buprenorphine may be given as a pre-sedative. If that is done, and buprenorphine also will be used as the post-operative analgesia

		medication, the first post-surgical dose will be appropriately timed relative to the pre-sedative dose. Duration 48 hours post-surgery, if used as a post-op analgesic. 8-12 hours, if used only as a pre-sedative. For Pilot Study: buprenorphine IM at a dose of 0.02 mg/kg or 0.03-0.04 mg/kg ~q12 hours for ~48 hours. May be repeated after 1 week with the lower /higher dose.
Name Convenia Purpose Antibiotic Other Use, Explain	Dose 8 mg/kg Route SC Other Route:	Frequency 1x Duration 14 days
Name Buspirone Purpose Other (describe in box below) Other Use, Explain Treatment of feeding disorder	Dose 1-10mg Route PO Other Route:	Frequency Up to 3 doses per day (i.e. 1hr before feeding or assessment) Duration 1 dose- entire period following spinal cord injury.
Name Mineral Oil Purpose Other (describe in box below) Other Use, Explain bowel evacuation	Dose 1 tsp- 1 Tbsp Route PO Other Route:	Frequency Mean time Duration until evidence of constipation resolution
Name Bitter apple spray Purpose Other (describe in box below) Other Use, Explain taste deterrent	Dose 1-2 sprays Route Topical Other Route:	Frequency Up to several times per day Duration Until evidence of excess grooming subsides.

Name Cefazolin Sodium Purpose Antibiotic Other Use, Explain Recommended for surgical prophylaxis in orthopedic or soft tissue procedures.	Dose 20-22 mg/kg Route IV Other Route:	Frequency Just before initial incision and repeated every 90-120 minutes Duration until incision closure
Name Glycopyrrolate Purpose Pre-anesthetic Other Use, Explain or intraoperative treatment	Dose 0.01 mg/kg Route IM Other Route: IV	Frequency 1x Duration at least 30 minutes prior to survival surgery; or as a vagolytic for intra-operative bradyarrhythmias
Name bupivacaine Purpose Analgesic Other Use, Explain	Dose 1-2 mg/kg Route IM Other Route:	Frequency 1x Duration 4-6 hours
Name mepivacaine HCL 2% Purpose Analgesic Other Use, Explain	Dose 0.5-2 mg/kg Route IM Other Route:	Frequency 1x Duration 2-2.5 hours
Name Polyethylene glycol 3350 powder (i.e. Miralax) Purpose Other (describe in box below) Other Use, Explain stool softener	Dose 1/8 - 1/4 teaspoon Route PO Other Route:	Frequency PRN Duration until evidence of constipation resolves

In the text box below, provide additional information on the use of the agents listed above. Examples may include use of certain analgesics pre-emptively, clarifying different anesthetic regimens for specific procedures, anesthetics used in combination, decision making process used to determine frequency or duration, etc.

Terminal Experiments: Sevoflurane or Isoflurane will initially be administered through box-induction then transitioned into mask inhalation after loss of consciousness. The animals will then be weaned onto sodium pentobarbital through a forelimb radial vein intravenous injection. Anesthetic doses and (supplemental doses as needed) will be given through the femoral IV catheter. Atropine sulfate will be given as needed to maintain mucous secretion. Decrease in total blood pressure or a reduced pulse pressure (from outside the normal physiological range) will prompt the use of lactated ringers solution into the femoral IV line. Arterial blood gas showing a metabolic acidosis (secondary to surgery, etc) will be treated with administration of sodium bicarbonate through the femoral IV line. Heparin pork will be placed in saline to clean the femoral IA line after blood collection for blood gas measurement. Lidocaine will be used before placement of the ear bars only 1 time during the experiment. Doxapram HCL, Baclofen, Kynurenic Acid, Isogubacine, Lidocaine, Cobalt Chloride, Substance P, Glutamate, Allostatin, Saporin, capsaicin, citric acid, acetic acid, and Codeine will be used as needed to suppress cough and/or swallow depending on the experimental protocol. Barium Sulfate will be used during the videofluoroscopic evaluation of swallow. It will be dissolved into a "meat-based" liquid (tuna, oysters, wet cat food, etc). It will be presented to the animal at a constant rate over a pressure transducer. This will allow for lick rate, frequency, and movement of the bolus to be recorded. The total time will be under <20 minutes. Survival Surgeries: Cefazolin Sodium will be delivered IV just before initial incision and reported every 90-120 minutes until incision closure. In addition to opioid administration the animal will receive local lidocaine, bupivacaine, or mepivacaine injection into the neck muscle prior to incision or closing or into the epidural space for pain management of the incision site. The local anesthetic will be given within the time period to obtain peak-plasma concentration of opioid administration. Amoxicillin combined with clavulanate acid (i.e. Clavamox) (12.5-25 mg/pound, po, bid) will be given for 7 days if a cat should present with blood in its urine. If blood persists or a persistent infection develops, additional treatment will be determined in consultation with a veterinarian. Laxatone and/or a supplement (such as mineral oil or Polyethylene glycol 3350 (i.e. Miralax)) may be added to the diet to encourage defecation. Acepromazine will be used as a pre-op anesthetic at least 30 minutes prior to surgery for 1 dose for the spinal cord injury surgeries. For the spay surgeries it will be combined with buprenorphine. Buspirone (BusPar) is an FDA approved medication commonly used in veterinary medicine for inappropriate urination. Its therapeutic effectiveness begins 1 hour after drug administration. It may be used to treat the swallow/laryngeal deficits after spinal cord injury up to 3 doses a day. It is easily crushed and placed in cat food for administration. Opioids are known to impact upper airway function, IM injection of buprenorphine reaches therapeutic effectiveness at ~1 hour. It may be used to track the effect of opioid administration pre and post SCI on laryngeal and swallow function as determined on the videofluoroscopy and laryngeal imaging. If the animal is hypoglycemic, LRS +/- 5% sucrose will be used to bring glucose back in range.

17.0 Anesthesia and Anesthetic Monitoring

17.1 Will animals be anesthetized for any reason OTHER THAN Euthanasia?

(•) Yes ( ) No

17.2 Animal Preparation for Anesthesia Select those that apply

- [x] Observation for normal behavior
- [ ] Pre-anesthetic diagnostics (e.g., hematology, serum blood chemistry panel)
- [ ] Overnight fasting (NON-RODENT MAMMALS ONLY).
- [x] Use of sedatives (describe in Anesthetics, Analgesics, and Other Therapeutics table).
- [x] Placement of non-medicated ophthalmic ointment in eyes.
- [x] Other (describe below):

Outline additional plans for pre-anesthetic evaluation or other animal preparation methods in the space below.

Food will be withheld the morning of the procedure(s) (4-6 hrs) and the animals fed after the procedure (s) later in the day.

17.3

Monitoring Anesthetic Depth Select those that apply

- [x] Body temperature measurement and support using temperature-measuring probe.
- [x] Use of intra-procedural fluids (describe in Anesthetics, Analgesics, and Other Therapeutics table).
- [x] Anesthetic depth checked at intervals no less than 15 minutes (describe other intervals below).
- [x] Anesthetic depth verified by withdrawal reflex (toe/tail pinch).
- [x] Other methods of anesthetic monitoring and animal support during anesthesia and/or surgery:

Provide additional information if needed.

Blood gas measurements may be taken every 60-90 minutes. Additionally if there is a question as to anesthetic level, a blood gas measurement will be performed for confirmation.

18.0 Surgical Preparation and Support

18.1 Will animals undergo surgical procedures?

(•) Yes ( ) No

18.2 Animal Preparation

- [x] Removal of hair from the incision site with clippers (describe use of razors or depilatories in the space below).
- [x] Preparation of surgical site with chlorhexidine or providone-iodine followed by a rinse with sterile water, saline, or alcohol; repeated at least 3 times.
- [ ] Other animal preparation procedures not describe above or in the "Anesthesia and Anesthetic Monitoring" section.

n/a

18.3 Aseptic Surgical Technique Select those that apply *Note*: survival surgery on ALL species must be performed using aseptic procedures. Surgical procedures on non-rodent mammals must be conducted in RRF-managed facilities intended for that purpose. [IACUC Policy]

- [x] Sterilize instruments via autoclave and/or ethylene oxide.
- [ ] Sterilize instruments via chemical (cold) sterilization (describe below).
- [x] Use of separate instruments between serial surgeries.
- [ ] Sterilization of instrument tips between surgeries using hot bead sterilizer.
- [ ] Sterilization of instrument tips between surgeries using chemical (cold) sterilants (describe below).
- [x] Use of sterile gloves.
- [ ] Use of tips-only technique.
- [x] Use of sterile drapes.
- [x] At least two-layer closure, using absorbable material to close muscle fascia and/or subcutaneous tissue.
- [x] Use of monofilament non-absorbable suture material or surgical staples (wound clips) for the skin incision.
- [x] Other (describe below):

Outline additional/alternative methods used (e.g., for non-survival surgery) or for maintaining asepsis.

There will not be any sutures that require removal.

18.4 Post Procedural Monitoring and Care Select all that apply

- [ ] Not applicable, animals will not recover from anesthesia
- [x] Constant monitoring of animals until fully ambulatory.
- [x] Complete surgical records, including anesthetic monitoring.

- ☐ Mark cage cards with date of surgery (rodents).
- ☑ Daily observation at least 7-14 days.
- ☐ Skin incision closure (sutures or wound clips) removal at 7-14 days (otherwise describe below).
- ☑ Continue and record post-operative care, including use of analgesics and daily observations.
- ☑ Use of postoperative fluids, antibiotics, or other therapeutics (described in Anesthetics, Analgesics, and Other Therapeutics table).
- ☑ Analgesics (described in Anesthetics, Analgesics, and Other Therapeutics table) will be provided for at least 48 hours. Criteria used to determine that additional analgesic use beyond 48 hours is described below.
- ☐ Non-pharmacological analgesia (e.g., cold packs, water baths) will be used (describe below).
- ☐ No analgesics will be used (provide justification for withholding analgesia below).
- ☐ Other (describe below):

Additional Post-Surgical Care.

Briefly describe additional post-procedural care in the space below. DO NOT list specific drug doses; they should be provided in the Anesthetics, Analgesics, and Other Therapeutics table.

All surgical procedures are performed under general anesthesia and a strong pain medication is used to alleviate post-operative pain. Surgery will be performed in a designated surgical room using strict sterile technique. If needed following surgery, due to problems with an IV line or intake, ~50 cc of fluid (sterile saline, ringers, or like solution) will be given SQ. If this is quickly absorbed, another ~50 cc will be given to prevent dehydration as a result of fluids lost and/or not taken in during, or acutely following, surgery. During the acute post-operative period and anytime that there is an acute health concern, animals will be monitored and provided with nursing care a minimum of 2-3x daily. Particularly following SCI, this may include manual bladder expression (accomplished by applying gentle, but firm pressure to the bladder through the abdominal wall - Crede method, as well as through peri-anal stimulation). Typically bladder function begins to recover within 2-4 days following the spinal procedures outlined in this protocol. If an animal should present with blood in its urine, Amoxicillin combined with clavulanate acid (i.e. Clavamox) will be given for 7 days. If blood persists or a persistent infection develops, additional treatment will be determined in consultation with a veterinarian. Constipation may be a result of almost any surgical procedure due to anesthetics and analgesics. Thus, animals also will be monitored for bowel movements and their colons palpated for retained feces daily during the early post-op period. Laxatone and/or a supplement (such as mineral oil) may be added to the diet to encourage defecation. Fiber supplements, like Metamucil, are avoided due to the animal's relatively low water intake and the potential for binding. If feces are not produced within a few days and the colon is very firm with feces, a warm water sudsy enema will be used. This is done using a lubricated Tom cat catheter and a 50-150 ml syringe filled with warm (~body temp) water and a very small amount of gentle soap (>1%, e.g. Dove). The catheter is inserted into the anus up to several inches and the solution gently pushed in until there is reflux. This usually produces results within minutes. However, as a result of lower intake post-surgery and sluggish bowels due to anesthesia and drugs, an animal may be allowed to go ~7-10 days post-surgery without a bowel movement if - upon palpation the bowel does not seem to be filled with hard stool, there is no pain with palpation and the animal seems to be generally active. This approach also would prevent us from giving an enema to an animal that had an undocumented bowel movement – meaning that someone cleaned the cage/litter pan and did not know, or forgot, to document the stool in the chart. Once the first bowel movement is produced, animals typically defecate effectively on their own. If problems persist, the veterinarian will be consulted. Bowel movements also may be facilitated by fluid intake. Thus, if there is any evidence of dehydration, animals may receive up to ~200 cc of warmed (body temp) subcutaneous fluids/day which also may facilitate a bowel movement as well as enhance hydration. Following the acute post-op period, animals will be checked on at least once a day to observe their general health (including alertness, movement, and skin integrity). In our experience, animals have tolerated incomplete lesions well and do not usually present with persistent health problems. All animals will be given analgesia for at least the first 48 hours post-op. Rarely will an animal present with pain after 48 hours when pain medication is discontinued. Signs of distress/pain may be indicated by excessive vocalization, struggling when handled, guarding, lethargy, hiding, abnormal grooming, poor appetite or a change in interaction style. If the animal shows any of these or other signs of discomfort or pain as/after analgesia is being/has been discontinued, analgesia will be extended for an additional 24 hours. If pain does not resolve, treatment will be discussed and determined in consultation with a veterinarian. Because sensation is partially disrupted below the lesion, animals are housed on thick soft bedding to prevent skin ulcers or peripheral nerve compression. This same sort of bedding also is used in the carrying cages which transport them between the housing and behavior room. Animals may lose weight following spinal cord injury or become plump post-injury, particularly if un-exercised. Weights and diets will be closely monitored and diet adjusted to maintain the health of the animals throughout the study. Although we have never had to euthanize an animal due to low weight or body condition score, if an animal were to fall below a 4 (5 is optimal) and did not respond to changes in diet, it would be considered for, and/or the RRC veterinarian consulted regarding the choice to move to, euthanasia. If an animal over-grooms an area causing prolonged irritation, a commercially available substance (e.g. bitter apple) that is safe for consumption but has a bad taste will be used on the area. If necessary, a special collar (eg. cone collar),

which will not allow the animal to access the site, will be used until the area is sufficiently healed. If needed a topical antibiotic ointment (e.g. Neosporin, triple antibiotic) or a liquid bandage will be used on the site. Occasionally we have normal and injured animals that will temporarily over-groom – this is not correlated with the spinal cord injury model used. Occasionally an animal will rub its distal limb consistently on a runway and create a small abrasion. If this occurs, the site will be cleaned, an antibiotic ointment applied and an adhesive bandage may be applied. Post-SCI, laminectomy sites must not be touched or palpated due to potential to cause unintended spinal damage. Animals also must be handled carefully and appropriately so that they may not twist, bump or move in any manner that may cause them unintended damage. A standard scruff hold should not be used. For the first few weeks post-SCI, laboratory personnel (including PI) readily will be available for moving/holding animals as needed for cage changing, veterinary assessment, bowel/bladder care (see above), etc, as well as to train any animal care staff in appropriate methods to handle cats with SCIs. All lab and veterinary staff will monitor the animals after surgery and written notes on the condition and overall progress of recovery will be made in the medical charts in order to keep an accurate and detailed description of the recovery progress of the animals and what treatments they are receiving. In some instances, integrity of the incision line may be lost and the skin partially re-open. Events associated with incision line re-opening are over grooming by the animal or its cage mate, bumping or rubbing of the incision site or surfacing (superficial emergence) of suture material. If this happens, the opening is typically small and will be treated through use of a topical antibiotic ointment, possible re-closure with an approved adhesive/suture (atypical) and/or use of an E collar to protect, prevent infection and promote healing. It should be noted that animals may independently move around the cage post-SCI without showing traditional ambulatory function. This is typical during the early acute period. Animals will be constantly/closely monitored post-op until body temperature is normal, they are able to independently assume a prone posture, sit up, move around, and are oriented. To briefly summarize, the potential post-op complications we will watch for are: constipation, interrupted bladder control, bladder infection, distress/pain, skin and peripheral nerve integrity, loss of incision line integrity and weight loss/gain.

18.5 Required Clinical Records

- ☐ Not Applicable: Protocol does not involve surgery.
- ☑ Individual animal health records will contain documentation of peri-operative surgical support, surgical procedures performed, anesthetic monitoring, post-operative monitoring and support (required for non-rodent mammalian species).
- ☑ Template peri-operative surgical records provided by RRF veterinary personnel will be used.
- ☑ Laboratory records will contain documentation of peri-operative surgical support, surgical procedures performed, anesthetic monitoring, post-operative monitoring and support.
- ☐ Other (describe below):

19.0 Privately-Owned Animals

19.1 Privately-Owned Animals

Does this Proposal include the use of any privately-owned animals?

○ Yes ◉ No

20.0 Non-Standard Housing, Food and Water (or Other Special Considerations)

20.1 Indicate which of the following, if any, pertain to this proposal

- ☑ Animals require special housing conditions (e.g., individual housing, special caging).
- ☑ Animals receive special food
- ☐ Animals receive special drinking water
- ☑ Animals will experience food or drinking water restriction or regulation
- ☑ Use of non-sterile or expired medical materials (disposable surgical supplies)
- ☐ Animals will be physically restrained for prolonged periods of time. Brief manual restraint for the purpose of performing routine clinical or experimental procedures (< 15 minutes for rodents, <30 minutes other mammals) need not be described unless the procedures will cause pain or distress.

20.2 **Special Housing** **Please provide a description and justification of special (non-standard) housing conditions below.**
NOTES: Individual housing: Description should address steps to compensate for isolated housing (e.g., periodic contact, visual/olfactory contact, human interaction, additional environmental enrichment.) Space Exception: Justification should include references/methods /performance criteria used to verify adequacy of space. Acclimation Period: Other than euthanasia and tissue harvest, IACUC Policy dictates acclimation periods of 72 hours for rodents, 72 hours for non-survival surgery or other procedures in non-rodents (7 days recommended), and 7 days for survival surgery or long-term experiments in non-rodents. Exceptions to these must be described in the table below.

Exception	Brief Description and Justification	Duration
Individual housing (see Note above)	Cats may be housed separately after surgery to monitor food intake,bowel and urine output as well as prevent cats from grooming incision lines of other cats. During this period the animals will be frequently handled, may be taken to the behavior room and may socialize with other cats when monitored.	Up to 7 days
Other Exceptions to Housing Policies (provide details)	Following spinal cord injury, the cats must be housed on a thick bedding of shredded newspaper (6-8") or thick foam (i.e. 4" egg crate or memory foam). This bedding must be maintained for the rest of the study to prevent skin ulcers and peripheral nerve compression. If sensory testing of the hindlimbs by the P.I. should show that sensation has 'fully' recovered (due to a treatment) then consideration may be given to reducing or removing the padding. Resting boards must be removed permanently from the cages of spinal cord injured cats. This must be done to prevent cats from 1) rubbing their incision sites which could further damage their spinal cords; 2) from jumping or rolling off of them and potentially hurting themselves; and 3) lying /sitting for extended periods on a the hard board surface on a sensory compromised limb/hindquarters which is likely to cause skin breakdown and/or nerve compression. Small litter pans must be used in the cages of spinal cord injured animals. These pans must be small enough to prevent the animal from laying in the pan. Like resting boards (above), the hard pan and litter can cause potential	Duration of the study post-SCI

	peripheral nerve compression and/or skin breakdown.	

20.3 Special Diets **Use of feeds beyond manufacturer-recommending expiration date should be included. Note: drugs, chemical agents, and other test articles also should be included in the Chemical Agents section later.**

Name/Manufacturer	Justification/Description
Diet Name a/d canine/feline critical care diet Manufacturer Hills	Justification for Diet This prescription diet may be mixed with other food during the first week post-surgery. This diet is typically used if a cat is not eating well or is at risk for losing significant weight. This food is designed to deal with the significant metabolic changes that may occur following surgery and support maintenance of lean body mass. This diet is particularly beneficial post-surgery for small cats or cats that don't eat well. If Hills a/d diet is not available then a comparable diet may be used (i.e. Royal Canin Recovery or Purina Pro Plan Veterinary CN, etc) If diet doesn't meet the nutritional needs for this species, provide a description of how the animals will otherwise be supported.

20.5 Food or Fluid Restriction or Regulation **Note: This section does NOT include a pre-surgical fast of less than 12 hours for rodents or overnight for other species. This DOES include fasting prior to blood or other tissue collection. Monitoring: Animal health monitoring procedures and frequency (physiologic or behavioral indices, including criteria for temporary or permanent removal of an animal from the experimental protocol (e.g., body weight, BUN, urine /fecal output, food/fluid consumption).**

Type and Duration	Justification/Monitoring/Assurance
	Justify the need for the restriction/regulation Upon entering the study, each cat will be evaluated using body condition score and weight. During training and at all points post-injury, cats will be weighed weekly to monitor weight. In addition to weekly weight, a body condition scoring chart will be used to monitor overall general health. An optimal body condition of the cats will have the ribs, lumbar vertebrae, pelvic bones and other bony structures easily palpable with slight fat cover, concave abdominal tuck, smooth hourglass shape to waist, abdominal fat pad minimal (for example see Purina body condition chart below). Post-injury, due to some muscle atrophy that occurs below the level of the spinal injury, condition scores will rely more heavily on assessment of the body areas above the lesion level. This injury-related atrophy, however, is relatively minimal due to lesion type (thoracic hemisection) and not sufficient to prevent voluntary movements associated with those muscles. The amount of food an animal receives daily will be based on the cat's

body condition score (scale of 1-9; Purina scale), weight gain/loss, and energy requirements. The goal is to provide the average caloric intake needed to meet an adult cat's requirements. The approach that will be used to determine each cat's caloric intake needs be based upon feline dietary energy requirements (RER). For example, adult spayed cats require a typical caloric (kcal) average of 1.2 x RER/day. If a cat is overweight (body condition score of >5), the typical calculation changes to 0.8 x RER. If a cat is underweight (body condition score >5) and does not begin to adjust towards normal (5) after a week of increased food; we will try different/additional palatable food types and/or contact an RRS veterinarian for input. If we have a cat that does not seem to care much for the general cat food used, we also will present this cat with additional options. Thus, although access to food will have a time and location restriction during training, the quantity (aka caloric value) provided will be appropriate for each adult cat and thus is not restricted. We have successfully used this approach for over 8 years on both NIH and VA funded studies. Although we have never had to euthanize a cat due to low weight or body condition score, if an animal were to fall below a 4/4+ and did not respond to changes in diet, it would be considered for, and/or the RRC veterinarian consulted regarding the choice to move to, euthanasia. Additionally, for the non-survival surgeries over-weight animals have more difficulty maintaining homeostasis and stable anesthetic levels- this leads to data of a lower quality, and may mean the animals has to be excluded from data analysis. The overall health of the animals is critical to understand the effects of the intervention appropriately.

Monitoring (see above for description)

Nestle PURINA body condition score daily to weekly.

Method of assuring adequate nutrition and hydration

Water will be available to cats at all times in their cages(ad lib). The amount of food an animal receives daily will be based on the cat's body condition, weight gain/loss, and energy requirements. The goal is to provide the average caloric intake needed to meet an adult cat's requirements. The approach that will be used to determine each cat's caloric intake needs will be based upon feline dietary resting energy requirements (RER). RER is calculated using either of the following 2 equation - although the 2nd one typically over estimates: RER in kcal/day = 70(BW) to the 0.75 power RER in kcal/day = 30 x (BW) + 70 BW = body weight in kg. For example, adult spayed cats require a typical caloric (kcal) average of 1.2 x RER/day. If a cat is overweight, the typical calculation changes to 0.8 x RER. Thus, although access to food will have a time restriction, the quantity (aka

Type of restriction or regulation

Food restriction/regulation

Duration

while in RRF

caloric value) provided will be appropriate for an adult cat and thus is not restricted. Further, if we have an underweight cat whose body condition does not begin to adjust towards normal after a week of increased food, we will try different/additional palatable and higher caloric/protein heavy food types and/or contact a veterinarian. If we have a cat that does not seem to care much for the general cat food used, we also will present this cat with additional options. The body condition score is more effective than weight monitoring in promoting a healthy body weight for each animal. An optimal body condition score (5) indicates the ribs, lumbar vertebrae, pelvic bones and other bony structures are easily palpable with a slight fat cover, concave abdominal tuck, smooth hourglass shape to waist, and a minimal abdominal fat pad (for examples see Purina body condition chart, http://placervillevet.com/feline%20body%20condition.htm). We use the 9 point Purina scale (vs 5 pt) for greater accuracy. Images can can be found at http://www.purina.com/cats/health/BodyCondition.aspx?print=1. Unfortunately the pictures would not import into this document. For cats that arrive at the facility with a body condition score of 5, a target and floor weight will be established following the general rule of no more than a 15% weight loss. For those cats that come in below or above a 5, initial target and floor weights will identified and re-evaluated weekly as cats move towards the body condition score of 5. Once a body condition score of 5 is achieved, the 15% weight loss criteria will be used as a floor weight. Body Condition Scores with their Descriptions are as follows: Too Thin- 1 Ribs visible on shorthaired cats; no palpable fat; severe abdominal tuck; lumbar vertebrae and wings of ilia easily palpated. 2 Ribs easily visible on shorthaired cats; lumbar vertebrae obvious with minimal muscle mass; pronounced abdominal tuck; no palpable fat. 3 Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind ribs; minimal abdominal fat. 4 Ribs palpable with minimal fat covering; noticeable waist behind ribs; slight abdominal tuck; abdominal fat pad absent. Ideal - 5 Well-proportioned; observe waist behind ribs; ribs palpable with slight fat covering; abdominal fat pad minimal. Too Heavy- 6 Ribs palpable with slight excess fat covering; waist and abdominal fat pad distinguishable but not obvious; abdominal tuck absent. 7 Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of abdomen; moderate abdominal fat pad. 8 Ribs not palpable with excess fat covering; waist absent; obvious rounding of abdomen with prominent abdominal fat pad; fat deposits present over lumbar area. 9 Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits.

20.6 Non-Sterile or Expired Medical Materials (disposable surgical supplies)

Describe the non-sterile or expired materials that will be used, and provide a justification for why they will be needed.

Non-sterile and expired medical materials will only be used in the non-survival procedures. E.g. gloves, gauze, cotton swabs, syringes, needles, suture, and instruments. After 60 minutes anything not aseptic can cause shifts in CBC's etc. and could potentially alter data when going for hours.

21.0 Collaborating Institutions

21.1 Select all that apply:

- ☐ This project involves the use of animals at another institution. Example: A colleague at another institution performs a procedure on animals that have been or will be used in a study at UofL.
- ☐ This project has contracted the production of custom monoclonal or polyclonal antibodies at a company or another institution. Custom polyclonal and monoclonal antibodies are those produced either from antigen provided by the contracting investigator ("custom" antibodies) or through the generation of a specific polypeptide that is then used to immunize animals to produce antibodies.
- ☐ This project is funded through a subaward, subgrant, or subcontract from another institution to perform some or all of the animal work described within this Proposal.
- ☑ None of the above.

Note: this section does **not** include collaborations in which you import a new strain from a collaborator.

22.0 Biological Agents

22.1 Indicate which Types of Biological Agents that will be administerd to animals. - *Examples: Mammalian cell lines; bacteria; other microbes; viruses; materials of human or non-human primate origin (e.g. antibodies etc.); toxins of biological origin (e.g., Complete Freund's Adjuvant, pertussis toxin).* These tables will be reviewed by the Biological Safety Office to determine the need for IBC Registration and/or applicable SASPs. - Select ALL that Apply

- ☐ Not Applicable: No Biological materials will be used in live animals
- ☑ Microbial Agents or Parasites (bacteria, viruses, protozoa, etc.)
- ☐ Cells or Tissues (cell lines, primary tisues or cells, etc.)
- ☐ Other Biological Material (antibodies, rDNA, toxins of biological origin such as Complete Freund's Adjuvant, pertussis toxin, etc.)

22.3 Microbiological Agents or Parasites (bacteria, viruses, protozoa, etc.) Include viral vectors, fungi, and parasites.

Name and ABSL	Desciption of organism and use	Select Agent
Name: Cholera Toxin Subunit B Animal Biosfatey Level N/A	Description of Organism: Cholera Toxin Subunit B is a non-toxic compound naturally derived from Vibrio cholerae; in research, however, a recombinant version is used to ensure purity. This also ensures that it is free of the toxic Subunit A. Describe use, (and note if the recipient is a Genetically-Modified Strain).	○ CDC ○ USDA ◉ Not Applicable

	Used as a neuronal tract tracer in a variety of applications, including tracing of rat forebrain afferents, projections of the parabrachial region, and neurons of the urinary bladder wall.	

22.5 **Testing of Biological Agents** NOTE: Policy, "Testing of Cell Lines and Other Biological Materials for Rodent Pathogens." **Are any of the above agents of rodent origin or may have been passaged through rodents?**

○ Yes ◉ No

23.0 Chemical Agents

23.1 Will animals be exposed *in vivo* to ANY other chemical agent not included in the "Anesthetics, Analgesics, and Other Therapeutic Agents" table above? This includes investigational drugs, test articles, or any other chemical agent introduced into the animal other than biological or radiological materials.

◉ Yes ○ No

23.2 List all Chemical Agents administered to animals that were not included in the Anesthetics, Analgesics, and Other Therapeutics table. Include all non-biological and non-radiological agents, regardless of the method of delivery (injection, food or water, inhalation, etc.). - Please note that biologically-derived toxins (e.g., pertussis toxin, CFA) and/or biohazardous agents (biologically-derived agents, and other agents that require IBC review) must be listed in the Biological Agents table to avoid delays in the review process. - A description of laboratory procedures involving the preparation of these chemical agents must be included in your laboratory's Chemical Hygiene Plan.

Uses may include test articles, agents used to induce disease (e.g., cancer), and investigational drugs.

Agent	Administration	Description
Name: 1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI) CAS Number 223769-64-0 ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use) Dose (mg/kg) 1-3 µls of 0.5-5% soln if injected. ~25 µls if gel foam application is used. Concentration (mg/mL) of the chemical in solution 0.5-5% soln Approximate Volume (mLs) 1-3 µls Frequency (e.g.twice daily) once	Duration (e.g once, every day for 3 weeks etc. once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps.
Name: Biotinylated Dextran Amine	Route	

(BDA) CAS Number No cas number ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Other (include in description of use) Dose (mg/kg) 0.25 -3uls Concentration (mg/mL) of the chemical in solution 1-5% solution Approximate Volume (mLs) 0,25 -3uls Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps
Name: Horseradish peroxidase (unconjugated or conjugated to wheat germ agglutin) CAS Number No CAS number ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use) Dose (mg/kg) ~ 2% solution Concentration (mg/mL) of the chemical in solution ~ 2% solution Approximate Volume (mLs) 1-10 µls Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps.
Name: Fast Blue CAS Number No CAS number ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous	Route Other (include in description of use) Dose (mg/kg) ~2% solution Concentration (mg/mL) of the chemical in solution ~2% solution Approximate Volume (mLs) 1-10 µls Frequency (e.g.twice daily)	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps

☐ Other (include in description of use)	Once	
Name: Diamindino Yellow (DiY) CAS Number 223769-64-0 ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use) Dose (mg/kg) ~3% solution Concentration (mg/mL) of the chemical in solution ~3% solution Approximate Volume (mLs) 1-10 µls Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps
Name: Sodium Nitrite CAS Number 7632-00-0 ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☐ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use) Dose (mg/kg) .01% Concentration (mg/mL) of the chemical in solution .01% Approximate Volume (mLs) 1ml Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Used during perfusion.
Name: Paraformaldehyde CAS Number 30525-89-4 ☑ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☑ Irritant	Route Other (include in description of use) Dose (mg/kg) 4% Concentration (mg/mL) of the chemical in solution 4% Approximate Volume (mLs)	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Intracardial perfusion

☐ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☐ Not Hazardous ☐ Other (include in description of use)	3-4L Frequency (e.g.twice daily) Once	
Name: 3,3'-Dioctadecyloxacarbocyanine Perchlorate (DiO) CAS Number 223769-64-0 ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use Dose (mg/kg) 1-3 µls of 0.5-5% soln if injected. ~25 µls if gel foam appl cation is used. Concentration (mg/mL) of the chemical in solution 0.5-5% soln Approximate Volume (mLs) 1-3 µls Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle inje tio placement u ing gelfoam or forceps.
Name: FluoroGold CAS Number 223769-64-0 ☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	Route Other (include in description of use) Dose (mg/kg) 1-3 µls of 0.5-5% soln if injected. ~25 µls if gel foam application is used. Concentration (mg/mL) of the chemical in solution 0.5-5% soln Approximate Volume (mLs) 1-3 µls Frequency (e.g.twice daily) Once	Duration (e.g once, every day for 3 weeks etc. Once Description of Use Label neurons. Intra-spinal /muscle injections or placement using gelfoam or forceps
Name: Cholera Toxin B (CTB) CAS Number 131096-89-4	Route Other (inc ude in description of use) Dose (mg/kg) 1-3 µls of 0.5-5% soln if	Duration (e.g once, every day for 3 weeks etc.

☐ Carcinogen/Mutagen /Teratogen ☐ Reproductive Toxin ☐ Toxicant ☐ Neurotoxin ☐ Irritant ☑ Investigational Drug/Agent ☐ Potential Hazards Not Yet Determined ☑ Not Hazardous ☐ Other (include in description of use)	njected. ~25 µls if gel foam application is used. Concentration (mg/mL) of the chemical in solution 0.5-5% soln Approximate Volume (mLs) 1-3 µls Frequency (e.g.twice daily) once	once Description of Use Label neurons. Intra-spinal /muscle injections, intra-pleural injection, placement using gelfoam or forceps.

24.0 Radiation or Other Physical Hazards

24.1 Will animals be exposed to radiation (e.g., isotopes, lasers, irradiators) or other physical hazards (e.g., loud noises)?

- ☐ Radioactive Material
- ☐ Cs-137 irradiator
- ☑ X-Ray radiation
- ☐ Other (magnet, lasers, noise, etc.)

24.2 List relevant hazard information.

List the building and room numbers where X-ray radiation producing machines will be used

Building and Room Number
[redacted]

25.0 Agent Administration and Return to RRF

25.1 Will live animals be returned to the RRF after exposure to hazardous substances (biological, chemical, or radioactive)?

- ☑ Not Applicable. No hazardous agents used or animals are not returned to the RRF
- ☐ Biological Exposure then return to RRF
- ☐ Chemical Exposure then return to RRF
- ☐ Radioactive Material Exposure then return to RRF

26.0 Euthanasia or Other Disposition

26.7 Please select all methods of euthanasia that will be employed in this proposal. - Non Rodent Mammal

- ☑ Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.
- ☑ General anesthesia as described in "Anesthetics, Analgesics, and Other Therapeutics," followed by an adjunctive physical method such as bilateral thoracotomy, exsanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.
- ☑ General anesthesia as described in "Anesthetics, Analgesics, and Other Therapeutics," followed by intravenous or intracardial injection of potassium chloride (KCl, at least 75-150 mg/kg).
- ☑ General anesthesia as described in "Anesthetics, Analgesics, and Other Therapeutics" followed by

Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver or kidneys) removal.

26.8 If methods of euthanasia other than those listed above will be employed, please describe their use in detail.

n/a

26.9 For animals that will not undergo euthanasia at the end of these studies, provide a description of their final disposition. If this includes assignment to another *Proposal*, identify the other *Proposal* (if known) and estimate the minimum time period before using the animal(s) in subsequent procedures.

n/a

27.0 Non-Pharmaceutical-Grade Agents

27.1 Are ANY of the agents, substances, drugs, test articles, etc. to be used in live animals chemical grade, that is, not pharmaceutical grade?

(•) Yes ( ) No

27.2 Describe the use and quality assurance practices for all non-pharmaceutical-grade agents. In the justification, please explain why pharmaceutical grade materials cannot, or should not, be used. Examples may include unavailability of an equivalent veterinary or human drug, a need for higher concentration, interference of vehicles/diluents, *etc.* Cost alone may not be a satisfactory reason. IACUC Policy

Chemical	Justification
Agent: codeine Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? (•) Yes ( ) No
Agent: baclofen Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the

physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? (•) Yes ( ) No
Agent: kynurenic acid Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? ( ) Yes (•) No
Agent: insogubacine Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? ( ) Yes (•) No
Agent: lidocaine	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. This will not be used for analgesic effects to maintain homeostasis, but only for experimental protocols in which non-standard concentrations are needed.

Is this chemical available as a pharmaceutical or USP grade?

(•) Yes ( ) No

Agent:

cobalt chloride

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Is this chemical available as a pharmaceutical or USP grade?

( ) Yes (•) No

Agent:

doxapram HCL

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Is this chemical available as a pharmaceutical or USP grade?

(•) Yes ( ) No

Agent:

Substance P

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

Glutamate

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Is this chemical available as a pharmaceutical or USP grade?

◉ Yes ○ No

Agent:

Allostatin

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Is this chemical available as a pharmaceutical or USP grade?

	◉ Yes ○ No
Agent: Saporin Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? ◉ Yes ○ No
Agent: 8-OH-DPAT Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4. Expected shelf-life (stability, expiration/discard timeframe that will be used): 1-3 days	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration. Is this chemical available as a pharmaceutical or USP grade? ○ Yes ◉ No
Agent: DOI Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.): Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.	Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use. For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

WAY-100635

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Only agents with certification of purity will be used, acsf or saline will be used to ensure physiologic compatibility, formulation will be filtered and pH to 7.4.

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non-survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

1-3 days

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiI)

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Agent:

Biotinylated Dextran Amine (BDA)

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

Horseradish peroxidase (unconjugated or conjugated to wheat germ agglutin)

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

Fast Blue

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

Diamindino Yellow (DiY)

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

3,3'- Dioctadecyloxacarbocyanine Perchlorate (DiO)

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

FluoroGold

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

○ Yes ◉ No

Agent:

Sodium Nitrite

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

Due to the fact that it is used in a deeply sedated, non-responsive animal within 1-3 minutes of death, we use a chemical grade solution we mix in the laboratory. We use an

This 0.1-1% solution is mixed from a powder and is not sterile.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

12 months at 4 degrees Celcius

overdose to vasodilate the vasculature immediately prior to introduction of the transcardial perfusion solution.

Is this chemical available as a pharmaceutical or USP grade?

(•) Yes ( ) No

Agent:

PFA

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

4% paraformaldehyde in a buffer system (pH 7.4) is used and is not sterile. A sterile option s not available.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

mixed on the day of use

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

This agent is introduced during the terminal transcardial perfusion

Is this chemical available as a pharmaceutical or USP grade?

( ) Yes (•) No

Agent:

Cholera toxin B

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

All tracers are mixed in a certified biological safety hood. All surfaces are sprayed down with alcohol or other appropriate solution to decontaminate surfaces, instruments are sterilized via steam sterilization (autoclave) and the person mixing the tracer wears sterile gloves. All consumables used to mix the tracer are sterile (Eppendorf tubes, centrifuge tubes, needles, syringes). All diluents are filtered using a 0.2 micron filter attached to a sterile syringe. Sterile saline or buffer is used to reconstitute the tracers. Upon reconstitution, the tracer is pulled up into a sterile micro- syringe and placed into a sterile tray for transport to the surgical facility.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Used the day it's reconstituted

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

The companies which supply these tracers will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used by our group and the publications in which their use is detailed in the neuroscience literature with experimental animal models are extensive. Further, they provide neuroanatomical details that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

( ) Yes (•) No

Agent:

Bovine Thrombin

Justification for use, including effectiveness (pharmacokinetics, etc.) and any potential animal welfare and scientific issues relating to its use.

Describe formulation including purity, methods of ensuring sterility, and physiological compatibiltiy (pH, pyrogenicity, osmolality, etc.):

Two options: 1) lypolized thrombin combined with sterile saline/water and filtered through micro filters just prior to application (5000 unit vial diluted to 500 units/ml). 2) high purity thrombin filled under sterile conditions.

Expected shelf-life (stability, expiration/discard timeframe that will be used):

Option 1: within 2 hours; Option 2: within 2 days of opening.

There has been increased difficulty getting pharmaceutical grade thrombin, due to issues with the manufacturer. The companies which supply this will not assure their sterility or their use outside of research purposes. However, the likelihood of microbial contamination is minimal and they have been successfully used. Further, they provide coagulation that cannot be achieved with other methods.

Is this chemical available as a pharmaceutical or USP grade?

(•) Yes ( ) No

28.0 Non RRF Study Site(s)

28.1 Will animals be transported to and used in rooms outside of the RRF?

(•) Yes ( ) No

28.2 Describe where animals will be taken, including the locations(s), procedures to be performed there, and the length of time that individual animals will be retained in those rooms.

Location	Procedures Performed	For Surgical Location ONLY
Building: [redacted] Room Number: [redacted] Duration: ☑ Day Use ☐ Overnight ☐ 24-72 Hours ☐ >72 Hours	☐ Euthanasia and Tissue Collection ☑ Behavior Assessment ☑ Imaging ☐ Hazardous Agent Use or Administration (describe) ☑ Non-Survival Surgery ☐ Survival Surgery ☐ Other Non-Surgical Procedures (describe)	Frequency of use: ☐ Heavy (daily) ☑ Moderate (weekly) ☐ Light **For locations where surgeries will be performed only:** Describe surgical support equipment available (e.g., gas anesthetic machines, ventilators, body temperature support). We have an anesthetic gas machine, CO2/O2 monitor, temperature probe controlled blanket, ventilator, gas mixer, and gasses.
Building: [redacted] Room Number:	☑ Euthanasia and Tissue Collection ☑ Behavior Assessment	Frequency of use: ☑ Heavy (daily) ☐ Moderate (weekly) ☐ Light **For locations where surgeries will be performed only:**

[redacted] Duration: ☑ Day Use ☐ Overnight ☐ 24-72 Hours ☐ >72 Hours	☑ Imaging ☐ Hazardous Agent Use or Administration (describe) ☑ Non-Survival Surgery ☐ Survival Surgery ☐ Other Non-Surgical Procedures (describe)	Describe surgical support equipment available (e.g., gas anesthetic machines, ventilators, body temperature support). Animals are initially "boxed" down in [redacted] because of the fume hood and after transfered to pentobarb are transfered to [redacted]. In reverse for perfusion/tissue collection animals are transfered back to [redacted] because of the fume hood collect for employee protections.
Building: [redacted] Room Number: [redacted] Duration: ☑ Day Use ☐ Overnight ☐ 24-72 Hours ☐ >72 Hours	☐ Euthanasia and Tissue Collection ☐ Behavior Assessment ☑ Imaging ☐ Hazardous Agent Use or Administration (describe) ☐ Non-Survival Surgery ☐ Survival Surgery ☐ Other Non-Surgical Procedures (describe) fluroscopy	Frequency of use: ☐ Heavy (daily) ☐ Moderate (weekly) ☑ Light **For locations where surgeries will be performed only:** Describe surgical support equipment available (e.g., gas anesthetic machines, ventilators, body temperature support).
Building: [redacted] Room Number: [redacted] Duration: ☑ Day Use ☐ Overnight ☐ 24-72 Hours ☐ >72 Hours	☐ Euthanasia and Tissue Collection ☑ Behavior Assessment ☐ Imaging ☐ Hazardous Agent Use or Administration (describe) ☐ Non-Survival Surgery ☐ Survival Surgery ☐ Other Non-Surgical Procedures (describe)	Frequency of use: ☐ Heavy (daily) ☑ Moderate (weekly) ☐ Light **For locations where surgeries will be performed only:** Describe surgical support equipment available (e.g., gas anesthetic machines, ventilators, body temperature support).

28.3 If animals will be housed outside the RRF overnight or longer, provide a justification and brief description of practices employed. ***Note that for locations where animals may be kept over 72 hours, a Satellite Housing Area Description (SHAD) is required.*** **If you already have an approved SHAD for the location(s) you will need to attach it as a "Study Document" in the Initial Review Submission Packet.**

Note: *Justification and a description of practices employed with animals retained outside of the RRF for over 12 hours must be described in the Special Housing section.*

29.0 Consideration of Alternatives

29.1 Indicate HIGHEST Pain Classification of Procedures in this protocol.

- ○ Class 0 - Animals will be acquired/held, but not used or manipulated in any way.
- ○ Class I - Studies in which animals will experience no pain or distress greater than that produced by routine injections or venipuncture and will therefore receive no pain-relieving agents.
- ◉ Class II - Studies in which there is a potential for pain or distress which is minimized or eliminated by anesthetics, analgesics, and/or tranquilizers. Examples include biopsy, endoscopy, vascular cut-down, footpad injections, use of adjuvants, implantation of chronic catheters, as well as survival and non-survival surgery.
- ○ Class III - Studies in which animals will experience pain or distress greater than that produced by routine injections or venipuncture and will not receive pain-relieving agents. Examples include exposure to agents or radiation levels that cause serious illness, research involving significant stress, or procedures involving prolonged restraint. A written justification (including supporting sources, journals, abstracts, etc.) for withholding pain-relieving agents must be provided in a following section.

29.2 List of Procedures **List all procedures potentially associated with more than minor pain or distress (*e.g.*, nephrectomy, craniotomy, forced exercise, use of Complete Freund's Adjuvant). This is meant to help you identify keywords needed in literature database searches for alternatives to the potentially painful or distressful procedures.**

Non-survival experiments:
Surgical procedures including: placement of catheters, tracheal cannulation, EMG placement, craniotomy, and alterations of respiratory state,
Anesthesia will be maintained during all procedures to eliminate pain/distress.
Lidocaine will be used to minimize the sympathetic response during placement of the ear bars.

Survival procedures:
No suitable alternatives have been identified to replace the use of live animals for the studies associated with this protocol. The lack of suitable alternatives was determined by continual review of the literature on a weekly basis. Searches used a variety of databases including Pub Med (which searches articles back to 1966) and current contents (broader, but searches only back to the early 90's), as well as search engines (i.e. google, yahoo) and the Johns Hopkins ALTWEB site. Typical searches included the following key words: spinal cord injury (SCI), SCI models, proteoglycans, regeneration, chondroitin sulfate, gycosaminoglycans, proteases, drug delivery, locomotion, in vivo, and in vitro. In addition to frequent searches, I, and members of my research group, attend meetings and give presentations at other scientific institutions (academic and private companies). During these meetings/trips, I have professional discussions with colleagues that cover many topics including viable research models. Additionally, while swallow has been assessed using videofluoroscopy in various rodent models of disease, aspiration has never been seen. Russell, et al. (Dysphagia, 28; 95-104; 2013) thoroughly discussed how the anatomical specifics of the rodent do not make aspiration or its use as an outcome measure possible.

We have chosen to use the cat model due to our expertise in this model, the specificity of established locomotor tests in the cat and the existing literature on SCI, locomotion and reflex in the cat. Our previous work and the existing literature give us interpretive power that is not possible in a less sentient species and allow us to directly compare any new work to our previous data. As indicated above under "B", the cat is an important translational model for issues involving SCI in humans. These include its spinal size with respect to growth requirements for re-connectivity, our work on CS GAGs (the target of the potential therapeutic agent, ch'abc) suggests that sulfation patterns are more similar between cat and human than rat and human, elegant motor control, similarity of spinal anatomy to the human, and seminal work regarding task specific training. In summary there are multiple importance and significant benefits of the animal model to be used and these currently cannot be achieved in other animal or non-animal models.

29.3 Consideration of Alternatives **Provide a written description of the methods (*e.g.*, literature database search) and sources (*e.g.*, databases, review articles, scientific meetings) used to determine that alternatives to painful procedures were not available. *Note*: Unless a compelling justification can be made without it, support your assurance by conducting a literature database search. - Note: the USDA Research Inspection Guide states that teaching exercises involving potential pain and distress (e.g., non-survival surgery) using animals should also consider alternatives such as veterinary mannequins, live tissue alternatives, and mechanical teaching devices. Protocols involving toxicity studies should also consider alternatives such as local lymph node assay, up-and-down procedures (see http://iccvam.niehs.nih.gov/about/overview/htm).**

Non survival experiments:
Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps described in Section 15 will be taken to re-established a surgical plane of anesthesia

and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end-tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone.
Survival Procedures:
Although we have never had to euthanize a cat due to low weight or body condition score, if an animal were to fall below a 4/4+ (see body condition scale below) and did not respond to changes in diet, it would be considered for, and/or the RRC veterinarian consulted regarding the choice to move to, euthanasia.

USDA Policy stipulates that for each search performed, you must provide the information requested in the table below. For additional information regarding performing such searches, see the **IACUC Information Sheet**. A representative in the Kornhauser Library is also available to assist you: **j0chen05@exchange.louisville.edu**

Keywords must include the procedure itself (*e.g.*, abdominal surgery, nephrectomy, thoracotomy, craniotomy, *etc.* Keywords should include terms for refinement as well as replacement for the painful procedure, such as analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare.

Database Name / Search Date	Keywords Used / Results
	List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above). Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare) Results: We directly deal with pain associated with surgical manipulations through the consistent use of pain medication following any surgical procedure. We also included the option in our protocol to extend the pain medication time frame in the unlikely event that we see pain behaviors after completion of the standard medication regimen. We also carefully monitor the animals' depth of anesthesia during all surgical procedures to assure that there is no perception of the procedure as it is occurring. Drugs used during all procedures are carefully chosen to adequately support the animal as well as assure that the research is translationally relevant. Many of the drugs available for sedation, anesthesia and pain control have neurotoxic or neuroprotective effects. These effects are critically considered and scrutinized as they would alter the

Database Name

MEDLINE

Date Search Performed

03/15/2021

Dates seached, if not ALL

1966-present

dynamics of the lesion being studied, its potential translational relevance, increase the variability of lesion magnitude across animals, and ultimately require an increase in the number of animals required to see an effect. For example, our surgical protocol uses a pre-sedative (acetylpromazine) with no apparent neurotoxic or protective effects and a gas anesthetic (isoflurane). Isoflurane (vs sevoflurane) is used for a variety of reasons, although the primary reason is a reduction in animal numbers. Isoflurane has been the standard anesthetic for these types of experimental surgeries in cats and for two experimental control groups we have already collected that will be directly compared to those in this protocol. This is important as both isoflurane and sevoflurane are good anesthetics for these types of procedures, but both have neurotoxic properties and are likely to affect lesion dynamics differently. One paper suggested a minimal potential benefit of more rapid induction with sevoflurane, but the findings are qualitative. Despite this and the fact that we have found that we obtain rapid induction with isoflurane, we use acepromazine prior to induction for surgeries. Careful consideration also is given with respect to the timing of drugs as well as their effects. An example is our use of buprenorphine. Buprenorphine is strong and effective for control of post-operative pain. However, due to its well-known neuroprotective properties it is critical that it is not introduced into the cat's system until after the spinal lesion has been made and the lesion dynamics are in progress, but will be given before the end of the procedure. (For the spay procedure buprenorphine is introduced before the surgery is initiated.) We will also be combining the opioid use with a local incisional nerve block in order to further reduce potential discomfort. Spayed female cats are used as males pose a significant problem with respect to bladder voiding post-spinal cord injury. Uric acid crystals often block the urethra in male cats and prevent urine exit and lead to morbidity and mortality if not remedied. Currently there are no good solutions. Pushing of the crystals back into the bladder with a catheter temporarily allows voiding, but blockage readily occurs again and again and risk for infection is high. Surgical interventions, such as bladder ostomy, are not trivial, introduce research variability, are not compatible with the training paradigm and increase infection risk. Nocita (bupivacaine liposome) is not indicated for incisional/procedural locoregional anesthetic techniquue that requires deep and complete sensory block in the area of administration, and the label does not recommend this use. Becuase Nocita (bupivacaine liposome) lasts up to 72 hours, this is actually be counter productive for our purposes as the risk for cardiac toxicity in the cat goes up due to prolonged exposure, especially if the liposomes rupture at a faster rate (or all at once... both are documented to have occurred), and if any inadvertently diffuses into the spinal cord the

cat would have a high epidural for 3 days. Also- The fast "unencapsulation" of bupivacaine liposome injectable suspension (BLIS) tends to occur if the drug is mixed with or comes in contact with hypotonic solutions (ie sterile water, etc), or acidic changes of the tissue pH, which could result in cardiac toxicity. This topic is reviewed in https://todaysveterinarypractice.com/nocita-dogs-cats/ and cited in the 4th edition of Plumbs Formulary.

List keywords (searches should be performed for alternatives to each of the potentially painful procedures listed in subsection 3 above).

Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare)

Database Name

PubMed

Date Search Performed

03/15/2021

Dates seached, if not ALL

All dates

Results:

Searches within this data base lead us to the same conclusions as searches in Pub Med. These conclusions indicate the importance and significant benefits of the surgical procedures to be used, that these currently cannot be achieved with other approaches, and that our drug regimen is the most effective for the studies. As indicated above under two specific search engines, no suitable alternatives to the surgical procedures /approaches involving spinal cord injuries or placement of tract tracers have been identified for the studies associated with this protocol. The lack of suitable alternatives was determined by continual literature review (typically a weekly basis). Searches used a variety of databases including Pub Med (which searches articles back to 1966) and current contents (broader, but searches only back to the early 90's), as well as search engines (i.e. google, yahoo) and the Johns Hopkins ALTWEB site. In addition to frequent searches and discussions with local colleagues, I, and members of my research group, attend meetings and give presentations at other scientific institutions (academic and private

	companies). During these meetings/trips, we have professional discussions with colleagues that cover many topics including surgical approaches, pain control and alternative, viable approaches. Several of my colleagues are either veterinarians or have appointments in a Veterinary School of Medicine.

29.5 **Humane Endpoints** **For some Class I and *all* Class II and III procedures, there is a potential for adverse effects. Humane endpoints are objective signs indicating a pain/distress level that warrants intervention (usually euthanasia), regardless of experimental timelines. These may be specific for each procedure or may be general for an experimental group or the entire *Proposal*. Often, basic "sick animal" signs such as inappetance or lethargy lasting over 24-48 hours or weight loss exceeding 10% are used. Other signs/criteria may be more appropriate for this study. [IACUC Policy and Pain Scoring Sheet Templates] - Make sure that your response**

1. **Precisely defines the humane endpoint, including assessment criteria**
2. **Describes the frequency of animal observation**
3. **Describes the response required upon reaching the humane endpoint**

Experimental Group or Procedure	Endpoint and Assessment Criteria	Frequency of Observation	Response
Non survival experiments:	Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps will be taken to re-established a surgical plane of anesthesia and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end-tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone.	At minimum every 15 minutes.	Additional anesthetic will be delivered or animal will be euthanized.
	Animals will be euthanized prematurely if they exhibit life threatening or painful health problems that are refractory to treatment. The spinal cord injuries they receive do not fall within the criteria for deciding to euthanize. In		

		isolation, change from baseline weight is not a reasonable criteria for euthanizing. Because a cat may enter the animal facility weighing more than their ideal weight, it is important to use a body condition scale in addition to weight in monitoring their health status. Thus, If a cat becomes a 4 (underweight) on the on the 9 point Purina Body Condition Score and measures to increase weight are not effective (i.e. greater access to food or food variety), a veterinarian will be contacted and measures taken to enhance appetite and increase weight. If weight loss progresses to a 3 (thin) a decision to euthanize will be made in consultation with the veterinarian. We believe it is extremely unlikely that any cats will progress to this point. However, a cat may temporarily drop into the 4/4+ range acutely post-injury when appetite might be suppressed by medication and muscle atrophy is occurring due to the injury. In the rare instance that a cat has internal bleeding that cannot be stopped during /following a spay procedure, the cat will be euthanized. In the rare instance that a cat has an unacceptable lesion magnitude or is a risk for handling (i.e. bites), the choice may be made to euthanize. Persistent loss of ambulation longer than 14 days.		
	Survival Procedures and following behavior testing		All animals are observed 1 to 3 times a day.	If events occur that lie outside of those listed, veterinarians will be consulted and /or the animal euthanized.

	Infection or pressure ulcers that is/are unresponsive to treatment, Evidence of a cerebrospinal fluid (CSF) leak (recognized as persistent clear fluid drainage from incision site), Hemodynamic instability or if the animal is not eating for 48 hours then the animal will be euthanatized, Body condition score of 2 It is possible for involuntary muscle spasms post SCI (seen in humans and other species) to affect behavior and the animal's willingness to eat. If this is observed, then the animal will be treated in consultation with a veterinarian. If other complications not foreseen arise, a CMRU veterinarian will be consulted and the animal will either be treated if the treatment will not interfere with the experiment or the animal will be euthanatized.		

30.0 Other Information for IACUC Review

30.1 Is there any additional information that may assist the IACUC in their review, *e.g.*, request for exemptions to IACUC policies not described elsewhere in this Proposal?

○ Yes ◉ No

31.0 End of Form

31.1 STOP To Submit Proposal click "Save & Continue," and complete the Initial Review Submission Packet Otherwise - Log Out or return to the sections you wish to revise.

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EXHIBIT C

FINAL

FOR OFFICE USE ONLY: IACUC Protocol # 4440 Application Received: 8/11/20 Routed: 8/11/20 Approved: 8/11/20 Expires: 8/10/23
USDA Covered X Exempt IBC # 1460

KANSAS STATE UNIVERSITY
University Research Compliance Office

Institutional Animal Care and Use Committee (IACUC)
Application for Approval Form

Please send your completed application to comply@k-state.edu

Version: Last Updated: 02/24/2020

ADMINISTRATIVE INFORMATION:

Responsible Individual/PI: Juergen Richt

Responsible Graduate Student (if applicable):

Title of Project/Course: Efficacy of experimental COVID-19 vaccines in cats.

Species/ Strain to be used: Felis catus / cats

Type of Application: ☑ New ☐ Renewal of IACUC# ☐ For a renewal, check both "New" & "Renewal"
☐ Addendum/Modification (complete modification section below)

Category: (check one box) ☐ Teaching ☐ Testing ☑ Research
☐ Other (if other, describe)

Funding Source: ☐ PHS/NIH ☐ Other Federal Agency List:
☐ State ☑ Other List: industry, DHS

Principal Investigator: Juergen Richt Degree/Title: DVM, PhD/Regents Distinguished Professor

Department: Diagnostic Medicine/Pathobiology Campus Phone: 785-532-3905

Campus Address: Mosier Hall, Room K-224, 1800 Denison Ave, Manhattan, Kansas

E-mail: jricht@vet.k-state.edu Alternate phone : 785-323-7970

Co-Principal Investigators:

Name: Igor Morozov Dept: Diagnostic Medicine/Pathobiology Degree/Title: DVM, PhD

Name: Dept: Degree/Title:

MODIFICATION:

If you are requesting a modification or a change to an IACUC approved protocol, please provide a concise description of all of the changes that you are proposing in the following block. Additionally, please highlight or bold the proposed changes in the body of the protocol where appropriate, so that it is clearly discernible to the IACUC reviewers what and where the proposed changes are. This will greatly help the committee and facilitate the review.

I. **NON-TECHNICAL SYNOPSIS** (Please provide a brief narrative description of proposal. This should typically be less than 75 words and be easily understood by nonscientists, e.g. '*We propose to test the effectiveness of a new class of anti-inflammatory drugs against arthritis that develops in the hips of dogs affected by congenital hip dysplasia*'):

The goal of the project is to evaluate efficacy of experimental vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in a cat animal model.

II. **BACKGROUND** (concise narrative review of the literature and basis for the study):

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China in late 2019 and has since become a global pandemic with more than 15 million confirmed cases and more than 623,000 fatalities attributed to the coronavirus disease (COVID-19) caused by SARS-CoV-2 (7/23/2020). Countermeasure development has become paramount to help prevent human SARS-CoV-2 infections (i.e. vaccines) and to treat COVID-19 patients (i.e. antiviral drugs). However, there are no licensed vaccines or treatments for COVID-19 available at this time and it is imperative that both, vaccines and treatments, need to be assessed in a preclinical animal model for efficacy before they can be used in humans.

Cats are susceptible to SARS-CoV and can be used as an animal model for testing of potential SARS-CoV-2 vaccine candidates. Dvelopment of vaccine for cats is also very important for the prevention and control of SARS-CoV-2 outbreaks. Limited studies with SARS-CoV-1 were performed in cats which demonstrated that cats are susceptible to experimental infection with SARS-CoV-1 and can shed and transmit virus (*Martina et al., Nature 425:915 [2003]; van den Brand et al., Vet Pathol. Jul;45:551-62 [2008]*). In the study conducted by *Martina et al.* (2003), cats did not develop clinical signs, but were shedding virus for up to 10 days post infection and transmitted virus to sentinel contact animals. *Van den Brand et al.* (2008) demonstrated that cats express the ACE2 receptor, which is used by SARS-CoV -1 and -2 to enter cells, in type 1 and type 2 pneumocytes and develop tracheobroncho-adenitis post SARS-CoV-1 challenge. In addition, Shi and coworkers (Shi et al., Science. DOI: 10.1126/science. abb7015 [2020]) recently described that cats are highly susceptible to SARS-CoV-2 infection and demonstrated virus transmission to sentinels.

In our first experiment with cats, we did not observe any obvious clinical signs post primary SARS-CoV-2 challenge and post re-infection. Significant transient shedding was observed in rectal, nasal and oropharyngeal swabs post primary challenge, which was cleared by day 21 post infection. The virus was easily transmitted to the 2 sentinel contact cats. By day 21 post initial infection, all cats (principals and sentinels) were free of virus in nasal, oropharyngeal and rectal swabs. After re-infection on day 21 pi, low amounts of viral RNA were shed from rectal, nasal and oropharyngeal orifices, and lung lesions were observed on day 4 post re-challenge (4DP2C). These data indicate that vaccines for cats are feasible and that the cat is a good model for testing of SARS-CoV-2 vaccines.

The purpose of our project is to use cat model for evaluation of efficacies of experimental coronavirus vaccines. Animal health company Zoetis is working on cat vaccine (recombinant subunit vaccine) and has developed a prototype which is currently being tested in cats to evaluate serological responses. One of the experiments in our project will be to evaluate the efficacy of Zoetis experimental vaccine for cats utilizing our SARS-CoV-2 cats challenge model (Study 1). Animals for this study will be vaccinated at Zoetis site.

III. **LITERATURE SEARCH FOR UNNECESSARY DUPLICATION**

(If your proposed activity is part of the formal veterinary teaching curriculum and is not research or testing, you may not have to perform a literature search for unnecessary duplication. A non-duplication explanation for teaching projects, for III.D., can be found at: https://www.k-state.edu/comply/iacuc/resources/protocol-development/teaching-projects/index.html.
A literature search for unnecessary duplication is required for all proposed research activities using animals.)

A. Date of literature search (should be within the last month): 07/21/2020

B. Search at least two appropriate databases and provide the years of coverage (i.e., PubMed (1950/current), CAB (1910/present)). A list of databases is available online at http://guides.lib.k-state.edu/sb.php?subject_id=38563:

1) PubMed (1950-current)

2) CAB Direct (1920/present);

3) Agricola (1970/present)

C. Keywords/Search Strategy:

((((SARS-CoV-2) AND (cat or feline))) AND virulence) AND pathogenicity
((((COVID19) AND (cat or feline))) AND immunity) AND vaccine

D. Please provide a concise narrative of the results of the searches relative to unnecessary duplication. You do not need to provide a copy of the actual search with your proposal, but it should be maintained for your records or available to the IACUC if requested. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu

Concise Narrative:

Search using key words " ((((SARS-CoV-2) AND (cat or feline))) AND virulence) AND pathogenicity " in various combinations resulted in 8 publications related to SARS COV-2. Two of them addressed the susceptibility of animals, including felines, to SARS-CoV-2, one paper addressed the topic of animal welfare during COVID19 pandemic, and two papers were on epidemiology of SARS-CoV-2.
Search using key words " ((((COVID19) AND (cat or feline))) AND immunity) AND vaccine " resulted in 6 publications discussing coronavirus vaccines in animals and animal models for COVID19. None of the papers mentioned development of the COVID19 vaccine for cats or using cats as a model for the vaccine testing.
Results of the search indicate that the proposed research is not a duplication of previous studies.

IV. OBJECTIVE\HYPOTHESIS (briefly state the objective of the study - and, if applicable, the hypothesis to be accepted or rejected):

To evaluate efficacy of experimental vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in cats.

V. MATERIALS AND METHODS:

V.A. Experimental Design and General Procedures (succinctly outline formal scientific plan for study):

Animal Details: Species, strain, sex, age, weight, number
Study Timeline: Arrival of animals, acclimation period, start of study, study time points, end of study
Biosample Details: Type, amount, method, location, needle size

Through vaccination phase of the study animals will be housed in animal facilities at Coles Hall (BSI-1 or BSL2). 3-5 days prior to SARS-CoV-2 challenge animals will be moved to BSL3-Ag rooms at the Biosecurity Research Institute. For some experiments, vaccination may be done at collaborator's facilities outside of KSU. In this case animals will be moved directly to the BRI BAL3-Ag facilities for SARS-CoV-2 challenge.

An outline of the **basic experimental study design** is provided below:

Group	No of Cats**	Vaccine	Challenge	Challenge Route
1	5	Vaccine A	SARS-CoV-2	IN*&Orally
2	5	Vaccine B	SARS-CoV-2	IN*&Orally
3	5	None	SARS-CoV-2	IN*&Orally

*IN - intratranasally.
** Group of five (n=5) is a normal sample size, but the study can be done with n=4 per group for external collaborators if

requested.

Briefly, 15 cats, approximately 5 months of age or older (all male, all female or mix), will be enrolled in the experiment. Upon arrival at the facilities, animals will be allowed to acclimate for 4 to 10 days prior to the experimental procedures. During acclimation time, the husbandry staff and the research team will spend time everyday handling the cats and practicing restraint, always ending with positive reinforcement using treats, pets and grooming. During this time the use of Feliway and catnip spray might also help with the acclimation. The length of the acclimation time will be determined by the cats' adjustment to new environment. Acclimation will be critical in decreasing the chances of escapes, bites and scratches. Once the cats are moved to BRI they also will be acclimated to become accustomed to the staff wearing the PAPRs. Personnel not experienced with handling of cats will be trained for proper handling and restraining of animals.

Post acclimation, Groups 1 and 2 (5 cats each) will be vaccinated with Vaccines A and B respectively. Group 3 (5 cats) will remain unvaccinated and serves as control group for SARS-CoV-2 challenge. Depending on the availability of animals, space/ rooms, caging and/or number of vaccines for testing, only 8-10 animals may be used in the study with one vaccinated group and one SARS-CoV-2 infected control group.

Vaccines will be formalin-inactivated whole virus (killed), subunit recombinant (recombinant proteins of SARS-CoV-2 S, E, N, M and fragments thereof), Peptides (peptides encoding immunogenic epitopes) and vector-based (NDV vector, VSV vector, Adenovirus vectors). Vaccination will be through intramuscular (IM), subcutaneous (SC), oral (PO) or intranasal (IN) routes with a single dose of vaccine or two doses of vaccine administered three to four weeks apart. A minor modification will be submitted prior to the start of animal study with specific details on the vaccine and vaccination route.

Vaccination will be with 20 - 100 µg of inactivated antigen/recombinant protein/peptide with adjuvant or with 10^4-10^7 TCID50 of vectored vaccine per animal administered in 0.5 - 1.0 ml. Before and through vaccination phase, cats will be bled via saphenous or jugular vein; prior to vaccination (between days -3 and 0) and on days 14 and 21 post single dose vaccination, or on days 14, 21, 28 and 35 for two dose vaccination. Prior to blood collection, cats will be sedated with Dexmedetomidine as described in Section VI.F. Atipamezole will be used to reverse the sedative effect of Dexmedetomidine at the end of the procedure (Section VI.F). Cats will be weighed prior to every sedation to provide accurate drug dosage, which will be recorded. During injection, the animal holder will be wearing heavy duty durable gloves with extended forearms to prevent accidental bites and scratches. On the day of the procedure food will be withdrawn in the morning and given to cats only after they completely recovered from the sedation. Ophthalmic ointment will be administered on the eyes of the cats each time the cat is either sedated or anesthetized so as to prevent any corneal ulcerations. Blood from animals will be collected by venipuncture from the jugular or saphenous vein using a 3 mL syringe and 1" x 21G needle. The area of blood collection will be shaved and treated with 70% alcohol prior to blood collection. Volume of collected blood will depend of the weight of animal and will not exceed 1% of Circulating Blood Volume (CBV) [approximately 1 mL per bleed from a 1.5 kg cat] per one time point and will not exceed 10% of the Circulating Blood Volume (CBL) within a 2-week period. Blood will be processed for serum and tested for serological responses against SARS-CoV-2 antigens by ELISA.

The challenge with SARS-CoV-2 will be two to three weeks after single dose vaccination or two to three weeks after administration of the second (booster) dose. The IN/oral challenge of cats will be performed as described in Section VI.I.6. Briefly, for challenge cat will be inoculated through intranasal (IN) and oral routes simultaneously with 10^6 $TCID_{50}$ of SARS-CoV-2 suspension. Half of the total dose (0.5 x10^6 TCID50) will be administered through each route in a volume of 0.5 - 1 mL. Prior to challenge, cats will be anesthetized with Dexmedetomidine, as described in section VI.F. Post SARS-CoV-2 challenge, cats will be observed daily for 14 - 17 days. Observed clinical signs include, but will not be limited to fever, anorexia, lethargy, respiratory distress, inappetence, depression, recumbency, coughing, sneezing, diarrhea, vomiting and others. Blood will be collected from all animals prior to infection on 0 day post challenge (DPC) and on 1, 7 and 14 DPC and at necropsy to monitor antibodies in serum. Nasal, oropharyngeal and rectal swabs will be also collected from sedated animals on the days of blood collection on 0, 1, 3, 5, 7, 10, 14 DPC and at necropsy. Prior to blood and swabs collection cats will be sedated with Dexmedetomidine as described in Section VI.F. Two animals of each group may be euthanized and necropsied on day 4 - 5 to monitor histopathology post challenge and virus in tissues. All remaining animals will be euthanized and necropsied at the end of the post challenge observation period on days 14-17 post challenge (extra days are added to distribute the work load at necropsy). At necropsy, blood and tissue samples (i. e. kidney, spleen, lung, bronchi, tonsils, intestine and lymph nodes) will be collected for virus isolation and/or PCR analysis for SARS-CoV-2 as well as histopathology.

Up to three studies in 3 years are requested with maximum of **45 cats**.

Design of Study 1:

For this study cats will be vaccinated by collaborators at their site with two dooses of the vaccine (recombinant subunit

COVID19 vaccine), and transferred to BSL-3Ag facilities at the BRI for acclimation and SARS-CoV-2 challenge. An outline of the experimental design is provided below:

Group	No of Cats	Vaccine	Challenge	Challenge Route
1	4	* SARS-CoV-2	SARS-CoV-2	IN&Orally
2	4	Control	SARS-CoV-2	IN&Orally

*SARS-CoV-2 recombinant subunit vaccine

After delivery to BRI cats will be placed in BSL-3Ag animal rooms in 2 large cages (4 animals per cage, groups co-mingled), and allowed to acclimate for 4 - 10 days prior to SARS-CoV-2 challenge. After acclimation all cats in the study will be challenged with SARS-CoV-2. Acclimation, challenge and post challenge observation and sample collection procedures will be the same as described above for **basic experimental study design, except that swabs will be collected on days 0, 2, 4, 6, 8, 10, 12 and 14.**

V.B. Photos, Videos and/or Audio Recordings (IACUC Guideline #6 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/6.html):

1. Will you be taking any photos, videos and/or audio recordings? ☑ Yes ☐ No

2. Please provide the necessary details to explain what you will be taking photos, videos and/or audio recordings of.

Photos of observation records; photos of gross pathological lesions at necropsy; photos and videos of sick animals in case if clinical signs are observed.

3. Please explain how the photos, videos and/or audio recordings will be used.

Photos will be used for record-keeping purposes and for use in scientific presentations and publications. Any sensitive material, such as videos of sick animals, will be reviewed by the AV prior to use and approved for release according to BRI established protocol.

4. Please describe how the photos, videos and/or audio recordings will be stored.

Photos and videos will be stored on secure folder on Q drive.

V.C. Non-animal Alternatives Considered (were non-animal alternatives considered - why are they not used?):

There are no non-animal alternatives to evaluate the efficacy of SARS-CoV-2 vaccine in animals.

V.D. Animal Model and Species/Strain Justification (Explain why animals are needed for your study. Give your rationale and justification for selecting this animal model or species):

Cats were selected because they were shown to be susceptible to SARS-CoV during the 2003 SARS-CoV epidemic and during current SARS-CoV-2 pandemic. The cats get infected and transmit the virus, and exhibit some minor clinical signs of infection.

V.E. Animals Requested -used in research testing or teaching (list genus and species/strain of animal model proposed):

Genus and Species:	Felis catus (cat)
Total number (by species) requested: (this should correspond to the sum of the animals listed in Section VI.A. below. The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the TOTAL number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.)	Felis catus (cat): 45
Source of animals (by species):	Felis catus (cat): Certified supplier, SPF colony

V.F. Justification of Animal Numbers / Data: Analysis: Research, testing, and teaching activities should be designed to provide a statistically significant result with a minimum number of animals. The specific method by which the number of animals was determined must be clearly stated. Statistical techniques and/or power analysis are appropriate in most cases to maximize the usefulness of the data generated from each animal. However, the IACUC acknowledges that the basis for an appropriate justification of animal numbers depends largely on the nature of the study itself. Prior experience and expertise with the model in question may be taken into account as well, but must be carefully documented in the protocol. The cost of the animal should not be considered as the primary justification for the use of a particular species or model. Consultation with a biostatistician or use of statistical software during the design phase of the experiment may be useful. This website may be helpful in performing a power analysis: http://statpages.org

Five basic types of studies are listed below, along with brief general guidelines for the justification of animal numbers appropriate for each type of study. These guidelines are intended to provide direction - any given study may not fall neatly into one of these five categories. **Select the appropriate box(es)** below and supply a narrative explanation that will clearly explain your rationale and justification for the number of animals proposed for your activity:

☐ 1. **Teaching Protocols:** (Animal numbers are determined by a specified student-to-animal ratio, which must be explained in the justification narrative. Animal numbers should be minimized to the fullest extent possible without sacrificing the quality of the hands-on teaching experience for students).

☐ 2. **Tissue Harvest Required for *In-vitro* Work and / or Antibody Production:** (Animal numbers are determined by the amount of tissue required and the number of individual animals needed to provide the appropriate amount of tissue, antibodies, etc. A detailed explanation of how the required number of animals was determined must be included in the justification narrative).

☐ 3. **Exploratory Study Requiring No Statistical Analysis - Qualitative**: (use of live animals to demonstrate success or failure of a desired goal, such as the production of transgenic mice): Animal numbers are justified based on the probability of success of the experimental procedure; a detailed explanation of how that probability was determined must be included in the narrative).

☐ 4. **Pilot Studies**: (Animal numbers are determined by the investigator's experience and personal judgment, and are typically small. Data collected in pilot studies are generally used to determine statistically relevant sample size calculations for future experiments).

☑ 5. **Studies Requiring Inferential Statistical Analysis**: (If possible, animal numbers are determined by statistical power analysis; the justification statement must include the specific test, i.e., ANOVA, student t-test, chi square, etc., used to determine sample size. Alternatively, minimum numbers of animals may be determined based on pertinent literature for comparable studies in which the desired effect sizes were shown to be statistically significant).

☑ a. **Statistical Test:**

Groups will be compared by analysis of variance ANOVA and t-test.

☑ b. **Literature Reference:**

1. Reference- (provide specific reference(s) for numbers justification)

(1) Brown, E G., H Liu, L Chang Kit, S Baird, and M Nesrallah. "Pattern of mutation in the

genome of influenza A virus on adaptation to increased virulence in the mouse lung: Identification of functional themes." Proceedings in the National Academy of Sciences 88.12 (2001): 6883-88. Web. 10 Mar. 2015.

(2) Brown, E G. "Increased Virulence of a Mouse-Adapted Variant of Influenza A/FM/1/47 Virus Is Controlled by Mutations in Genome Segments 4, 5, 7, and 8." Journal of Virology 64.9 (1990): 4523-33. Web. 10 Mar. 2015.

(3) : Kim Y, Liu H, Galasiti Kankanamalage AC, Weerasekara S, Hua DH, Groutas WC, Chang KO, Pedersen NC. Reversal of the Progression of Fatal Coronavirus Infection in Cats by a Broad-Spectrum Coronavirus Protease Inhibitor. PLoS Pathog. 2016 Mar 30;12(3):e1005531. doi: 10.1371/journal.ppat.1005531. eCollection 2016 Mar. Erratum in: PLoS Pathog. 2016 May;12(5):e1005650. PubMed PMID: 27027316; PubMed Central PMCID: PMC4814111.

2. Narrative Justification- (provide a succinct justification / rationale for using the reference(s) to determine the numbers proposed in the activity)

Brown et al. 1990 and 2001, used the Spearman-Karber method for LD50 calculation, used 5 animals per group for statistically valid interpretation of the results.
Kim et al. 2016, used 4 animals per group to test antiviral compound against Feline Infectious Peritonitis (FIP) virus (coronavirus) in cats with statistically significant outcome. Collaborators in the study confirmed, that four cats per group proposed in Study 1 design will allow statistically significant interpretation of results with sufficient power, especially in case if vaccine is efficacious in reducing clinical manifestation of disease.

☐ 6. **Other:** (This applies if your activity does not fit into one of the other categories. If you check this option, you must provide a detailed and defendable description of the rationale for the number of animals proposed for your activity).

VI. HUMANE CONSIDERATIONS:

A. Pain Category (for your proposal, please estimate the number of animals in each applicable pain category below to the best of your knowledge - it may be appropriate to list animals in more than one pain category, i.e. controls in Cat. C, infected animals in Cat. D or E. If more than one species is requested, provide pain category estimates on all species requested. We are required to report this animal use and pain category information annually to the USDA).

USDA Pain and/or Distress Category

Please estimate the number of animals in your proposed activity that would fall into one or more of the following three pain and/or distress categories. It is common to have animals listed in more than one category - for example, an uninfected control versus a challenge group. The cumulative total number for the three Pain Categories should equal the total number of animals requested in Section V.D.

SPECIES #1 (common name):	Cat (feline)	
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	45
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

(If you are using more than one species in this activity, also complete the following section)

SPECIES #2 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	
SPECIES #3 (common name):		
Pain Category B (bred, conditioned, or held for use)	**# of animals**	
Pain Category C (*No or Momentary Pain and/or Distress)	**# of animals**	
Pain Category D (**Alleviated Pain and/or Distress)	**# of animals**	
Pain Category E (***Unalleviated Pain and/or Distress)	**# of animals**	

If more species are used, please list them on an attached sheet.

* List animals in USDA Pain Category B that are being bred, conditioned or held for use.

* List animals in USDA Pain Category C that will undergo no activity that will produce pain and/or distress, or procedures similar to those that might routinely be performed on humans by a physician without provision of anesthesia or analgesia, i.e. injections, phlebotomy, ear tagging, etc. If you only listed animals in category B or C, you may skip Sections VI.B-F below and resume with Section VI.G.

** List animals in USDA Pain Category D that will undergo procedures where pain-alleviating methods are used, such as anesthesia, analgesia. Surgical patients would fall into this category, even if the procedure were terminal. If you placed animals in Category D or E, you must carefully complete Section VI. B-D below

*** List animals in USDA Pain Category E that will experience unalleviated pain and/or distress. This should be considered only when the use of a pain alleviating strategy would seriously compromise the validity of the study, and/or no other option is available or possible. If you place animals in Category D or E, you must carefully complete Section VI.B-D below.

The IACUC approves protocols for a period of 3 years, so the number(s) listed here should represent the **TOTAL** number of animals requested for a project up to a three-year period- and not simply reflect annual usage projections.

VI.B. Alternatives to Painful Procedures (If you have animals listed in Pain Category D or E above, you must provide the following information. The Animal Welfare Act requires that you provide a narrative description of methods used and sources searched to ensure that you have verified that alternatives are not available to prevent unnecessary pain and distress. The Animal Welfare Information Center (AWIC) has a site that gives tips for performing this search https://www.nal.usda.gov/awic/alternatives-literature-searching. Gayle Willard, Dir, Vet Med Library is the IACUC consultant. Please contact her if you need assistance. Phone 2-6006; email: gwillard@vet.ksu.edu).

1. **Date of literature search** (should be within the last month): 07/21/2020
2. **Search at least two appropriate databases and provide the years of coverage** (i.e., PubMed (1950/current), CAB (1910/present). A list of databases is available online at https://guides.lib.k-state.edu/sb.php?subject_id=38563:

1) PubMed (1950-present)

2) CAB Direct (1920-present)

3)

3. **Keywords/Search Strategy:**

Replacement: Animal use alternatives, alternatives, in vitro, cell culture, simulation or audiovisual or model or digital, cat, feline, COVID-19, SARS-CoV-2, computers, alternative, tissue culture, artificial.

Refinement: Pain or distress of animals after challenge with SARS-CoV-2 will be alleviated through monitoring of clinical signs and humane euthanasia of animals exhibiting severe clinical signs.

4. **Concise Narrative:**

Replacement: The search resulted in numerous articles pertaining to the searched fields. The articles discussed virus etiology and animal response to infection and other characteristics of the virus. There were some articles on simulation models relevant to outbreak surveillance, virus transmission models and preventive strategies. No articles were found that discussed the use of alternative methods to evaluate efficacy of the vaccines in animals.

Refinement: Our study will utilize individuals properly trained in the care and use of experimental animals with immediate notification of CMG veterinarians when animals are exhibiting decreased health or attitude. Sedation will be used to minimize pain and distress during IN and oral challenge of cats. Sedation will also be used in cats prior to blood and swab samples collection. Cats will be anesthetized prior to final bleed and euthanasia. Sedation for sample collection was approved by attending veterinarian, Dr. Denver Marlow.

VI.C. Painful Procedure Justification (How do you plan to minimize unnecessary pain and/or distress? You must provide strong justification for having animals in Category D or E above):

Evaluation of the efficacy of SARS-CoV-2 vaccine in cats requires experimental exposure of animals of the vaccinated and control groups to SARS-CoV-2. A proper monitoring and documentation of the onset of clinical signs post challenge is one of the critical tasks for these studies. Clinical observation parameters of animals with clinical signs are provided in the AMPs, including intervention points for euthanasia to avoid unnecessary pain and suffering in case if the animals in the study observed with severe clinical signs.

VI.D. Attending Veterinarian Consultation: ☑ **Yes** ☐ **No**

Name: Denver Marlow Date of Consult: 07/24/2020

If you have animals listed in Pain Category D or E in paragraph VI.A. above, the AWA requires that you formally consult with the IACUC attending veterinarian (AV) or his designee on all aspects of pain and / or distress management. This must be done prior to submission of the proposal to the IACUC / URCO. (Reference IACUC Guideline #22 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/22.html. To facilitate scheduling the AV consultation, please contact Ms. Shirley Whitney in the CMG office (103 Coles Hall, 532-5640, or swhitney@vet.k-state.edu) *Important note: the AV consult is not the IACUC review of your proposal. Please understand that the IACUC committee is autonomous and members will likely ask different questions they deem appropriate during the actual committee review.

VI.E. Prolonged Restraint: ☐ **Yes** ☑ **No** (Describe and justify any plans for prolonged restraint >15 min. Reference IACUC Guideline #2 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/2.html)

VI.F. **Pain or Distress Alleviation** - Will you be administering drugs or compounds for sedation, anesthesia or analgesia as a premedication or for anesthetic induction or maintenance? ☑ **Yes** ☐ **No** **(If "YES", all animals receiving the drug or compound will need to be placed in USDA Pain Category D.)**

1. List all drugs or compounds being used for sedation, anesthetic or analgesia during the course of your procedure. Included drug/compound name, dosage, route and frequency.

Drug/Compound	Dosage	Route	Frequency
Dexmedetomidine (Dexdomitor®; 0.5 mg/ml)	0.02-0.04 mg/kg (0.04-0.08 ml/kg)	IM	For sedation prior to intranasal/oral inoculation, blood and samples collection, or prior to euthanasia.
Atipamezole HCl (Antisedan®; 0.5 mg/ml)	0.2-0.4 mg/kg (0.04-0.08 ml/kg)	IM	For reversal of Dexmedetomidine sedation for intranasal/ oral inoculation, blood and samples collection.
Telazol® 100 mg/ml	9 - 12 mg/kg	IM	For anesthesia prior to euthanasia only.

2. How will you monitor the animal to ensure the animal is properly anesthetized?

Cats: Test responsiveness by checking toe pinch reflex and palpebral reflex (blinking).

VI.G. **Surgery** ☐ **Yes** ☑ **No** If No, please skip to the next section, VI.H. Animal Monitoring.

(Reference IACUC guidelines #4 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/4.html, #10 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/10.html)

1. **Procedure** (Describe surgical procedures planned)

2. **Location** (Where is the surgical procedure to be performed?)

3. **Surgeon/Qualifications** (Who will perform procedures? List their training and qualifications.)

4. **Multiple Survival Surgery Procedures** ☐ **Yes** ☐ **No** (If yes, please provide justification)

(Reference IACUC guideline #7 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/7.html)

5. **Non-Survival Surgery Procedures** ☐ **Yes** ☐ **No**

VI.H. Animal Monitoring - In order to evaluate potential for pain and/or distress, the KSU IACUC requires an approved plan of how pain or distress will be minimized and documentation of how observations of animals will be recorded. All procedures performed upon an animal should be listed on an **Animal Monitoring Plan (AMP)** form. The AMP form along with the **Animal Observation Record (AOR)** detail how you will observe your animals and what actions you will take in order to minimize pain or distress associated with your research project. Examples of procedures needing an AMP include surgical procedure, animals that undergo anesthesia, animals experimentally infected with an infectious disease, experimental vaccination, or animals inoculated with potential tumor forming cells. Exceptions to the use of the AMP and AOR would be simple procedures with minimal physiological effect upon the animal, examples of which include blood collection, or injection of therapeutic drugs.

If an AMP is included in your approved IACUC document, it is your responsibility as the PI to assure that the AMP activities will be used as described in the approved protocol. It it is your responsibility to be able to provide documentation for the activities called for in the AMP.

1. Does this protocol require the use of the AMP and AOR? ☑ **Yes** ☐ **No**
   (Checking "YES" will make the AMP form appear on the next page)
2. Is an AMP completed? ☑ **Yes** ☐ **No**
3. Indicate where the AMP will be kept (i.e. animal room posted on wall, lab or barn office).

[redacted]

Animal Monitoring Plan

Protocol #:		PI:	Juergen Richt	PI Contact #:	785-532-2793
Animal/Group ID:		Species:	Felis catus (cat);	Animal Location:	[redacted]
Procedure:	Vaccination; Challenge with SARS-CoV-2			Date of Procedure:	TBD

I. Post-Procedure Care (if applicable)

A. List all drugs/medications to be given following the procedure (include name, dose, route, and frequency)

Drug/Medications	Dose	Route	Frequency
NA			

B. List all other care to be provided following the procedure and note frequency.

Post-Procedure Care	Frequency
None	

II. Observations

A. Observation Frequency: Post vaccination: observe daily for general health, and for at least 3 days post vaccination for reactions at injection site (until resolved).
Post challenge: Observe once daily for clinical signs. If clinical signs are observed, consult CMG veterinarian if observations has to be increased to twice a day with at least 8-10 hours between checks. If no clinical signs are observed for 2 consecutive days (48 hours) for all animals, then frequency can go back to once a day. CMG veterinarians will be notified of any clinical signs observed.

B. When will the animal be returned to its cage/pen:

N/A

C. List the parameters to be monitored, criteria to monitor for and directions for recording, and the appropriate action to be taken if necessary.

Parameter	Monitoring Criteria	Intervention
Injection site reactions (post vaccination)	Bleeding, redness, swelling, lumps or skin ulceration/necropsies at the site of injection	If modcrate to severe injection site reactions (swelling or ulceration) are observed, CMG will be contacted to assess if animal may be treated or must be euthanized.
Body Temperature (post challenge)	Measure of rectal temperature. Temperature above 102.5 F will be considered as fever.	None anticipated but if very high (>105F for >24h hours) along with clinical signs (anorexia, depression/ lethargy, respiratory distress, anorexia and etc.) a CMG veterinarian associated with the project will be consulted if the animal has to be euthanized.
Appetite (post challenge)	Observe intake of food by the animal.	Animal will be offered special food (treat) during observance period and notes taken on those that are eating offered feed, if animal does not eat offered treats and shows other clinical signs of illness, a CMG veterinarian will be consulted if the animal has to be euthanized.
Respiratory Signs (post challenge)	Observe for sneezing, coughing, labored breathing, nasal discharge	If severe clinical signs of respiratory distress are observed, a CMG veterinarian will be consulted if the animal has to be euthanized.
Digestive Signs	Observe for diarrhea, vomiting	If severe clinical signs of digestive distress are observed, a CMG veterinarian will be consulted if the animal has to be euthanized.
Activity /Attitude (post challenge)	Depression, decreased alertness and/or responsiveness	If severe signs are observed, depression/lethargy contact CMG veterinarian associated for monitoring and/or treatment plan.
Body weight	Body weight will be taken during sampling times on a special scale. Any significant change (>10%) in body weight will be reported to the CMG veterinarians and will be consulted to determine monitoring plan, treatment plan or euthanasia.	

III. Contact Information:

	Name	Telephone Number
PI	Juergen A. Richt	O: 785-532-2793 C: 785-323-7970
Co-Investigator	Igor A. Morozov	O: 785-532-4255 C: 785-323-7966
Co-Investigator		
Veterinarian	CMG clinical veterinarian - Sally Olson	C: 785-410-7513

In the event that the investigators or the responsible veterinarian cannot be reached or if you have concerns about an animal's care, please contact the KSU Attending Veterinarian (785-532-5648).

VI.I. Animal Manipulations:

1. **List all other drugs and compounds** that you will be administering other than those listed above in Pain or Distress Alleviation (Section F), on the Animal Monitoring Plan (Section H) or in Euthanasia (Section J.8). Include drug, dosage, route and frequency. **If any drugs/compounds are to be stored at the Veterinary Health Center Pharmacy, please consult Landa Colvin-Marion, (785)532-4127 lcolvin@k-state.edu, prior to ordering to ensure adequate storage space/requirements.**

Drug/Compound	Dosage	Route	Frequency
SARS-CoV-2 10^6 TCID50	1.0-2.0 mL (0.5x10^6 TCID50) per each route	IN/Oral	Once
Opthalmic lubricant	1-2 drops	Ocular	Each time cat is sedated
Inactivated COVID19 vaccine	500-1000 ul (20-100 ug)	IM or SC	Twice
Recombinant subunit COVID 19 vaccine	500-1000 ul (20-100 ug)	IM or SC	Twice
Peptide COVID19 vaccine	500-1000 ul (20-100 ug)	IM or SC or IN or PO	Once or Twice
Vectored COVID19 vaccine	10^4-10^7 TCID50 administered in 100 - 200 ul	IM or SC or IN or PO	Once or Twice

2. If any of the above drugs/compounds are to be prepared in your lab, please explain the preparation and storage process.

SARS-CoV-2 will be propagated in cell culture and stored at -80oC until use.

3. **List any rooms where procedures with animals are done (excluding housing and surgery).** Locations for procedures such as behavior testing, treadmill training, blood draws, injections, gavage, etc. should be listed in this chart. If procedures are performed within CMG facilities, the "CMG assigned".

Building/Room Number	Procedure
[redacted]	Oral & IN virus challenge, collection of blood, swab samples
[redacted]	Vaccination, collection of blood.

4. **Biosamples:** ☑ **Yes** ☐ **No** (list type & amount, i.e., phlebotomy, minor biopsies, ascitic fluids, etc.)

Blood:
Blood post vaccination: animals will be bled via saphenous or jugular vein prior to vaccination (between days -3 and 0) and on days 14 and 21 post single dose vaccination, or on days 14, 21, 28 and 35 for two dose vaccination.
Volume of collected blood will depend of the weight of the animal and will not exceed 10% of Circulating Blood Volume (CBL) within a 2-week period [approximately 2 ml per single bleed from a 3 kg cat]. Up to 40-50 ml of blood will be collected at final bleed prior to euthanasia by venipuncture from the jugular vein. Cats will be anesthetized prior to blood collection and euthanasia.

Swabs:
Nasal, oropharyngeal and fecal swabs will be collected from cats on DPC 0, 1, 3, 5, 7, 10 and 14 during the blood collection time points when the animals are sedated.
Swabs will be collected using tip swabs. For nasal and oral swabs collection swab will be inserted into the mouth or the nostril as far as possible and rubbed a few times to remove epithelial cells. After collection, swabs will be placed into the tube with 3 ml of the collection media (on ice) for further processing.

5. **Tissue Sharing:** ☐ **Yes** ☑ **No** (detail any tissue sharing you plan with other investigators)

6. **Other Procedures:** (list any other procedures you might perform on animals in this project)

Challenge of Cats (IN&Orally): For intranasal challenged cats will be sedated with Dexmedetomidine, and 0.5 - 1.0 ml of virus suspension will be slowly administered in each nostrils using a 1 - 3 ml syringe. Then a similar dose of the virus will be administered orally using a syringe. The cat is held vertically with it's head up for approximately ten seconds and then the animal will be laid on the floor inside the cage. Before exiting the room post challenge, personnel will monitor animals until full recovery from the anesthesia which is until the animals are ambulatory or sternal and able to hold their heads up.

7. **Adjuvants:** ☑ **Yes** ☐ **No** (explain any adjuvant use. Reference IACUC guideline #12 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/12.html)

Montanide ISA W/O, O/W and polymer-based adjuvants, commercially available from Seppic Inc., New Jersey, US, or similar products. Modification will be submitted for each study with specifics of the adjuvant used.

8. **Chemical Grade Drugs:** ☐ **Yes** ☑ **No** (If you plan to use a chemical grade please list and provide a scientific explanation for its use; Reference IACUC guideline # 19 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/19.html)

VI. J. Veterinary Care:

1. **Animal Housing:** (Provide specific information on where the animals will be housed for your activity.)
PLEASE INCLUDE ROOM NUMBER IF KNOWN

☐ **CVM/CMG**		☐ **LACS(Biology)**	
☐ **Bluemont Hall**		☐ **Justin Hall**	
☑ **BRI/PRH**	[redacted]	☐ **ASI Facilities**	
☐ **LARC**		☑ **Coles Hall**	CMG assigned
☐ **Mosier Hall**		☐ **Other (specify room or area)**	

2. **Social/Paired Housing:** (Social animals should be housed in stable pairs or groups of compatible individuals unless they must be housed alone for experimental reasons or because of social incompatibility. "The Guide" 8th Edition):

☑ **Yes** ☐ **No** My animals will be housed in stable pairs or compatible groups?

If no, please provide an adequate justification for an exception to this guidance.

Animals will be group housed

3. **Special Husbandry Considerations:** (Animals will be housed in designated animal rooms/areas, unless approved by the IACUC. Detail special husbandry requirements, i.e. special diets, micro-isolators, etc.):

Animals will be housed in designated animal rooms in individual cages or group housed inside of large cages. Enrichment items, such as balls and small toys will be provided for cats.

4. **Animal Surveillance:** (Who observes the animals daily for health problems?)

Research personnel

5. **Veterinary Clinical Care:** (Who will you contact if there is a health problem requiring veterinary care?)

CMG veterinarian

6. **Wire Bottom Rodent Caging:** If you are using rodents, do you propose to house them in wire-bottom cages?

☐ **Yes** ☐ **No** (If yes, you must explain the rationale for the use of wire bottom cages scientifically. See IACUC Guideline #14 https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/14.html)

☑ **N/A**

7. **Study Endpoint** (Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The IACUC must consider the selection of the most appropriate endpoint(s). This requires careful consideration of the scientific requirements of the study, expected and possible adverse effects research animals may experience (pain, distress, illness, etc.), the most likely time course and progression of those adverse effects, and the earliest most predictive indicators of present or impending adverse effects. Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity as endpoints since they minimize pain and distress. **The use of death of the animal as an endpoint is strongly discouraged and must be justified to the IACUC - Reference IACUC guideline # 13** https://www.k-state.edu/comply/iacuc/aop-assurances/guidelines/13.html. Please describe the endpoint of your study):

The endpoint of the study is euthanasia on the last day of the study at the end of the post challenge observation period (between days 14 - 17 post challenge). Animals that are clinically ill and with severe clinical signs post challenge will be euthanized. Moribund animals will be euthanized immediately.

8. **Euthanasia:** (Reference the AVMA Guidelines for the Euthanasia of Animals: 2020 Edition, link available on the KSU IACUC or the AVMA website, https://www.avma.org/KB/Policies/Pages/Euthanasia-Guidelines.aspx)

Will animals be euthanized as a part of your protocol? ☑ **Yes** ☐ **No**

i. **Method** (include drug, dosage, and route)

Prior to euthanasia, cats will be anesthetized. Cats will be anesthetized with 12 mg/kg of Telazol administered IM or, alternatively, with 0.4 mg/kg of Dexmedetomidine administered IM. Up to 40 ml of blood will be collected at final bleed prior to euthanasia from jugular or saphenous vein or by IC. Euthanasia performed with Pentobarbital Sodium (Trade name Fatal plus, 390 mg /mL), dose 85-100 mg/kg (approx. 1-1.2 ml/10 lbs.) administered IV to effect. Alternatively, cats may be euthanized through intracardial injection (IC).

ii. **Name of person(s) responsible for performing the euthanasia.**

Juergen Richt, Igor Morozov, Daniel Madden, Chester McDowel

9. **Animal Disposition** (what is your plan for the animals after the study is over?)

☑ **Euthanasia** ☐ **Adoption** ☐ **Long-term holding**

☐ **Transfer to another investigator with approved or pending protocol.**

Name:

☐ **Other**

VII. Investigator & Technician Qualifications/Training: List all persons involved in your activity below - excluding CMG and LACS personnel - and their professional training. **Include years of experience with the specific species and procedures listed in this proposal.**
Contact the University Research Compliance Office, 532-3224 for information or guidance on animal care and use training

Name	Training and experience with the species and procedures listed in this protocol
Juergen Richt	DVM, PhD, 5 years of working with cats as a practicing veterinarian, 2 recent cat studies at the BRI; IBC and IACUC online training modules; BRI specific training
Igor Morozov	DVM, PhD, at least 3 years of experience of working with cats during vaccine trials; IBC and IACUC online training modules; BRI specific training
Taeyong Kwon	DVM, MS, Research Assistant; limited work experience with cats (2 animal studies at the BRI); IACUC and IBC online training modules; BRI specific training
Natasha Gaudreault	PhD, Research Assistant Professor; no cat work experience; IACUC and IBC online training modules; BRI specific training.
Jessie Trujillo	DVM, PhD, Senior Research Associate; no cat work experience; IBC and IACUC training modules; BRI specific training.
Sabarish Indran	DVM, Post Doc; limited work experience with cats (2 animal studies at the BRI); IACUC and IBC online training modules; BRI specific training.

Daniel Madden	DVM, Graduate Research Assistant; DVM directed training; significant experience of cat handling, restraining and vaccinating during vet school; two cat studies at the BRI; IBC and IACUC online training modules; BRI specific training.
Dashzeveg Bold	DVM,Graduate Research Assistant; limited work experience with cats (2 animal studies at the BRI). One on one training from CMG or project DVM; IBC and IACUC training modules; BRI specific training
Velmurugan Balaraman	Post Doc Fellow; limited work experience with cats (2 animal studies at the BRI); IBC and IACUC training modules; BRI specific training
David Meekins	Post Doc Fellow;limited work experience with cats (2 animal studies at the BRI). IBC and IACUC training modules; BRI specific training.
Bianca Libanori Artiaga	PhD, Post Doc Fellow, Limited work experience with cats (2 animal studies at the BRI). IBC and IACUC training modules; BRI specific training.
[redacted]	MS, Research Assistant; Limited experience working with cats (2 animal studies at the BRI). IACUC and IBC online training modules; BRI specific training
[redacted]	BS. Research Assistant; Limited experience working with cats (2 animal studies at the BRI). IBC and IACUC online training modules, BRI specific training.
Chester McDowell	DVM, MS. Graduate Research Assistant. Significant experience of cat handling, restraining and vaccinating during vet school; 2 cat studies at the BRI; IACUC and IBC online training modules; BRI specific training.
Yonghai Li	DVM, PhD; no experience working with cats; IACUC and IBC online training modules; BRI specific training
Jemie Henningson	DVM, PhD, Diplomate American College of Veterinary Pathologists; IBC and IACUC training modules; BRI specific training. Studies pathologist (necropcies).
William "Bill" Wilson	PhD, Microbiologist/Animal Scientist, more than1 year of cat handling experience (several studies, including two studies at the BRI in 2020); IBC and IACUC training modules; BRI specific training

The IACUC is required to review and approve changes in personnel for research or teaching involving animals. **Consequently, you must inform the IACUC (via protocol modification) of any changes in animal care research personnel that may occur in your activity. Additionally, you must ensure that new personnel involved in your activity are qualified, have completed the mandatory animal care and use training, and are enrolled in the occupational health and safety program.

☑ **Yes** ☐ **No** **Will personnel be trained in humane handling of this species?**

☑ **Yes** ☐ **No** **Are all personnel enrolled in the KSU Animal Worker Occupational Health and Safety Program?**

(If no, forms can be downloaded from http://www.k-state.edu/research/comply/iacuc/ohsp or you may contact the University Research Compliance Office (2-3224) for information.)

☐ **Yes** ☑ **No** **Will you need animals for protocol-related training purposes, i.e., experimental or surgical technique development or refinement, etc.?** If yes, please specify the technique or procedure to be performed during training (you may reference detailed description in another section of the proposal if appropriate):

Number of animals required to accomplish the proposed training (be sure to include the number of animals requested for training purposes in the total number of animals listed in Section V.D., and Section VI.A.):

Please indicate how training is/will be accomplished:

☑ **Yes** ☐ **No** **Training and/or orientation with P.I., CMG or LACS personnel**

☐ **Yes** ☑ **No** **Instruction by supervising animal caretaker**

☑ Yes ☐ No **Viewing of instructional videos**

☑ Yes ☐ No **Other (please specify)**

Training on handling, restraining and blood collection from cats.

☑ Yes ☐ No **Individual Technical Procedure Training Form.**

if you marked no, explain below how you are going to document training or technical competence for personnel to perform the procedure(s) proposed.

If you are proposing to use a technical, manipulative, or invasive procedure on animals as part of your activity, it is a requirement that you document the competence of your staff to perform the proposed procedure. Documentation of training is necessary for all personnel for specific animal use procedures such as handling, stomach tubing, euthanasia, injections, biopsy, phlebotomy, restraint, etc. This formal training documentation should be maintained in the laboratory or close by and be readily available for IACUC, USDA, AAALAC, OLAW and research compliance review as appropriate. It is the PI's responsibility to ensure that adequate training is performed, and documented. If you need assistance with training for technical procedures, contact the attending veterinarian (532-5648) or the university veterinarian (532-3224) for advice or assistance.

VIII. Hazardous or Potentially Hazardous Material Use: (explain if you are using hazardous or potentially hazardous materials in your study)

1. ****Biological, Infectious or Toxic agents** ☐ No ☑ Yes (list)

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

2. ****Recombinant or synthetic nucleic acid molecules** ☑ No ☐ Yes (list)

3. **Hazardous chemicals** ☑ No ☐ Yes (list)

4. **Radioisotopes** ☑ No ☐ Yes (list)

5. **Other (example: Nanoparticles)** ☑ No ☐ Yes (list)

6. ****Select Agents:** Are you using or planning to use agents listed in the Federal Select Agent Program. (http://www.selectagents.gov/SelectAgentsandToxinsList.html)?

☑ No ☐ Yes (list)

The Federal Select Agent Program (www.selectagents.gov), a joint program of the Centers for Disease Control and Prevention (CDC), and the USDA Animal and Plant Health Inspection Service (APHIS), oversees the activities of possession, use and transfer of biological agents and toxins that have the potential to pose a severe threat to public, animal or plant health, or to animal or plant products.

If you plan to use or are using any of the viruses, bacteria, fungi, rickettsial agents, or toxins on the select agent list, please contact the K-State Responsible Official for select agent use at the Biosecurity Research Institute (785-532-3248), or the URCO (785-532-3224) for information.

(If "yes" you must have a Registration Document from the Institutional Biosafety Committee)**

IBC Registration Document # 1460 **Approval Date** 04/09/2020

IX. DEA Controlled Substances: Guide For the Care and Use of Laboratory Animals "All those involved in animal care and use must comply with federal laws and regulations regarding human and veterinary drugs and treatments."

☒ Yes ☐ No Are you using any DEA controlled substances in this protocol? If "Yes" please list them below.

Pentobarbital sodium, Telazol

Name of DEA Registration Certificate Holder Juergen Richt

X. Extramural Funding: (It is critical that animal care and use procedures detailed in the IACUC protocol are consistent with external funding proposals documents. Discrepancies between the two documents in animal care and use procedures could jeopardize individual and/or institutional funding and compliance. If you make changes, or they are required by the IACUC, it is your responsibility to ensure that grant or funding agencies are informed.)

☑ Yes ☐ No All animal care and use procedures described in this proposal are consistent with those described in external funding applications/documents. If no is checked, please contact the URCO (532-3224).

☐ **N/A**

XI. Clinical Research:

☐ **Yes** ☑ **No** Does this protocol involve client owned animals?

If yes, have you attached a copy of the client consent form to be used? ☐ **Yes** ☐ **No**

Please visit: URCO - IACUC - Resources - Protocol Development https://www.k-state.edu/comply/iacuc/resources/index.html For client consent form examples/templates.

If the protocol is associated with the veterinary health center, please consult Kris Richardson, (785)532-3046 krichardson@vet.k-state.edu, **prior to submitting a client consent form to the IACUC committee for review.**

Date of Consult with Kris Richardson

XII. USDA Regulated Activities: (Is your activity regulated by provisions of the Animal Welfare Act?) Contact the URCO or the attending veterinarian if you need clarification.

Regulated animals would include: - Any live or dead dog, cat, monkey, guinea pig, hamster, rabbit, or warm-blooded animal used for biomedical research, teaching, testing, experimentation, or exhibition purposes. Exemptions to this definition are listed below.

Exempt or non-USDA regulated animals would include: (1) lab rats and mice (*Mus / Rattus*) bred for use in research, (2) birds, (3) horses not used for (biomedical) research purposes, and (4) other farm animals such as, livestock or poultry, used or intended for use as food or fiber, or improving animal nutrition, breeding, management, production efficiency, or for improving food or fiber quality.

☑ **Yes -** My activity involves species **COVERED** by the definition of animal in the Animal Welfare Act.

☐ **No -** My activity involves animals that are **EXEMPT** from coverage by the USDA

☐ **Both** - My activity involves both covered and exempt species.

XIII. Wildlife or Field Investigation:

☐ **Yes** ☑ **No** Does your activity involve the use or observation of nondomesticated vertebrate species under field conditions?

If "Yes," please answer the following:

☐ **Yes** ☐ **No** Does your wildlife field activity require any international, federal, state or local permits? If "Yes" please provide copies of permits.

☐ **Yes** ☐ **No** Are you using any relevant professional society guidelines that are available for your wildlife field activity?

If "Yes," please list:

Online Required Training

TRAINING REQUIREMENTS HAVE RECENTLY CHANGED

The IACUC requires mandatory training prior to protocol approval. Training is now offered through the Collaborative Institutional Training Initiative (CITI) Program. Instructions to register and access training are found on the URCO website: http://www.k-state.edu/research/comply/

Use the check boxes below to select the training courses that apply to this protocol. If you have any questions about training, contact URCO at comply@k-state.edu, or (785) 532-3224.

Mandatory Training

Required for all Principal Investigators, research staff and students

☒ Responsible Conduct of Research ☒ Working with the IACUC

Required (Provost-mandated) for all full time K-State employees

☒ Export Compliance

Species-specific training (check all that apply to this protocol)

☐ Swine ☐ Cattle ☐ Rat ☐ Mouse ☐ Guinea Pig ☐ Hamster ☐ Ferret

☐ Dog ☒ Cat ☐ Horse ☐ Gerbil ☐ Sheep or Goat ☐ Rabbit ☐ Zebrafish

☐ Fish (except zebrafish) ☐ Amphibians ☐ Wildlife (except fish) ☐ Farm Animals or Agricultural Animals

Required procedure-specific training (check all that apply to this protocol)

☐ Survival Surgery

☐ Rat or Mouse, Category D or E procedures

☐ Antibody Production

All new personnel or personnel with expired training are required to register for CITI and take the new training requirements. If you previousiy completed oniine IACUC modules, your training status will remain current until it expires. URCO will verify training from the previous system as well as the new system prior to approval of any protocol.

POST APPROVAL MONITORING: The URCO has a Post-Approval Monitoring (PAM) program to help assure that animal care and use activities are performed in accordance with provisions or procedures approved by the IACUC. Accordingly, the URCO staff will arrange PAM visits as appropriate to assess compliance with approved activities.

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LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

EXHIBIT D

Protocol 42593 Amendment 3.1

Approval date	03/18/2024
Expiration date	08/11/2026

1. Basic Information

1. Elements ID

For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

2. eACUC Number (Automatically Assigned)

42593

3. Principal Investigator

Pitts, Teresa Greene

Job title	PROF, ASOC
Department	Speech Lang & Hearing Sci
Division	Health Sciences
Business unit	University of MO-Columbia

4. Protocol Title

1) Regulation of swallow and the effects of high cervical spinal cord injury and 2) Influence of Opioids on the Brainstem Respiratory Network Central and Peripheral Regulation of Laryngeal Adduction

5. Triennial Re-write

Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

○ Yes ● No

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

Species	Strain/ Stock/Breed	Age/ Weight	Pain/Distress Category	Authorized	Ordered	Received	Adjustment	Available
Cat	Any breed	adult	USDA Category D	91				91
Total Cats:				91	0	0	0	91
Guinea Pig	any breed	adult	USDA Category D	300				300
Total Guinea Pigs:				300	0	0	0	300

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

○ Yes ● No

3. Proposal Overview

1. Purpose

Purpose of the study:

The long-term goal of this project is to determine the role of tracheal, pharyngeal, and spinal sensory feedback in the regulation of cough and swallow.

Our central hypothesis is that an aspiration event produces a series of coughs and swallows which are expressed in various behavioral interaction patterns, and that there are decipherable rules that regulate the various patterns of expression. Cough and swallow are airway protective behaviors. The pharyngeal phase of swallow prevents aspiration of oral material (saliva, food and liquid), by epiglottal movement, laryngeal adduction, and clearing the mouth and pharynx. Cough is an aspiration-response behavior that clears material from the airway. Coordination of these behaviors is vital to protect the airway from further aspiration-promoting events, such as a swallow occurring during the inspiratory phase of cough. The peripheral inputs, operational characteristics, and primary strategies that coordinate cough and swallow are unknown. This lack of information impedes understanding of the deficits in airway protection, with co-occurrence of dystussia and dysphagia, which occurs with diseases such as Parkinson's disease and Alzheimer's disease.

The Specific Aims of the project are: 1) Identify the operational principles that govern the coordination of cough and swallow motor patterns following activation of tracheal and pharyngeal afferents during an

aspiration event; 2) Develop a predictive computational distributed network model of the central influence of tracheal sensory pathways on the expression of cough and swallow; 3) Identify the role of neurons in the reticular formation in processing tracheal and pharyngeal afferent feedback on cough and swallow; 5) Identify the effects of cervical spinal cord disruption on the swallow motor pattern; and 6) Determine the therapeutic efficacy of 5-HT1A agonists on recovery of swallow function after spinal cord injury.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

A variety of neuromuscular/ neurotraumatic diseases result in impaired airway protection including breathing, swallow and/or swallow function.

Impairment of these airway protective behaviors results in an increase in pulmonary infections due to aspiration. Pulmonary complications related to inadequate airway defense are the leading cause of death in patients with spinal cord injuries and Parkinson's disease. The long-term goal of this project is to determine brainstem and spinal mechanisms that control the regulation of airway protection. Our central hypothesis is the brainstem is reliant on feedback from the spinal cord to regulate airway protective behaviors. Loss of this information creates mis-timed swallows, laryngeal dysregulation and increases aspiration risk. The cat and guinea pig models are essential to the study. These animals exhibit the same cough and swallow reflex as humans, even when they are anesthetized.

Currently there are no effective treatments for swallow disorders beyond the limited effects of exercise. This work provides the foundation to translate experimental models into clinical effects.

3. Lay Term Description of Experimental Design

To put something in layman's terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This section should be written so that someone with a **10th grade science education can easily understand the project.**

There are several disorders that can hurt a person's ability to swallow properly and to coordinate swallow with breathing. The airway is normally protected by several reflexes, which include swallow and airway closure. When the airway is not properly protected, food and liquids can invade, and can enter the lungs to cause breathing distress and dangerous infections (pneumonia). Such complications are the leading cause of death in patients with spinal cord injuries and Parkinson's disease. The long-term goal of this project is to determine the processes in the lower brain and spinal cord that control the regulation of airway protection. Our main hypothesis is that the lower brain relies on input from the spinal cord to regulate airway protection. Loss of this information creates mis-timed swallows, disorder of muscles at the opening of the airway, and increases risk of food and liquids entering the lungs. It is necessary for us to use cats or guinea pigs in these experiments because, unlike rodents, they have the all of the same cough and swallow reflexes as humans, even when they are anesthetized. Currently there are no effective treatments for swallow disorders beyond the limited effects of exercise. This work provides the foundation to translate animal work into strategies that can help humans.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

The substances used for the general procedure are as follows: Sevoflurane or Isoflurane will initially be administered through box-induction then transitioned into mask inhalation after loss of consciousness. The

animals will then be weaned onto sodium pentobarbital through a forelimb radial vein intravenous injection (no catheter). Later, as part of the experimental protocol, the femoral vein will be catheterized, and anesthetic doses and (supplemental doses as needed) will be given through the femoral IV catheter. Atropine sulfate will be given as needed to maintain mucous secretion. Decrease in total blood pressure or a reduced pulse pressure (from outside the normal physiological range) will prompt the use of lactated ringers solution into the femoral IV line. Arterial blood gas showing a metabolic acidosis (secondary to surgery, etc) will be treated with administration of sodium bicarbonate through the femoral IV line. Heparin pork will be placed in saline to clean the femoral IA line after blood collection for blood gas measurement. Lidocaine will be used before placement of the ear bars only one time during the experiment. Doxapram HCL, Baclofen, Kynurenic Acid, Isoguvacine, Lidocaine, Cobalt Chloride, Substance P, Glutamate, Allostatin, Saporin, capsaicin, citric acid, acetic acid, and Codeine will be used as needed to suppress cough and/or swallow depending on the experimental protocol.

1) Modulation of airway reflexes -- terminal electrophysiology experiments: Adult cats or guinea pigs may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats or guinea pigs will be anesthetized to a surgical level of anesthesia. Electromyographic recordings of swallow and breathing muscle activity will be made during stimulation of airway reflexes including cough and swallow. Crainiotomy and/or laminectomy will be performed. Stimulations will be performed again. Microinjection and/or infusion of experimental compounds may be administered, then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e.g., 1 hour, 2 hours, 3 hours and 4 hours). In some animals we may elect to stimulate the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. In some animals an acute spinal cord injury will be performed and then the stimulations will be repeated (as described above). At the end of the experiment, anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo perfusion. In some animals, cats or guinea pigs will be given buprenorphine prior to the terminal electrophysiology procedure. Condition A: 40 cats and 110 guinea pigs will undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition B: 30 cats and 70 guinea pigs will undergo an acute spinal cord injury and undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition A (40) + Condition B (30) = 70 cats - 25 cats already completed = a total of 45 cats requested. Condition A (110) + Condition B (70) = 180 guinea pigs requested (none yet completed).

2) Neuron Recording -- terminal electrophysiology experiments: Adult cats or guinea pigs may have their swallow (via videofluoroscopy) or laryngeal function tested prior to the terminal experiments outlined below. This will occur at least 2 days prior to the terminal procedure. Adult cats or guinea pigs will be anesthetized to a surgical level of anesthesia. Electromyographic recordings of swallow and breathing muscle activity will be made during stimulation of airway reflexes including cough and swallow. Crainiotomy and/or laminectomy will be performed. Recording electrode/s will then be placed in targeted areas of the brainstem and/or spinal cord to extracellularly record from neurons of interest. Then stimulations including: cough, swallow, alteration in respiratory state, or laryngeal cooling will be assessed at standard time points depending on the half-life of the compound (e.g., 1 hour, 2 hours, 3 hours and 4 hours). In some animals we may elect to stimulate the superior laryngeal nerve or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended. In some animals swallow/cough may be suppressed. In some animals an acute spinal cord injury will be performed and then the stimulations will be repeated (as described above). At the end of the experiment, anesthetic level will be checked and confirmed for a surgical level of anesthesia and the experimental animal will then either be euthanized or undergo

perfusion. This protocol will use 50 cats - 4 animals already completed = a total of 46 cats requested, and 120 guinea pigs requested (none yet completed).

4. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

In vivo, there are multiple neural circuits and large variety of cells, muscles, and nerves which contribute to the control of airway protective behaviors. The cat and guinea pig are models used for assessing coordination of breathing and airway behaviors, allowing us to make relevant comparisons within the field. Similarly, the airway protective muscles exhibit a number of functional and anatomical similarities to humans. We will work to reduce the number of animals by assessing multiple behaviors and completing multiple protocols on each animal used. No suitable alternatives have been identified to replace the use of live animals for the studies associated with this protocol. The lack of suitable alternatives was determined by a continual review of the literature on a weekly bases.

2. Justify Animal Species

Justify the choice of species for your study.

Adult cats and guinea pigs are used for three reasons: (a) While swallow has been assessed using videofluoroscopy in various rodent models of disease, aspiration has never been seen. Russell, et al. (Dysphagia, 28; 95-104; 2013) thoroughly discussed how the anatomical specifics of the rodent do not make aspiration or its use as an outcome measure possible. (b) The cat has been used extensively as a model system for studying vertebrate motor control, including cough, which is a critical respiratory-related defense with similarities to swallow. The extensive literature on feline motor control during breathing and cough, including our own studies, supports the importance of this model for translation. Relative to other species, a large amount of information exists on the physiology and pharmacology of airway protection and vagal afferents in the cat and guinea pig. Further, because the rat lacks airway protective behaviors such as cough and/or vomiting, use of cats and guinea pigs affords greater translational potential for the current study. (c) The complex instrumentation during some of the terminal electrophysiology procedures requires that larger animals be used.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total numbers should match the requested numbers in the species section.

- Animal Numbers Justification
- The Logical Determination of "N" in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

This protocol is for 91 cats and 300 guinea pigs altogether.

Modulation of airway reflexes - terminal electrophysiology:
Three conditions will be needed to complete this specific aim. Condition A: 40 cats and 110 guinea pigs will undergo airway reflex testing and modulation with administration of compounds via infusion or

microinjection. Condition B: 30 cats and 70 guinea pigs will undergo an acute spinal cord injury and undergo airway reflex testing and modulation with administration of compounds via infusion or microinjection. Condition A (40) + Condition B (30) = 70 cats. Condition A (110) + Condition B (70) = 180 guinea pigs.

We have completed 15 cats from Condition A and 10 cats from condition B. These have been removed removed from the cat sample size calculation. For a total of 45 cats. These sample size calculations are based on % change in our previous studies in EMG, with an alpha level of $p< 0.05$ and a power of 0.9.

Neural Recording - terminal electrophysiology:
The historical yield for these types of experiments is that 5% of total neuron pairs produce features that suggest functional interactions between the neurons. Given the complex nature of this network, as well as the fact that we will study a population of neurons about which there is little published information, we anticipate approximately 1700 pairs with evidence of functional interactions. Therefore, we estimate that the minimum number of neuron phenotypes that we will encounter in these experiments is 19, representing (19)(18)/2=171 possible combinations. Allowing for at least ten examples of each yields a total of 3,400 pairs with significant functional interactions. This number represents 5% of the total pairs required, which is 3,400/0.05=68,000 pairs. We anticipate a yield of approximately 350 pairs per cat or 68,000 pairs/350 pairs per cat=194 total cats for the project. Yields will be lower for guinea pigs due to the smaller area available for placing electrode arrays. Fifty cats and 120 guinea pigs will be used to complete this protocol. Should our yields per animal be higher than anticipated, fewer than 50 cats and 120 guinea pigs will be used. We have completed 4 cats from this experiment, for a total of 46 cats and 120 guinea pigs needed.

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5. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

○ Yes ● No

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

● Yes ○ No

A. Description of Transportation

Describe how animals will be moved, including caging/transport carriers used (covered microisolator cages, dog/cat carrier, etc.), type of transport used (i.e. personal car or van), and projected transport time. (Note: Animals should be transported in a temperature controlled environment.)

Animals will be transported in commercial cat carrier cages using personal cars in which temperature is controlled. Projected transport time is less than 10 minutes.

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

○ Yes
○ No
● Overnight only

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

☐ Single housing of social species
☐ Wire-bottom cages
☐ Special diet/water
☐ Extended time to weaning
☐ Extended time between cage changes
☐ Alternative light cycles
☐ Out of range temperatures
☐ Cage-size exceptions
☐ Other

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6. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

● Yes ○ No

A. Sample Type

Type of sample(s):

Blood

B. Sample Volume

Volume of sample(s):

1 mL per sample for cat; 0.15 mL per sample for guinea pig: arterial blood gas sampling during terminal electrophysiology experiments

C. Sampling Frequency and Duration

Frequency of collection and for how long:

Every 60-90 minutes for the duration of the experiment (1-18 hrs)

D. Sampling Method

Method of collection:

Femoral artery catheter

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

	Procedure	Description of procedure	Building name	Room number or area
1	Laryngeal Imaging	In some animals we will choose to image their vocal folds (larynx). Animals will be anesthetized and a laryngoscope will be placed to lower the epiglottis to view the vocal folds. Then a flexible endoscope with video capabilities will be run along the laryngoscope. Video will be taken on the larynx during breathing, and some animals will alter respiratory state (as described above). Video will be recorded through out the procedure. In some animals we may give buprenorphine IM ~ 1hour before beginning the evaluation. Animals will be returned to the Veterinary Medicine facility after the procedure.	[redacted]	Room not yet assigned - will be done in a lab

7. Substances Used in Animals

1. Substances Used in Animals

List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
1	Doxapram HCL	0.001-30mg/ Kg	IV, IA (femoral or vertebral artery), Microinjection	1-10x per hour; 1-3 hours	No	No
2	Baclofen	0.03-1,000ug/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
3	Codeine	0.001-30mg/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
4	Cobalt Chloride	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
5	Isoguvacine	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
6	Capsaicin	0.016-50 µM	IV, IA (femoral or vertebral), Microinjection, aerosolized in ethanol and saline and placed in the upper and or lower airways	1-10x per hour; 1-3 hours	No	Yes
7	Citric Acid	0.125-32%	IV, IA (femoral or vertebral), Microinjection, aerosolized in ethanol and saline and placed in the upper and or lower airways	1-10x per hour; 1-3 hours	No	Yes
8	Substance P	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
9	Glutamate	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
10	Allostatin	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
11	Saporin	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No

	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
12	Kynurenic Acid	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour; 1-3 hours	No	No
13	8-OH-DPAT	0.03-300ug/Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour, or via infusion to maintain a steady concentration; 1-18 hours (duration of experiment)	No	No
14	WAY-100635	0.03-3000ug/ Kg	IV, IA (femoral or vertebral), Microinjection	1-10x per hour, or via infusion to maintain a steady concentration; 1-18 hours (duration of experiment)	No	No
15	Buspirone	1-10mg	PO	Up to 3 doses per day (i.e. 1hr before feeding or assessment); or 1 dose prior to terminal experiment	No	Yes
16	Artificial Cerebrospinal Fluid	0.01-1mL	CNS microinjection or intravertebral artery	1-10x per hour; 1-3 hours	No	Yes
17	Saturated KCl	3 cc	IV	1 dose; 1x per procedure	No	Yes
18	Acetic Acid (vinegar)	1-5%	Topical on mucosa in the upper and/ or lower airway	1-10x per hour; 1-3 hours	No	Yes
19	Thrombin (bovine)	10-1000 units/ mL	Applied to surface of skin, muscle or bone	To control bleeding when cauterization is not an option; applied until bleeding is controlled	No	No
20	Buprenorphine	0.01-0.05 mg/ kg	IM	For an opioid study: buprenorphine IM at a dose of 0.02 mg/kg or 0.03- 0.04 mg/kg ~q12 hours for ~48 hours. May be repeated after 1 week with the lower/ higher dose.	No	Yes

	Substance	Amount/ Dose/Volume	Route	Frequency/Duration	Hazard	Pharmaceutical Grade
21	Carbon dioxide	up to 10%	Inhalation	once initially, repeated as indicated by hypercapnic challenge protocol	No	No
22	Sodium Nitrite	0.1-1% solution / 1 cc of 1% solution	IV	Used only once during terminal perfusion	No	No
23	Paraformaldehyde	4% solution / 3-4 L	Intracardial perfusion	Used only once during terminal perfusion	Yes	No

2. Non-Pharmaceutical Grade Substances

For those substances that are marked "no" as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

Only agents with certification of purity will be used, artificial cerebrospinal fluid or saline will be used to ensure physiologic compatibility, and formulations will be filtered and pH adjusted to 7.4.

For drugs that are microinjected or given as an "experimental" pharmacological agent in non- survival procedures: For these purposes it is necessary to have very small concentrations and have the drug dissolved into artificial cerebral spinal fluid. This is not commercially available in pharmaceutical grade. Often the experimental procedure calls for administration of the drug through various routes in the same animal and for comparison sake, the same lot of drug is needed for all routes of administration.

We have been unable to access pharmaceutical-grade thrombin, but thrombin is rarely used in these terminal experiments.

Sodium Nitrite and paraformaldehyde are used in a deeply sedated, non-responsive animal within 1-3 minutes of death. Thus, we use chemical grade solutions that we mix in the laboratory. We use an overdose of Sodium Nitrite to vasodilate the vasculature immediately prior to introduction of the transcardial perfusion solution of 4% Paraformaldehyde in a buffer system (pH 7.4). A sterile option for Paraformaldehyde is not available.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., "All substances" or "non-hazardous substances") if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

	Substance	Gloves	Eye Protection	Lab Coat	Face Mask	Fume hood	Biosafety cabinet	Double-Gloves	Other	Other PPE
1	All substances	☑	☐	☑	☐	☐	☐	☐	☐	
2	Paraformaldehyde	☑	☑	☑	☑	☑	☐	☐	☐	

Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

8. Hazardous Materials

1. Will you use any Biological Hazards?

○ Yes ● No

2. Will you use any Chemical Hazards?

● Yes ○ No

A. Chemical Hazards

List all chemical hazards that will be used in live animal work.

	Agent or type of hazard	Receiving species	Dose	Route/Volume of Admin.	Frequency of Admin.	Other
1	Paraformaldehyde	cat	4% solution / 3-4 L	Intracardial perfusion	Used only once during terminal perfusion	
2	Paraformaldehyde	guinea pig	4% solution / 1-3 L	Intracardial perfusion	Used only once during terminal perfusion	

B. Is this an FDA Approved Drug?

○ Yes ● No

C. Chemical Hazard - Anticipated Effect(s)

List any anticipated effect(s) of hazardous chemical on animal.

This is used near the time of death during terminal perfusion.

D. Chemical Hazard Housing/Procedure Sites

	Agent	Receiving Species	Building	Room or area	Housing	Procedure
1	Paraformaldehyde	cat	Dalton (DCRC)	310	☐	☑
2	Paraformaldehyde	guinea pig	Dalton (DCRC)	310	☐	☑

E. Chemical Hazard - Animal Identification

Explain how animals treated with a chemical hazard will be identified (ex. cage card, ear tag, etc.)

☐ Cage Card

- ☐ Chip
- ☐ Door Sign
- ☐ Other

F. Chemical Hazard - By-Products/Presence

- ☐ None
- ☐ Feces/Urine/Bedding
- ☐ Saliva
- ☐ Blood
- ☐ Aerosols
- ☐ Animal Bite/Scratch
- ☐ Animal Carcasses/Tissues
- ☐ Surgical Site Wound or Sore
- ☐ Other

G. Chemical Hazard - Personal Protection Equipment (PPE)

PPE to be worn when handling chemical hazards include: (ex. safety glasses, surgical mask, etc.)

	Chemical Agent	Gloves	Eye Protection	Lab Coat	Double-Gloves	Face Mask	Fume hood	Other	Other PPE
1	Paraformaldehyde	☑	☑	☑	☐	☑	☑	☐	

H. Additional Information

List additional information, i.e., special precautions for pregnant women, immunocompromised individuals, special handling, or storage, etc.

3. Will you use any Radiation Hazards?

○ Yes ● No

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9. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

● Yes ○ No

2. Preanesthetic Agent(s)

List preanesthetic agents here

No Preanesthetic Agents...

3. Anesthetic Agent(s)

List anesthetic agents here. **Do not list isoflurane here, it will be listed later in the form.**

	Species	Agent	Controlled Substance	Dose/ Volume	Route	Frequency of Admin.
1	Cat	Sevoflurane	No	0.1-5%	Inhalation	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment)
2	Cat	Sodium pentobarbital (Nembutal or pharmaceutical grade generic)	Yes	35 mg/ kg	IV	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment). Initial dose is 35 mg/kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose will always be given
3	Cat	Lidocaine HCl 2%	No	2-4 mg/ kg	Topical, subcutaneous	Subcutaneous: once injected around each ear for nerve block; Topical: anesthesia of the larynx or trachea; 30 minute duration of effect.
4	Guinea Pig	Sevoflurane	No	0.1-5%	Inhalation	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment
5	Guinea Pig	Sodium pentobarbital (Nembutal or pharmaceutical grade generic)	Yes	20 mg/ kg	IV	As needed to maintain stable anesthetic depth; 1-18 hours (duration of experiment). Initial dose is 20 mg/kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose will always be given
6	Guinea Pig	Lidocaine HCl 2%	No	2-4 mg/ kg	Topical, subcutaneous	Subcutaneous: once injected around each ear for nerve block; Topical: anesthesia of the larynx or trachea; 30 minute duration of effect.

4. Isoflurane Use

A. Will you use isoflurane?

● Yes ○ No

B. What species?

cat, guinea pig

C. How will it be administered?

- [x] Vaporizer with nose cone
- [ ] Vaporizer with endotracheal tube
- [x] Induction box connected to vaporizer
- [ ] Open drop method (bell jar, etc.)
- [ ] Other

5. Non-Pharmaceutical Grade Anesthesia

If non-pharmaceutical grade anesthesia must be used, strong scientific justification must be provided. In addition, describe how the solution will be made and stored. Also, describe how pH, osmolarity, and sterility will be kept in acceptable physiological ranges. Please see ACUC policies for Anesthesia and Non-pharmaceutical Anesthesia and ACUC Guidelines for pentobarbital, avertin, inactin, and urethane/chloralose.

6. Monitoring and Life Support

Monitoring and life support systems to be utilized to ensure adequate depth of analgesia or anesthesia and to prevent overdose:

Body temperature measurement and support using temperature-measuring probe.
Use of intra-procedural fluids.
Anesthetic depth checked at intervals no less than 15 minutes.
Anesthetic depth verified by withdrawal reflex (toe/tail pinch, eye blink, and/or jaw tone).
Monitoring heart and respiratory rates.

7. Post Anesthetic Recovery

Complete description of post-anesthetic recovery monitoring and care:

Animals will be anesthetized with isoflurane for laryngeal imaging tests. After the testing and withdrawal of anesthesia, animals will be monitored until they are fully alert and ambulatory, prior to returning to housing.

8. Pharmaceutical Analgesia

○ Yes ● No

9. Non-pharmacologic control of pain

○ Yes ● No

10. Paralytic Agents

○ Yes ● No

11. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

● Yes ○ No

12. Antibiotics and Other Agents

List other agents such as antibiotics and other emergency drugs

	Species	Agent	Dose/ Volume	Route	Frequency of Admin.
1	Cat	Lactated Ringers Solution +/- 5% sucrose	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
2	Cat	Normal saline	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; also used to flush IV and IA lines; 1-18 hrs (duration of experiment)
3	Cat	Atropine Sulfate	0.01-10 mg/ kg	IV or IM	Before tracheostomy and as needed based on blood pressure measurement; 1-18 hours (duration of experiment)
4	Cat	Sodium Bicarbonate	0.2 x kg x base deficit	IV	As need based on base deficit from blood gas measurements; 1-18 hours (duration of experiment)
5	Cat	Heparin Pork	1MU/mL	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
6	Guinea Pig	Lactated Ringers Solution +/- 5% sucrose	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment)
7	Guinea Pig	Normal saline	5-10 mL/kg/ hr	IV or IA	As needed based on the animal's physiology; also used to flush IV and IA lines; 1-18 hrs (duration of experiment)
8	Guinea Pig	Atropine Sulfate	0.01-10 mg/ kg	SC, IV, or IM	Before tracheostomy and as needed based on blood pressure measurement; 1-18 hours (duration of experiment)
9	Guinea Pig	Sodium Bicarbonate	0.2 x kg x base deficit	IV	As need based on base deficit from blood gas measurements; 1-18 hours (duration of experiment)
10	Guinea Pig	Heparin Pork	1MU/mL	IV or IA	As needed based on the animal's physiology; 1-18 hrs (duration of experiment

10. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

● Yes ○ No

2. Surgery: Pre-surgical Prep

Describe pre-surgical preparation of the animals. Include information about fluid/food restriction, skin, and instrument prep.

Food will be withheld the morning of the surgery (4-6 hrs). The requirements for terminal surgery will be followed, including shaving the surgical site and using clean instruments.

3. Surgical Procedures

List surgical procedures (include incision location and size, tissue(s) manipulated, and closure methods and materials).

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
1	Alteration in Respiratory State	While under anesthesia, in some animals we will choose to observe how changes in the breathing pattern will effect the EMGs or neurons of interest. We will hyperventilate, (hyperventilation is the method in which we create a hypocapnic condition in the animal) the animal by putting them on a ventilator and increasing the respiratory rate (we will then run an arterial blood gas to insure the hypercapnia has been reached then conduct the cough protocol. We will also be subjecting these animals to a hypercapnic or hypoxic condition to observe this modified physiological state . This is done by supplementing the spontaneously breathing animal with up to 10% CO2.	Terminal	[redacted]	[redacted]
2	Blood collection	Every 60-90 minutes 1cc of blood will be collected from the femoral artery cannula and run for blood-gas measurement. The line will be flushed with heparinized saline. A total of 8- 22cc's of blood. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
3	Cough stimulation	Cough will be stimulated by placement of a 4-5 inch piece of small tubing caudal into the trachea to the level of the carina or rostral into the larynx and slowly rotating. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
4	Cough-swallow stimulation	The described cough-swallow stimulation will be overlapped to produce cough and swallow in sequence. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
5	Craniotomy	An occipital craniotomy will be performed and the caudal portion of the cerebellum will be removed to allow access to the brainstem. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
6	Electrical Simulation of the superior laryngeal nerve	In some animals we may elect to stimulate the superior laryngeal nerve (SLN) or the surface of the skin between the thyroid notch and the clavicle for the purposes of eliciting and/or manipulating cough and swallow. A bilateral electrode will be placed around one or both of the SLN or on the skin without cutting/ damaging the nerve. Stimulation will be at <4V and at a range from 5-500 Hz. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
7	Electromyography placement	Parasternal intercostal, diaphragm (costal and crural), laryngeal, abdominal, and pharyngeal muscles will be exposed and electrodes placed for EMG recordings of muscle activity during cough and swallow. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
8	Microinjection into the brainstem and/or spinal cord	In some animals we will microinject compounds that are known antitussives or alter areas known to participate in the control of cough and swallow into the areas of interest in the brainstem or spinal cord. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
9	Multi-electrode array	Up to three multi-electrode arrays will be placed in the brainstem or spinal cord. Electrodes will be advanced until the number of simultaneously recorded neurons is maximized for each array. Once the number of well-isolated neurons is maximized, recordings will be obtained during breathing for approximately 30 minutes. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
10	Placement of catheters	An incision will be made in the medial aspect of the right hindlimb and catheters (made of polyethylene tubing) will be placed in the artery and vein for additional venous access for fluid maintenance, supplemental anesthesia and arterial pressure observation. An incision will be made through the left armpit, the axillary artery is exposed and cannulated to allow for infusion of compounds into the vertebral artery. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
11	Placement of ear bars	In only non-survival experiments, while the animal is under general anesthesia, a local anesthetic will be infiltrated into the skin around the ear canal for analgesia. Ear bars will be placed bilaterally and the head affixed to the stereotaxic apparatus.	Terminal	[redacted]	[redacted]
12	Swallow stimulation	A 3 cc syringe will be attached to a 1 inch piece of PE-90 polyethylene tubing. 3 cc's of a water bolus will be injected into the mouth. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
13	Acute Spinal Cord Injury	The spinal column will be exposed by making an incision through the skin and then the muscle overlying the vertebra. A bilateral laminectomy will be performed to expose the underlying spinal level. The dura will be slit longitudinally and the dorsal columns and dorsal root entry zones visualized to identify the spinal midline. The spinal cord will either be: completely severed, over severed, or under severed, using iridectomy scissors or a midline incision will be made. More than one cut may be made. Any fibers left adhering to the ventral or lateral dura may be gently lifted with suction and cut with iridectomy scissors. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]

	Procedure	Description of procedure	Survival or Terminal	Building Name	Room Number or area
14	Distension	In some animals the esophagus, trachea, pharynx, stomach, and/or larynx may be distended by a balloon to test the effects of mechanoreceptors activity on cough and swallow. This may be combined with the above described cough and swallow protocols. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
15	Swallow/cough suppression	In some animals we may bathe the trachea, esophagus, larynx, and/or pharynx in capsaicin, citric acid, and/or acetic acid. This may be combined with the above described cough and swallow protocols. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
16	Laminectomy	Removal of bone covering spinal segment of interest. This is while the animal is under general anesthesia.	Terminal	[redacted]	[redacted]
17	Perfusion	Trans or intra-cardial perfusion may be performed. Initially, animals will be anesthetized with isoflurane or sevoflurane. Once anesthetized, Sodium Pentobarbital (IP, IV or IA) will be given. As needed, animals will be given one or more supplemental doses of sodium pentobarbital (typically 25% of initial dose) to achieve or maintain a deep plane of anesthesia (IA, IP or IV). They will then be given 1 cc of a blood thinner (e.g. 10% heparin, IV) followed ~20 minutes later by a vasodilator (e.g. ~1 cc of 1% sodium nitrite). Immediately following administration of sodium nitrite, they may be perfused transcardially with 0.9% saline typically followed by a fixative (eg. 4% paraformaldehyde and/or 1.25%-4% glutaraldehyde in a buffer solution (pH 7.4)). It is critical that the heart continue beating until the perfusion of fluids has begun in order to prevent collection of blood in the vascular system which interferes with post-mortem histological procedures. This is why Nembutal is the drug of choice and other euthanasia options are not good options as they rapidly stop the heart. By providing a bolus of Nembutal IP after the animal is initially anesthetized, a deep plane of anesthesia is easily maintained and the heart continues beating for a long time which allows completion of the transcardial perfusion steps required. The tissues of interest (including spinal cord, brain, leg bones, muscles, etc) will be removed after perfusion. If the heart is not beating strongly enough, an option may be exercised to introduce the blood thinner and/or vasodilator directly into the left ventricle immediately prior to starting the intracardial perfusion of saline.	Terminal	[redacted]	[redacted]

4. Surgery: Post-operative Care

Describe post-operative care (include **both short and long-term care**; monitoring, surgical wound care including suture removal, and list drugs and doses anticipated to be used).

N/A

5. Surgery: Special Needs

Special needs of the animals following surgery:

N/A

6. Surgery: Length of Time Alive

Length of time animals will be kept alive following surgery:

N/A

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11. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

Non-survival experiments: Surgical procedures include placement of catheters, tracheal cannulation, EMG placement, craniotomy, and alterations of respiratory state. Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps will be taken to re-established a surgical plane of anesthesia and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end- tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone. This will be monitored at minimum every 15 minutes. Should the adverse event occur, the response will be to halt the procedure and deliver additional anesthetic or euthanize the animal.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

Anesthesia will be maintained during all procedures to eliminate pain/distress. Lidocaine will be used to minimize the sympathetic response during placement of the ear bars.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

○ Yes ● No

12. Disposition of Animals

1. Animal Disposition

Check all that apply

- ☐ Adoption (See MU adoption policy)
- ☐ Market
- ☑ Euthanasia
- ☑ Transfer to different project, PI, or another institution
- ☐ Returns to breeding colony, herd, source, owner, or wildlife site
- ☐ Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, "Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue."

A. Method of Euthanasia

Select the method of euthanasia

- ☐ Inhalant agent
- ☐ Physical Method without Anesthesia
- ☑ Physical Method with Anesthesia
- ☑ Noninhalent Pharmaceutical Agent

B. Euthanasia Descriptions

	Species	Agent/Method	Dose/Volume	Route
1	Cat	Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
2	Cat	General anesthesia as described for experiments, followed by an adjunctive physical method such as bilateral thoracotomy, exsanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.	Doses according to general anesthesia with sodium pentobarbital, sevoflurane, isoflurane, Beuthanasia aka Euthanasia III Solution	IV or inhaled
3	Cat	General anesthesia as described for experiments, followed by intravenous or intracardial injection of potassium chloride (KCl, at least 75-150 mg/kg).	3 cc of saturated KCl following general anesthesia	IV
4	Cat	General anesthesia as described for experiments, followed by Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
5	Guinea Pig	Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver, or kidneys) removal.	Initial dose is 20 mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV
6	Guinea Pig	General anesthesia as described for experiments, followed by an adjunctive physical method such as bilateral thoracotomy, exsanguination or vital organ (brain, heart, lungs, liver, or kidneys) removal, decapitation, or perfusion.	Doses according to general anesthesia with sodium pentobarbital, sevoflurane, isoflurane, Beuthanasia aka Euthanasia III Solution	IV or inhaled

	Species	Agent/Method	Dose/Volume	Route
7	Guinea Pig	General anesthesia as described for experiments, followed by intravenous or intracardial injection of potassium chloride (KCl, at least 75-150 mg/kg).	1-2 mEq/kg (equivalent to 75-150 mg KCl/ kg) following general anesthesia	IV
8	Guinea Pig	General anesthesia as described for experiments, followed by Barbiturate injection (IV), overdose to effect. Death will be ensured by careful physical examination and an adjunctive physical method such as bilateral thoracotomy or exsanguination / vital organ (brain, heart, lungs, liver or kidneys) removal.	Initial dose is 35mg /kg to obtain a surgical level of anesthesia, except if there are signs of significant respiratory depression (e.g. very low respiratory rate or very high tolerated CO2 levels) are observed. Supplemental dose will be given to maintain a stable anesthetic level. For euthanasia or perfusion the full dose (35 mg/kg) will always be given rapidly without concern for respiratory depression.	IV

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

D. Scientific Justification for Use

- [x] AVMA Approved Method
- [ ] Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

- [x] Bilateral pneumothorax
- [ ] Cervical dislocation
- [ ] Decapitation
- [x] Exsanguination
- [x] Removal of vital organs

☐ Other

13. Project Information

1.

Associate	Role	Responsibilities	OHSP Training	Animal Care & Use	Survival Surgery	P&D Training
Pitts, Teresa Greene [redacted]	Principal Investigator Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ Mar 3, 2023	☑ Mar 3, 2023	☑ Mar 3, 2023	⊖
[redacted]	Co-Investigator Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ May 1, 2023	☑ May 1, 2023	⊖	⊖
[redacted]	Key Personnel Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ Feb 6, 2024	☑ Feb 5, 2024	☑ Feb 6, 2024	⊖
[redacted]	Key Personnel Authorized to order animals Access to view cages Editor	Surgery Euthanasia P&D assessment	☑ May 30, 2023	☑ May 26, 2023	☑ May 26, 2023	⊖
[redacted]	Key Personnel Access to view cages	Surgery Euthanasia P&D assessment	☑ Mar 6, 2024	☑ Mar 6, 2024	☑ Mar 6, 2024	⊖

2. Training and Qualifications

Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	Pitts, Teresa Greene	> 15 years experience with cats	Dr. Pitts will perform all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/ or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone	Dr. Pitts has over fifteen years of experience using the cat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats in acute and chronic experiments. She has continued to work extensively with anesthetized cats and rats. She has successfully completed the all online module trainings.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
2	[redacted]	Board-certified ABVP canine feline practice (since 2003) with > 30 years experience with cats and other animals	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/ or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone	[redacted] is veterinarian that is AVBP certified in canine feline practice. He has over thirty years of experience with animals including cats, and has used the cat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. He is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats and rats in acute and chronic experiments. He has continued to work extensively with anesthetized cats and rats. He has successfully completed the all online module trainings.	Part-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
3	[redacted]	> 10 years experience with cats	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotaxtic System, Spinal Cord Injury (SCI), Euthanasia via Barbiturate Overdose and KCl Administration, Euthanasia via Transcardial Perfusion, Lung Volume Determination, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Collection of Stomach Acid and Contents, Brainstem and/ or Spinal Cord Microinjection, Electrical Stimulation, Oral Administration of Buspirone.	[redacted] has over ten years of experience using the rat as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia of cats and rats in acute and chronic experiments. She has continued to work extensively with anesthetized cats and rats. She has successfully completed the all online module trainings.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
4	[redacted]	>15 years with mammals	[redacted] may perform and /or assist with all procedures. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotactic System, Spinal Cord Injury (SCI), Euthanasia via Transcardial Perfusion, Tissue Collection, Lung Volume Determination, Agonist/Antagonist IV Injection Protocol, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Brainstem and /or Spinal Cord Microinjection, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal/ Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Electrical Stimulation, and Oral Administration of Buspirone	[redacted] has over fifteen years of experience using rodents as an experimental model in studies involving the respiratory, airway function, cardiovascular, and central nervous systems. She is familiar with animal handling, anesthesia (injectable and inhalation), and euthanasia in acute and chronic experiments. She has continued to work extensively with anesthetized rodents, and will also perform the same procedures with cats. She previously served on an institutional biosafety committee. She has successfully completed the all online module trainings.	Full-time employee

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
5	[redacted]	Still in training	[redacted] will initially assist with procedures and will be supervised until she can perform them independently. Procedures include: Anesthesia Induction and Maintenance, Catheter Placement and Transition of Anesthetic Maintenance, Tracheal Cannulation, EMG Placement, Swallow Stimulation, Placement into a Stereotactic System, Spinal Cord Injury (SCI), Euthanasia via Transcardial Perfusion, Tissue Collection, Lung Volume Determination, Agonist/Antagonist IV Injection Protocol, Craniotomy and Brainstem Exposure, Dorsal Laminectomy, Brainstem and /or Spinal Cord Microinjection, Micromanipulation of the Laryngeal Muscles, Superior Laryngeal Nerve Isolation and Recording, Laryngeal/ Esophageal or Tracheal Distention, Topical Administration of Solutions in the Airway, Hypercapnia Effect on Respiratory State, Subcutaneous Injection, Electrical Stimulation, and Oral Administration of Buspirone	[redacted] will initially assist with procedures and will be supervised until she can perform them independently. She has successfully completed the all online module trainings.	Undergraduate student

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- [x] PHS (NIH, CDC, FDA, NSF, NASA)
- [ ] DoD
- [ ] VA
- [ ] AHA
- [ ] USDA
- [ ] Foundation/Industry
- [ ] Internal

☐ Other

14. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

● Yes ○ No

2. USDA Covered Species

Does this protocol utilize animals covered by the Animal Welfare Act or assigned to Category E? (AWA covered species include all warm blooded animals except birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research, horses not used for research purposes, and other farm animals.)

● Yes, includes USDA covered species or Category E ○ No

3. Includes USDA covered species or Category E

Search for Animal Alternatives

In the literature search and in the written narrative, replacement by non-animal systems, reduction in numbers of animals and refinement of experimental methods (the three R's) must be addressed.

Provide at least two sources of information: one of these sources must be a scientific literature database; documented expert consultation may be used as one source of information.

If you are in the School of Medicine and need assistance with this item, please contact Rachel Alexander, HSL Research Support Librarian, at AlexanderRL@health.missouri.edu. Others can contact the Zalk Veterinary Medical Library, at MU CVM VetMed Library for help.

See also:

https://www.nal.usda.gov/awic/sample-searches
https://library.missouri.edu
Literature Search Help

A. Source 1: Literature Database

Complete the information below:

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	05/17/2023	MEDLINE	1966-present	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, buspirone, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare
2	2/20/2024	MEDLINE	1966-present	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, guinea pig, cavies, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, guinea pig, cavies, laminectomy, hemilaminectomy / laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, buspirone, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare

B. Source 2: Literature Database

For the second source you may use a literature database search or an expert consultation (see following question).

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	05/17/2023	PubMed	All dates	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, cat, feline, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, cat, feline, laminectomy, hemilaminectomy /laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare)
2	2/20/2024	PubMed	all years	KEYWORDS: Tracheostomy, craniotomy, abdominal surgery, placement of EMG anesthesia, distress, guinea pig, cavies, tracheal stimulation, laryngeal stimulation, swallow testing, laryngeal reflex testing, cough stimulation, alterations in respiratory state, CO2 exposure, placement of catheters, placement of ear bars, spinal cord, central nervous system, spinal cord injury (SCI), SCI models, regeneration, locomotion, training, activity-dependent, in vivo, in vitro, guinea pig, cavies, laminectomy, hemilaminectomy / laminectomy, spay, ovariohysterectomy, anesthesia, analgesia, propofol, buprenorphine , pentobarbital, buprenorphine SR, meloxicam, ketoprofen, carprofen, xylazine, neurosteroid, isoflurane, sevoflurane, halothane, acetylpromazine, atropine, bus-irons, BusPar, DOI, hypercapnia induction agents, neuroprotective, neurotoxic, restraint, shaving, intraspinal injection *analgesia (*also with the derivatives analges*, anesthe* or anaesthe*, advers*, monitor*, pain*, distress*, stress*, welfare)

C. Source 2: Expert Consultation (alternative)

For the second source you may use a literature database search or an expert consultation. Documented expert consultation may be used as one source of information.

No Sources...

D. Animal Alternatives Narrative

Based on the information from the sources above, provide a written narrative of alternatives to procedures that may potentially cause more than momentary or slight pain or distress. The narrative should be such that the ACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

If a possible alternative was identified or is known, but will not be employed, discuss why.

Non-survival experiments include surgical procedures including: placement of catheters, tracheal cannulation, EMG placement, craniotomy, and alterations of respiratory state. Anesthesia will be maintained during all procedures to eliminate pain/distress. Lidocaine will be used to minimize the

sympathetic response during placement of the ear bars.

We have chosen to use the cat model due to our expertise in this model, the specificity of established locomotor tests in the cat and the existing literature on SCI, locomotion and reflex in the cat. Airway protection has been extensively studied in the guinea pig model, as rodents do not possess the full complement of airway protective reflexes, particularly cough. Our previous work and the existing literature give us interpretive power that is not possible in less sentient species and allow us to directly compare any new work to our previous data and the data of others in our field. The cat is an important translational model for issues involving SCI in humans. These include its spinal size, more similarity between recovery in cat and human than rat and human, elegant motor control, similarity of spinal anatomy to the human, and seminal work regarding task specific training. There are multiple importance and significant benefits of the animal models to be used and these currently cannot be achieved in other animal or non-animal models.

RESULTS OF LITERATURE SEARCHES:

We directly deal with pain associated with surgical manipulations through the consistent use of pain medication following any surgical procedure. We also included the option in our protocol to extend the pain medication time frame in the unlikely event that we see pain behaviors after completion of the standard medication regimen. We also carefully monitor the animals' depth of anesthesia during all surgical procedures to assure that there is no perception of the procedure as it is occurring. Drugs used during all procedures are carefully chosen to adequately support the animal as well as assure that the research is translationally relevant.

The conclusions we have drawn from literature searches indicate the importance and significant benefits of the surgical procedures to be used, that these currently cannot be achieved with other approaches, and that our drug regimen is the most effective for the studies. As indicated above under two specific search engines, no suitable alternatives to the surgical procedures /approaches involving spinal cord injuries have been identified for the studies associated with this protocol. The lack of suitable alternatives was determined by continual literature review (typically a weekly basis). Searches used a variety of databases including Pub Med (which searches articles back to 1966) and current contents (broader, but searches only back to the early 90's), as well as search engines (i.e. google, yahoo) and the Johns Hopkins ALTWEB site. In addition to frequent searches and discussions with local colleagues, I, and members of my research group, attend meetings and give presentations at other scientific institutions (academic and private companies). During these meetings/trips, we
have professional discussions with colleagues that cover many topics including surgical approaches, pain control and alternative, viable approaches. Several of my colleagues are either veterinarians or have appointments in a Veterinary School of Medicine.

HUMANE ENDPOINTS:

For terminal surgical procedures: Since all procedures are being performed under general anesthesia, the only potential adverse even would be if the level of anesthesia the animals are under is found to be too light then the procedure will be stopped and the steps will be taken to re-established a surgical plane of anesthesia and a bolus of pentobarbital will be administered to deepen the anesthetic level. The primary anesthetic level indicator is end-tidal CO2, secondarily is the reflex response from: a) toe pinch, b) eye blink, and c) jaw tone; this will be observed at least every 15 minutes; Additional anesthetic will be delivered or animal will be euthanized.

15. Investigator Assurances

1. ABSL-2 Assurance

I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

- ○ Yes, I will meet the requirements of this statement.
- ○ No, I will not meet the requirements of this statement.
- ● Not Applicable

2. Investigator Assurances

- ☑ 1. The information provided herein is accurate to the best of my knowledge.
- ☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.
- ☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.
- ☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.
- ☑ 5. I assure that I am not unnecessarily duplicating previous experiments.
- ☑ 6. I have considered alternative methods to using animals.
- ☑ 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

2024-06-13 10:23:29 -0500

Protocol 41056 Application 1.3

Approval date	03/17/2023
Expiration date	03/17/2026

1. Basic Information

1. Elements ID

   For existing protocols, enter the ID assigned to this protocol in Topaz Elements.

2. eACUC Number (Automatically Assigned)

   41056

3. Principal Investigator

Ganta, Roman Reddy

Job title	MCKEE ENDOWED PROFESSOR
Department	Veterinary Pathobiology
Division	Veterinary Medicine
Business unit	University of MO-Columbia

4. Protocol Title

   Tick-borne rickettsial diseases; pathogenesis and vaccine development

5. Triennial Re-write

   Is this protocol a triennial re-write of a protocol that was previously approved at the University of Missouri?

   ○ Yes ● No

2. Species Section

1. Please note, the total number of animals requested is the amount of animals you will need for a 3 year period. This number should include all experimental animals plus animals used for colony maintenance (breeders and offspring produced that are not used for experiments). These numbers should match the amounts in the Justify Animal Numbers section. If this is a triennial re-write these amounts should also include any animals on the previous protocol that will be transferred to the new protocol.

Species	Strain/ Stock/ Breed	Age/ Weight	Pain/Distress Category	Authorized	Ordered	Received	Adjustment	Available
Cattle	Holstein	6-12 months	Undefined (non-covered species only)	1				1
			USDA Category E	20				20
Total Cattles:				21	0	0	0	21
Dog	Beagle	6-10 months	USDA Category D	346				346
			USDA Category E	52				52
Total Dogs:				398	0	0	0	398

2. Phenotypic consequences

Describe any phenotypic consequences of the genetic changes to the animals and the outcome of these consequences (e.g. whether or not any change in animal welfare or husbandry is anticipated).

No Phenotypic consequences...

3. Wild Animals

Are WILD ANIMALS to be used or studied?

○ Yes ● No

4. Client-Owned Animals

Are CLIENT-OWNED animals to be used or studied?

○ Yes ● No

3. USDA Category E

1. Justification for Withholding Drugs

Provide scientific justification for withholding pain/distress-relieving drugs:

Non-vaccinated infection control animals in the RMSF study (project 3) will serve as controls to aiding to differentiate how effective the vaccine will be. If we start giving treatments to the control animals, we will not be able to make true comparisons of vaccine-associated protection, which is the primary goal of the study.

Primary objective of project 4 is is to define Heartwater disease parthenogenesis in the US cattle. We will need to record the infection severity as measured by clinical signs following infection with Ehrlichia ruminantium pathogen. Therefore, offering drugs to reduce the clinical signs will prevent us from assessing the disease severity.

2. Monitoring Pain and Stress

Explain how the level of pain or physical stress will be monitored (include the frequency of monitoring).

We will monitor all animals more closely; twice a day from the time we will fist observe clinical signs. In the event animals begin to show a severe disease symptoms, we will promptly contact the assigned veterinarian for guidance. Accordingly, we may initiate supporting care such offering subcutaneous fluid therapy and/or other care as recommended, but not providing antibiotic treatments.

3. Point of Euthanasia

Define the point at which the animals will be euthanized.

The decision to euthanize animals will be as per the animal health status monitored and subject to recommendation of the veterinarian assigned to the project. Importantly, we will actively seek guidance regarding health status changes of animals and a decision will be made with a high priority given in providing humane treatment of animals.

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4. Proposal Overview

1. Purpose

Purpose of the study:

To support the federally funded research grant proposals current in progress:

We currently have three active NIH funded R01 applications involving the use of animals; these studies involve the use of the canine host. We also have an active USDA cooperative agreement grant. This study will involve the use of cattle.

1) NIH R01 grant # AI070908 (title: Vector and host contributions to the regulation of E. chaffeensis gene expression), we will need to perform in vivo screening of Ehrlichia chaffeensis mutants to identify genes essential for pathogens survival in vertebrate and tick hosts.

2) NIH R01 grant # AI152418 (title: Vaccines against Ehrlichia and Anaplasma species infections), canine host will be used to define the value of a modified live vaccine studies protecting against tick-borne rickettsial infections by Ehrlichia chaffeensis, Ehrlichia canis and Anaplasma phagocytophilum. Three primary goals (experiments) of this project are to; 1) evaluate the value of modified live attenuated vaccine (MLAV) to define the duration of immunity against wild type infection challenge through blood stream and tick transmission; 2) determine if immunity to MLAV protects against genetically distinct E. chaffeensis strains; and 3) to evaluate similar MLAVs from related Ehrlichia and Anaplasma species for their usefulness as a live attenuated vaccine protecting against infections. Goals of the first experiment are already accomplished during the year 1 and 2 funding, while experiments 2 and 3 are yet to be accomplished.

3) NIH R01 grant # AI152417 (title: Rocky Mountain Spotted fever vaccine development), we proposed to investigate the utility of whole cell inactivated vaccine to prevent Rocky Mountain spotted fever in dogs. This project major goals (experiments) involve the use of canine host; 1) evaluate inactivation methods for preparing WCA-S (Sheila Smith strain) and adjuvants in defining the vaccine protection; 2) evaluate the duration of immunity; 3) evaluate protection against tick-transmitted challenges; and 4) evaluate WCA protection against R. rickettsii heterologous strain infection challenges.

4) Ehrlichia ruminantium is an important foreign animal disease pathogen of ruminants as the infections with it in non-endemic regions can inflict major morbidity and mortalities. This sub-Saharan African pathogen is also well established in parts of the Caribbean islands. The goals of this proposal are to; 1) investigate heartwater disease pathogenesis in cattle resulting from an important tick-borne foreign animal disease pathogen in ruminants, Ehrlichia ruminantium; and 2) test if E. ruminantium can be transmitted by

Amblyomma maculatum; the tick previously identified as a competent vector and having wide distribution in southeastern parts of the USA.

2. Value

Please provide the information necessary to allow the ACUC to evaluate the objectives of the study against potential animal welfare concerns.

The studies in all four projects are independent and are critical for advancing our understanding of important tick-borne diseases impacting dogs, people and ruminants. The first project goals are to perform mutational analysis and in vivo screening to identify genes essential for the Ehrilchia chaffeensis pathogenesis in vertebrate and tick hosts. The second proposal aims to evaluate modified live vaccines against tick borne diseases in dogs and people resulting from E. chaffeensis, E. canis, and Anaplasma phagocytophilum. The 3rd project evaluates an inactivated whole cell antigen-based vaccine to confer protection against Rocky Mountain spotted fever (RMSF) which is a major fatal disease in dogs and people. The 4th project investigates pathogenesis of an important foreign animal tick-borne disease of ruminants. There are no non-animal alternatives for these tick-borne diseases. The objectives of the studies are the first to define pathogenesis and vaccine development in physiologically relevant animal models. All studies will be performed in accordance with the animal welfare regulations and the studies aim to develop the most effective methods to protect animals from several important tick-borne diseases which are more common in companion animals, agricultural animals and in people.

3. Lay Term Description of Experimental Design

To put something in layman's terms is to describe a complex or technical issue using words and terms that the average individual (someone without professional training in the subject area) can understand. This section should be written so that someone with a **10th grade science education can easily understand the project.**

The studies in all four projects are independent and are critical for advancing our understanding of important tick-borne diseases impacting dogs, people and ruminants. The first project goals are to perform mutational analysis and in vivo screening to identify genes essential for the Ehrilchia chaffeensis pathogenesis in vertebrate and tick hosts. We will generate large pools of E. chaffeensis transposon mutants in support of this objective. The second proposal aims to evaluate modified live vaccines against tick borne diseases in dogs and people resulting from E. chaffeensis, Ehrlichia canis, and Anaplasma phagocytophilum. We recently developed a modified live attenuated vaccine which confers protection against infection challenge by direct blood-borne infection and against tick-transmission challenge. Specifically in the current project, we aim define the protection against heterologous strains of E. chaffeensis, and similarly test homologous modified live vaccines to protect dogs against E. canis and A. phagocytophilum infections. The 3rd project evaluates an inactivated whole cell antigen-based vaccine (WCAV) to confer protection against Rocky Mountain spotted fever (RMSF) which is a major fatal disease in dogs and people. In our prior studies, we reported the best protect from WCAV and in the current study, we will assess various formulations of vaccine and length of protection using the best vaccine formulation; both against blood-borne infection, then test protection against tick transmission and finally against heterologous strains of the pathogen. The 4th project investigates pathogenesis of an important foreign animal tick-borne disease of ruminants; the heartwater disease caused by Ehrlichia ruminantium. This study investigates the risk of cattle from E. ruminantium by direct needle infection and from a tick native to the mainland USA, Amblyomma maculatum.

4. Scientific Description of Experimental Design

In language a scientific colleague can understand, provide a step-by-step, general description of the animal experiments you will perform including experimental groups and timing of procedures and manipulations. For complicated experimental designs, including a flow chart, diagram, or table in the Attachments section is recommended to help the ACUC understand what is proposed. DO NOT describe details of the procedures here as such details are requested later in the form.

Project 1) Active NIH grant # R01 AI070908: Vector and host contributions to the regulation of E. chaffeensis gene expression

Brief summary: Perform mutational analysis and in vivo screening to identify genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts. We will generate large pools of E. chaffeensis transposon mutants in support of this objective. Our funding was approved to generate 200 mutant organisms. These mutants will then be screened to define the pathogenesis using the canine infection model; three experiments were proposed to accomplish this goal.

Background: The family Anaplasmataceae contains several obligate, intracellular, Gram-negative bacteria which include species of the genera Ehrlichia and Anaplasma and responsible for causing infections in dogs and people, as well as in several other vertebrate hosts. We recently performed mutational analysis and demonstrated that mutations in three different genes of E. chaffeensis caused attenuated growth of the organism in vivo (Cheng et al. 2013). These data formed the basis for our funded NIH-R01 grant application having the three specific aims. Aim 3 requires the use of animal studies, i.e., to perform mutational analysis and in vivo screening to identify additional genes essential for the E. chaffeensis pathogenesis in vertebrate and tick hosts. We have completed part the proposed experiments of this aim already at K-State as per an IACUC approval (Wang et al. 2020). This application will focus on the remaining proposed portion of the experiment. Dog is chosen as the infection model for the proposed experiments because it is an incidental host in acquiring E. chaffeensis similar to humans. Moreover, our several recent experimental studies demonstrated that this host serves as an excellent infection model, where the pathogen infection causing a very mild disease and the infection persists in (Nair et al. 2016). Our experimental infection studies demonstrated that dogs develop only mild fever (rise in only up to 1.5oC body temperature), while maintaining persistent infections with detectable hematological changes, host response and having milder histopathological changes.

Experimental plan:
Animal details. We will use about 6-month-old beagle breed dogs (representing both sexes equally) weighing approximately 8-10Kg for all of our studies. Animals will be purchased from a USDA approved vendor and acclimated for one week prior to introduction into the study. The study timeline and end points are described under each experiment.

Experiment involving animals: We proposed to screen 200 E. chaffeensis mutants in the canine host. As of now, we completed screening 60 mutants as 6 pools by infecting three dogs each with about 10 mutants in each pool. A total of 18 were used under this objective as part of the current protocol at K-State. In this protocol, we will expect to screen 14 pools (maximum) of mutants to complete the project goals. Each pool of up to 10 mutants will be used and in three independent animals (n=3) per pool which totals 42 animals. The infection status will be assessed twice a week for two months. Nymphal ticks (typically about 250) will be allowed to acquisition feed on animals starting from day 5 post infection. Tick cells (containers that hold ticks) will be placed on dogs and covered with sheep soc (made of Nylon Spandex for easy flexibility) (Sheepman Supply co. or something similar) by following the procedures similar to those done on deer, except that there is no need for anesthetize the dogs. For these experiments, the backs of the animals will be shaved with veterinary clippers. A custom designed tick containment chamber (modified top of Nalgene jar containing screw cap lid) will be glued to polyvinyl membrane with a center circular opening. The chamber will then be glued to animals with industrial adhesive (commercially available). The chambers have round bottom smooth surface and once glued, the chambers remain attached for several weeks until polyvinyl membrane is lifted off the skin with the hair growth. To ensure that the chambers are tightly attached, tick infestations will be performed only after about 24 h following the attachment of the chambers. We will monitor for the retainment of the chambers on the animals, as well as their firm attachment. If dogs attempt to remove the chambers, we will place Elizabethan neck collars to restrict grooming. The chambers will be covered with sheep sox. To perform the tick infestation, lids of the chambers will be unscrewed, ticks will be placed inside, and the chambers will then be tightly closed with the lids and animals will be covered back with sheep sox. About 7 days following tick attachment, ticks will be collected by opening the chamber lids.

We will evaluate ticks from each animal following the molting to adult stage to assess which mutants are acquired by ticks. Together, the assessments of blood (10 ml blood drawn twice a week from cephalic veins for the first two weeks and then on once a week) and tick sampling will help us determine which genomic regions of E. chaffeensis that are critical for the in vivo growth in an incidental host model with important implications in extending the observations in understanding pathogenesis in people (total dogs for this sub-experiment are 42).

Animal monitoring plan: After infection, animals will be observed twice daily with once daily monitoring the body temperatures. Although we do not anticipate serious clinical signs in this study, a possibility of animals developing an unrelated illness cannot be ruled out. In such instances, an attending veterinarian will be consulted for appropriate action particularly if exhibiting depression, lethargy for more than24 hours, and/or changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer. All animals will also be monitored for hematology and the presence of bacteria assessed by molecular methods, such as by PCR and culture recovery methods, as well as by blood smear analysis.

Blood sampling and other procedures: In the experiments, animals will be kept for 60 days each to monitor the mutant E. chaffeensis circulation in blood. Blood sampling will be done twice a week from cephalic veins (10 ml each) for the first two weeks and then once a week thereafter. Total blood draws will be 11 times per animal. About 6- to 8-month-old dogs of the breed 'Beagle' will be used for these experiments. For convenience, we will either use all males or all females in each experimental group, while maintaining equal numbers of males and females throughout the study. The weight of each animal will be about 15 to 20 pounds. Diphenhydramine (Benadryl) (1mg per pound) will be orally administered to all animals about 30 minutes prior to inoculation with Ehrlichia. (The stock concentration to be used is 2.5 mg/ml; 6 to 8 ml per animal or 15-to-20-pound dogs.) Benadryl is administered to prevent any possible anaphylactic shock resulting from injection of organisms containing traces of serum or other animal products likely present in the culture media.

At the end of the study: At the completion of the study, dogs will be transferred to another study or will be adopted out after a four-week treatment with doxycycline. This infection is very common in dogs and pose milder disease and so it will not be a concern to either the dogs or to pet owners. The infection with E. chaffeensis is very common in dogs and poses milder disease (Bowman et al., 2009 and Beall et al. 2012). It will not be a concern to either the dogs or to pet owners. Thus, subjecting to adaptation or transferring to other research projects are fully justified. These animals will be transferred to other projects within the university as per the needs of a project(s) or may also be opened up for the adaption if such option is not available.

References:

Cheng C, Nair ADS, Indukuri VV, Gong S, Felsheim RF, Jaworski D, Munderloh UG and Ganta RR. Targeted and random mutagenesis of Ehrlichia chaffeensis for the identification of genes required for in vivo infection. PLoS Pathog. 2013 Feb;9(2):e1003171. doi: 10.1371/journal.ppat.1003171. Epub 2013 Feb 14.

Wang Y, Nair ADS, Alhassan A, Jaworski DC, Liu H, Trinkl K, Hove P, Ganta CK, Burkhardt N, Munderloh UG and Ganta RR. Multiple Ehrlichia chaffeensis genes critical for its persistent infection in a vertebrate host are identified by random mutagenesis coupled with in vivo infection assessment. Infect Immun. (2020) 88(10) DOI: 10.1128/IAI.00316-20.

Nair AD, Cheng C, Ganta CK, Sanderson MW, Alleman AR, Munderloh UG, Ganta RR. Comparative experimental infection study in dogs with Ehrlichia canis, E. chaffeensis, Anaplasma platys and A. phagocytophilum. PLoS One. 2016 Feb 3;11(2):e0148239. doi: 10.1371/journal.pone.0148239

Bowman, D., Little, S. E., Lorentzen, L., Shields, J., Sullivan, M. P., & Carlin, E. P. (2009). Prevalence and geographic distribution of Dirofilaria immitis, Borrelia burgdorferi, Ehrlichia canis, and Anaplasma phagocytophilum in dogs in the United States: results of a national clinic-based serologic survey. Vet

Parasitol, 160(1-2), 138-148.

Beall MJ, Alleman AR, Breitschwerdt EB, Cohn LA, Couto CG, Dryden MW, Guptill LC, Iazbik C, Kania SA, Lathan P, Little SE, Roy A, Sayler KA, Stillman BA, Welles EG, Wolfson W, Yabsley MJ. Seroprevalence of Ehrlichia canis, Ehrlichia chaffeensis and Ehrlichia ewingii in dogs in North America. Parasit Vectors. 2012 Feb 8;5:29. doi: 10.1186/1756-3305-5-29. doi:10.1186/1756-3305-5-29

Project 2) Active NIH grant # R01 AI152418: Vaccines Against Ehrlichia and Anaplasma Species Infections

Brief summary: Tick-borne pathogens belong to the genera Ehrlichia and Anaplasma continue to emerge as a major public health concern during the last 3-4 decades. They include the emerging diseases; human monocytic ehrlichiosis, human ewingii ehrlichiosis, and human granulocytic anaplasmosis caused by Ehrlichia chaffeensis, Ehrlichia ewingii, and Anaplasma phagocytophilum. We recently reported the development of a modified live attenuated vaccine (MLAV) inactivating an important gene (ECH_0660) against E. chaffeensis that conferred protection against infection challenge from blood transfusion and from infected ticks (Nair et al. 2015 and McGill et al. 2016). Goals of this funded project are 1) to evaluate the duration of protection offered from E. chaffeensis MLAV against wild type infection challenge through blood stream and tick transmission; 2) to determine if immunity to the vaccine protects against genetically E. chaffeensis strains; and 3) to evaluate similar MLAV from related Ehrlichia and Anaplasma to protect against infections.

Background: Rickettsial diseases caused by pathogens of the Anaplasmataceae family, including members of the genera Ehrlichia and Anaplasma, are responsible for frequent infections in people over the past three decades and are a leading cause of tick-borne infections in humans throughout the USA and many parts of the world. These pathogens also infect diverse vertebrate hosts, although also are causing a milder disease in majority of host species. These pathogens have evolved strategies to evade host immunity and cause persistent infections. Through our recently established mutagenesis experiments, we created E. chaffeensis mutants that contained insertions causing functional gene disruptions. An insertion mutation in the ECH_0660 gene resulted in the pathogen's rapid clearance from two vertebrate hosts (Cheng et al. 2013). Vaccination with this mutant induced a strong host response and offered complete protection against blood stream and tick transmission infection with wild-type E. chaffeensis one month after vaccination (Nair et al. 2015 and McGill et al. 2016). Previously, we performed molecular characterization of several E. chaffeensis isolates and reported that the isolates represent three distinct genetic groups (Cheng et al. 2003). We proposed the following three specific aims (all three involves the use of animals): 1) Evaluate the duration of immunity offered by the ECH_0660 gene mutant live attenuated vaccine (MLAV) against wild type infection challenge through blood stream and tick-transmission. 2) Evaluate the protection of the MLAV against genetically distinct E. chaffeensis strains. 3) Evaluate mutants in related Ehrlichia and Anaplasma species for their efficacy as live attenuated vaccines in conferring protection against the pathogens' infection into blood stream and by tick-transmission. As part of the completed research during the last two years, we completed the goals of aim 1, thus, we propose in executing experiments planned as part of aims 2 and 3 which we call as experiments 1 and 2.

Experimental plan:

Animal details. We will use about 6-month-old beagle breed dogs (representing both sexes equally) weighing approximately 8-10Kg for all of our studies. Animals will be purchased from a USDA approved vendor and acclimated for one week prior to introduction into the study. The study timeline and end points are described under each experiment.

Experiment 1: Evaluation of cross protection induced by MLAV against different E. chaffeensis strains
Experiment 1a) Comparison of Arkansas isolate-derived MLAV protection against St. Vincent and Jax infection

challenges by I.V. and tick-transmitted infection

This experiment will have 8 groups (n=6); groups 1-4 will receive the Arkansas isolate derived MLAV intravenously, while Groups 5-8 will serve as infection controls. Groups 1 and 2 will receive I.V. infection challenge one month after vaccination with wild type St. Vincent and Jax culture infection challenges, respectively. As per our prior published data, infection challenge following one month of vaccination with attenuated mutant induce sufficient host immune response in offering complete protection against blood stream infection and tick transmission challenges with wild-type E. chaffeensis (1, 2). Groups 3 and 4 will be similar to Groups 1 and 2, except that the infection challenges will be performed by tick-transmission. Groups 5 and 6 (n=6) will serve as non-vaccinated controls but will be challenged via I.V. and Groups 7 and 8 (n=6) transmitted will be challenged tick- transmitted challenge with the St. Vincent or Jax isolates, respectively similar to groups 1-4 above.

Table 1. Experimental design to test Arkansas isolate-derived MLAV protection against St. Vincent and Jax infection challenges by I.V. and tick-transmitted infection
Group Vaccine # of animals* Infection challenge.
1 Arkansas MLAV 1X I.V. 6 (3F+3M) I.V.E. chaffeensis (St. Vincent)
2 Arkansas MLAV 1X I.V. 6 (3F+3M) I.V.E. chaffeensis (Jax)
3 Arkansas MLAV 1X I.V. 6 (3F+3M) tick transmission E. chaffeensis (St. Vincent)
4 Arkansas MLAV 1X I.V. 6 (3F+3M) tick transmission E. chaffeensis (Jax)
5 Infection Control 6 (3F+3M) I.V.E. chaffeensis (St. Vincent)
6 Infection Control 6 (3F+3M) I.V.E. chaffeensis (Jax)
7 Infection Control 6 (3F+3M) tick transmission E. chaffeensis (St. Vincent)
8 Infection Control 6 (3F+3M) tick transmission E. chaffeensis (Jax)

*48 animals

Experiment 1b) Comparison of the St. Vincent isolate-derived MLAV protection against Arkansas and Jax infection challenge by I.V. infection and by tick-transmission.
In this experiment, the St. Vincent isolate mutant MLAV will be used as the vaccine, and infection challenges will be performed with wild type I.V. infection and tick transmission with Arkansas and Jax isolates of E. chaffeensis. This experiment will have 4 vaccinated groups (n=6) and four non-vaccinated groups (n=6); groups 1-4 will receive the St. Vincent isolate derived MLAV intravenously, while Groups 5-8 will serve as infection controls. Infection challenges will be performed with wild type I.V. infection and tick transmission with Arkansas and Jax isolates. Groups 1 and 2 will receive I.V. infection challenge one month after vaccination with wild type Arkansas and Jax culture infection challenges, respectively. Groups 3 and 4 will be similar to Groups 1 and 2, except that the infection challenges will be performed by tick-transmission. Groups 5 and 6 (n=3) will serve as non-vaccinated controls and will receive I.V. infection challenge with the Arkansas or Jax isolate cultures, respectively. Groups 7 and 8 (n=3) will be challenged via tick-transmitted challenge with the Arkansas or Jax isolate infected ticks. Since we have sufficient number of control animals in the previous experiments, we reduced the number of control animals (n=3) in this study.

Table 2. Experimental design to test St. Vincent isolate-derived MLAV protection against Arkansas and Jax isolates infection challenges by I.V. and tick-transmitted infection
Group Vaccine # of animals* Infection challenge.
1. St. Vincent MLAV 1X I.V. 6 (3F+3M) I.V.E. chaffeensis (Arkansas)
2. St. Vincent MLAV 1X I.V. 6 (3F+3M) I.V.E. chaffeensis (Jax)
3. St. Vincent MLAV 1X I.V. 6 (3F+3M) tick transmission E. chaffeensis (Arkansas)
4. St. Vincent MLAV 1X I.V. 6 (3F+3M) tick transmission E. chaffeensis (Jax)
5. Infection Control 3 (F or M) I.V.E. chaffeensis (Arkansas)
6. Infection Control 3 (F or M) I.V.E. chaffeensis (Jax)
7. Infection Control 3 (F or M) tick transmission E. chaffeensis (Arkansas)
8. Infection Control 3 (F or M) tick transmission E. chaffeensis (Jax)

*36 animals

Experiment 1c) Comparison of the Jax isolate-derived MLAV protection against Arkansas and St. Vincent infection challenge by I.V. infection and by tick-transmission.
In this experiment, the Jax isolate mutant MLAV will be used as the vaccine, and infection challenges will be performed with wild type I.V. infection and tick transmission with Arkansas and St. Vincents isolates. This sub-aim will have four vaccinated groups (n=6) and all four groups will receive the MLAV and will also include four non-vaccinated control groups (n=3). Groups 1 and 2 will receive I.V. infection challenge one month after vaccination with wild type Arkansas and St. Vincents culture infection challenges, respectively. Groups 3 and 4 will be similar to Groups 1 and 2, except that the infection challenges will be performed by tick-transmission with the respective isolate infections. Groups 5 and 6 (n=3) will serve as non-vaccinated controls challenged via I.V. and Groups 7 and 8 (n=3) will be challenged via tick-transmitted challenge with Arkansas or St. Vincent isolates, respectively, similar to groups 3 and 4.

Table 3. Experimental design to test Jax isolate-derived MLAV protection against Arkansas and St. Vincent isolates infection challenges by I.V. and tick-transmitted infection

Group Vaccine # of animals Infection challenge .
1. Jax MLAV 1X I.V. 6 (3F 3M) I.V.E. chaffeensis (Arkansas)
2. Jax MLAV 1X I.V. 6 (3F 3M) I.V.E. chaffeensis (St. Vincent)
3. Jax MLAV 1X I.V. 6 (3F 3M) tick transmission E. chaffeensis (Arkansas)
4. Jax MLAV 1X I.V. 6 (3F 3M) tick transmission E. chaffeensis (St. Vincent)
5. Infection Control 3 (F or M) I.V.E. chaffeensis (Arkansas)
6. Infection Control 3 (F or M) I.V.E. chaffeensis (St. Vincent)
7. Infection Control 3 (F or M) tick transmission E. chaffeensis (Arkansas)
8. Infection Control 3 (F or M) tick transmission E. chaffeensis (St. Vincent)

*36 animals

Experiment 2) Evaluation of related Ehrlichia and Anaplasma species MLAV for their efficacy in conferring protection against wild type infection in the blood stream and by tick-transmission.
Experiment 2a): Evaluation of Ecaj_0381 disrupted MLAV's ability to confer protection against E. canis infection by I.V. into blood stream and by tick transmission.
This study will have two vaccination groups (n=6) and both groups will receive the same E. canis MLAV. Group 1 will receive I.V. infection challenge, while Group 2 will receive tick-transmission infection one month after vaccination. Groups 3 and 4 (n=3) will serve as non-vaccinated controls, which will receive infection challenges similar to Groups 1 and 2. For the control groups also we will use n=6.

Table 4. Experimental design to test E. canis MLAV protection against E. canis infection challenges by I.V. and tick-transmitted infection

Group Vaccine # of animals* Infection challenge.
1. E. canis MLAV 1X I.V. 6 (3F 3M) I.V.E. canis (wild type)
2. E. canis MLAV 1X I.V 6 (3F 3M) tick transmission E. canis (wild type)
3. Infection Control 6 (M or F) I.V.E. canis (wild type)
4. Infection Control 6 (M or F) tick transmission I.V.E. canis (wild type)

*24 animals

Experiment 2b): Evaluation of Aph_0634 disrupted MLAV's ability to confer protection against A. phagocytophilum infection challenge by I.V. infection into blood stream and by tick transmission.

In this study, A. phagocytophilum Aph_0634 mutant MLAV will be used as the vaccine similar to the previous experiment. Infection challenges will be performed with a human isolate of A. phagocytophilum (HGA2) using the wild type cultured organisms for I.V. infection and using infected ticks. As in the previous experiment, this study will include two vaccination groups (n=6) and two non-vaccinated control groups (n=6).

Table 5. Experimental design to test A. phagocytophilum MLAV protection against A. phagocytophilum infection challenges by I.V. and tick-transmitted infection

Group Vaccine # of animals* Infection challenge .
1. A. phagocytophilum MLAV 1X I.V. 6 (3F 3M) I.V.A. phagocytophilum (wild type)
2. A. phagocytophilum MLAV 1X I.V 6 (3F 3M) tick transmission A. phagocytophilum (wild type)
3. Infection Control 6 (M or F) I.V.A. phagocytophilum (wild type)
4. Infection Control 6 (F or M) tick transmission A. phagocytophilum (wild type)

*24 animals

Mutant Live Attenuated Vaccines (MLAVs): The MLAVs contain either modified E. chaffeensis, E. canis or A. phagocytophilum in vitro cultured mutant organisms washed with PBS and resuspended in PBS at a dose rate of 2X108 organisms/mL. Vaccines will be administered as I.V. (1 mL/animal).

Infection challenge dose: Infection challenges will be performed with 2X108 bacteria grown in appropriate cell culture by I.V. inoculation method; we chose this dose as we previously reported in an infection model utilizing this dose (Nair et al., 2016). E. canis organisms will be quantified in the culture; the culture will be centrifuged to concentrate and remove the culture media and resuspended into 1x PBS to a final concentration of 2X108 bacteria per 1 ml for use in inoculation experiments.

Intravenous injections: Each dog will receive 1 ml of the inocula into left or right cephalic vein using a 23 G butterfly needle. The vaccination site will be aseptically prepared by shaving hair (approximately 2cm2) and cleaning with 70% ethanol. To prevent any possibility of developing anaphylactic reactions, Benadryl (diphenhydramine) will be administered 30 min prior to any intravenous vaccine or challenge inoculum administration.

Tick transmission challenge: Infection challenge with tick transmission will be done as per our published protocol. Twenty-five adult infected tick pairs (25 males and 25 females) will be allowed to transmission feed on vaccinated dogs for 7 days. Engorged nymphs (obtained from a commercially available source) will be infected with E. chaffeensis, E. canis or A. phagocytophilum by needle inoculation and allowed to molt to the adult stage (Cheng et al. 2015 and Jaworski et al., 2016). To prepare for a tick transmission experiment, we will prepare a tick containment cell for each dog. In our system, we will use containment chambers constructed from the tops of Nalgene jars that are each fitted with a screen and polyvinyl gasket that will be directly glued (3M Scotch-Weld 4799 adhesive) to the shorn back of a dog. Dogs are manually held for the application of the tick containment cell. The shaved area will be approximately 4 inches in diameter and to either the right or left side of the dog over the midback area. The placement of containers will be done 24 hours prior to tick infestation. In addition, the dogs will be fitted with a collar to restrict grooming near the containment chamber. Tick infestations will be accomplished by placing 25 female and 25 male ticks on each dog. We will count ticks to be used for each dog carefully. The transfer of ticks to dogs will be performed by unscrewing the screened top of the container and placing the ticks on the dog. The top of the chamber will be re-secured immediately, and dogs will be returned to individual housing. The dog will be restricted from group play during the 7-day period that the tick containment cells are present. Dogs, tick containment chambers and tick attachments will be monitored daily until all ticks are removed from dogs. Extreme care will be taken, and all ticks will be counted (live or dead) when partially fed ticks are removed on day 7. The Nalgene top of the container will be removed from the polyvinyl gasket and the gasket will be removed by shaving. The dogs will be monitored for an additional four weeks.

Animal monitoring plan: After infection with live vaccines and after infection challenges, animals will be observed twice daily with once daily monitoring the body temperatures. Body weights will be measured twice a week. Although we do not anticipate serious clinical signs in this study, a possibility of animals developing an unrelated illness cannot be ruled out. In such instances, an attending veterinarian will be consulted for appropriate action particularly if exhibiting depression, lethargy for more than24 hours, and/or changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer. All animals will also be monitored for hematology and the presence of bacteria assessed by molecular methods, such as by PCR and culture recovery methods, as well as by blood smear analysis.

Blood sampling: All blood collections will be done from jugular, or anterior cephalic or lateral saphenous veins using 20 or 22 gauge needles.

Vaccination phase: About 20 ml of blood (10 ml in ACD tube and 10ml in EDTA tube) will be collected once a week during vaccination phase. In addition, 1 ml of whole blood in EDTA tube will also be obtained for performing CBC analysis (once a week) for one month following vaccination. One ml of blood in EDTA tube will also be collected and used for checking the infection status twice a week for the first month. In experiment 1, after the first month of vaccination, about 20 ml blood will be collected once in every two weeks until challenge.

Challenge phase: About 20 ml of blood (10 ml in ACD tube and 10ml in EDTA tube) will be collected once a week until end point. In addition, 2 ml blood in EDTA tubes will be collected twice a week for assessing the systemic bacterial load and 1ml blood will be collected for CBC analysis. If dogs exhibit high fever or other clinical symptoms, additional 1 ml blood may be collected a third time in a week to monitor the infection status.

At the end of the study: At the completion of the study, dogs will be transferred to another study or will be adopted out after a four-week treatment with doxycycline. The infections with E. chaffeensis, E. canis and A. phagocytophilum are very common in dogs and pose milder disease and will not be a concern to either the dogs or to pet owners. Thus, subjecting to adaptation or transferring to other research projects are fully justified.

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Project 3) Active NIH grant # R01 AI152417: Rocky Mountain Spotted fever vaccine development

Brief summary: Rocky Mountain spotted fever remains a life-threatening tick-borne disease of people and continues to be a public health concern in the USA and several North, Central and South American countries. During the last two decades, reported RMSF cases continue to rise in parts of North America. This NIH funded application investigates RMSF vaccine development using a relevant animal-tick-pathogen infection model (dog and tick). At the completion of the project, we expect to have a fully developed vaccine useful in devising strategies to control the disease.

Background: Tick-transmitted rickettsial diseases of the genera Anaplasma, Ehrlichia, and Rickettsia remain a growing public health concern in the USA and many parts of the world. The diseases include one of the oldest known rickettsial diseases, Rocky Mountain spotted fever (RMSF) caused by Rickettsia rickettsii. RMSF remains a serious disease of people and dogs for about a century and continues to be a public health concern in the USA and several North, Central and South American countries resulting from a tick bite (Alvarez-Hernandez et al., 2017; Piranda et al. 2008; Labruna et al., 2009; Piranda et al., 2011; Drexler et al., 2017; Hatcher et al. 2018; Londono et al. 2019;) [4, 7-19]. Clinical signs of RMSF include fever, headache, nausea, vomiting, muscle pain, lack of appetite, and rash. The disease can progress rapidly to a life-threatening illness in untreated patients, resulting in high mortality rates ranging from 30-80% [4, 20]. During the last two decades, reported RMSF cases continue rising in parts of North America (Drexler et al. 2017; Tinoco-Gracia et al. 2018). Since dogs develop disease similar to people, a vaccine to prevent the disease in this host will most likely be effective in controlling the disease spread from wildlife, ticks and also infections from dogs to people. We recently demonstrated that whole cell inactivated antigens of R. rickettsii offer complete protection against virulent infection challenge in the canine host (Alhassan et al.; 2019). Our prior published work offers the strongest justification for the proposed detailed investigation for which we received NIH grant funding. The following are the proposed objectives.

1) Evaluate inactivation methods for preparing WCA-S (Sheila Smith strain) and adjuvants in defining the vaccine protection.
2) Evaluate the duration of immunity
3) Evaluate protection against tick-transmitted challenges.
4) Evaluate WCA protection against R. rickettsii heterologous strain infection challenges.

Experimental plan:
Three different inactivation methods will be used to prepare WCA-S (whole cell inactivated antigen from Sheila Smith strain); heat, formalin and hydrogen peroxide.

Animal details: Purpose bred beagle dogs (4-6 months old of both sexes), weighing approximately 8-10 kg, obtained from a Class A USDA vendor, will be housed in indoor climate-controlled facilities with ad libitum food and water and adequate spacing to allow regular exercise activities. They will be acclimated for one week prior to introduction into the study. The study timeline and end points are described under each experiment.

Experiment 1: Evaluate inactivation methods for preparing WCA-S and adjuvants in defining the vaccine protection.

Vaccine assessments with WCA prepared by three different inactivation methods and using three different adjuvants: In our recent study, we used 70 µg of heat inactivated whole cell antigens of R. rickettsii Shelia Smith strain diluted in PBS with final concentration of 2.5% Montanide™ Gel.This experiment will be performed similarly; 9 vaccination groups will be included (n=6 for each group; 3 males and 3 females). One group will receive only adjuvant (n=6 and two animals each per adjuvant) and then will be subjected to infection challenge to serve as infection controls. (Total number animals for this experiment will be 60.)

We will not include uninfected controls as we have ample data generated previously using such controls. Vaccines prepared with three inactivation methods (heat, formaldehyde and H2O2) and with three different adjuvants (Montanide gel, QS-21 saponin and Aluminum hydroxide) will be used in this experiment. Similarly,

adjuvant only preparations will be administered to control groups. The vaccination protocol will be similar to our recent publication with a priming vaccination on day 0, booster vaccination on day 21 and I.V. infection challenge with 105 R. rickettsii Shelia Smith strain organisms recovered from embryonated chicken eggs on day ~50 (Alhassan et al., 2019). Infection progression will be monitored for 30 days. All dogs in all groups will be monitored daily for health, clinical and behavioral changes, and twice weekly for hematological changes by complete blood count analysis. Body weights will be measured once a week. Body temperatures will be measured twice a week during the vaccination phase and daily following infection challenges. Temperature assessments will be done at similar times each day. Blood sampling will be performed as per the description in our recent publication for CBC analysis, to evaluate T- and B-cell responses, and to monitor bacterial burden of circulating R. rickettsii. At the end of the experiment, the animals will be euthanized in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) using a commercial euthanasia solution. A full necropsy will be performed, and tissue samples will be assessed for gross pathology and histopathology, as in (Alhassan et al., 2019). While our preference is to do all the groups at one time, we will be able to do this experiment in two phases if we are limited by the constraints of the facilities available for housing. (Note: depending on the resource availability and personnel management, we may opt to perform this experiment as two parts.)

Note: A minor modification will be submitted prior to experiment 1 to provide the exact details of which formulations are to be used once the results have been obtained.

Table 1.
Group Vaccine vaccination date* # of animals **. Infection Challenge***
1. (Heat & Montanide gel) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
2. (Heat & QR-21 saponin) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
3. (Heat & Aluminum hydroxide) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
4. (Formaldehyde & Montanide gel). Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
5. (Formaldehyde & QR-21 saponin). Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
6. (Formaldehyde & Aluminum hydroxide) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
7. (H2O2 & Montanide gel) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
8. (H2O2 & QR-21 saponin) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
9. (H2O2 & Aluminum hydroxide) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii
10. Infection control (2 per adjuvant) Day 0 and 21 6 (3F+3M) ~day 50 I.V. 105 R. rickettsii

*All vaccinations will be performed subcutaneously.
**60 animals
**The infection challenge will be performed for all 10 groups with Shelia Smith strain of R. rickettsii.

Experiment 2: Assess the duration of immunity of WCA-S prepared using the optimum vaccine formulation.

In this experiment, we will investigate the duration of immunity induced by WCA-S. We will select the best vaccine formulation (inactivation method and adjuvant) as per the results identifying the most efficacious in Experiment 1. The criteria for selecting the best vaccine formulation will be based on the data assessments comparing the protection in clearing the clinical disease coupled with immune response determined by comparing the results of three different vaccine preparations and adjuvants. All data will be assessed and discussed by our research team to reach this conclusion. If all three vaccines will yield similar results, then we will add economic costs to determine our chose for the next set of experiments.

Note: A minor modification will be submitted after the completion of Experiment 1, and prior to any remaining experiments, to provide which vaccine formulation will be used for the experiments.

We selected four time points for assessing the protection following the booster vaccination: 2, 4, 8 and 12 months. This experiment will include four groups (n=6) and a control group (n=4) to serve as non- vaccinated infection challenge group for comparing the protection. The reason that n=4 will be sufficient to serve in the

control group as by this point we will have sufficient knowledge regarding RMSF in the dog model, which will be based on our prior work as well as the results generated from our previous experiment. (Total animals for this experiment will be 28.) Vaccination protocol will be followed as in the previous experiment. Similarly, all assays to assess the bacterial clearance, host immune responses, hematological parameters and pathological assessments will be followed as per the previous experiment, except that the infection challenge times will be different for each group. Peripheral blood and sera will be collected from the animals from all groups immediately prior to each challenge, as well as on different days post R. rickettsii challenge to evaluate cellular and humoral memory responses throughout the course of the study

Table 2.
Group Vaccine. Vaccine* days. # of animals** Infection challenge*** .
1. Vaccine formulation Day 0 and 21 6 (3F+3M) 12 months after vaccination; I.V. 105 R. rickettsii
2. Vaccine formulation Day 0 and 21 6 (3F+3M). 8 months after vaccination; I.V. 105 R. rickettsii
3. Vaccine formulation Day 0 and 21 6 (3F+3M). 4 months after vaccination; I.V. 105 R. rickettsii
4. Vaccine formulation. Day 0 and 21 6 (3F+3M). 2 months after vaccination; I.V. 105 R. rickettsii
5. Infection Control (no vaccination) 4 (2F+ 2M) infection with groups 1-4; I.V.105 R. rickettsii

*All vaccinations will be performed subcutaneously.
**28 animals
***The infection challenge will be performed with the Shelia Smith strain of R. rickettsii.

Experiment 3: Evaluate protection against tick-transmitted challenges.

In this experiment, we will investigate the efficacy of the WCA-S vaccine against tick-transmitted challenge with R. rickettsii Sheila Smith strain. We will use the optimized vaccine formulation (with inactivation and adjuvant formulation) for this experiment. Three groups of dogs will be used in the tick- transmission challenge experiments. Two groups will be used for tick transmission challenge (n=6), while the third group will be used for I.V. infection challenge. We will reduce the number of dogs to 4 in the 3rd group, as we anticipate having sufficient data already in place regarding the efficacy of the WCA vaccine against I.V. infection challenge. (Total number of animals for this experiment will be 12.) The 1st and 3rd group will receive WCA primary and booster vaccinations as described above. The 2nd group will serve as the non-vaccinated and tick-transmission infection control group. Infection challenge will be performed one month after the final WCA immunization, or as per the optimum time point established in our time course experiment described above. Dogs in groups 1 and 2 will receive tick transmitted infection challenge by allowing 25 pairs of R. rickettsii-infected adult D. variabilis ticks to feed on the dogs for a week. The third group will receive an I.V. infection challenge with 105 R. rickettsia organisms. All assays to assess the vaccine protection will be similar as in the previous experiments.

We will use engorged D. variabilis nymphal ticks (within 24 - 48 h post blood meal) obtained from a commercial vendor {we typically use BEI Resources (Manassas, VA) and the Tick Rearing Facility of Oklahoma State University (Stillwater, OK)} to inject with chicken egg embryo-derived R. rickettsii organisms suspended in PBS at a concentration of 100 bacteria per micro liter. Needle puncture inoculation (with 26-gauge needle) will be placed into the ventral side of the ticks. Ticks will then be allowed to molt to adult stage at room temperature by exposure to 14 h light and 10 h dark cycle in a 96% humidity chamber [118]; we followed this protocol as part of several earlier studies. About 10 randomly selected ticks will be assessed for the infection rates using individually isolated genomic DNAs as templates for the nested PCR targeting to AdR2 gene of R. rickettsii [22]. This method, however, may not yield infected ticks and is the reason we proposed experiments in this application seeking approval to generate infected ticks following acquisition feeding on R. ricketsii-infected dogs (described in the experimental section).
Table 3.
Group Vaccine* # of animals** Infection challenge with Sheila Smith strain***
1. WCA vaccine; 0 and 21 days 6 (3F+3M) after 1 month; tick transmission R. rickettsii
2. Infection controls. 6 (3F+3M). tick transmission R. rickettsii

3. WCA vaccine; 0 and 21 days. 4 (2F+2M) after 1 month; infection by I.V.105 R. rickettsii

*All vaccinations will be performed subcutaneously.
**16 animals
**The infection challenge will be performed with the Shelia Smith strain of R. rickettsii.

Experiment 4: Evaluate WCA protection against R. rickettsii heterologous strain infection challenges.

Experiment 4.1: Compare Sheila Smith strain derived WCA protection against Morgan strain infection challenge. In this experiment, WCA will be prepared using the Sheila Smith strain R. rickettsii and primary and booster vaccinations will be performed as per previous experiment. The infection challenge will then be performed using the heterologous, virulent R. rickettsii Morgan strain by I.V. infection and tick transmission. This experiment will include four groups (n=6); Groups 1 and 2 will be vaccinated and Groups 3 and 4 will serve as non-vaccinated controls. Groups 1 and 3 will be challenged via I.V. infection with 105 R. rickettsii Morgan strain organisms, while Groups 2 and 4 will be challenged via tick-transmission using R. rickettsii Morgan strain infected D. variabilis. (Total number of animals for this experiment will be 24.) Infected ticks will be generated as outlined previously. All parameters to assess the bacterial clearance, host immune responses, hematological responses and pathological assessments will be performed as described under aim 1.

Table 4.1.
Group Vaccine* # of animals** Infection Challenge
1. Sheila Smith WCA vaccine. 6 (3F+3M) I.V.105 R. rickettsii (Morgan)
2. Sheila Smith WCA vaccine. 6 (3F+3M) tick transmission R. rickettsii (Morgan)
3. Infection Control. 6 (3F+3M) I.V.105 R. rickettsii (Morgan)
4. Infection Control. 6 (3F+3M) tick transmission R. rickettsii (Morgan)

*All vaccinations will be performed subcutaneously.
**24 animals

Experiment 4.2: Compare Morgan strain-derived WCA protection against Sheila Smith strain infection challenge.
Approach: All proposed experiments in this sub-aim will be similar to the previous sub-aim, except that we will use the Morgan strain to prepare the WCA vaccine, and dogs will be challenged with the Sheila Smith strain of R. rickettsii. For groups 3 and 4, we will use only 4 animals each, as we expect to have sufficient data related to this kind of controls. (Total number of animals for this experiment will be 20.)

Table 4.2.
Group Vaccine* # of animals**. Infection Challenge
1. Morgan WCA vaccine 6 (3F+3M) I.V.105 R. rickettsii (Sheila Smith)
2. Morgan WCA vaccine 6 (3F+3M) tick transmission R. rickettsii (Sheila Smith)
3. Infection Control 4 (2F+2M) I.V.105 R. rickettsii (Sheila Smith)
4. Infection Control 4 (2F+2M) tick transmission R. rickettsii (Sheila Smith)

*All vaccinations will be performed subcutaneously.
**20 animals

Experiment 4.3: Compare Iowa strain derived WCA protection against Sheila Smith and Morgan strains' infection challenges.
Approach: The experimental design and assessments to monitor vaccine protection will also be similar to the previous two experiments. Here, we will use Iowa strain for preparing the WCA. The experiment will include 8 groups; four groups will receive the Iowa strain WCA vaccine, and four groups will serve as non-vaccinated controls. For the four non-vaccinated control groups, we will have four animals each. We believe that n=4 will

be sufficient for non-vaccinated infection control groups as we will have ample data from similar controls from previous two sub aims. Vaccinated groups will have 6 dogs each. (Total number of animals for this experiment will be 40.) Two groups will receive the Morgan strain infection via I.V. or by tick-transmission; the remaining two groups will be challenged with the Sheila Smith strain via I.V. or tick-transmission.

Table 4.3
Group Vaccine* # of animals** Infection Challenge
1. Iowa WCA vaccine 6 (3F+3M) I.V.105 R. rickettsii (Morgan)
2. Iowa WCA vaccine 6 (3F+3M) tick transmission R. rickettsii (Morgan)
3. Iowa WCA vaccine 6 (3F+3M) I.V.105 R. rickettsii (Sheila Smith)
4. Iowa WCA vaccine 6 (3F 3M) tick transmission R. rickettsii (Sheila Smith)
5. Infection Control 4 (2F + 2M) I.V.105 R. rickettsii (Morgan)
6. Infection Control 4 (2F + 2M) tick transmission R. rickettsii (Morgan)
7. Infection Control 4 (2F + 2M) I.V.105 R. rickettsii (Sheila Smith)
8. Infection Control 4 (2F + 2M) tick transmission R. rickettsii (Sheila Smith)

*All vaccinations will be performed subcutaneously.
**40 animals

Subcutaneous injections: Dogs receiving WCA vaccines in all the above outlined experiments will be administered subcutaneously. Total of 70 micro grams of antigen will be mixed with an adjuvant in a final volume of 500 micro liters (0.5 ml) and the entire vaccine will be administered once and at one site at the back of an on animal after shaving the inoculation site.

Infection challenges: Each dog will receive 1 ml the inoculum into left or right cephalic vein using a 23 G butterfly needle. The infection site will be aseptically prepared by shaving hair (approximately 2cm x 2cm) and cleaning with 70% ethanol. To prevent any possibility of developing anaphylactic reactions, Benadryl (diphenhydramine) will be administered 30 min prior to any intravenous vaccine or challenge inoculum administration.

Tick transmission challenge: Infection challenge with tick transmission will be done as per our published protocol. Twenty-five adult tick pairs (25 males and 25 females) infected with Sheila Smith strain or Morgan strain (as per the experiments outlined above) will be allowed to transmission feed on vaccinated dogs for 7 days. To prepare for a tick transmission experiment, we will prepare a tick containment cell for each dog. In our system, we will use containment chambers constructed from the tops of Nalgene jars that are each fitted with a screen and polyvinyl gasket that will be directly glued (3M Scotch-Weld 4799 adhesive) to the shorn back of a dog. Dogs are manually restrained for the application of the tick containment cell. The shaved area will be approximately 4 inches in diameter and to either the right or left side of the dog over the mid back area. The placement of containers will be done 24 hours prior to tick infestation. In addition, the dogs will be fitted with a collar to restrict grooming near the containment chamber. Tick infestations will be accomplished by placing 25 female and 25 male ticks on each dog. We will count ticks to be used for each dog carefully. The transfer of ticks to dogs will be performed by unscrewing the screened top of the container and placing the ticks on the dog. The top of the chamber will be re- secured immediately, and dogs will be returned to individual housing. The dog will be restricted from group play during the 7-day period that the tick containment cells are present. Dogs, tick containment chambers and tick attachments will be monitored daily until all ticks are removed from dogs. Extreme care will be taken, and all ticks will be counted (live or dead) when partially fed ticks are removed on day 7. The Nalgene top of the container will be removed from the polyvinyl gasket and the gasket will be removed by shaving. The dogs will be monitored for an additional four weeks.

Animal monitoring plan: After Rickettsia rickettsii infection with I.V. and tick transmission following vaccinations and in control groups, all animals will be monitored twice daily with once daily monitoring the body temperatures. Body weights will also be measured twice a week. While we do not anticipate serious

clinical signs for the vaccinated groups, all non-vaccinated infection controls are expected to develop a severe clinical disease. Onset of signs for I.V. may occur within three days while tick transmission may take about a week. The clinical signs will include high fever, edema, lethargy and lack of appetite. We will closely monitor the animals' health and promptly communicate with the attending veterinarian for appropriate action particularly if exhibiting depression, lethargy for more than 24 hours, and/or changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer. All animals will also be monitored for hematology and the presence of bacteria assessed by molecular methods, such as by PCR and culture recovery methods, as well as by blood smear analysis.
Blood sampling: All blood collections will be done from jugular, or anterior cephalic or lateral saphenous veins using 20-22 gauge needles.

Vaccination phase: About 20 ml of blood (10 ml in ACD tube and 10ml in EDTA tube) will be collected once a week during vaccination phase for the first 30 days and then every two weeks thereafter. In addition, 1 ml of whole blood in EDTA tube will also be obtained for performing CBC analysis once a week) for one month following vaccination. One ml of blood in EDTA tube will also be collected and used for checking the infection status twice a week for the first month. In experiment 1, after the first month of vaccination, about 20 ml blood will be collected once in every two weeks until challenge.

Challenge phase: About 20 ml of blood (10 ml in ACD tube and 10ml in EDTA tube) will be collected once a week until end point. In addition, 1 ml blood in EDTA tubes will be collected alternate days for 10 days for assessing the systemic bacterial load and 1 ml blood will be collected for CBC analysis. From day 11 to 21, blood sampled twice a week for CBC and bacterial analysis. If any dogs exhibit high fever or other clinical symptoms, additional 1 ml blood may be collected on the days of clinical signs to monitor the infection status.

Euthanasia and tissue sample collection: All dogs will be sacrificed following the assessment four-week assessment following the infection challenge. Before euthanasia, approximately 50 ml blood will be collected from vein puncture. Euthanasia will be performed in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA). Specifically, commercial euthanasia solution, Fatal-Plus®, of volume 0.22 ml/kg (86 mg/kg of pentobarbital) will be administered I.V. after the terminal bleed. The following tissue samples will be collected postmortem; spleen, liver, lymph nodes, lung, brain and bone marrow and they will be used for final detailed assessment of infection and gross pathology status.

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Project 4) Active USDA cooperative agreement grant:

Brief summary: Ehrlichia ruminantium is the disease-causing agent for an important tick-transmitted foreign animal disease, Heartwater. The goals of this project are to test if the pathogen can be transmitted by an indigenous US vector tick; Amblyomma maculatum (Gulf Coast tick). Secondly, we propose to investigate if tick feeding, and salivary gland secretions can enhance virulence of E. ruminantium in cattle.

Background: Ehrlichia ruminantium, a tick-borne rickettsial bacterium, causes Heartwater disease in ruminants resulting in a severe vascular endothelial damage throughout sub-Saharan Africa and parts of the Caribbean (Marcelino et al. 2016). Subacute and subclinical forms of the disease inflict significant morbidity, while peracute and acute forms can cause high mortalities [2]. The disease severity varies greatly depending on ruminant species, the animal breeds and their geographic origins, and also for different E. ruminantium strains (Kasari et al.2010). Nearly two centuries ago, E. ruminantium and a major tick vector, Amblyomma variegatum (the tropical bont tick, also known as the Senegalese tick) from Sub-Saharan Africa were introduced to certain Caribbean islands (Vachiéry et al. 2008). In our earlier studies, we reported the first molecular evidence to confirm the origins of E. ruminantium in the Caribbean to be from parts of northern Africa; Senegal and Sudan (Reddy et al. 1996). Despite the long presence of E. ruminantium (over two centuries) in three Caribbean islands in close proximity to each other (Guadeloupe, Antigua and Marie Galante) (Kelly et all, 2011), there is no obvious evidence of the pathogen spread and severe outbreaks (Barré et all 1995). However, the presence of the pathogen and a vector in parts of the Caribbean, coupled with the availability of potential indigenous vectors, such as Amblyomma maculatum (Gulf Coast tick), are identified as a major threat to the US ruminants. For the first time, we recently established a Heartwater research program on the mainland USA and performed the first infection study with seven different E. ruminantium strains (Nair et al. 2021). All sheep exhibited clinical signs characteristic of Heartwater disease, which included labored breathing, depression, coughing and nasal discharges. Gross pathology and histopathology observations in the animals were also consistent for Heartwater. However, the animals did not develop a severe form of disease. Specifically, we only observed subacute and subclinical disease with no progression to a fatal outcome (Nair et al. 2021). Much remains to be defined relative to the potential threat of the disease to the ruminants on the mainland USA.

The goals of this project are as follows: 1) Test if E. ruminantium can be transmitted by A. maculatum, the tick having wide distribution in southeastern parts of the USA. 2) Investigate if tick feeding and salivary gland secretions can enhance virulence of E. ruminantium in cattle.
Animals in use for all experiments: Steers of 6–12-month-old; 21 steers total will be obtained from a vendor.

Experiment 1: Determine if needle injected ticks will transmit E. ruminantium. We will generate infected ticks by following the needle infection method which we developed for other related ticks and rickettsial pathogens. We will use the infected adult A. maculatum (up to 25 pairs) for transmission experiments in one

group of four steers to measure virulence. Up to 25 pairs of needle infected ticks will be allowed to feed to repletion upon each animal. Infection assessment will be followed for 60 days.

Experiment 2: Determine if saliva/salivary glands (saliva extracts) mixed with cultured E. ruminantium will enhance virulence.

2A: Uninfected A. maculatum adult ticks (n=100) will be partially fed 4-6 days and will be removed before repletion (which typically takes about 10-15 days) on an uninfected steer (n=1). Salivary extracts will be collected from the ticks. The steers will be either adopted out, transferred to another project, or sold back to a farm.

2B: We will mix saliva extracts with E. ruminantium cultured organisms for use in infection experiments in the following four groups of animals (n=4):

Group 1. Mix saliva extracts + cultured E. ruminantium (2 x 10^8 bacteria) and use it for subcutaneous inoculations (SQ).
Group 2. Cultured E. ruminantium (2 x 10^8 bacteria) using SQ inoculation alone.
Group 3. Mix saliva extracts + cultured E. ruminantium (2 x 10^8 bacteria) and use it for IV inoculation.
Group 4. Cultured E. ruminantium IV inoculation alone (2 x 10^8 bacteria). IV infections will be performed in 2 ml volume of the inocula into jugular veins or
subcutaneous injections as per animal grouping.

Sample Collection: For both experiments 1 and 2, blood samples will be collected from the jugular veins using a 20-gauge needle. Blood will be collected twice per week starting 2 days prior to the start of the experiment; 10 ml for use in monitoring CBC, culture and DNA analysis and for immunological studies. Two ml each of additional blood sampling will be done daily when animals exhibit fever and clinical signs. At the time of euthanasia, up to 100 mL will be collected from the jugular vein.

Acquisition feeding of ticks for both experiments 1 and 2: To determine if E. ruminantium can be acquired by A. maculatum, nymphs will be allowed to feed on all four groups of animals when we begin to see clinical signs or between 7 to 14 days post infection challenges. Ticks will be allowed to attach for feeding on steers (about 500 naïve nymphal ticks). Ticks will be allowed to secure complete blood meals and then allowed to molt to adult stages. Infection rates in the molted ticks will then be assessed by nested PCR analysis. During tick feeding, animals will be housed separately in pens as necessary and as per the CMG recommendation. Tick cells will be placed on steers. For these experiments, the backs of the animals will be shaved with veterinary clippers. A stockinette sleeve or hard capsule (cell) will be glued to the backs of steers. The firm attachment will be verified after about 24 h and prior to allowing ticks to feed. The cell will remain attached for several weeks. We will monitor twice daily for the retainment of the cell on the animals, as well as its firm attachment. To perform the tick infestation, ticks will be placed inside the cells and closed with the rubber bands or screw cap lid. Ticks will be collected following opening of the cell. We will evaluate ticks from each animal following the molting to adult stage to assess E. ruminantium acquisition by ticks. We will try to account for all ticks on each animal by counting live and dead ticks.

Animal housing during tick feeding: Steers will be individually housed for the tick feeding experiments. Individual housing of the pens are necessary to prvent grooming of animls attempting to remove tick cells. Animals will be allowed to return to co-housing at the completion of tick feeding experiments, i.e., upon final tick removal which will take about 7-10 days.

Animal monitoring plan: After infection, animals will be observed twice daily with once daily monitoring the body temperatures. Upon the onset of symptoms, daily collections of 2 ml blood will be initiated. An attending veterinarian will be consulted for appropriate action if the animals appear seriously ill, such as exhibiting depression, lethargy for more than24 hours, changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer, increased heart rate of respiration, or any neurological

symptoms.

After infection challenge: All animals in all groups will be monitored for clinical signs, hematology and the presence of bacteria assessed by molecular methods, such as by PCR and culture recovery methods, as well as by blood smear analysis for the rickettsemia. All animals will be monitored for behavioral changes and any changes in their eating patterns. Body temperature will be measured daily for first two weeks and once a week thereafter until the end point of the study. Any abnormal changes noted in animals will be discussed with the CMG-assigned veterinarian for follow up action plans.

Euthanasia and tissue sample collection: All steers will be sacrificed at the end of the study by following the captive-bolt stunning method by a certified veterinarian (possibly by a VHC clinician; to be identified). Before euthanasia, approximately 100 ml blood will be collected. Euthanasia will be performed in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA). The following tissue samples will be collected postmortem; spleen, liver, lymph nodes, lung, brain and bone marrow and they will be used for final detailed assessment of infection and gross pathology status.

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5. Justify

1. Justify Use of Animals in your Research

Justify the use of animals for your experimental goals. **DO NOT** describe details of the experimental design or justify animal numbers here.

There are no non-animal alternatives for all four proposed projects. Investigations focused on pathogenesis and vaccine development studies require the use of animals, particularly those naturally acquire infections are the best to define and develop effective methods of control.

2. Justify Animal Species

Justify the choice of species for your study.

Projects 1 and 2) Dog is the perfect animal model for such studies because it acquires E. chaffeensis, E. canis and A. phagocytophilum infections naturally like humans; both canines and humans are incidental hosts for the tick-borne diseases. Moreover tick transmission studies can be done in this animal model similar to those likely occurring naturally in this host species. Dogs develop persistent infections with all three pathogens. Clinical signs with the infections in the canine host are minor. The Beagle breed is chosen for the studies because it is the most commonly reported breed for similar studies in the literature and moreover, it is easy to work with this breed. Finally, this dog breed is commercially available for use in experimental studies.

Project 3) RMSF pathogen, Rickettsia rickettsii, causes infections in dogs and people naturally from infected Ixodid (hard) ticks. We previously demonstrated that dogs develop severe form of the RMSF in the canine host (Beagle breed) and that the WCAV confers complete protection against the infection challenge. Canine model is an ideal host for defining various aspects, including assessing host-vector-pathogen interactions and vaccine potential. The beagle is chosen for this study because it is the most commonly reported breed for similar studies in the literature and moreover, it is easy to work with this breed. Finally, this breed of dog is commercially available for use in experimental studies.

Project 4) Cattle are known to acquire Ehrlichia ruminantium infections naturally in endemic regions. Thus, they are highly susceptible to Heartwater disease and is ideally suited to define if the disease can be a risk for the US cattle industry.

3. Justify Animal Numbers

Justify numbers of animals to be used (attach timeline or flow chart and power analysis, if possible, to describe study groups). This section should include a description of animals used for colony maintenance (breeders and all offspring produced) as well as a description of experimental animal numbers. Total numbers should match the requested numbers in the species section.

- Animal Numbers Justification
- The Logical Determination of "N" in Animal Experimentation
- Non-Statistical Approach for Calculating the Optimum Number of Animals Needed in Research
- Statistics and the Issue of Animal Numbers in Research
- JUSTIFY ANIMAL NUMBERS EXAMPLE

Sample size calculation was performed to identify necessary sample size to distinguish between treatment groups accounting for repeated measures over time. Type 1 error at 5% and type 2 error rate set at 20% (80% power). Calculations were performed for differences in percent of T-cells producing interferon, PCR positives assessed by conventional and real time PCR assays, and to measure antibody levels. The largest sample size required was to detect differences requiring 6 dogs in each group to detect the expected differences in pathogenesis, pathogen persistence monitoring, and to differentiate between vaccinated animals and non-vaccinated controls over time. We also will include both sexes to account for variations resulting from sex as a variable. If an experiment is repeated multiple times, then the number of animals will

be reduced to account for prior data as the way of justifying the reduced numbers; more details provided in the experimental design section.

6. Animal Husbandry

1. Facilities

In which animal facility will animals be housed?

	Facility
1	[redacted]
2	[redacted]

2. Housing Outside of Facility

Will animals be housed anywhere other than a designated animal housing facility for more than 12 hours (e.g., a laboratory)?

○ Yes ● No

3. Transportation Between Animal Housing/Use Facilities

Will animals be transported with a private vehicle between animal housing/use facilities?

○ Yes ● No

4. Non-Standard Husbandry

A. Does this protocol contain any Prolonged Physical Restraint?

See: ACUC Physical Restraint policy

○ Yes ● No

B. Does this protocol contain any Food/Fluid Regulation?

See: ACUC Food and Fluid Restriction policy

○ Yes
● No
○ Overnight only

C. Does this protocol contain Multiple Survival Surgical Procedures?

See: ACUC Multiple Survival Surgical Procedures policy

○ Yes ● No

D. Does this protocol contain any of the following Non Standard Husbandry?

☑ Single housing of social species
☐ Wire-bottom cages
☐ Special diet/water
☐ Extended time to weaning
☐

Extended time between cage changes

- ☐ Alternative light cycles
- ☐ Out of range temperatures
- ☐ Cage-size exceptions
- ☐ Other

i. Explain non-standard husbandry and list the length of time the animal will undergo non-standard husbandry.

When performing tick infestation studies, animals will need to be individually housed in their own pens, but at close proximity to each other. This will be important to minimize the damage to the tick cells placed on animals, while not adversely impacting the socialization of animals. Typically, tick cells will be on the animals up to about 7-10 days.

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7. Description of Non-Surgical Procedures

1. Sample Collection

Will samples, such as blood or tissues, be collected from live animals? (Include sampling for genotyping.)

● Yes ○ No

A. Sample Type

Type of sample(s):

Mostly blood samples will be collected. In the event of animals requiring termination, such as in the RMSF and in heartwater disease infection studies (projects 3 and 4), tissue samples will be collected from several sources to define gross lesions, histopathological assessments and to look for the presence of pathogen by molecular or cell culture methods. These details were included in the project description.

B. Sample Volume

Volume of sample(s):

Sample volumes will be variable which vary from 1 ml to 20 ml. We provided additional details in the scientific project description section.

C. Sampling Frequency and Duration

Frequency of collection and for how long:

Maximum of 20 ml blood sampling occurs at times and when this happens it will only a once a week. Some times the blood volumes are 10 ml per draw and twice a week. Many times, 1 ml blood will be sampled. These volumes will be similar for dog and cattle studies we proposed. We do not anticipate drawing more than 40 ml of blood a week per animal.

D. Sampling Method

Method of collection:

Blood samples will be collected typically from jugular veins of dogs and cattle. We will also be sampling from cephalic and saphenous veins at times. The blood collections will not be carried out via intracardiac stick.

2. Induced or Spontaneous Neoplasia

Will induced or spontaneous neoplasia occur in live animals?

○ Yes ● No

3. Non-Surgical Procedures

	Procedure	Description of procedure	Building name	Room number or area
1	Tick transmission challenge in dogs	Infection challenge with tick transmission will be done as per our published protocol. Twenty-five adult infected tick pairs (25 males and 25 females) will be allowed to transmission feed on vaccinated dogs for 7 days. Engorged nymphs (obtained from a commercially available source) will be infected with E. chaffeensis, E. canis or A. phagocytophilum by needle inoculation and allowed to molt to the adult stage (Cheng et al. 2015 and Jaworski et al., 2016). To prepare for a tick transmission experiment, we will prepare a tick containment cell for each dog. In our system, we will use containment chambers constructed from the tops of Nalgene jars that are each fitted with a screen and polyvinyl gasket that will be directly glued (3M Scotch-Weld 4799 adhesive) to the shorn back of a dog. Dogs are manually held for the application of the tick containment cell. The shaved area will be approximately 4 inches in diameter and to either the right or left side of the dog over the midback area. The placement of containers will be done 24 hours prior to tick infestation. In addition, the dogs will be fitted with a collar to restrict grooming near the containment chamber. Tick infestations will be accomplished by placing 25 female and 25 male ticks on each dog. We will count ticks to be used for each dog carefully. The transfer of ticks to dogs will be performed by unscrewing the screened top of the container and placing the ticks on the dog. The top of the chamber will be re-secured immediately, and dogs will be returned to individual housing. The dog will be restricted from group play during the 7-day period that the tick containment cells are present. Dogs, tick containment chambers and tick attachments will be monitored daily until all ticks are removed from dogs. Extreme care will be taken, and all ticks will be counted (live or dead) when partially fed ticks are removed on day 7. The Nalgene top of the container will be removed from the polyvinyl gasket and the gasket will be removed by shaving. The dogs will be monitored for an additional four weeks.	[redacted]	to be decided
2	Tick feeding experiments with cattle	Acquisition feeding of ticks for both experiments 1 and 2: To determine if E. ruminantium can be acquired by A. maculatum, nymphs will be allowed to feed on all four groups of animals when we begin to see clinical signs or between 7 to 14 days post infection challenges. Ticks will be allowed to attach for feeding on steers (about 500 naïve nymphal ticks). Ticks will be allowed to secure complete blood meals and then allowed to molt to adult stages. Infection rates in the molted ticks will then be assessed by nested PCR analysis. During tick feeding, animals will be housed separately in pens as necessary and as per the CMG recommendation. Tick cells will be placed on steers. For these experiments, the backs of the animals will be shaved with veterinary clippers. A stockinette sleeve or hard capsule (cell) will be glued to the backs of steers. The firm attachment will be verified after about 24 h and prior to allowing ticks to feed. The cell will remain attached for several weeks. We will monitor twice daily for the retainment of the cell on the animals, as well as its firm attachment. To perform the tick infestation, ticks will be placed inside the cells and closed with the rubber bands or screw cap lid. Ticks will be collected following opening of the cell. We will try to account for all ticks on each animal by counting live and dead ticks.	Middlebush Farm	to be decided

8. Substances Used in Animals

1. Substances Used in Animals

List the substances you will give the animals here (including vehicles given to controls, hazards, radiation, etc.):

	Substance	Amount/Dose/ Volume	Route	Frequency/ Duration	Hazard	Pharmaceutical Grade
1	Diphenhydramine	1mg per pound	oral	once before I.V. infections or vaccinations	No	Yes
2	Adjuvants	2.5% Montanide™ Gel	subcutaneous	twice	No	Yes
3	In vitro cultures of Ehrlichia, Anaplasma and Rickettsia species	variable	I.V.	once	Yes	No
4	Naive and rickettsial bacteria infected ticks	25 pairs of adults of both sexes or 250 nymphs (for dogs) or 500 nymphs (cattle)	on the shaved surface of the skin	once	Yes	No
5	QS-21 saponin	1 mg	subcutaneous	twice	No	Yes
6	aluminium hydroxide	2%	subcutaneous	twice	No	Yes

2. Non-Pharmaceutical Grade Substances

For those substances that are marked "no" as pharmaceutical grade, list a justification in the space below. Also, include instructions for how they will be mixed to maintain sterility and adjust pH.

3. In vitro cultures of Ehrlichia, Anaplasma and Rickettsia species used for infection studies will be obtained from our laboratory and are always grown in sterile culture conditions. Further, all procedures involving recovering the cultures will also be carried out using sterile experimental conditions.

4. Ticks are natural ectoparasites of animals. We will purchase them from a well-established tick rearing laboratory or maintained by us in the laboratory. It is not possible to obtain pharmacological grade ticks.

3. Substances Used in Animals Personal Protective Equipment (PPE)

PPE is needed to safely handle most materials in the laboratory. In general, a minimum of gloves and lab coat should be used. Other substances would require more PPE such as eye protection, respiratory protection, fume hood, etc. Please notify laboratory members if there are any special precautions that need to be taken when working with the above substances.

Describe the PPE required to handle these substances. You may group substances (e.g., "All substances" or "non-hazardous substances") if all or some use the same PPE. Please list any substances needing alternative or additional PPE separately. You do not have to include additional PPE needed for work with hazards as that will be described in the Hazards section, however, you may include here as well if you wish.

	Substance	Gloves	Eye Protection	Lab Coat	Face Mask	Fume hood	Biosafety cabinet	Double-Gloves	Other	Other PPE
1	In vitro cultures of Ehrlichia, Anaplasma and Rickettsia species	☑	☐	☑	☐	☐	☐	☑	☐	
2	Naive and rickettsial bacteria infected ticks	☑	☐	☑	☐	☐	☐	☑	☐	

Hazardous Agent

If you marked "yes" under Hazard, please complete the "Hazardous Materials" Section that follows.

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9. Hazardous Materials

1. Will you use any Biological Hazards?

● Yes ○ No

A. Biological Hazard

List all biological hazards that will be used in live animal work.

	Agent or type of hazard	Donor species	Receiving species	Dose	Route/ Volume of Admin.	Frequency of Admin.	Other
1	In vitro cultures of Ehrlichia, Anaplasma and Rickettsia species	N/A	Canine and bovine	2-5X10^8 organisms/ mL	I.V.	Once	
2	Naive and rickettsial bacteria infected ticks	N/A	Canine and bovine	1-2X10^5 organisms/ mL	N/A	Once	

B. IBC Protocol Number (if applicable for recombinant DNA or biological materials)

List your IBC Approval Number or attach your current IBC application. (Include attachments in the attached files section.)

IBC application is submitted and currently under review.

☐ Unsubmitted

☑ Submitted

☐ Approved

C. Biological Hazard - Anticipated Effect(s)

List any anticipated effect(s) of biological hazards on animal.

In project 1, E. chaffeensis random mutant organisms will be used to infect dogs. Naive nymphal stage ticks will be used to acquisition feed on dogs.

In project 2, modified live attenuated vaccine (MLAV) of E. chaffeensis and similarly, E. canis and A. phagocytophilum MLAV will be used for testing the vaccine efficacies. Infection challenges will be performed with in vitro cultured live organisms or using infected ticks. All three pathogens cause only mild disease as detailed in the project description section.

In project 3, rickettsia rickettsii cultured organisms will be used for the infection experiments before or after vaccinations. Non-vaccinated and the pathogen infected animals will develop a severe disease which can be fatal. A severe form of the disease requires close monitoring and observation and guidance of a veterinarian. We expect vaccinated animals to be healthy.

In project 4, cattle will be infected with Ehrlichia ruminantium. The pathogen may or may not cause severe disease, although we will anticipate the likelihood of developing severe clinical signs.

In all projects, we will work closely with an attending veterinarian to ensure that animals are cared humanely.

D. Biological Hazard - Housing/Procedure Sites

Where do you anticipate housing/working with animals receiving hazardous or potentially hazardous biological agents? Coordinate with the facility manager then list building and room numbers below.

	Agent	Receiving species	Building	Room or Area	Housing	Procedure
1	Tick transmission of Ehrlichia ruminantium	bovine	[redacted]	Housing and procedures	☑	☑
2	I.V. and tick transmission infections of Ehrlichia, Anaplasma and Rickettsia species	canine	[redacted]	Housing and procedures	☑	☑

E. Biological Hazard - Animal Identification

Explain how animals treated with a biological hazard will be identified (ex. cage card, ear tag, etc.)

- ☐ Cage Card
- ☐ Chip
- ☑ Door Sign
- ☐ Other

F. Hazardous Agents or By-Products /Presence

The biological hazard or by-products may be present in which of the following?

- ☐ None
- ☐ Feces/Urine/Bedding
- ☐ Saliva
- ☑ Blood
- ☐ Aerosols

- ☐ Animal bite/scratch
- ☑ Animal carcasses/tissues
- ☐ Surgical site wound or sore
- ☐ Other

G. Biological Hazard - Personal Protection Equipment (PPE) and Engineering Controls

PPE to be worn when handling biological hazards. LIDR ABSL-3 includes protective suit, shoe covers, double gloves, full-face PAPR.

	Biological Hazard	Gloves	Eye Protection	Lab Coat	Double-Gloves	Face Mask	Biosafety cabinet	LIDR ABSL-3	Other	Other PPE
1	Naive and rickettsial bacteria infected ticks	☑	☑	☑	☑	☐	☑	☐	☐	

H. Additional Information

List additional information, i.e., special precautions for pregnant women, immunocompromised individuals, special handling, or storage, etc.

2. Will you use any Chemical Hazards?

○ Yes ● No

3. Will you use any Radiation Hazards?

○ Yes ● No

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10. Anesthetic Procedures, Pain Control, Other Clinical Drugs

1. Anesthetics, Preanesthetics & Tranquilizers

Will any anesthetics, preanesthetics, or tranquilizers be used?

○ Yes ● No

2. Pharmaceutical Analgesia

○ Yes ● No

3. Non-pharmacologic control of pain

○ Yes ● No

4. Paralytic Agents

○ Yes ● No

5. Antibiotics and Other Agents

(Include any emergency drugs, fluids, etc. here)

● Yes ○ No

6. Antibiotics and Other Agents

List other agents such as antibiotics and other emergency drugs

	Species	Agent	Dose/Volume	Route	Frequency of Admin.
1	Dog	Doxycyclin	10 mg/kg	oral	once per day for four weeks

11. Description of Surgical Procedures

1. Surgical Procedures

Will there be any surgical procedures?

○ Yes ● No

12. Potential Pain or Physical Stress

Potential Pain and/or Distress

Note: Animal Welfare Act regulations define a painful procedure as "any procedure that would reasonably be expected to cause more than slight or momentary pain ... in a human being to which that procedure was applied, that is, pain in excess of that caused by injections or other minor procedures." Procedures reasonably expected to cause pain in the absence of anesthetics or pain relieving drugs should be considered to have the potential to cause pain even with the use of such drugs.

1. Potential Side-Effects and Adverse Health Effects

Describe any potential side-effects or anticipated adverse health effects of all procedures listed in the preceding sections: animal husbandry, description of non-surgical procedures, anesthetic procedures, and surgical procedures.

In projects 1 and 2, clinical signs following infection challenges with Ehrlichia chaffeensis, Ehrlichia canis or Anaplasma phagocytophilum typically include only mild fever (rise in only up to 1.5 C above body temperature). Although lethargy and joint pain are possible, based on our past research experience, we do not anticipate seeing these signs with the infections.

Clinical signs of RMSF in dogs (project 3) may include fever, nausea, vomiting, muscle pain, lack of appetite, edema, and rashes. The disease can progress rapidly to a life-threatening illness within two weeks in naive animals.

Clinical signs of Heartwater disease in cattle resulting from Ehrlichia ruminantium (project 4) may result in significant morbidity. A sudden rise in high fever (107° F) coupled with the loss of appetite, depression and increased respiratory rate are likely. Neurological disorders may follow the respiratory signs which may include excessive chewing movements, incoordination, head tilting, rigid posture and staggered walking with a high-stepping gait. Animals may also exhibit convulsions or be unable to get up. These nervous signs may progress to mortality within one to two days. It is also possible that the animals may not exhibit any nervous signs before progressing to life threatening illness.

Adjuvants in project 3 might possibly induce a reaction. We will closely monitor the animals for such reactions and will follow the guidance of a clinical veterinarian.

2. Assurance of Limited Discomfort and Pain

Describe how it is assured that discomfort and pain are limited to that which is unavoidable for the conduct of this experimentation.

Projects 1 and 2: Ehrlichia and Anaplasma species infections in dogs animals will be observed twice daily with once daily monitoring the body temperatures. Although we do not anticipate serious clinical signs in this study, a possibility of animals developing an unrelated illness cannot be ruled out. In such instances, an attending veterinarian will be consulted for appropriate action particularly if exhibiting depression, lethargy for more than24 hours, and/or changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer.

Project 3: After Rickettsia rickettsii infection with I.V. and tick transmission following vaccinations and in control groups, animals will be monitored twice daily with once daily monitoring the body temperatures. While we do not anticipate serious clinical signs for the vaccinated groups, all non-vaccinated infection controls are expected to develop a severe clinical disease. Onset of signs for I.V. may occur within three days while tick transmission may take about a week. The clinical signs will include high fever, edema, lethargy and lack of appetite. We will closely monitor the animals' health and promptly communicate with the attending veterinarian for appropriate action particularly if exhibiting depression, lethargy for more than 24 hours, and/or changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer. Infection control group animals developing severe disease will be requiring euthanasia to alleviate the pain and suffering. We will be following the guidance of the veterinarian regarding when this decision needs to be made. In the event, the animals will be euthanized in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) using a commercial euthanasia solution.

Project 4: Ehrlichia ruminantium infections in cattle will be observed twice daily with once daily monitoring the body temperatures. Upon the onset of symptoms, daily collections of 2 ml blood will be initiated. An attending veterinarian will be consulted for appropriate action if the animals appear seriously ill, such as exhibiting depression, lethargy for more than24 hours, changes in appetite lasting also for more than 24 hours, fever above 104°F for two days or longer, increased heart rate of respiration, or any neurological symptoms. While it is unclear if cattle develop a severe disease with E. ruminantium, in the event we do observe cattle infected with the pathogen develop severe disease, they will be requiring euthanasia to alleviate the pain and suffering. We will be following the guidance of the veterinarian regarding when this decision needs to be made. In the event, such cattle will be euthanized in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) by captive bolt method.

3. Pain and Distress Form

Is there a Pain and Distress form associated with this protocol?

See: Painful or Distressful Procedures

● Yes ○ No

Please attach the form in the attachments section of this protocol.

A. Which experimental groups, procedures, or animals require the Pain and Distress form?

Project 3 involving non-vaccinated dogs receiving infection by needle infection and tick transmitted challenge with Rickettsia rickettsii.

Project 4 involving the assessment of parthenogenesis in cattle following infection with Ehrlichia ruminantium.

Note: Files were attached with the previous submission.

13. Disposition of Animals

1. Animal Disposition

Check all that apply

- [x] Adoption (See MU adoption policy)
- [ ] Market
- [x] Euthanasia
- [x] Transfer to different project, PI, or another institution
- [ ] Returns to breeding colony, herd, source, owner, or wildlife site
- [ ] Other

2. Euthanasia

Euthanasia Statement

As noted in the Guide, "Euthanizing animals is psychologically difficult for some animal care, veterinary, and research personnel, particularly if they perform euthanasia repetitively or are emotionally attached to the animals being euthanized (Arluke 1990; NRC 2008; Rollin 1986; Wolfle 1985). When delegating euthanasia responsibilities, supervisors should be sensitive to this issue."

A. Method of Euthanasia

Select the method of euthanasia

- [ ] Inhalant agent
- [x] Physical Method without Anesthesia
- [ ] Physical Method with Anesthesia
- [x] Noninhalent Pharmaceutical Agent

B. Euthanasia Descriptions

	Species	Agent/Method	Dose/Volume	Route
1	Dog	Euthanasia will be performed in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (AVMA).	Fatal-Plus®, of volume 0.22 ml/kg (86 mg/kg of pentobarbital) will be administered.	I.V. injection
2	Cattle	Captive bolt method	N/A	stunner fires a retractable bolt against the animal's head, primarily into the animal's brain

C. Additional Explanation of Euthanasia Procedures

Include any additional explanation of euthanasia procedures here.

Animals will be checked for the lack of heart beat and breathing to confirm the euthanasia procedure worked accordingly.

D. Scientific Justification for Use

- [x] AVMA Approved Method
- [ ] Not AVMA Approved Method

E. Secondary (Physical) Means of Assuring Euthanasia

- [ ] Bilateral pneumothorax
- [ ] Cervical dislocation
- [ ] Decapitation
- [ ] Exsanguination
- [x] Removal of vital organs

☐ Other

14. Project Information

1.

Associate	Role	Responsibilities	OHSP Training	Animal Care & Use	Survival Surgery	P&D Training
Ganta, Roman Reddy [redacted]	Principal Investigator Authorized to order animals Access to view cages		☑ Jan 27, 2023	☑ Feb 1, 2023	☑ Feb 2, 2023	⊖
[redacted]	Co-Investigator Authorized to order animals Access to view cages	Euthanasia P&D assessment	☑ Jan 11, 2023	☑ Jan 18, 2023	☑ Jan 19, 2023	☑ Jan 23, 2023
[redacted]	Co-Investigator Authorized to order animals Access to view cages	Surgery Euthanasia P&D assessment	☑ Jan 18, 2023	☑ Jan 19, 2023	☑ Jan 19, 2023	☑ Jan 23, 2023
[redacted]	Co-Investigator Authorized to order animals Access to view cages		☑ Jan 19, 2023	☑ Jan 20, 2023	☑ Jan 20, 2023	☑ Jan 23, 2023
[redacted]	Co-Investigator Authorized to order animals Access to view cages		☑ Jan 25, 2023	☑ Feb 2, 2023	☑ Feb 2, 2023	⊖
[redacted]	Co-Investigator Authorized to order animals Access to view cages		☑ Jan 18, 2023	☑ Jan 19, 2023	☑ Jan 26, 2023	☑ Jan 23, 2023
[redacted]	Co-Investigator Authorized to order animals Access to view cages		☑ Jan 18, 2023	☑ Jan 18, 2023	☑ Jan 20, 2023	☑ Jan 23, 2023

2. Training and Qualifications

Provide a description of the training and qualifications for each individual listed above under Protocol Associates. Provide adequate detail to allow the ACUC to determine if the individual has adequate training and experience with the species and procedures to perform their role proficiently. If they do not have prior training or experience, how will this be obtained?

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
1	Ganta, Roman Reddy	dogs, sheep, and cattle	Handling, bleeding, vaccine and tick experiments, and measuring temperature.	10 years with dog work in all listed procedures Four months of working with sheep for handling and bleeding, I.V. infections Two months of working with cattle; support help with animal handling	Full-time employee
2	[redacted]	Cattle, sheep, rabbits, barnyard fowls, and wildlife animals	Animal husbandry handling, blood sampling, temperature measurements, surgical procedures, vaccine and tick studies, and euthanasia.	Served as a registered veterinary technician[redacted] 2017– 2022 Animal husbandry (etc) 10+ years Veterinary practice (technician) work with , small, exotic, and wildlife animals 3 years Trapping, hunting, and wildlife management on rural farm 10+ years. Cattle in research – 2 years Sheep in research – 1 year Dogs in research – 2 years Mice in research – 1 year Surgical experience (veterinary practice) many species – 3 years Tick and vaccine studies with animals; dogs, sheep and cattle - about 6 months with each species Euthanasia for two years.	Grad student/ Professional student
3	[redacted]	Cattle and swine	Cattle; Less than a year of experience, collecting blood, performing routine health checks Swine; Less than a year of experience, Collecting blood, taking temperature, weighing, performing routine health checks	Three months each for all the listed procedures	Grad student/ Professional student
4	[redacted]	Cattle, sheep and mice	Cattle: temperature measurement, report clinical signs, help collecting blood samples Sheep: handling, bleeding, temperature measurements Mouse: handling, mice mating, dissection, Peritoneal injection, bleeding (terminal blood collection : cardiac puncture), collect of organs, euthanize using carbon dioxide chamber	Cattle 2 years Sheep; 6 weeks Mouse 4 years	Grad student/ Professional student

	Associate	Experience with research animals:	Which procedures will this person perform?	Experience with each procedure:	Employment Status
5	[redacted]	cattle, sheep, goat, dogs, cats, donkeys, horses, and pigs	Animal handling, blood and tissue sample collection, animal health monitoring, surgeries, euthanasia and necropsies.	As a trained veterinarian (DVM equivalent) and also worked in clinical practice with 10 years of experience on all listed procedures	Postdoc fellow/ Resident
6	[redacted]	dogs, sheep, and cattle	Dog - handling, temperature and blood sampling Cattle - help with blood sample collections	Dog - 2 years handling, temperature and blood sampling Cattle - 1 year	Full-time employee
7	[redacted]	dogs, sheep, and cattle	Animal handling, blood sample collection, tick studies, and animal health monitoring	Dogs; 8 years of experience with all the above procedures. Cattle; 2 years for the listed procedures Sheep; 2 years also for the above listed procedures	Full-time employee

Training Requirements

Note: The ACUC required Basic Training can be found at: https://research.missouri.edu/acqa/. This training must be updated every three years in order to receive protocol approval.

Note: It is the Principal Investigator's responsibility to ensure that all persons listed in Protocol Associates above participate in the MU Occupational Health and Safety Program. See Section 7:020 MU Business Policy and Procedures Manual for details. For enrollment procedures visit the OHSP website.

3. Funding Source

What is the funding source for this project? (Note: If funded internally or by a non-peer-reviewing agency, a peer review of scientific merit may be required.)

- [x] PHS (NIH, CDC, FDA, NSF, NASA)
- [ ] DoD
- [ ] VA
- [ ] AHA
- [x] USDA
- [ ] Foundation/Industry
- [ ] Internal

☐ Other

15. Refinements or Literature Search

Attach relevant files in the attached files section.

1. Painful Procedures

Any procedure that may potentially cause more than momentary or slight pain or distress requires a literature search for animal alternatives.

Are you performing any procedures that may potentially cause more than momentary or slight pain or distress?

● Yes ○ No

2. USDA Covered Species

Does this protocol utilize animals covered by the Animal Welfare Act or assigned to Category E? (AWA covered species include all warm blooded animals except birds, rats of the genus Rattus, and mice of the genus Mus, bred for use in research, horses not used for research purposes, and other farm animals.)

● Yes, includes USDA covered species or Category E ○ No

3. Includes USDA covered species or Category E

Search for Animal Alternatives

In the literature search and in the written narrative, replacement by non-animal systems, reduction in numbers of animals and refinement of experimental methods (the three R's) must be addressed.

Provide at least two sources of information: one of these sources must be a scientific literature database; documented expert consultation may be used as one source of information.

If you are in the School of Medicine and need assistance with this item, please contact Rachel Alexander, HSL Research Support Librarian, at AlexanderRL@health.missouri.edu. Others can contact the Zalk Veterinary Medical Library, at MU CVM VetMed Library for help.

See also:

https://www.nal.usda.gov/awic/sample-searches
https://library.missouri.edu
Literature Search Help

A. Source 1: Literature Database

Complete the information below:

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	December 120 2022	Pubmed	1950 to current	For project 1) Searched Ehrlichia chaffeensis AND mutagenesis AND pathogenesis with or without the word dog For project 2) The following words in several combinations were searched; vaccine OR vaccines OR attenuated live vaccines AND Anaplasma AND Ehrlichia AND dogs For project 3) vaccine OR vaccines OR attenuated live vaccine OR attenuated live vaccines AND dog OR dogs OR canine AND Rickettsia OR Rocky Mountain spotted fever OR Rickettsia rickettsii AND Rocky Mountain spotted fever vaccine For project 4) Searched the following combinations and other variations of the words; (((Salivary Glands) OR (Salivary Gland)) OR (saliva)) AND ((((((heartwater) OR (heartwater disease)) OR (ehrlichia ruminantium)) OR (cowdria ruminantium)) AND (((cattle) OR (ruminant)) OR (ruminants))) AND ((((((amblyomma) OR (amblyomma maculatum)) OR (Gulf coast tick)) OR (gulf coast ticks)) OR (tick, gulf coast)) OR (ticks, gulf coast)))

B. Source 2: Literature Database

For the second source you may use a literature database search or an expert consultation (see following question).

	Date of Search	Name of Database	Years Covered by Search	Keywords and Search Strategy
1	December 20, 2022	CAB Direct	1920 to present	For project 1) Searched Ehrlichia chaffeensis AND mutagenesis AND pathogenesis with or without the word dog For project 2) The following words in several combinations were searched; vaccine OR vaccines OR attenuated live vaccines AND Anaplasma AND Ehrlichia AND dogs For project 3) vaccine OR vaccines OR attenuated live vaccine OR attenuated live vaccines AND dog OR dogs OR canine AND Rickettsia OR Rocky Mountain spotted fever OR Rickettsia rickettsii AND Rocky Mountain spotted fever vaccine For project 4) Searched the following combinations and other variations of the words; (((Salivary Glands) OR (Salivary Gland)) OR (saliva)) AND ((((((heartwater) OR (heartwater disease)) OR (ehrlichia ruminantium)) OR (cowdria ruminantium)) AND (((cattle) OR (ruminant)) OR (ruminants))) AND ((((((amblyomma) OR (amblyomma maculatum)) OR (Gulf coast tick)) OR (gulf coast ticks)) OR (tick, gulf coast)) OR (ticks, gulf coast)))

C. Source 2: Expert Consultation (alternative)

For the second source you may use a literature database search or an expert consultation. Documented expert consultation may be used as one source of information.

No Sources...

D. Animal Alternatives Narrative

Based on the information from the sources above, provide a written narrative of alternatives to procedures that may potentially cause more than momentary or slight pain or distress. The narrative

should be such that the ACUC can readily assess whether the search topics were appropriate and whether the search was sufficiently thorough.

If a possible alternative was identified or is known, but will not be employed, discuss why.

For project 1 PubMed search yielded 13 citations and 7 of them represent the work we previously published. The remaining 6, included a review, and are unrelated to the work proposed in our study. There is no evidence of duplication of our current work with any published research including our previous research. CAB Direct search with the similar word search yielded only three citations and two of which were our previous articles and a review. Again, we found no evidence for duplication.

For project 2, despite the use of several combinations of the listed words yielding 278 citation on the PubMed search, there was no evidence of any published work reporting any data on similar topics as we planned in the current study. Specifically, description of vaccine development, particularly using the live attenuated versions of Ehrlichia and Anaplasma pathogens impacting people or dogs are non-existing. CAB Direct for a similar search did not result in the detection of published research related to our proposed goals.

For project 3, Pubmed search resulted in 92 articles; 23 of which are related to vaccine studies in the past. Our recent publication on the topic is among the identified publications (Alhassan et al. 2019, Infect Immun. 2019 Jan 24;87(2):e00628-18. doi: 10.1128/IAI.00628-18). This article summarizes all the work prior to our study. Notably, the prior research focused mostly on inactivated vaccines did not translate in outcomes research for the RMSF vaccine development. The review article Richards [Expert Rev Vaccines. 2004 Oct;3(5):541-55. doi: 10.1586/14760584.3.5.541] is among the articles found. It summarizes the importance of our study as it stated that the vaccine studies in the past century to prevention of rickettsial diseases did not yield any rickettsial vaccines manufactured and/or licenses. Also stated that "Early rickettsial vaccines were difficult, expensive and very hazardous to produce." Based on all these analyses, it is evident that the only significant publication related to vaccine studies is our recent publication. The current project, thus, extends our previous published work in developing vaccine that will likely be valuable for application for controlling the RMSF in dogs and possibly in people in the near future. Cab Direct found four results which included our above listed publication (Alhassan et al. 2019) and the remaining articles are unrelated the proposed project goals. Our prior publication indeed is the basis for expanding research on the current funded NIH grant for which this search was performed.

For project 4, a maximum of 9 citations were identified, but none of the publications were directly relevant to the project description we proposed. Thus, we will not be duplicating any prior studies.

---

16. Investigator Assurances

1. ABSL-2 Assurance

I will provide training to the husbandry/veterinary staff at least 48 hours prior to exposing animals to a biohazard regarding (but not limited to): the health hazards and symptoms of the biohazard(s) being used; husbandry related research specific SOP's (e.g. handling live exposed animals and contaminated cages); and animal/carcass disposition.

- ● Yes, I will meet the requirements of this statement.
- ○ No, I will not meet the requirements of this statement.
- ○ Not Applicable

2. Investigator Assurances

☑ 1. The information provided herein is accurate to the best of my knowledge.

☑ 2. Procedures involving vertebrate animals will be performed only by trained or experienced personnel, or under the direct supervision of trained or experienced persons.

☑ 3. Any change in the care and use of vertebrate animals involved in this protocol, will be promptly forwarded to the MU ACUC for review; such changes will not be implemented until the committee's approval is obtained.

☑ 4. The number of animals proposed is the minimum necessary to conduct valid experimentation.

☑ 5. I assure that I am not unnecessarily duplicating previous experiments.

☑ 6. I have considered alternative methods to using animals.

☑ 7. I understand that animal housing must be coordinated with the facility veterinarian and/or facility manager and that approval of this protocol does not guarantee space to house animals.

2023-03-17 11:53:49 -0500

University of Missouri

Animal Care Quality Assurance

[redacted]

March 17, 2023

Subject: IACUC APPROVAL

Dear Dr. Ganta,

Your MU animal use protocol **Protocol 41056 Application 1.3** entitled *"Tick-borne rickettsial diseases; pathogenesis and vaccine development"* was approved by the IACUC on March 17, 2023 and will expire on March 17, 2026.

Sincerely,

R. Scott Rector

Scott Rector, Ph.D.
Chair, Animal Care and Use Committee
Division of Research, Innovation & Impact

[redacted]

Pain and Distress Form

PI: Ganta Protocol #: 41056 Species: Bovine

Expected Clinical Signs (phenotype, disease, response to manipulations, etc.): *Ehrlichia ruminantium* may cause severe vascular endothelial damage in ruminants. Disease severity varies depending on ruminant species, breed, geographic origin, and strain of bacteria. Possible clinical signs include high fever, loss of appetite, depression, and increased respiratory rate. Neurologic signs such as excessive chewing movements, incoordination, head tilt, rigid posture, staggered walked or convulsions are also possible.

Scoring Initiation (criteria or time when scoring will start): Scoring will start the day following infection (either via injection or tick exposure) with *E. ruminantium.*

Scoring Frequency and Duration:

Frequency: If score < 0.5, score once daily
If score ≥ 0.5, score twice daily

Duration: Scoring will be performed until euthanasia. After the first 14 days, if the body temperature is normal, body temperature frequency may be adjusted to once a week.

If total score ≥ 0.8 or neurologic signs present, contact veterinarian
If total score ≥ 1.0, euthanize animal unless veterinarian permits a recheck*

*If an animal's total score is ≥1.0, the animal will be euthanized, or an OAR veterinarian must be notified to evaluate the animal. If the animal is determined to be in stable condition by the veterinarian, a recheck of the animal may be performed 8 hours later, or at an interval recommended by the veterinarian.

Observation	Score	Details
Attitude	0.0	BAR (Bright/Active/Responsive)
	0.2	Quiet but alert and rouses/responds when approached or touched
	0.6	Lethargic, slower to rouse/respond, may vocalize or be reluctant to stand
	1.0	Recumbent and minimally responsive
Temperature	0.0	<103.5°F
	0.2	≥103.5 but <105°F
	0.4	≥105 but <106°F
	1.0	≥106°F
Respiration	0.0	Normal respiratory rate and depth
	0.4	Increased respiratory rate and/or effort, occasional coughing
	0.6	Labored breathing, and/or nasal discharge, frequent coughing
Appetite	0.0	Eating and drinking normally, appears hydrated (skin does not tent)
	0.2	Decreased food consumption, but appears hydrated (skin does not tent)
	0.8	Minimal food consumption and/or appears dehydrated (skin tents)

Pain and Distress Form

PI: Ganta Protocol #: 41056 Species: Bovine

Date	Animal #	Attitude Score	Temperature Score	Respiration Score	Appetite Score	Total Score	Action Taken	Scorer's Initials

Pain and Distress Form

PI: Ganta Protocol #: 41056 Species: Canine

Expected Clinical Signs (phenotype, disease, response to manipulations, etc.):

Rickettsia rickettsii can cause severe disease in non-vaccinated animals. Possible clinical signs include fever, nausea, vomiting, muscle pain, loss of appetite, edema, and skin rashes. The disease can progress rapidly to a life-threatening illness within two weeks in naïve animals.

Scoring Initiation (criteria or time when scoring will start): Scoring will start the day following infection (either via injection or tick exposure) with *R. rickettsii.*

Scoring Frequency and Duration:

Frequency: If score < 0.5, score once daily
If score ≥ 0.5, score twice daily

Body weights will be performed at least once a week. On all other days, a body condition score (BCS) may be used to assess animal for evidence of weight loss.

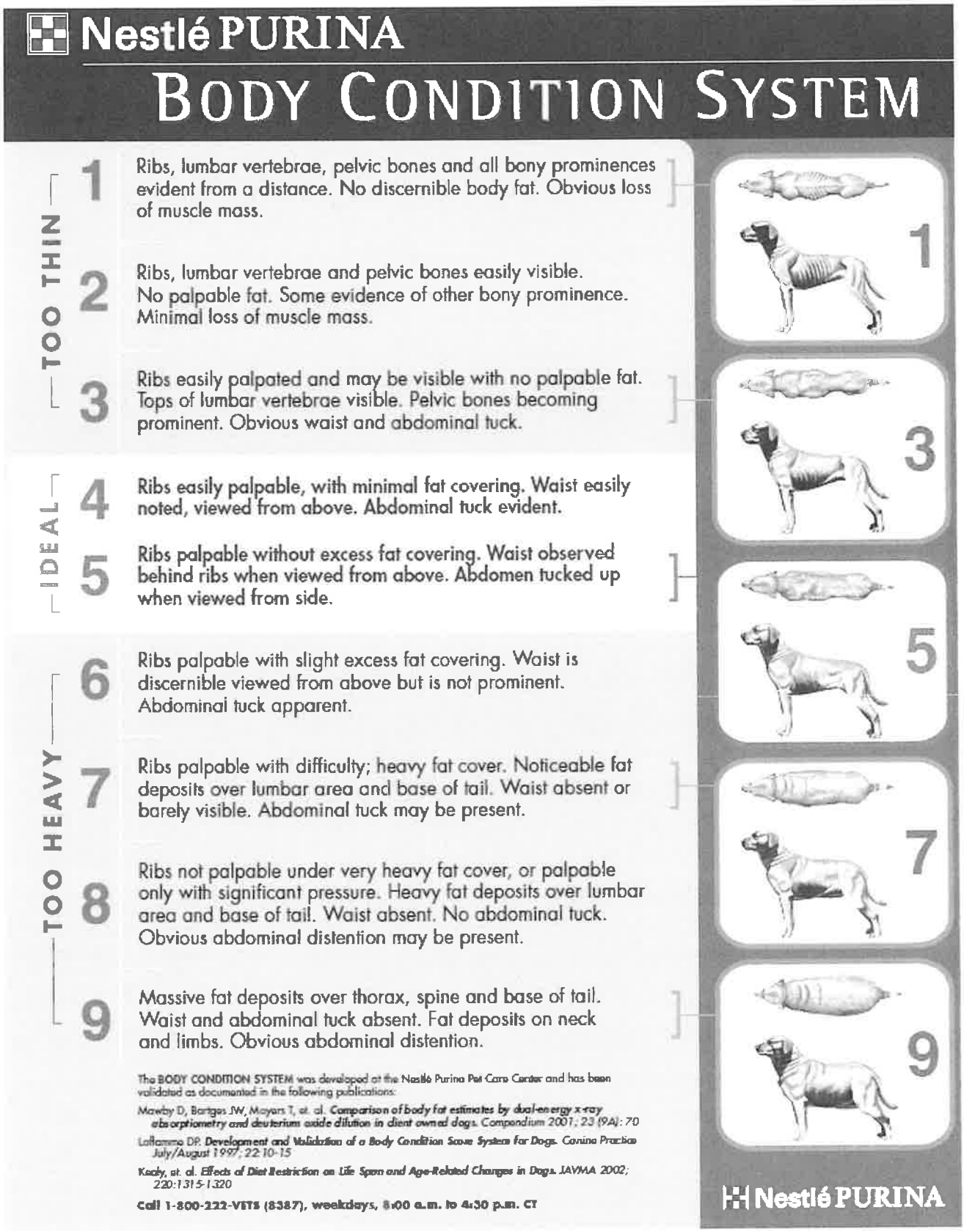
Duration: Scoring will be performed until euthanasia. After the first 14 days, if the body temperature is normal, body temperature frequency may be adjusted to once a week.

If total score ≥ 0.8, contact veterinarian
If total score ≥ 1.0, euthanize animal unless veterinarian permits a recheck*

*If an animal's total score is ≥1.0, the animal will be euthanized, or an OAR veterinarian must be notified to evaluate the animal. If the animal is determined to be in stable condition by the veterinarian, a recheck of the animal may be performed 8 hours later, or at an interval recommended by the veterinarian.

Observation	Score	Details
Attitude	0.0	BAR (Bright/Active/Responsive)
	0.2	Quiet but alert and rouses when approached or touched
	0.6	Lethargic, slower to rouse, may vocalize or be reluctant to stand
	1.0	Recumbent and minimally responsive
Weight Loss or Body Condition Score (BCS)	0.2	<5% weight loss OR BCS 4-9 / 9 (ideal body condition or overweight)
	0.6	10-20% weight loss OR BCS 3 / 9 (thin, bones can be felt with slight pressure and may be visible)
	1.0	>20% weight loss OR BCS ≤ 1-2 / 9 (very thin, bones can be felt easily and are visible)
Temperature	0.0	<103.5°F
	0.2	≥103.5 but <105°F
	0.4	≥105 but <106°F
	1.0	≥106°F
Appetite	0.0	Eating and drinking normally, appears hydrated (skin does not tent)
	0.2	Decreased food consumption, but appears hydrated (skin does not tent)
	0.8	Minimal food consumption and/or appears dehydrated (skin tents)

Figure 1. Body Condition Scoring in Dogs



Pain and Distress Form

PI: Ganta Protocol #: 41056 Species: Canine

Date	Animal #	Attitude Score	Weight Loss or BCS Score	Temperature Score	Appetite Score	Total Score	Action Taken	Scorer's Initials

ELECTRONICALLY FILED
3/26/2026 10:17 AM
43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

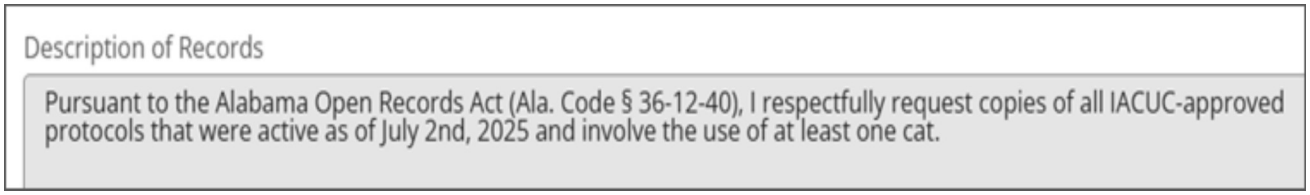
EXHIBIT E

From: **Madelyn Bingham** mjb0174@auburn.edu
Subject: FW: Open Record Request Acknowledgment - IACUC Protocols
Date: October 10, 2025 at 8:38 PM
To: KM@whitecoatwaste.org

MB

From: Open Records <openrec@auburn.edu>
Date: Wednesday, July 16, 2025 at 1:07 PM
To: Madelyn Bingham <mjb0174@auburn.edu>
Subject: Open Record Request Acknowledgment - IACUC Protocols

Our office has received your open records request, and we are currently reviewing the request. You can expect a response within 15 business days unless it is determined more time is required to provide a substantial response, in which case you will be notified of an extension via email.



Sincerely,
Auburn University Open Records

ELECTRONICALLY FILED
3/26/2026 10:17 AM
43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

EXHIBIT F

From: **Madelyn Bingham** mjb0174@auburn.edu
Subject: FW: Auburn University Open Records Request Decision
Date: October 10, 2025 at 8:41 PM
To: KM@whitecoatwaste.org

MB

From: Open Records <openrec@auburn.edu>
Date: Thursday, September 18, 2025 at 2:28 PM
To: Madelyn Bingham <mjb0174@auburn.edu>
Subject: Auburn University Open Records Request Decision

Madelyn,

Your request for an open record from Auburn University has been **denied.**

Please review the comment below for more details.

Approver Comments: After a diligent search, we have not been able to locate any records responsive to your request subject to release under the Alabama Open Records Law. Sincerely, Auburn University Open Records Officer

After reading the comment, if you feel this was made in error feel free to submit a new request with updated information.

Thank you,

Auburn University General Counsel

ELECTRONICALLY FILED
3/26/2026 10:17 AM
43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

EXHIBIT G

From: Madelyn Bingham
To: Kailey Mauro; Jared@whitecoatwaste.org
Subject: FW: Auburn University Open Records Request Decision
Date: Tuesday, October 28, 2025 11:00:31 AM

From: Open Records <openrec@auburn.edu>
Date: Tuesday, October 28, 2025 at 7:39 AM
To: Madelyn Bingham <mjb0174@auburn.edu>
Cc: Jaime Hammer <jsh0073@auburn.edu>
Subject: RE: Auburn University Open Records Request Decision

Hello Madelyn,

Upon review the records sought are not subject to release under the Alabama Open Records Law. The Alabama Open Records Law allows residents the opportunity to inspect public records subject to appropriate protections for private, confidential, privileged, and other nonpublic information. *(AL Code S36-12-43)* State and federal laws, as well as case law, provide protection for scientific research materials, documents, and records, which are not the same as agency policymaking documents, and may qualify as confidential, privileged, or nonpublic in nature. Research materials, documents, and records, including but not limited to, research protocols, drafts, communications, peer review commentary, preliminary results, underlying data, or study methodologies, etc., are not subject to release under the Alabama Open Records Law if the records sought are prepublication preliminary research documents; have not been publicly disseminated, published, copyrighted, or patented; contain intellectual property, trade secrets, or other confidential material; are received in confidence by a public officer; or the release of the records sought will unduly interfere or hinder the discharge of a public officer's duties or government business.

Sincerely,
Auburn University Open Records Officer

From: Madelyn Bingham <mjb0174@auburn.edu>
Sent: Monday, October 27, 2025 4:59 PM
To: Open Records <openrec@auburn.edu>
Cc: Jaime Hammer <jsh0073@auburn.edu>
Subject: Re: Auburn University Open Records Request Decision

Hi,

My name is Madelyn Bingham, and I'm writing to follow up on your response to my recent open records request. I am a recent Auburn graduate and have great interest in how the federal government, mainly the National Institutes of Health, is spending taxpayer dollars to fund animal experiments at my alma mater. That is why I submitted my Alabama Open Records Act request for "all IACUC-approved protocols

that were active as of July 2, 2025 and involve the use of at least one cat."
Auburn's response to my request is unclear. It said, "we have not been able to locate any records responsive to your request subject to release under the Alabama Open Records Law." This is ambiguous as to whether there are no records subject to my request, or there are records, but they are being withheld. The response's general reference to the Open Records Law and no specific exception leaves me no way of knowing why my request was denied. Providing a reasonably specific reason for denying a request seems like a bare-minimum standard under the Open Records Law and is generally what is expected when a citizen asks their government for transparency.

I believe the records I requested exist. In Auburn's most recent report to United States Department of Agriculture's Animal and Plant Health Inspection Service (enclosed), Auburn reported having 52 cats who were used for experiments and 82 cats who were held for use in future experiments. Auburn is also conducting experiments on cats in a research collaboration with Northwestern University, according to National Institutes of Health Project/Grant number R01NS115571. This project or grant has a current end date of May 31, 2026. Additional details about this grant can be found here: https://reporter.nih.gov/search/sYLzk8y3Q0q4XfyFKi-SWQ/project-details/10849633#description. These experiments and the cat colony maintained both require protocols, and Auburn's website confirms that its IACUC is required to review protocols for experiments involving animal testing: https://cws.auburn.edu/OVPR/pm/compliance/iacuc/home.

I ask that Auburn please reconsider its response and provide the requested records, or alternatively, at least clarify its response so that I can tell (1) whether the records exist and (2) if they exist and are being withheld, a reasonably specific reason for your denial of my request. I would ask that Auburn send me a clarified response or the records themselves within 7 days.

Best,

Madelyn Bingham

From: Open Records <openrec@auburn.edu>
Date: Thursday, September 18, 2025 at 2:28 PM
To: Madelyn Bingham <mjb0174@auburn.edu>
Subject: Auburn University Open Records Request Decision

Madelyn,

Your request for an open record from Auburn University has been **denied**.

Please review the comment below for more details.

Approver Comments: After a diligent search, we have not been able to locate any records responsive to your request subject to release under the Alabama Open Records Law. Sincerely, Auburn University Open Records Officer

After reading the comment, if you feel this was made in error feel free to submit a new request with updated information.

Thank you,

Auburn University General Counsel

ELECTRONICALLY FILED
3/26/2026 10:17 AM
43-CV-2026-900213.00
CIRCUIT COURT OF
LEE COUNTY, ALABAMA
MARY B. ROBERSON, CLERK

EXHIBIT H

RUSHTON ◇ STAKELY
Lawyers Since 1890 MONTGOMERY ◆ BIRMINGHAM

J. Evans Bailey
Direct: 334-206-3170 E-mail: ebailey@rushtonstakely.com

December 10, 2025

Via email openrec@auburn.edu
Auburn University
Open Records Request
152 S. College Street
Auburn, Alabama 36849

To Whom It May Concern:

My name is Evans Bailey, and I represent Madelyn Bingham, an Auburn graduate, related to her recent open records request to Auburn University ("Auburn") regarding protocols associated with laboratory testing on cats.

As I understand it, sometime in July 2025, Madelyn requested "all IACUC-approved protocols that were active as of July 2, 2025 and involve the use of at least one cat." After acknowledgement and an extension, Auburn told Madelyn "we have not been able to locate any records responsive to your request subject to release under the Alabama Open Records Law" on September 18, 2025. After Madelyn pushed back on this vague and confusing response, Auburn provided a longer, but not necessarily more enlightening, explanation for its decision on October 28, 2025. It reads, in relevant part:

> State and federal laws, as well as case law, provide protection for scientific research materials, documents, and records, which are not the same as agency policymaking documents, and may qualify as confidential, privileged, or nonpublic in nature. Research materials, documents, and records, including but not limited to, research protocols, drafts, communications, peer review commentary, preliminary results, underlying data, or study methodologies, etc., are not subject to release under the Alabama Open Records Law if the records sought are prepublication preliminary research documents; have not been publicly disseminated, published, copyrighted, or patented; contain intellectual property, trade secrets, or other confidential material; are received in confidence by a public officer; or

> the release of the records sought will unduly interfere or hinder the discharge of a public officer's duties or government business.

This response remains flawed in that it provides no specific references to the supposed state and federal laws or case law on which it relies. An average citizen, or even a lawyer, has no way of knowing if what the response says is true about supporting authority for Auburn's position because no citations are provided.

On the other hand, there is strong support for the notion that the Institutional Animal Care and Use Committee (IACUC) protocols Madelyn requested are public records subject to disclosure. Alabama law defines public writings (or public records) as both (1) "all written, typed or printed books, papers, letters, documents and maps made or received in pursuance of law...in the transactions of public business and shall also include any other public record authorized by law" under Ala. Code Sec. 41-13-1, and (2) "such a record as is reasonably necessary to record the business and activities required to be done or carried on by a public officer so that the status and condition of such business and activities can be known by our citizens" from *Stone v. Consolidated Publishing Co.*, 404 So. 2d 678, 681 (Ala. 1981).

The IACUC is a federally mandated committee under the Animal Welfare Act that oversees animal research programs (including cat research), facilities, and procedures, for research facilities, like Auburn. See 7 U.S.C. § 2143 & 9 C.F.R. 2.31. The United States Department of Agriculture regulations require IACUCs to ensure that animal testing meets certain criteria related to personnel, housing, procedures, rationale, objectives, minimization of pain and distress, safety factors, and final disposition of the test subjects. *Id.* These criteria are put into protocols (also called applications) before testing or research begins, which the IACUC for a particular institution either approves or disapproves and then re-evaluates as research continues. As Auburn's website notes, its IACUC "reviews all animal use protocols, reviews the animal care and use program, and monitors university animal facilities to ensure compliance with standards and regulatory requirements." https://research.auburn.edu/research-administration/compliance/iacuc/index.php (accessed 11/24/25) (underline added).

Similarly, research funded by the National Institute of Health is subject to the Public Health Service Policy on Humane Care and Use of Laboratory Animals, which requires institutions to implement programs for activities involving animals

based on the *Guide for the Care and Use of Laboratory Animals*. It also requires a written Animal Welfare Assurance, describing training offered to scientists and laboratory personnel in animal care and in methods that minimize the number of animals used and minimize animal distress. https://olaw.nih.gov/policies-laws/phs-policy.htm (accessed 11/24/2025). These requirements stem from the Health Research Extension Act of 1985, Public Law 99-158, "Animals in Research" (November 20, 1985).

In short, the protocol(s) that Madelyn requested clearly meet the definition of public records under the Alabama Open Records Act because they are made or received in pursuance of law and record the business and activities required to be done or carried on by a public officer. Federal law mandates that Auburn both have an IACUC and that it review and approve protocols for compliance with federal law.

Other states and the federal government have made these protocols public. Below is a ShareFile link which contains:

3 federal protocols for cat, dog, and nonhuman primate experiments
Kansas State University cat protocol
University of Georgia cat protocol
University of Louisville cat protocol
University of Missouri-Columbia cat and dog protocols.

https://rushtonstakley.sharefile.com/d-s83b66a5fab614c69831835d257b45e4e

In addition, the ShareFile link also contains cat veterinary and acquisition records which the University of Alabama has made public.

Further demonstrating that these protocols are subject to disclosure, extensive information regarding an Auburn cat protocol active as of July 2, 2025, and therefore subject to this request, is already in the public domain. The federal funding application for the experiments—conducted in conjunction with Northwestern University, Tufts University, and University of Massachusetts Medical School—reveals: the project abstract, narrative, methods, and goals, and highly detailed descriptions of the experiments that will be conducted at Auburn in Douglas Martin's lab, the facilities where they will be conducted, who will conduct them, the equipment to be used, the cats to be experimented on, the intended animal care, the justification for using them, when they will be euthanized, and the federal funds

requested. Auburn also has breeding colonies to supply cats for these and other experiments, which themselves necessitate active protocols for as long as the university maintains them. The protocols related to these experiments and breeding colonies could hardly be less confidential, privileged, or worthy of trade secret protections.

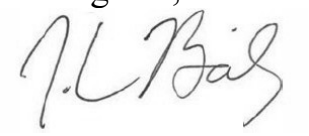
Auburn's decision not to produce the requested protocol(s), in any form or fashion, is clearly a violation of the Alabama Open Records Act. On behalf of Madelyn, I hereby demand that Auburn reconsider its position and produce the protocols on or before January 5, 2026.

While Auburn has cited confidentiality, privilege, trade secrets, and numerous other possible exceptions to the Alabama Open Records law, it is clear based on the publicly available protocols and the funding application included herewith that the entirety of a protocol is not made up of such materials. If such materials are present in the requested protocol(s), they can be redacted and the remainder produced. *See Allen v. Barksdale*, 32 So. 3d 1264 (Ala. 2009); Op. Att'y Gen. Ala. No. 2006-134, 2006 Ala. AG LEXIS 97 (Aug. 17, 2006); Op. Att'y Gen. Ala. No. 2008-073, 2008 Ala. AG LEXIS 43 (Apr. 21, 2008). Any redactions, or the failure to produce the protocol(s) entirely, should be supported in the form of a privilege or confidentiality log that comports with Rule 26 of the Alabama Rules of Civil Procedure and provides Madelyn with some level of specificity so she can judge whether to challenge Auburn's assertions. *Something Extra Publ'g, Inc. v. Mack*, 350 So. 3d 663, 671 (Ala. 2021) (Stewart, J., concurring). Indeed, if Madelyn is forced to litigate this matter, the burden will be on Auburn to establish that exceptions to the Open Records Law have merit. *Chambers v. Birmingham News Co.*, 552 So. 2d 854, 856-57 (Ala. 1989).

I also demand that Auburn provide the name of its "Auburn University Open Records Officer" within the same timeframe. Like with its initial response to Madelyn's request, Auburn has been purposefully evasive in not naming the individual who is responsible for responding to Madelyn's request. A google search for "Auburn University Open Records Officer" leads to this web page which identifies the "Office of General Counsel" and an email address: https://auburn.edu/administration/general-counsel/openrecords.php. As Auburn's general counsel knows, Auburn the institution is an instrumentality of the state and cannot be sued. *Rigby v. Auburn Univ.*, 448 So. 2d 345, 347 (Ala. 1984). However, its agents and employees can be sued to force them to comply with the law. It

appears Auburn has purposefully avoided publicly naming its Open Records officer to draw unsuspecting citizens into filing suits against an immune entity. Please provide the name of the individual responsible for responding to Madelyn's requests by January 5, 2026.

Regards,



J. Evans Bailey

JEB/ttf

c: Jaime S. Hammer, Esq.
General Counsel