IACUC Protocols and Review Documents

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

March 25, 2009

MEMORANDUM FOR DR. [redacted] DEPARTMENT OF PEDIATRICS

SUBJECT: IACUC Approval of Protocol - Triennial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) via Designated Member Review on March 25, 2009:

Animal Protocol Title: "Control of Blood Pressure and Cardiac Function in the Pig"

USUHS Protocol Number: PED-09-420

Expiration Date: March 24, 2012

Supporting Grant(s) Number: 302100

Name of Principal Investigator: Dr. [redacted]

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact [redacted] to schedule a pre-protocol planning meeting [redacted] This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.

[redacted]
Chair, Institutional Animal
Care and Use Committee

cc:
Office of Research

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

PROTOCOL NUMBER: PED-09-420

PROTOCOL TITLE: Control of Blood Pressure and Cardiac Function in the Pig.

GRANT TITLE (if different from above): N/A

USUHS PROJECT NUMBER: N/A this is a teaching protocol funded by TRS

FUNDING AGENCY: USUHS

EARLIEST ANTICIPATED FUNDING START DATE: funding is ongoing

PRINCIPAL INVESTIGATOR: [redacted]

(b)(6)

Pediatrics (Department) [redacted] (Office/Lab Telephone) 20 Mar 09 (Date)

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

(b)(6)

Professor and Chair (Title) [redacted] (Telephone) 20 Mar 09 (Date)

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

(b)(6)

Pediatrics (Department) [redacted] (Telephone) 20 Mar 09 (Date)

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

(b)(6)

LAM (Department) [redacted] (Telephone) 20 Mar 09 (Date)

USUHS Form 3206- Animal Study Proposal Form Instructions

USUHS / DOD – SPONSORED ANIMAL RESEARCH PROPOSALS MUST USE THIS STANDARDIZED FORMAT

Reference DOD Directive 3216.1 & USUHS Instruction 3203

****

Specific information requested in the following animal-use protocol template is a result of requirements of the Animal Welfare Act regulations (AWAR), the Guide for the Care and Use of Laboratory Animals, and other applicable Federal regulations and DOD directives.

****

This document is intended to be an aid in the preparation of a USUHS DOD – sponsored animal use proposal. The instructions and written explanations provided for individual paragraphs (ref. animal-use protocol template in AR 40-33 / USUHSINST 3203, Appendix C) are coded as hidden text. To see the instructions and examples for each section, select the "**Show/Hide ¶**" button on your tool bar. To print the hidden text, select "Print" on the drop down file menu. Under the "Options" button, select "Hidden text" under the "Include with document" section. Use of a word processor makes completion of this template a "fill-in-the-blanks" exercise. Please provide all response entries in the following font: Arial, Regular, 12, Black. Please do NOT submit this page of instructions with your animal protocol submission.

With the exception of title headings, each paragraph and subparagraph in the following template must have a response. Portions of the template that are not applicable to your particular protocol, i.e., no surgery or no prolonged restraint, should be marked "**N/A**". There are no space limitations for the responses.

Pertinent standing operating procedures or similar documents that are readily available to your IACUC may be referenced to assist in the description of specific procedures. It is critical that only animal studies or procedures documented in an IACUC – approved protocol be performed at your facility. Additionally, Principal Investigators, or other delegated research personnel, should keep accurate experimental records and be able to provide an audit trail of animal expenditures and use that correlates to their approved protocol.

ANIMAL PROTOCOL NUMBER:
PED-09-420
PRINCIPAL INVESTIGATOR:
[redacted]
ANIMAL PROTOCOL TITLE: Control of Blood Pressure and Cardiac Function in the Pig.

GRANT TITLE (if different from above): N/A

USUHS PROJECT NUMBER: N/A

CO-INVESTIGATOR(S): All LAM staff
[redacted] Department of Anatomy, Physiology and Genetics
TECHNICIANS(S): All LAM vet techs, surgical techs, veterinarians.

I. NON-TECHNICAL SYNOPSIS:

With the guidance and direction of university faculty, medical students will perform procedures and observe some of the most important factors responsible for the control of blood pressure and cardiac function. Students will be asked to locate and identify major blood vessels and nerves. They will observe the effects of injecting cardiovascular drugs and vagal nerve stimulation on blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) in the presence and absence of the strongest cardiovascular reflex mechanism. Students will place catheters into different compartments of the cardiovascular system in a manner performed clinically in humans from which they can observe the blood pressures from all chambers of the heart, systemic circulation and the pulmonary circulation. Students will observe the beating heart inside the chest cavity. Students will also perform open-chest cardiac massage.

II. BACKGROUND:

II.1. Background:

It is well-established that students learn more and retain more information when that information can be presented in a real-life situation. Laboratories, including the study of live animals, have long been accepted and used to support the instruction of science. The procedures described in this protocol are aimed at enhancing the knowledge and experience of our medical students prior to their first clinical expenses.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched:
Although using animals for teaching is duplicative in its nature, I searched the following for ideas in the 3 R's: BRD , CRISP and PubMed
II.2.2. Date of Search:
BRD (3/18/09), CRISP (3/18/09) PubMed (3/18/09)
II.2.3. Period of Search:
BRD (1998-2007), PubMed (1950 - present), CRISP (1972-2009)

II.2.4. Key Words and Search Strategy:
Cardiovascular and (pig or swine or porcine) and (teaching or education)

II.2.5. Results of Search:

BRD: No hits were retrieved until I broadened the search to just "cardiovascular and education or teaching or training". This yielded 10 hits involving pigs. Three of which were past approvals of this protocol.7 were training protocols from military medical centers, validating the value of the use of pigs in cardiovascular training and education.

PubMed: 141 citations were found. Most citations dealt with exercise training and effects on the cardiovascular system. Nearly all used the pig as a model of the cardiovascular system in humans. We identified 13 citations describing the use of the pig as a teaching model for cardiovascular training and assessment. The most interesting (Gupta S., et al, *Adv Physiol Educ,* June;29(2): 118-27, 2005) describes the use of a model very similar to that described here to educate second year medical students. The authors state "...has proven effective in reinforcing the fundamental principles of cardiovascular physiology and pharmacology." They report that there is greater than 90% participation in this optional exercise.

CRISP:- 6 hits but none relevant. 1 was interesting in that it was a Pharmacology short course using instrumented dogs and rabbits to demonstrate fundamentals of cardiovascular physiology and neuropharmacology

III. OBJECTIVE\HYPOTHESIS:

The purpose of this laboratory exercise is to teach, through direct experience and demonstration, dynamic cardiovascular anatomy and some common dynamic cardiovascular functions in the live animal. This first-hand experience should help the student understand and remember these basic cardiovascular functions. The students will also experience, for the first time, patient to patient (animal to animal) variability in physiological responses to stimuli. They will also appreciate the complexities of anesthesia and some drug-drug interactions and how animal to animal variability further complicates these complexities. More specifically the student will:

1. To learn how to locate and isolate the external jugular vein, carotid artery and the vagus nerve in a live animal
2. To learn how to catheterize the external jugular vein.
3. To analyze the cardiovascular response to an increase in blood pressure with and without the vagus nerves intact.
4. To analyze the cardiovascular response to an inotropic drug.
5. To analyze the cardiovascular effects of severing and stimulating the vagus nerves.
6. Place catheters into the left ventricle, superior vena cava, right atrium, right ventricle and pulmonary artery and identifying the pressures characteristic of each of these compartments of the cardiovascular system.
7. To appreciate the strength and potential of the heart by making direct observations of the heart in response to a contractility-enhancing drug.
8. To learn the ability of external stimuli to alter the heart's performance by, directly electrically pacing the heart, atria and ventricles.
9. Create myocardial ischemia and appreciate the changes in electrocardiogram and appearance of the heart.
10. Cause fibrillation and directly observe defibrillation of the heart.
11. To appreciate the ability of the heart by performing direct cardiac massage in the fibrillated heart.

IV. MILITARY RELEVANCE:

This learning experience will prepare our medical students to be more effective clinicians. This laboratory experience is the first in which students will observe live tissue. They will begin to build their knowledge of cardiovascular physiology toward the understanding of trauma medicine and surgery. Since our medical students become the medical officers of the future, we are preparing them for a career in military medicine. In order to achieve this goal, we must provide the best learning experience possible for those who will take care of our military personnel and their dependents.

V. MATERIALS AND METHODS:

V.1. Experimental Design and General Procedures:

- Animals will be sedated, intubated and anesthetized to a surgical plane.
- Instructors will place catheter sheaths in the right common carotid artery and the external jugular vein. They will also isolate the right vagus nerve. After observing videotape demonstration presented by the PI and the instructor's isolation of the external jugular vein and the vagus nerve, students will isolate the left vagus nerve and catheterize the left external jugular vein with small (PE-240) catheter. This jugular catheter will be used for injection of drugs.
- Students will inject a pressor drug (phenylephrine) and an inotropic agent (dobutamine) and observe changes in blood pressure, heart rate and electrocardiogram.
- Students will cut and stimulate vagus nerves and observe changes in blood pressure, heart rate and electrocardiogram.
- The pressor will be reinjected to observe changes in blood pressure, heart rate and electrocardiogram with baroreceptor reflex loop eliminated.
- Through the catheter sheaths, the students will pass catheters via the common carotid artery into the left ventricle and via the external jugular vein into the superior vena cava, right atrium, right ventricle, and pulmonary artery. Students will observe pressures in nearly every compartment of cardiovascular system.
- With the assistance of the instructor, students will open the chest with a midline sternal incision. Students will observe all major thoracic blood vessels and the beating heart in the open chest of a live animal.
- Students will mechanically stimulate the heart to produce ectopic beats. They will also electrically stimulate the heart pacing it from the atrium and the ventricle. They will over stimulate the heart to cause fibrillation. Students perform opened chest cardiac massage. Students will also observe as instructors defibrillate the heart.
- Students will then place a ligature around the left anterior descending coronary artery and tie it off and observe appearance and color of the heart and the changes in the electrocardiogram due to myocardial infarction.
- Animals will then be euthanized.
- If time permits students will be allowed to remove the heart and lungs and dissect the heart and observe the chambers, the valves, papillary muscles, etc. in a fresh heart.

V.1.1. Exercise 1:

Two training exercises will be performed. These exercises are aimed at training new instructors (cardiology staff and fellows) and USUHS, staff including veterinarians, in the cardiovascular physiological concepts, anatomical features of the pig vascular system, surgical procedures and goals and objectives of the exercise. Two pigs will be needed for each of the sessions. Thus, 4 pigs will be used in this section.

V.1.2. Exercise 2:
There will be approximately 172 students participating in the main teaching exercise. We have found that 4 students per animal is optimal for student participation and learning. We lose approximately 10% of the animals ordered to difficulties in anesthesia induction and surgical preparation. Thus, we will need 49 animals for this exercise.

Exercise 1	4 pigs
Exercise 2	49 pigs

V.2. Data Analysis:
Students are required to analyze the data obtained and submit a report to the PI on the findings and results of each of the procedures performed. Reports include analysis of the physiological concepts learned. **There is no need for statistics.** However, I include the anonymous responses of the classes of 2007 and 2012 to the question: Was the pig lab helpful in your understanding of cardiovascular physiology?

	2007	2008
	Class of 2010	Class of 2011
Very helpful	141	118
Helpful	22	32
No Opinion	2	4
A little helpful	4	6
Not at all helpful	1	6

V.3. Laboratory Animals Required and Justification:

V.3.1. Non-animal Alternatives Considered:
Since it is the objective of this exercise to demonstrate cardiovascular function and basic principles in a live animal, non-animal alternatives are inappropriate. However, many non-animal learning experiences are provided for our students. For example, videotapes, CD-ROM exercises, web-based exercises, and computerized case reports are available to enhance our students learning experience. These are provided to support the animal experience not to replace it.
In collaboration with the Department of Anesthesiology, we have developed an algorithm for the Patient Simulation Laboratory, which simulates some of the principles taught in the animal laboratory. All students are offered the opportunity to participate in the patient simulation laboratory as an adjunct to the animal lab to further reinforce the physiological concepts of the animal lab. Since there is no available time in the dense medical student curriculum, this exercise must be offered outside the current curriculum time, in Dean's time. To encourage students to participate extra credit is offered for their participation. To earn the extra credit students will answer a short questionnaire geared to assess whether the students thought the simulation was an academically beneficial experience.

One of the more important lessons of the animal laboratory is that all physiologic functions and responses are not absolutely predictable and identical (patients and animals don't always read the textbook) but are well defined only for populations of animals and humans. We use procedures that are generally predictable but all responses in biological systems vary somewhat. Individual responses vary from animal to animal and human to human. Computer models and patient simulators strip out this biologic variation. For example, an appropriate dose of phenylephrine raises blood pressure in all animals. However, it will raise BP 15 mmHg in some and 50 mmHg in others. The dynamic character of the responses will vary even more from animal to animal. The lesson is that phenylephrine increases BP but the same dose will elicit different responses depending on the animal and its physiologic state. These different physiologic states and responses are discussed with the students. Patients are the same in biologic variation. Computer models and simulators don't teach us these important things.

V.3.2. Animal Model and Species Justification:
This teaching exercise previously utilized dogs as the model of choice. Although the dog was an excellent model for teaching cardiovascular physiology and demonstrating cardiovascular principles, social pressures have encouraged us to evaluate other models to demonstrate cardiovascular principles. The swine was selected as our instructional model because of cardiovascular system's similarity to man in size, appearance and physiology. Therefore, we can use catheters designed and marketed for human use and provide the medical students with an experience similar to that that they will see clinically. In addition, the swine has become a frequently used research animal. Therefore, a large volume of physiological data has already been collected and published in the swine. We were able to use this published data to design experimental procedures that would produce reproducible results in the teaching environment.

V.3.3. Laboratory Animals

		Species #1	Species #2
V.3.3.1.	Genus & Species:	*Sus Scrofa Domestica*	
V.3.3.2.	Strain/Stock:	Yorkshire	
V.3.3.3.	Source/Vendor:	Animal Biotech Industries (ABI)	
V.3.3.4.	Age:	Age appropriate to weight	
V.3.3.5.	Weight:	15-25 kg	
V.3.3.6.	Sex:	Male: 27/year Female:26/year	
V.3.3.7.	Special Considerations:	Pigs from these sources should be free	

of pseudotuberculosis
and brucellosis and
Swine flu free

V.3.4. Number of Animals Required (by Species): Pigs: 159

V.3.5. Refinement, Reduction, Replacement (3 Rs):

V.3.5.1. Refinement:
We have refined the method of catheterizations used in this protocol. We now use catheter sheaths that are placed in the carotid artery and jugular veins. This eliminates the need to perform femoral cut downs, a procedure that is frequently associated with bleeding resulting in collection of poor data and even some loss of animals. We also demonstrate the location of the carotid artery, jugular vein and vagus nerve in a video and tag them in each animal to facilitate student dissection and isolation of each. We have evaluated an alternative anesthetic regime and have found that replacing i.v. pentobarbital with i.v. Telazol and xylazine results in a more consistent baroreceptor response to the pressor.

V.3.5.2. Reduction:
The protocol described in this application represent a consolidation of protocols previously use in two separate labs. Thus, we have halved the number of animals used. We also offer the tissue to other investigators for harvest after completion of our lab. For example: liver, eyes, hearts, skeletal muscle and skin have been harvested in previous years. In addition, investigators have used animals from this laboratory to study organs and tissues with ultrasound technology to either collect normative data or to prove a concept.

V.3.5.3. Replacement:
We have switched from dogs to pigs as our model to avoid the use of companion animals. Animals cannot be totally replaced for reasons stated above.

V.4. Technical Methods: See section V.4.3.2

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals:

	Species #1	Species #2
V.4.1.1.1.1. Column C:		
V.4.1.1.1.2. Column D:	159	
V.4.1.1.1.3. Column E:		

V.4.1.2. Pain Relief / Prevention:

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization:
Animals will be sedated with Telazol (approx: 4-6.5 mg/kg) and Xylazine (dose: 4-6.5 mg/kg) i.m. with a 21g butterfly needle /catheter. An intravenous drip of 5% dextrose will be set up via an ear vein with an 18g angiocath through which all additional medications will be given. Animals will be intubated and placed on a ventilator. A surgical plane of anesthesia will be achieved with bolus doses of Telazol (2.5-3.5 mg/kg) and Xylazine (2.5-3.5 mg/kg) given intravenously. Level of pain sensation will be assessed by hoof pinch-retraction response. Anesthesia will be given to eliminate the retraction. Anesthesia will be maintained with additional bolus doses of Telazol (2.5 -3.5 mg/kg) and Xylazine (2.5-3.5 mg/kg) given intravenously. Prior to opening the chest a bolus dose of sodium pentobarbital (30 mg/kg) will be given intravenously. Pain and distress will be monitored by veterinary staff, faculty instructors and students. If anyone perceives that animals are in any distress, a pain assessment is made by the veterinary staff and additional doses give as needed. Veterinary staff circulate in labs during the entire procedure to monitor pain and distress and carry anesthesia to administer as needed. LAM veterinarians may also use sodium pentobarbital and/or isoflurane anesthesia in animals that cannot be adequately anesthetized with telazol and xylazine.

Altenatively:
Propofol – For anesthesia maintenance after induction with Telazol/xylazine combination. Propofol (10 mg/ml) will be administered as a constant IV infusion utilizing the previously placed ear vein catheter. One 20 ml ampoule will be added directly to a 500/1000 ml bag of 5% dextrose in saline or 0.9% saline and administered at a constant drip rate to provide a dose of 12-20 mg/kg/hr. Dose/rate will be adjusted according to depth and character of anesthetic plane as monitored by investigative staff or LAM personnel. If maintenance infusion of propofol is found to be insufficient for maintaining anesthesia, augmentation of anesthesia will be accomplished with maintenance doses of pentobarbital as previously outlined (2.5-5 mg/kg).
To reduce movement and the decrease the amount of Propofol needed to 6 mg/kg/hr or less, fentanyl (3 μg/kg/hr) or a derivative (sufentanil, carfentanil @ 0.03 – 3 μg/kg/hr) to the may be added to the Propofol infusion.

V.4.1.2.2. Pre- and Post-procedural (not surgery) Provisions:
N/A
V.4.1.2.3. Paralytics:
N/A
V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched:
Although we feel that we use the most up-to-date analgesia under consultation with and guidance of the Department of Anesthesiology, I searched the following databases: AWIC: Altweb (Anesthesia and analgesia), and AGRICOLA
V.4.1.3.2. Date of Search:
Altweb: (3/19/09), and AGRICOLA (3/19/09)

V.4.1.3.3. Period of Search:
Altweb (1990 - present), and AGRICOLA: (1986-present)
V.4.1.3.4. Key Words of Search:
(Pain or anesthesia or analgesia) and (cardiovascular surgery or cut-downs or thoracotomy) and (pig or swine or porcine) and animal
V.4.1.3.5. Results of Search:
AGRICOLA; yielded 1 citation An article 2008 on the use of ultra sound in catheterization under general anesthesia. Articles indicate that the assessment of analgesia we use is standard and the anesthetic, analgesic method is sufficient to maintain the pigs in a pain-free state during the procedure, while maintain relatively good cardiovascular reflexes.

Altweb: using all terms yielded no citations
Altweb, using pigs, swine or porcine: Yielded 164 citations half dealt with guinea pigs. Narrowed to pig swine or porcine NOT guinea yielded 18 the majority dealt with anesthetic interactions and mechanisms. A few examined different protocols on cardiovascular function. The only useful article was Ko, JCH et al, Lab Anim Sci, 1995, 45(3): 290-294 It concluded that boosting the recommended Xylazine dose to equal that of Telazol produced better analgesia without prolonging recovery, changing heart rate or blood pressure significantly. This paper guided us to the dose we use. Wenzel V, Comp Med. 2000 Dec;50(6):644-8 discussed the variability of CV responses under general anesthesia but did not suggest one better than our regimen.

Using just vascular cutdowns or cardiac catheterization or venous cutdowns:
Altweb: yielded 3 hits 1995-1996 by Skarda-RT, et al. dealing with cats and dogs only. Animals in these studies were morbid and received therapeutic catheterizations. Considerations of anesthetics and analgesics were made based on the risks, given the individual morbidities.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification:
N/A
V.4.2. Prolonged Restraint:

V.4.3. Surgery

V.4.3.1. Pre-surgical Provisions:
Animals will be fasted overnight (standard veterinary practice) for animals undergoing surgical procedures the next day. Water access not limited.
V.4.3.2. Procedure:
Animals will be sedated with Telazol (approx: 4-6.5 mg/kg) and Xylazine (dose: 4-6.5 mg/kg) i.m. An intravenous drip of 5% dextrose will be set up via an ear vein. Animals will be intubated and additional doses Telazol and Xylazine intravenously to achieve a surgical plane of anesthesia.

After induction of a surgical plane of anesthesia, instructors will place catheter sheaths in blood vessels of the pig: the right common carotid artery and the right external jugular vein. Instructors will also isolate the right vagus nerve and place a loose suture around it

for students to observe. Students will connect the carotid artery catheter, marked with red tape, to the pressure transducer and begin recording arterial pressure in the pig.

Students will: Inspect the catheters and blood vessels and the vagus nerve. Using the landmarks from the right side, make an incision on the left side. Dissect and isolate at least 2 cm of the vagus nerve and the external jugular vein in the left side of the neck. Place a loose suture around the vagus nerve as a marker. Place the catheter provided into the external jugular vein as shown in the video. This catheter will be used for injections of pharmaceuticals.

Before continuing, the instructor will inspect their setup for correct catheter placement and nerve isolation.

Administration of the pressor agent and inotropic agent:
Students will:
Calculate the amount (12.5 µg/kg) of pressor agent to be given to their animal, inject the calculated dose and rapidly, flush the injection with 5 mls of normal saline. The dose inotropic agent to be given is also 12.5 µg/kg of body weight. When blood pressure has returned to preinjection value, the student inject the inotropic agent followed by a rapid flush of normal saline. The changes in BP, heart rate and ECG will be observed.

Stimulation of the vagus nerve:
Students will:
Tie two sutures close together around each vagus nerve as rostral as possible. Cut the vagus nerve between the sutures leaving 2 free cm to place the stimulating electrodes on later and compare the blood pressure, heart rate and ECG to the values that existed before they cut the vagus nerve.
Students will then stimulate the vagus nerve and observe heart rate and BP changes.

Students will:
Resuscitate their animal if necessary, with the assistance of the instructor. If mean arterial BP does not recover to above 75 mmHg they should take measures to increase the animals blood pressure with the assistance of their instructors (add phenylephrine to the iv bag and titrate pressure to 75 mmHg by adjusting drip rate.

Re-inject pressor agent in animal with baroreflex severed:
Students will:
Using the same effective pressor dose as above, inject the pressor again and observe cardiovascular response in the absence of the blood pressure reflex.

Place special catheters

Left ventricular catheter:
Students will :
Be given a pigtail catheter (used clinically to catheterize the left ventricle in humans). This catheter will be inserted into the sheath already placed in the carotid artery and advanced into the left ventricle as is done clinically. Note the differences between aortic and ventricular pressure.

Pulmonary artery catheter:

Students will:

Be given a wedge pressure catheter, insert the tip into the jugular vein sheath and advance it 15 cm. Inflate the balloon. Slowly advance the catheter until right atrial pressure is noted. Continue to advance the catheter until right ventricular pressure is noted. Continue to advance the catheter until it floats into the pulmonary artery. Continue advancing the catheter until wedge pressure has been observed as is done clinically.

Thoracotomy:

After administration of sodium pentobarbital (30 mg/kg) iv the students will:

Make a midline incision the entire length of the sternum and extend the incision at least to the end of xiphoid process. Being careful to stay on midline and avoiding muscle and blood vessels, continue incision all the way to the sternum. Expose at least one cm of the sternum to the left of midline by scraping away muscle insertions with their scalpel blade. Ask their instructor to observe while they cut the sternum with the electric bone saw. Observe the heart within the pericardial sac. Make a nick in the pericardium. Carefully extend the hole to expose the entire heart. Identify all chambers of the heart. Identifying the major vessels going to and coming from heart. Observe the sequence of events in the cardiac cycle, ie. the sequence of contractions of the atria and the right and left ventricles. Observe the coronary arteries and veins. With their fingers, compare the feel of the atria and the right and left ventricles. Note and be able to explain the difference in color between the right and left sides of the heart. Snap the ventricle with their finger to elicit an extra contraction.

Re-inject inotropic agent:

Students will:

While observing the heart, inject the inotropic agent again. Observe the changes in the heart size, shape and rate.

Direct stimulation of the heart:

Students will:

1. AFTER CONSULTING WITH INSTRUCTOR Students will: set the stimulator to give repetitive impulses at a frequency below existing heart rate. Apply electrodes to an atrium (AVOID THE VENTRICLES!) and progressively increase the frequency of stimulation until the heart rate is captured. Then increase the stimulations to dictate the "pace" of the heart.
2. Repeat the above experiment by slowly increasing the rate of repetitive stimulation with electrodes on the ventricle, until fibrillation occurs. Examine the fibrillating ventricle. Attempt opened-chest cardiac massage by cupping the ventricles in the palm of the hand. Squeeze the heart then relax allowing the heart to fill (about one squeeze per second).

The instructor will demonstrate the technique of de-fibrillation.

If they have successfully defibrillated their animal, the student, using the needle and suture (00) provided, tie off the left anterior dissenting coronary artery producing a myocardial infarction. Watch the color of the heart, blood pressure and ECG.

If time allows, students will remove the heart and lungs and dissect them to examine the chambers of the heart in relation to the major blood vessels and open the chambers to examine the heart valves, papillary muscles and chamber interiors in fresh tissue..

N/A

V.4.3.3. Post-surgical Provisions:

V.4.3.4. Location: MDL MS-I labs (A20- 09, 13, 17, 21, 25, 29, 31)

N/A

V.4.3.5. Surgeon: Medical students supervised by qualified USUHS staff who have participated in the training laboratory.

N/A

V.4.3.6. Multiple Major Survival Operative Procedures:

V.4.3.6.1. Procedures:

N/A

V.4.3.6.2. Scientific Justification:

N/A

V.4.4. Animal Manipulations:

V.4.4.1. Injections:

Animals will be injected intravenously through the already placed catheter. Injections will be phenylephrine (12.5 – 25 µg/kg) and dobutamine (12.5 – 25 µg/kg).

V.4.4.2. Biosamples:

No biosamples are planned at this time. However, we welcome investigators to utilize tissue from these animals. We will do whatever we can, in coordination with the IACUC, to accommodate requests for tissue.

V.4.4.3. Adjuvants:

N/A

V.4.4.4. Monoclonal Antibody (MAbs) Production:

N/A

V.4.4.5. Animal Identification:

Animals will be identified by cage cards and individual animal numbers that LAM assigns.

V.4.4.6. Behavioral Studies:

N/A

V.4.4.7. Other Procedures:

No other procedures are anticipated at this time. However, as we will with tissue sharing we will accommodate reasonable requests, in coordination with the IACUC, to utilize animals to gain other valuable scientific information.

V.4.4.8. Tissue Sharing:

No tissue sharing is planned at this time. However, we welcome investigators to utilize tissue from these animals. We will do whatever we can, in coordination with the IACUC, to accommodate requests for tissue. Previously we have shared eyes, muscle, hearts, liver and skin with other protocols.

V.4.5. Study Endpoint:

All animals initially sedated will be use in the exercise and therefore, will be euthanized at the completion of the all procedures. The endpoint of the exercise is completion of the maneuvers described above. If animals survive fibrillation and myocardial ischemia, they will be euthanized an overdose of pentobarbital combined with potassium chloride. Direct observation of the heart will confirm death. The chest will be open already to assure respiration does not restart. Carcasses will be appropriately contained and disposed of by the LAM staff.

V.4.6. Euthanasia:

Animals will be euthanized with Euthasol (390 mg pentobarbital and 50 mg phenytoin / ml) (1 ml/10 lbs intracardiac). Direct observation of the heart will confirm death. The chest will be open already to assure respiration does not restart. Carcasses will be appropriately contained and disposed of by the LAM staff. Other euthanasia solutions that are acceptable according to the 2007 AVMA Guidelines on Euthanasia may be used as replacements for Euthasol.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations: Except as noted below, routine animal husbandry will be provided in accordance with LAM Husbandry SOPs for each species in this protocol.

Pigs are housed in large pig runs usually with others until used. Pigs can roam and can perform normal social behaviors. Pigs thrive while housed at USUHS. They generally gain weight and remain healthy.

V.5.1.1. Study Room: MDL MSI Labs

Building(s) A Room Number(s) 20- 09, 13, 17, 21, 25, 29, 31

V.5.1.2. Special Husbandry Provisions:

Food Restriction:	Yes	_____	No	NO
Fluid Restriction:	Yes		No	
V.5.1.3. Exceptions:		_____		NO

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care:

Animals are observed at least once a day by the attending veterinary staff. Veterinary techs visit daily on medical rounds

V.5.2.2. Emergency Veterinary Medical Care: All emergency, weekend, and holiday care is provided by two animal husbandry technicians, one or more veterinary technicians, and an on-call veterinarian. Essential husbandry procedures and health rounds are conducted by LAM personnel once daily during weekend and holidays.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Except as indicated below, all animals on this protocol will be provided with routine environmental enrichment in accordance with LAM SOPs and IACUC Policies. Examples include nestlets and tunnels for rodents; balls, toys and food enrichment treats for large animal species.

V.5.3.2. Enrichment Restrictions:
None

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

STUDY PERSONNEL QUALIFICATIONS/TRAINING

Protocol activity or procedure (e.g., tail vein injections, euthanasia)	Name of person performing activity	Qualifications of person performing activity (e.g., research technician, 2 yrs experience)	Specific training in this activity or procedure (e.g., rodent handling class, 1999)
All	[redacted] Ph.D (b)(6)	PI, 30 years	WRAIR experimental pig handling course, 1992 Rodent and Lagomorph DOD Laboratory Animal Workshop and the DoD Swine Techniques (10/2000, 5/2005) Completed the) and Animal Care and Use for Principal Investigators (ACUP), 10/2001 courses given by the Division of Veterinary Medicine at Walter Reed Army Institute of Research (WRAIR).
Anesthesia induction	[redacted] Ph.D	PI, 30 years	500 pigs, 500 lambs, 20 sheep, 240 dogs, 50 cats, 100 rats
intubations	[redacted]	PI, 30 years	1351 various species (VS)
Anesthesia induction	LAM staff	Surgical and Animal Techs, various	Various
intubations	LAM staff	Surgical and Animal Techs, various	Various

cutdowns	(b)(6)	PI, 30 years	2410 VS
Left Ventricle catheterizations		PI, 30 years	1600 VS yes, this includes rats
euthanasias		PI, 30 years	1710 VS
Necropsies to determine proper catheter placements and general healthy appearance of brain heart and lungs.		PI, 30 years	1110 VS

1. **VII. BIOHAZARDS/SAFETY:** Personnel will appropriate protective clothing, face mask, and gloves when handling pigs. If inhalational anesthesia is used, waste gases will be passively scavenged using a charcoal filter system.

VIII. ENCLOSURES:

IX. ASSURANCES:

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Training: I verify that I have attended the USUHS Investigator/Animal User Training Course

(b)(6)

20 MAR 09
Date

G. Training: The following personnel will attend the next USUHS Investigator/Animal User Training Course:

_____ _____

_____ _____

H. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" that the DOD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible and conducting humane and lawful research.

(b)(6)

20-Mar-09
Date

I. Painful Procedure(s):

I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress **WILL** be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(b)(6)

20 March 2009
Date

X. PROTOCOL ABSTRACT:

It is well-established that students learn more and retain more information when that information can be presented in a real-life situation. Laboratories, including the study of live animals, have long been accepted and used to support the instruction of science. The procedures described in this protocol are aimed at enhancing the knowledge and experience of our medical students prior to their first clinical expenses.

With the guidance and direction of university faculty medical, students will perform procedures and observe some of the most important factors responsible for the control of blood pressure and cardiac function. Students will be asked to locate and identifying major blood vessels and nerves. They will observe the effects of injecting cardiovascular drugs and vagal nerve stimulation to on blood pressure (BP), heart rate (HR) and electrocardiogram (ECG) in the presence and absence of the strongest cardiovascular reflex mechanism. Students will place catheters into different compartments of the cardiovascular system in a manner performed clinically in humans from which they can observe the blood pressures from all chambers of the heart, systemic circulation and the pulmonary circulation. Students will observe the beating heart inside the chest cavity. Students will also perform open-chest cardiac massage.

A. Animal Protocol Number:

B. Animal Protocol Title: Control of Blood Pressure and Cardiac Function in the Pig.

C. Principal Investigator: [redacted] Pediatrics

D. Performing Organization: Uniformed Services University

E. Funding: USU/TRS

F. Objective and Approach:

The purpose of this laboratory exercise is to teach, through direct experience and demonstration, dynamic cardiovascular anatomy and some common dynamic cardiovascular functions in the live animal. This first-hand experience should help the student understand and remember these basic cardiovascular functions. The students will also experience, for the first time, patient to patient (animal to animal) variability in physiological responses to stimuli. They will also appreciate the complexities of anesthesia and some drug-drug interactions and how animal to animal variability further complicates these complexities. More specifically the student will:

1. To learn how to locate and isolate the external jugular vein, carotid artery and the vagus nerve in a live animal
2. To learn how to catheterize the external jugular vein.
3. To analyze the cardiovascular response to an increase in blood pressure with and without the vagus nerves intact.
4. To analyze the cardiovascular response to an inotropic drug.
5. To analyze the cardiovascular effects of severing and stimulating the vagus nerves.
6. Place catheters into the left ventricle, superior vena cava, right atrium, right ventricle and pulmonary artery and identifying the pressures characteristic of each of these compartments of the cardiovascular system.

7. To appreciate the strength and potential of the heart by making direct observations of the heart in response to a contractility-enhancing drug.
8. To learn the ability of external stimuli to alter the heart's performance by, directly electrically pacing the heart, atria and ventricles.
9. Create myocardial ischemia and appreciate the changes in electrocardiogram and appearance of the heart.
10. Cause fibrillation and directly observe defibrillation of the heart.
11. To appreciate the ability of the heart by performing direct cardiac massage in the fibrillated heart.

G. Indexing Terms (Descriptors):

Cardiovascular teaching, education or training, animals, pig, swine

3

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

April 16, 2008

MEMORANDUM FOR DR. [redacted] DEPARTMENT OF PREVENTIVE MEDICINE AND BIOMETRICS

SUBJECT: IACUC Approval of Protocol – Triennial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) on April 16, 2008:

Animal Protocol Title: "Filarial Infected Jirds for Teaching (Gerbils)."

USUHS Protocol Number: PMB-08-342

Expiration Date: April 15, 2011 (b)(6)

Supporting Grant(s) Number: N/A 302100/ PM

Name of Principal Investigator: Dr. [redacted]

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact [redacted] to schedule a pre-protocol planning meeting [redacted]. This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.

(b)(6)

Chair, Institutional Animal
Care and Use Committee, USUHS

cc:
Office of Research

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

FEB 20 AM 9:00

PROTOCOL NUMBER: PMB-08-342

PROTOCOL TITLE: **"Filarial Infected Jirds for Teaching (Gerbils)"**

GRANT TITLE (if different from above): N/A

USUHS PROJECT NUMBER: N/A

FUNDING AGENCY: N/A

EARLIEST ANTICIPATED FUNDING START DATE: N/A

PRINCIPAL INVESTIGATOR: John H. Cross, Ph.D

John H. Cross, Ph.D	PMB	295-3139	13 Feb 08
Principal Investigator Signature	Department	Office/Lab Telephone	Date

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

Research Unit Chief / Dept. Head Signature Typed Name:	Title	Telephone	Date

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

N/A			
Statistician Signature Typed Name:	Department	Telephone	Date

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

	LAM	295-3315	
Attending/Consulting Veterinarian Signature Typed Name:	Department	Telephone	Date

ANIMAL PROTOCOL NUMBER: PMB-05-342

PRINCIPAL INVESTIGATOR: John H. Cross, Ph.D

ANIMAL PROTOCOL TITLE: N/A

GRANT TITLE (if different from above): N/A

USUHS PROJECT NUMBER: N/A

CO-INVESTIGATOR(S): N/A

TECHNICIANS(S): None

I. NON-TECHNICAL SYNOPSIS:

The Mongolian gerbils or Jirds are infected with the filarial parasite *Brugia malayi* by the intraperitoneal route. The life cycle of the parasite is completed in the peritoneal cavity and microfilaria or baby worms produced can be easily recovered from the peritoneal cavity. The diagnosis of human infections with *Brugia malayi* is made by detection and the identification of the microfilariae in the blood. Peritoneal exudates aspirated from the belly containing microfilariae is given to medical students so that they can learn about these diagnostic stages. The baby worms can be seen alive, smeared onto a microscope slide, stained and examined microscopically.

II. BACKGROUND:

II.1. Background:

This protocol does not involve research, it is strictly a teaching exercise. It is used to instruct students in making a diagnosis of filariasis. The infected gerbils are purchased from a supplier in Georgia and are kept in LAM until used for teaching.

II.2. Literature Search for Duplication:

Agricola, Biomedical Research Database (BRD). Librarian did a FEDRIP and MEDLINE search. There were 51 hits dealing with jirds and *Brugia malayi*. It was stated that this was a teaching protocol and no other alternatives were found that would take the place of using animals. A search in the NORINA database on 15 April 05 yielded no results. A query was made at the National Agriculture Library and it was said you do not really need to do a search for a teaching protocol.

II.2.1. Literature Source(s) Searched:

II.2.2. Date of Search:

14 April 05; 15 April 05

II.2.3. Period of Search:

14 April 05; 15 April 05

II.2.4. Key Words and Search Strategy:
Gerbil, Jird, *Brugia malayi*

II.2.5. Results of Search:
A search of BRD revealed 10 hits representing 5 protocols. 2 of this described mosquito feeding and filarial research; 1 described phlebotomy and 2 described teaching and training protocols. It is a teaching protocol and is duplicative in nature. Gerbil models are widely used especially in immunology and molecular biology. It is not known how widely this is used in teaching. USUHS gives the most extensive course in Parasitology than other universities. The gerbil-filarial system is used widely experimentally but in our case, it is strictly for teaching.

II. OBJECTIVE\HYPOTHESIS:

The objective is to provide teaching material for students – to provide diagnostic elements for the disease known as filariasis. The use of living material makes a greater impression than using dead material.

III. MILITARY RELEVANCE:

Filarial infections are transmitted by mosquitos in most tropical areas of the world and it's a disease that was acquired during WWII and Vietnam War.

V. MATERIALS AND METHODS:

V.1. Experimental Design and General Procedures:

The two filarial infected jirds are purchased from commercial vendor. They both have adult and larval worms in the peritoneal cavity. When needed, peritoneal fluid containing worms are withdrawn from the peritoneal cavity using 27 – gauge 1.5" sterile needle attached to a 5 ml syringe. If necessary, 2-3 ml of sterile normal saline will be introduced into the peritoneal cavity prior to withdrawal if there is not sufficient peritoneal exudates. Only one jird is used. The second jird is held in reserve in case one jird dies from unrelated causes. There is a long lag time between ordering and delivering of infected animals due to the lengthy infection procedure. The peritoneal exudates will be diluted in saline and normally microfilariae will stay alive for quite sometime. Some of this material is smeared on slides for staining by the students. The rest is used for direct examination to microscopically observe the motility of the microfilaria. The jirds are used in three parasitology courses taught in January, March, and July. They will be euthanized by CO2 after the last class Human blood is not required for these parasites nor the use of pig's blood.

V.1.1. Experiment 1: N/A

V.1.2. Experiment 2: N/A

V.2. **Data Analysis**: N/A

V.3. **Laboratory Animals Required and Justification**:

Although cats and mice can serve as hosts (as suggested by reviewer), cats are companion animals and realistically not applicable and would cost more to house. Mice are poor host to provide the numbers of microfilaria needed, furthermore intraperitoneal infections are not as good as in gerbils. Peritoneal infection with *Brugia* are internationally used in drug trials as the parasite is easily recovered. Infection in other animals are in the lymphatic channels and the parasite is not easily recovered. The gerbil is the ideal host for our purpose; the infection are productive. The microfilariae are easily recovered and the animals are easy to handle.

V.3.1. Non-animal Alternatives Considered:

B. malayi will only develop in animals. In-vitro culture of the parasite is considered as a non-animal alternative but filariae will not survive in-vitro.

1. **V.3.2. Animal Model and Species Justification:** Although cats and mice can serve as hosts (as suggested by reviewer), cats are companion animals and realistically not applicable and would cost more to house. Mice are poor host to provide the numbers of microfilaria needed, furthermore intraperitoneal infections are not as good as in gerbils. Peritoneal infection with *Brugia* are internationally used in drug trials as the parasite is easily recovered. Infection in other animals are in the lymphatic channels and the parasite is not easily recovered. The gerbil is the ideal host for our purpose; the infection are productive. The microfilariae are easily recovered and the animals are easy to handle.

V.3.3. Laboratory Animals

	Species #1	Species #2
V.3.3.1. Genus & Species:	*Meriones unguiculatus*	
V.3.3.2. Strain/Stock:	N/A	
V.3.3.3. Source/Vendor:	TRS-Inc, 295 Research F Athens GA 30604	
V.3.3.4. Age:	Adults, 8-10 weeks old.	
V.3.3.5. Weight:	Appropriate with age.	
V.3.3.6. Sex:	Males; infections take more readily	
V.3.3.7. Special Considerations:	Require animals with an intraperitoneal infection of *Brugia malayi*.	

V.3.4. **Number of Animals Required (by Species):** Two gerbils are needed per year. The need for the 3 year cycle would be 6 animals.

V.3.5. **Refinement, Reduction, Replacement (3 Rs):**

V.3.5.1. **Refinement:** N/A

V.3.5.2. **Reduction:** Only using two animals for three courses. No other reduction alternatives were considered.

V.3.5.3. **Replacement:** We considered using in-vitro culture of parasites, but they do not survive in-vitro. No other replacement alternatives were considered.

V.4. **Technical Methods:**

V.4.1. **Pain / Distress Assessment:**

V.4.1.1. **APHIS Form 7023 Information:**

V.4.1.1.1. **Number of Animals:**

	Species #1	Species #2
V.4.1.1.1.1. **Column C:**	6 Mongolian gerbils	
V.4.1.1.1.2. **Column D:**		
V.4.1.1.1.3. **Column E:**		

V.4.1.2. **Pain Relief / Prevention:** N/A

V.4.1.2.1. **Anesthesia/Analgesia/Tranquilization:** Research personnel will monitor the gerbils at least once a day for the first 3-4 days following procedural manipulation. Post-procedural observations will be made in the animals' home cage. Animals exhibiting signs of illness or discomfort will be treated as described in section V.5.2.1. (Routine Vet Care), and /or as recommended following consultation with the veterinary staff.

V.4.1.2.2. **Pre- and Post-procedural (not surgery) Provisions:** N/A

V.4.1.2.3. **Paralytics:** N/A

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures: N/A

V.4.1.3.1. Sources Searched: N/A

V.4.1.3.2. Date of Search: N/A

V.4.1.3.3. Period of Search: N/A

V.4.1.3.4. Key Words of Search: N/A

V.4.1.3.5. Results of Search: N/A

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification: N/A

V.4.2. Prolonged Restraint: N/A

V.4.3. Surgery: N/A

V.4.3.1. Pre-surgical Provisions: N/A

V.4.3.2. Procedure: N/A

V.4.3.3. Post-surgical Provisions: N/A

V.4.3.4. Location: N/A

V.4.3.5. Surgeon: N/A

V.4.3.6. Multiple Major Survival Operative Procedures: N/A

V.4.3.6.1. Procedures: N/A

V.4.3.6.2. Scientific Justification: N/A

V.4.4. Animal Manipulations: N/A

V.4.4.1. Injections: N/A

V.4.4.2. Biosamples: Peritoneal taps will be performed as described in the materials and methods section

V.4.4.3. Adjuvants: N/A

V.4.4.4. Monoclonal Antibody (MAbs) Production: N/A

V.4.4.5. Animal Identification: Gerbils will be identified by name cards on their cage

V.4.4.6. Behavioral Studies: N/A

V.4.4.7. Other Procedures: N/A

V.4.4.8. Tissue Sharing: N/A

V.4.5. Study Endpoint:
The study endpoint for gerbils is survival through the end of the instructional period, after which they will be euthanized. While not expected as a consequence of the procedures outlined in this study, gerbils found to be critically ill or moribund for any reason will be euthanized immediately.

V.4.6. Euthanasia: Animals will be euthanized by the Center for LAM personnel at the end of the teaching program using cylinderized CO2 in compliance with the most current report of the AVMA Panel of Euthanasia. Euthanasia will be performed in the LAM CAF. Animals will be placed in a chamber that is not overcrowded. With animals in the chamber, the flow rate will displace at least 20% of the chamber volume per minute. Gas flow will be maintained for at least one minute after apparent clinical death. Animals will be verified as dead (lack of heart beat, lack of respirations) before removal of the chamber. Bilateral thoracotomy will be performed following this procedure to ensure complete death.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations: Except as noted below, routine animal husbandry will be provided in accordance with LAM Husbandry SOPs for each species in this protocol.

V.5.1.1. Study Room:
Building(s) B Room Number(s) G104

V.5.1.2. Special Husbandry Provisions:

Food Restriction: Yes N/A No _____

Fluid Restriction: Yes N/A No _____

V.5.1.3. Exceptions: N/A

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care: The PI or his research technician will observe the gerbils at least once a day for 3 or 4 days following peritoneal taps. Center for LAM personnel will observe the gerbils at least once a day for general health and husbandry conditions. Any gerbil observed to be lethargic, loosing weight, or exhibiting

any other signs of illness will be evaluated by the PI and/or veterinary staff then treated or euthanized as deemed appropriate.

V.5.2.2. Emergency Veterinary Medical Care: All emergency, weekend, and holiday care is provided by two animal husbandry technicians, one or more veterinary technicians, and an on-call veterinarian. Essential husbandry procedures and health rounds are conducted by LAM personnel once daily during weekend and holidays.

V.5.3. Environmental Enrichment: All emergency, weekend, and holiday care is provided by two animal husbandry technicians, one or more veterinary technicians, and an on-call veterinarian. Essential husbandry procedures and health rounds are conducted by LAM personnel once daily during weekend and holidays.

V.5.3.1. Enrichment Strategy: Except as indicated below, all animals on this protocol will be provided with routine environmental enrichment in accordance with LAM SOPs and IACUC Policies. Examples include nestlets and tunnels for rodents; balls, toys and food enrichment treats for large animal species.

V.5.3.2. Enrichment Restrictions:

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

STUDY PERSONNEL QUALIFICATIONS/TRAINING

Protocol activity or procedure (e.g., tail vein injections, euthanasia)	Name of person performing activity	Qualifications of person performing activity (e.g., research technician, 2 yrs experience)	Specific training in this activity or procedure (e.g., rodent handling class, 1999)
Peritoneal taps	[redacted]	Parasitologist 50 yrs experience	Rodent handling
Transport animal from LAM to lab and return	[redacted]	Parasitologist 50 yrs experience	Utilizing animals for Research or Teaching 03/17/05
Euthanasia	Center for LAM personnel	1.5 – 25 years experience with CO2 euthanasia; veterinary technicians, husbandry technicians, veterinarians	Investigator training, On-the-Job training. Have performed 100s to 1000s of CO2 euthanasia.

2. **VII. BIOHAZARDS/SAFETY:** PPE gloves, mask and labcoats worn during extraction of peritoneal fluid from gerbils.

VIII. ENCLOSURES: N/A

IX. ASSURANCES:

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Training: I verify that I have attended the USUHS Investigator/Animal User Training Course.

(b)(6) _____ 12/2/08
Date

G. Training: The following personnel will attend the next USUHS Investigator/Animal User Training Course:

_____ _____

_____ _____

H. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" that the DOD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible and conducting humane and lawful research.

(b)(6) _____ 12/2/08
Date

I. Painful Procedure(s):

I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress **WILL or WILL NOT** be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

_____ _____

Principal Investigator Signature Date

X. PROTOCOL ABSTRACT:

A. Animal Protocol Number: PMB-05-342

B. Animal Protocol Title: Filarial Infected Girbils for Teaching

C. Principal Investigator: [redacted]

D. Performing Organization: USUHS

E. Funding: N/A

F. Objective and Approach: To obtain microfilaria to train students in diagnosing filariasis. Infected gerbils will be commercially obtained and peritoneal fluid with microfilaria extracted as needed.

G. Indexing Terms (Descriptors): *Meriones unguiculatus*, gerbil,jird *Brugia malayi*

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

[redacted] Animal Care and Use Committee
(301) 295-3579
Fax: (301) 295-1964

December 26, 2007

MEMORANDUM FOR [redacted] DEPARTMENT OF SURGERY

SUBJECT: IACUC Approval of Protocol - Triennial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) on December 19, 2007:

Animal Protocol Title: "MS-III Surgical Skills and Review Laboratory (Swine Model)."

USUHS Protocol Number: SUR-07-521

Expiration Date: December 18, 2010

Supporting Grant(s) Number: 302100

Name of Principal Investigator: Dr. [redacted]

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact [redacted] to schedule a pre-protocol planning meeting [redacted] This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.

[redacted] (b)(6)

Chair, Institutional Animal
Care and Use Committee

cc:
Office of Research

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

SEP 4 AM 6:59

PROTOCOL NUMBER: SUR-07-521

PROTOCOL TITLE: **MS-III Surgical Skills and Review Laboratory (Swine Model).**

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER: N/A

FUNDING AGENCY: USUHS

EARLIEST ANTICIPATED FUNDING START DATE: N/A

PRINCIPAL INVESTIGATOR: **John E. Hutton Jr., MD, FACS**

(b)(6) _____ Surgery (Department) _____ (Telephone) _____ (Date)

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

(b)(6) _____ Department Chairman (Title) _____ (Telephone) 8/29/07 (Date)

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

N/A (teaching course) (Statistician Signature) _____ (Department) _____ (Telephone) _____ (Date)
Typed Name:

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

(b)(6) _____ LAM (Department) _____ (Telephone) 18 Dec 07 (Date)

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

10/15/07

PROTOCOL NUMBER:

PROTOCOL TITLE: MS-III Surgical Skills and Review Laboratory (Swine Model).

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER: N/A

FUNDING AGENCY: USUHS

EARLIEST ANTICIPATED FUNDING START DATE: N/A

PRINCIPAL INVESTIGATOR: (b)(6) **MD, FACS**

Principal Investigator Signature	Surgery Department	Telephone	Date

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

Research Unit Chief / Dept. Head Signature Typed Name:	Department Chairman Title	Telephone	Date

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

N/A (teaching course) Statistician Signature Typed Name:	Department	Telephone	Date

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

Attending/Consulting Veterinarian Signature Typed Name:	LAM Department	Telephone	Date

ANIMAL PROTOCOL NUMBER:

PRINCIPAL INVESTIGATOR: [redacted] **MD, FACS**

ANIMAL PROTOCOL TITLE: MS-III Surgical Skills and Review Laboratory (Swine Model)

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER: N/A

CO-INVESTIGATOR(S): [redacted]

(b)(6)

TECHNICIANS(S):

I. NON-TECHNICAL SYNOPSIS: The MS-III Surgical Skills and Review Laboratory is an 8 hour transition from the classroom to the reality of the surgical suites in our Medical Centers. This includes an introduction into the total operating floor environment under strict and well-experienced senior staff supervision. Operating room discipline is emphasized, from preparation and draping the animal, scrubbing and gowning of the operating room personnel, handling of the instruments, aseptic techniques, and hands on didactic instruction from the surgical staff. This course uses anesthetized pigs to demonstrate these techniques and skills to medical students. The exercise emphasizes the team approach to the operating room environment and will remain under strict and well-experienced senior staff supervision.

II. BACKGROUND:

II.1. Background: In recent years the thrust to condense the students first year of clinical exposure (MS-III level) to the surgical specialties has distracted from their handling the body's organs and tissues, especially within the abdominal and thoracic cavities. Our brief but highly focused introductory course in surgical exposure and technical skills has greatly enhanced the students' perspective of surgical procedures and techniques which are best learned prior to their introduction into the operating room environment. This affords the student the best opportunity to more fully participate within the surgical arena and to optimize the experience in the circumscribed time allotted. The course has been highly successful in accelerating the students' capabilities, confidence, and interest in the field of surgery and its subspecialties.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched: EMBASE, AGRICOLA, BRD, Federal Research In Progress (FEDRIP), and MEDLINE.

II.2.2. Date of Search: 10 August 2007

II.2.3. Period of Search: November 1978 -May 2007

II.2.4. Key Words and Search Strategy: Education and training and surgery and swine.

II.2.5. Results of Search: Teaching courses are by nature duplicative. However, the USUHS students trained on this protocol do not otherwise receive this type of training in their medical education.

III. OBJECTIVE\HYPOTHESIS: This course introduces third year USUHS medical students to the basics of surgical technique at the start of their clinical surgery rotation and prepares the students for entry in to the operating room while protecting the health and safety of the patient.

IV. MILITARY RELEVANCE: The mission of all USUHS graduates is unique in that there is a strong likelihood that early in their career they will be assigned as a General Medical Officer, and often in a role which may be remote and independent of immediate senior specialty consultation. Under such circumstances, especially if deployed with a field unit, the Medical Corps Officer must have a surgical orientation above and beyond that afforded by the civilian medical institutions. Experience in the handling of living tissues and organs, methods of achieving hemostasis, understanding anatomical relationships, and estimating the gravity of wounds from their external presentation is essential, and is remarkably enhanced by the students' exposure in our surgical laboratory. All military physicians should be skilled in suturing, suture ligation of bleeding vessels, creating adequate exposure, decompression and drainage of chest injuries (hemo/pneumothorax), and categorizing patients according to the gravity of their wounds and the urgency required for evacuation and treatment (triage).

The experience afforded by this course, coupled with mandatory completion of the Advanced Trauma Life Support Course during their fourth year, is essential in the preparation of the military physician's readiness for the obligations that are incumbent in their role when supporting our soldiers under the worst of circumstances.

V. MATERIALS AND METHODS:

V.1. Experimental Design and General Procedures:

V.1.1. Experiment 1:

The Surgical Rotation for the MSIII students has an introductory phase of a 2-day duration and includes an experience in the Simulation Center and Animal Laboratory. The latter is a one-day experience for each of the two groups, A and B, comprised of 20 to 24 students each. -

The students are subjected to the discipline of aseptic techniques, and scrubbing and gowning, as they will when participating in the operating rooms of their assigned hospitals. The Operating suites are fully equipped, and contain 7 operating tables, each attended by a board certified surgeon or a senior resident from one of our major medical centers, who will continue to be of influence to the students during their clinical experience in the outpatient clinics, on the wards and in the operating rooms.

Initially, each anesthetized animal (anesthesia described in V.4.1.2.1) will be placed in the supine position, "prepped" and draped in the standard fashion. Pigs will have an ear vein or other peripheral vessel catheterized using a 22-18 gauge catheter once anesthetized.

The students will alternate roles during the procedures – as surgeon, assistant and instrument technician ("scrub nurse"). The procedures are as follows:

1. Midline incision, zyphoid to symphysis.
2. Abdominal exploration, emphasizing anatomical relationships, vascular elements, peritoneal and retroperitoneal organs, and supporting ligaments.
3. Anterior Gastrostomy, 4.5 inches in length, inspection of internal (mucosal) surfaces, internal palpation of esophagogastractric, and pyloric sphinctors, performance of a pyloromyotomy.
4. Cholecystectomy, inspection of the Foramen of Winslow and the hepatobiliary structures.
5. Splenectomy – Emphasizing isolation of the splenic pedicle, ligation of vascular structures, and identification and ligation of the short gastric vessels.
6. Resection of 10 cm of colon or jejunum and reanastamosis.
7. Cystotomy (urinary Bladder), visualization of ureters and trigones, and bladder closure.
8. Abdominal closure, emphasizing the importance of including fascia for strength of repair.

After completion of the midline incision repair, the animal will be placed in the right lateral decubitus posture, and the left chest will be prepped and draped in the standard fashion for a left thoracotomy.

Using the tip of the scapula as a reference point, the 4th rib will be identified, and a line will be drawn over the 4th Intercostals space (ICS) from just below the left scapula to the sternum. Access to the thoracic cavity will be made through

the 4th or 5th ICS or by excising the 4th or 5th rib depending on the preference of the preceptor's instruction. Once opened, and with the rib retractor in place, the intrathoracic contents will be explored, noting the heart, great vessels, lung, diaphragm, aortic arch, phrenic and vagus nerves, sympathetic ganglia, azyqous veins, and descending aorta. The procedure will be performed as follows:

1. The tip of the lung will be grasped with the appropriate clamp and the left main bronchus identified.
2. The lung will be postured so that the bronchus is visible, and the dissection begun, elevating the vascular structures carefully, and individually isolating and ligating the left pulmonary arteries and veins. This should clear the left main bronchus of vascular structures.
3. The left main bronchus should be clamped using a non-crushing instrument leaving stump of no greater that 1cm.
4. With a clamp applied, the bronchus is divided and the lung removed from the field.
5. The bronchus is closed with an interrupted or a running suture, the clamp removed and the thoracic cavity filled with saline to test for leaks, and if present, additional suturing may be necessary.
6. After completion of the pneumonectomy the descending aorta is exposed and partially occluded with an appropriate partial occluding clamp (Satinsky). Various defects will be created in the anterior wall of the aorta to simulate traumatic injury and then repaired with the appropriate suture, patch, or interpositional graft. The clamp is then removed and sponges applied for a few minutes until the homeostasis is complete. Additional clamping and suturing may be necessary.
7. Now completed, the chest will be closed with heavyweight suture with or without the assistance of a rib approximator.
8. Each student will then be instructed in the placement and technique of inserting and removing a decompressing chest tube.

V.1.2. Experiment 2: N/A

V.2. Data Analysis: N/A

V.3. Laboratory Animals Required and Justification:

V.3.1. Non-animal Alternatives Considered: Non-animal alternatives were considered, including; simulators, computer modeling, plastic substitutes and manikins. None of these are acceptable substitutes for teaching students in a true operative setting. This swine lab is in addition to the other available alternatives. Students do make use of a simulation center. However, military medicine is unique in that any physician may be expected to perform surgery in combat at any time in his/her career regardless of his/her specialty. The use of live animals gives this unique population of physicians the experience they need to step onto a battlefield and perform with confidence.

V.3.2. Animal Model and Species Justification: The swine was selected as the animal model since their anatomy is very similar to humans. The surgical approach for the above mentioned surgical procedures is also very similar to the procedures used in humans and the live animal model will simulate the most realistic operating room experience for the students. Eight animals will be needed for each iteration, in order that each of the students is to adequately learn aseptic preparation (scrubbing, gowning, draping, etc), including hemostasis, exposure, packing, tissue and instrument manipulation. The pig model provides the ideal size and tissue similarity to best reflect an operating room experience. In our current review (literature search) we find no alternative but to use animals of the type and size described. By the students' consensus, this remains one of the most valuable experiences during their third year.

V.3.3. Laboratory Animals :
We will be using 16 animals per course. The course will be held 4 times a year: 16x4=64.
For a period of 3 years: 64x3= 192 animals are needed.

	Species #1	Species #2
V.3.3.1. Genus & Species:	Sus scrofa domestica	
V.3.3.2. Strain/Stock:	Yorkshire	
V.3.3.3. Source/Vendor:	LAM-approved vendor	
V.3.3.4. Age: 4-5mo		
V.3.3.5. Weight: 40-50 lbs		
V.3.3.6. Sex: Female		
V.3.3.7. Special Considerations: None		

V.3.4. Number of Animals Required (by Species): 192

V.3.5. Refinement, Reduction, Replacement (3 R's):
V.3.5.1. Refinement: This is a non-survival surgical laboratory. Once the animals are under anesthesia they will never be allowed to recover and will be euthanized while under anesthesia. Therefore, no animal will experience any pain or distress.

V.3.5.2. Reduction: Animals are, and have been, available for tissue sharing at the end of the surgical procedures. Several investigators within the Department of Surgery, as well as investigators from other USUHS Departments, have taken the opportunity to utilize organs/tissues from our course animals. The animal may now be euthanized, with exception of one or two which may be utilized by the Cardiology Service in a research project (see enclosure) in which a fresh heart is removed from the animal in the

presence of the Cardiac researchers who pack the organ in ice and transport it to their lab for immediate analysis. Post euthanasia, a group o f MS I and II students will enter the lab, and with supervision, will create incisions of up to 15 cm in length, and receive hands on instruction in suturing and closure of the wounds. In addition, by performing multiple procedures per animal and assigning 3 students per animal, we maximize the amount of exposure and experience from each of our anesthetized animals. Periodically, the Graduate School of Nursing request post euthanized animals for instruction in airway access though the cricothyroid ligament (membrane). Approximately 12 nurse anesthesia students will observe a prosection of the airway including surgical exposure of the thyroid cartilage, the cricrothyroid ligament, cricoid cartilage and the upper trachea. They will practice percutaneous cricothyroidotomies and insertion of tracheal tubes in the tracheas of the euthanized animals. Requests for tissue sharing with approval will be made available to the IACUC Executive Secretary

V.3.5.3. Replacement: Non-animal alternatives were considered, including; simulators, computer modeling, plastic substitutes and manikins. We will make use of these models in addition to the pigs. However, military medicine is unique in that any physician may be expected to perform surgery in combat at any time in his/her career regardless of his/her specialty. The use of live animals gives this unique population of physicians the experience they need to step onto a battlefield and perform with confidence. Therefore, non-animal alternatives alone are not acceptable substitutes for teaching military medical students in a true operative setting.

V.4. Technical Methods:

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals: 192

	Species #1	Species #2
V.4.1.1.1.1. Column C:	N/A	
V.4.1.1.1.2. Column D:	192	
V.4.1.1.1.3. Column E:	N/A	

V.4.1.2. Pain Relief / Prevention:

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization: Telazol (4-6mg/kg) IM and xylazine (2-4 mg/kg) IM injection either as a mixture in a single syringe or separated by separate injections 5 minutes apart. After intubation, pigs will be maintained on isoflurane gas (1-3%) delivered by a precision vaporizer in oxygen (1 -2 liters/minute). They will be monitored for adequate plane of anesthesia approximately every 15-20 minutes. Adjustments to gas concentration and/or flow rate may be made after approval by a LAM veterinarian in the event an animal is at a plane of anesthesia that is too light or too deep. Alternatively, if isoflurane is unavailable, a #20 catheter will be inserted into

an ear vein and Pentobarbital (30mg/kg, dosed to effect) will be administered. The jugular vein will cannulated and the anesthetic plane with IV Pentobarbital (30mg/kg) will be maintained.

V.4.1.2.2. Pre- and Post-procedural (not surgery) Provisions: Animals will be fasted the night prior to surgery. Induction anesthesia by VSD personnel will be accomplished as described earlier. No post-operative care is required since the animals will be euthanized at the end of the surgical procedures.

V.4.1.2.3. Paralytics: None

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched: MEDLINE, AGRICOLA, Biosis Preview, FEDRIP, BRD, NORINA

V.4.1.3.2. Date of Search: MEDLINE 10 August 2007; AGRICOLA, FEDRIP, BRD 13 August 2007; NORINA 03 October 2007

V.4.1.3.3. Period of Search: March 1968 –October 2007

V.4.1.3.4. Key Words of Search: Pain and/or Surgery and/or Swine and/or Training

V.4.1.3.5. Results of Search: Minimal distress will be encountered during initial restraint and will be alleviated with anesthetic agents as previously described. The anesthetics chosen provide for adequate anesthesia and analgesia and the highest level of quality veterinary care.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification: N/A.

V.4.2. Prolonged Restraint: N/A.

V.4.3. Surgery:

V.4.3.1. Pre-surgical Provisions: Animals will be fasted the night prior to surgery.

V.4.3.2. Procedure: The students will alternate roles during the procedures – as surgeon, assistant and instrument technician ("scrub nurse"). The procedures are as follows:

1. Midline incision, zyphoid to symphysis.
2. Abdominal exploration, emphasizing anatomical relationships, vascular elements, peritoneal and retroperitoneal organs, and supporting ligaments.
3. Anterior Gastrostomy, 4.5 inches in length, inspection of internal (mucosal) surfaces, internal palpation of esophagogastractric, and pyloric sphinctors, performance of a pyloromyotomy.

4. Cholecystectomy, inspection of the Foramen of Winslow and the hepatobiliary structures.
5. Splenectomy – Emphasizing isolation of the splenic pedicle, ligation of vascular structures, and identification and ligation of the short gastric vessels.
6. Resection of 10 cm of colon or jejunum and reanastamosis.
7. Cystotomy (urinary Bladder), visualization of ureters and trigones, and bladder closure.
8. Abdominal closure, emphasizing the importance of including fascia for strength of repair.

After completion of the midline incision repair, the animal will be placed in the right lateral decubitus posture, and the left chest will be prepped and draped in the standard fashion for a left thoracotomy.

Using the tip of the scapula as a reference point, the 4th rib will be identified, and a line will be drawn over the 4th Intercostals space (ICS) form just below the left scapula to the sternum. Access to the thoracic cavity may be made through the 4th or 5th ICS or by excising the 4th or 5th rib depending on the preference of the preceptor's instruction. Once opened, and with the rib retractor in place, the intrathoracic contents will be explored, noting the heart, great vessels, lung, diaphragm, aortic arch, phrenic and vagus nerves, sympathetic ganglia, azyqous veins, and descending aorta. The procedure as follows:

1. The tip of the lung will be grasped with the appropriate clamp and the left main bronchus identified.
2. The lung will be postured so that the bronchus is visible, and the dissection begun, elevating the vascular structures carefully, and individually isolating and ligating the left pulmonary arteries and veins. This should clear the left main bronchus of vascular structures.
3. The left main bronchus should be clamped using a non-crushing instrument leaving stump of no greater that 1cm.
4. With a clamp applied, the bronchus is divided and the lung removed from the field.
5. The bronchus is closed with an interrupted or a running suture, the clamp removed and the thoracic cavity filled with saline to test for leaks, and if present, additional suturing may be necessary.
6. After completion of the pneumonectomy the descending aorta is exposed, partially occluded with an appropriate partial occluding clamp (Satinsky). Various defects will be created in the anterior wall of the aorta to simulate traumatic injury and then repaired with the appropriate suture, patch, or interpositional graft. The clamp is then removed and sponges applied for a few minutes until the homeostasis is complete. Additional clamping and suturing may be necessary.
7. Now completed, the chest will be closed with heavyweight suture with or without the assistance of a rib approximator.
8. Each student will then be instructed in the placement and technique of inserting and removing a decompressing chest tube.

V.4.3.3. Post-surgical Provisions: No post-operative care is required since the animals will be euthanized at the end of the surgical procedures.

V.4.3.4. Location: Veterinary Surgery Division operating rooms in the Center for Laboratory Animal Medicine

V.4.3.5. Surgeon (s): MS-III students

V.4.3.6. Multiple Major Survival Operative Procedures: N/A

V.4.3.6.1. Procedures: N/A

V.4.3.6.2. Scientific Justification: N/A.

V.4.4. Animal Manipulations:

V.4.4.1. Injections: Telazol (4-6mg/kg) IM and xylazine (2-4 mg/kg) IM injection either as a mixture in a single syringe or separated by separate injections 5 minutes apart using 20-23 gauge needles.

V.4.4.2. Biosamples: Samples could be collected and will vary based on potential requests from other researchers wishing to share tissue or fluids from the animals used in this protocol.

V.4.4.3. Adjuvants: None

V.4.4.4. Monoclonal Antibody (MAbs) Production: N/A

V.4.4.5. Animal Identification: Cage cards

V.4.4.6. Behavioral Studies: None

V.4.4.7. Other Procedures: None

V.4.4.8. Tissue Sharing: Samples could be collected and will vary based on potential requests from other researchers wishing to share tissue or fluids from the animals used in this protocol.

V.4.5. Study Endpoint: Animals will be euthanized at completion of surgical procedures while still under anesthesia.

V.4.6. Euthanasia: Current AVMA guidelines will be followed. While under anesthesia, VSD will administer a lethal dose of 1ml/10 lbs body weight of sodium pentobarbital IV or IC. Euthanasia will be confirmed by observing the stopping of the heart or by listening to the chest for absence of heart beat and breath sounds.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations: Except as noted below, routine animal husbandry will be provided in accordance with LAM Husbandry SOPs for each species in this protocol.

V.5.1.1. Study Room:

Building(s) A Room Number(s) 164

V.5.1.2. Special Husbandry Provisions:

Food Restriction: Yes x No

Fluid Restriction: Yes No x

V.5.1.3. Exceptions: None

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care:

V.5.2.2. Emergency Veterinary Medical Care: All emergency, weekend, and holiday care is provided by two animal husbandry technicians, one or more veterinary technicians, and an on-call veterinarian. Essential husbandry procedures and health rounds are conducted by LAM personnel once daily during weekend and holidays.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Except as indicated below, all animals on this protocol will be provided with routine environmental enrichment in accordance with LAM SOPs and IACUC Policies. Examples include nestlets and tunnels for rodents; balls, toys and food enrichment treats for large animal species.

V.5.3.2. Enrichment Restrictions: None

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

STUDY PERSONNEL QUALIFICATIONS/TRAINING

Protocol activity or procedure (e.g., tail vein injections, euthanasia)	Name of person performing activity	Qualifications of person performing activity (e.g., research technician, 2 yrs experience)	Specific training in this activity or procedure (e.g., rodent handling class, 1999)
Supervision of medical students performing procedures	[redacted] MD, FACS	Over 30 years, over 10 years with swine model	Prior to 2001
	[redacted]	Over 30 years, over 10 years with swine model	Prior to 2001

Anesthesia induction and maintenance	LAM veterinary experience ranges from 3 months to over 15 years of delivering anesthesia to pigs.	All LAM staff completes investigator training as soon as possible after arrival.

VII. BIOHAZARDS/SAFETY: All personnel will protect themselves by wearing gloves, masks, and either a lab coat or scrubs when handling the pigs.

VIII. ENCLOSURES: None.

IX. ASSURANCES:

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration and made the proper coordination's regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Training: I verify that I have attended the USUHS Investigator/Animal User Training Course.

(b)(6) 27 Aug '07
Date

G. Training: The following personnel will attend the next USUHS Investigator/Animal User Training Course:

_____ _____

_____ _____

H. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" that the DOD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible and conducting humane and lawful research.

(b)(6) 27 Aug '07
Date

I. Painful Procedure(s):

I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress **WILL** be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

_____ _____

Principal Investigator Signature Date

X. PROTOCOL ABSTRACT:

A. Animal Protocol Number:

B. Animal Protocol Title: MS-III Surgical Skills and Review Laboratory (Swine Model)

C. Principal Investigator: [redacted] MD, FACS

D. Performing Organization: USUHS

E. Funding: USUHS

F. Objective and Approach: The MS-III Course, termed the Surgical Skills and Review Laboratory, is an eight hour transition from the classroom to the reality of the surgical suites in our Medical Center. This includes an introduction into the total operating floor environment under strict and well-experienced senior staff supervision. Operating room discipline is emphasized, from preparation and draping the animal, scrubbing and gowning of the operating room personnel, handling of the instruments, aseptic techniques, and hands on didactic instruction from the surgical staff. The exercise emphasizes the team approach to the following procedures: celiotomy, abdominal exploration, cholecystectomy, cecectomy, gastrostomy, splenectomy, cystotomy, bowel resection and anastomosis, thoracotomy, lung resection and large vessel (aorta) disruption and repair. The students alternate roles as: surgeon, first and second assistants, and instrument technician practicing the various skills of dissection, suturing, knot tying, gaining anatomic exposure, techniques of achieving hemostasis with clamping, packing and electro-cautery, and selecting the appropriate type and size suture material, and reaction to sudden hemorrhage from the interruption of larger vascular structures.

G. Indexing Terms (Descriptors): Surgery, education, training, animal, swine, alternative teaching

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

Institutional Animal Care and Use Committee
[redacted]

July 21, 2008

MEMORANDUM FOR RECORD

SUBJECT: Minor Modification for Dr. [redacted] Department of Surgery, Protocol Number SUR-07-521, entitled, "MS-III Surgical Skills and Review Laboratory (Swine Model)."

On July 21, 2008, Dr. [redacted] Department Surgery, requested a minor modification for Protocol Number SUR-07-521, entitled, "MS-III Surgical Skills and Review Laboratory (Swine Model)."

Dr. [redacted] request to add tissue sharing to the above protocol is approved. This approval is considered temporary and will be validated at the next full meeting of the USUHS IACUC.

[redacted]
(b)(6)
Animal Care and Use Committee, USUHS

cc:
File
[redacted]

Minor Modification

We would like to modify protocols, SUR-07-521, SUR-07-625, and SUR-06-573 to supply swine ears and feet taken from deceased swine as part of tissue sharing. They are to be used for surgical training of medical students. The normal supply of these tissues is the local butcher, and the University purchases them. Obtaining them from existing protocols will save the University additional funding. The tissues are normally thrown out with the animal carcass. Our staff veterinarian, [redacted] DVM, will perform the removal of the tissues from the animal carcasses.

The tissue sharing that we propose is a refinement because the tissues will be taken from deceased animals, so no pain will be encountered by the animals. It is a reduction because animals that are used for other protocols will supply the tissues instead of using other animals from the butcher as tissue donors.

Please consider our request for a modification to the aforementioned protocols.

[redacted]

7.21.08 [redacted]

[redacted] 8.4.08

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814–4712
www.usuhs.mil

Institutional Animal Care and Use Committee
[redacted]

April 20, 2009

MEMORANDUM FOR RECORD

SUBJECT: Minor Modification for Dr [redacted] Department of Surgery, Protocol Number SUR-07-521, entitled, "MS-III Surgical Skills and Review Laboratory (Swine)"

On April 20, 2009, [redacted] Department Surgery, requested a minor modification for Protocol Number SUR-07-521, entitled, "MS-III Surgical Skills and Review Laboratory (Swine)."

[redacted] request to add two procedures at the end of the Surgical Skills class in the above protocol is approved. This approval is considered temporary and will be validated at the next full meeting of the USUHS IACUC.

[redacted]
(b)(6)
and Use Committee, USUHS

cc:
File
[redacted]

[redacted]
Chair, Institutional Animal
Care and Use Committee, USUHS

Dear [redacted]

SUBJECT: **Minor Modification**

Animal Protocol Number and title: **SUR-07-521**: MS-111 Surgical Skills and Review Laboratory (Swine Model)

PI: [redacted]

We would like to request an addendum to our existing protocol to include the following procedures:

Cricothyrostomy: The landmarks utilized in performing the procedure are the thyroid cartilage and the cricoids cartilage. These should be identified by palpation over the trachea in the anterior neck of the swine, much as in the human, prior to skin preparation with antiseptic solution. The swine trachea is much deeper (3cm) and more mobile than that of humans. Between the cartilaginous structures lies the cricothyroid membrane which is the "target" of this surgical procedure. A one inch horizontal incision through the skin into the subcutaneous tissue is performed. Now, re-indentify the thyroid and cricoids cartilages and make a stab incision with the # 11 blade horizontally into the cricothyroid membrane. Withdraw the scalpel, invert it, and insert the handle of the scalpel in the stab wound in the cricothyroid membrane in the horizontal plane and rotate it to the sagittal plane. This could create an opening between the cricoids and thyroid cartilages. Insert your fingertip into this opening to dilate slightly and ensure you are n the trachea and have not created a false passage over, the trachea. Insert your endotracheal tube or tracheostomy tube into the trachea. Inflate the cuff and ventilate the swine with a bag-value device attached to the tube.

Emergency Tracheostomy: Hyperextend the neck. Prep and drape the anterior neck. The larynx should be identified by palpating the anterior neck in the midline. The trachea is fixed by grasping the anterolateral portion of the larynx between the thumb and forefinger of the operator's left hand. An incision is then made from the inferior margin of the larynx to just superior to the suprasternal notch, carrying the incision through the subcutaneous tissue by blunt dissection. The tissue overlying the pretrahel fascia is retracted with skin, using small skin retractors. The inferior margin of the cricoids cartilage is identified and a small horizontal incision is made immediately inferior to the cricoids cartilage through the pretracheal fascia. Utilizing blunt finger dissection, moving the pretracheal fascia and thyroid isthmus inferiorly, expose the first three tracheal rings. Next make a vertical incision trough the second and third tracheal rings. Insert a hemostat or tracheal dilator in the trachea and insert the endotracheal or tracheostomy tube into the trachea. Once the airway is established, any bleeding may then be controlled in an orderly fashion. Close the incision as necessary with 5-O nylon sutures, tying-off any small bleeders with absorbable sutures.

These procedures do not increase the number of animals or the study outcome. These additional procedures would be done at the end of the Surgical Skills Class.
Thank you for your consideration.

[redacted]
(b)(6)
Chief, Division of General Surgery, USUHS

Proposal for Animal Tissue Labs, Graduate School of Nursing Students

The Graduate School of Nursing (GSN) requests that approximately 25-40 Family Nurse Practitioner (FNP), Perioperative Clinical Nurse Specialist (CNS) and Psychiatric Mental Health Nurse Practitioner (PMH) students be allowed to participate in annual live tissue, animal model trauma and surgical skills training as a part of the operational curriculum. Our hope is to rejoin similar training for medical students that is already conducted by the Department of Surgery.

Background/History

GSN nurse anesthesia students have participated in trauma and skills training in a live-tissue, animal model in conjunction with medical students. The intent of the training was to instill confidence in the students that they would be able to perform Advanced Trauma Life Support (ATLS) type skills in austere field conditions, particularly emergency airway access and intervenous access via interosseus or cutdown technique.

A collaborative agreement between the Department of Surgery and the GSN resulted in an addendum to the animal protocol that provided the opportunity for live-tissue training. After the medical students completed their training in the animal labs here at the university, GSN students would be offered the opportunity to perform ATLS procedures on the same animals, providing a similar experience without the expense or requirement for additional animals.

This practice was stopped because of the practical logistical considerations associated with bringing graduating Nurse Anesthesia students back to the university from their training sites. It was not a reflection of the training or the relationship between the GSN and the Department of Surgery. The training was highly regarded by students and was simply stopped for administrative reasons.

Current Deployment Issues

In 2009, the United States will enter the 7th year of the Global War on Terror. Ongoing operational requirements have placed a prolonged burden on uniformed physicians and physician's assistants who have traditionally provided trauma care on the battlefield. These providers have had repeated deployments and over time there has been a pronounced increase in the attrition rates.

In 2005, the Army responded to the shortfall by provisionally assigning Army FNPs to traditional physician and physician assistant roles. Army FNPs began serving with operational units at the Forward Operating Bases (FOBs) and with Brigade Combat Teams (BCTs).

The original intent was to limit FNP response to far forward/remote operations to prevent them from engaging in the care of trauma patients, as FNPs are not traditionally prepared to manage acute trauma, but this management strategy is predicated upon a linear battlefield. In

current operations. trauma care has not fit a traditional echelon-oriente ı doctrine: FNPs are now providing trauma care on a regular basis.

Current trauma training of Army Nurse Practi ioners

Currently, there is no unique operational training required for Army, Navy, or Air Force FNPs, CNSs or PMHs. The only requirements are the bare minimum, basic predeployment tactical training required by the individual services.

In 2004, the Army instituted the Trauma Combat Medical Care (TCMC) Course at Ft. Sam Houston. Designed for and run by Army physician assistants, this five day refresher course reinforces trauma management of combat casualties under tactical conditions. It contains 16 hours of didactic training and 42 hours of hands-on training to include a live-tissue model.

The current MEDCOM policy "highly encourages" providers who are forward deploying to attend and complete TCMC. Many Army FNPs are provided the opportunity to attend the course prior to deploying on a case-by-case, space available basis although it is not a requirement. The course is open to the Air Force and Navy Family Nurse Practitioners, but it is infrequently utilized by these providers and is generally not utilized by CNSs or PMHs.

Experiences of Army Nurse Practitioners

In an effort to support the request for live-tissue training for all Uniformed Services University of Health Sciences FNP, CNS, and PMH students, active duty Army FNPs were asked to submit their thoughts on combat trauma management experiences they have had:

[redacted]

> Live-tissue provides invaluable training that simply cannot be replicated by any other training aid. I just returned from a 13 month deployment to Iraq. I lived and worked at a small combat outpost (COP) for the duration, where I was the sole-provider. I treated more than 40 trauma victims in a 5 month period. These trauma patients were primarily improvised explosive device blast victims, some gunshot wounds, mortars, etc. If you need to secure an airway with an endotracheal tube prior to evac, due to your patient crumping as the bird is touching down on your hot landing zone, you do NOT want your first try ever to be on a 10 year old boy with a head injury. (Which we had.)
>
> ... I am sure you know that dropping a chest tube in a SIM man does not come close to dropping one in a large animal. We had hands-on practical experience with LIFE SAVING procedures that could not have been reproduced by any other means.

...Bottom line: NPs need to be able to do these procedures. It saves lives.

[redacted]

I had the opportunity to go through animal lab training at Fort Sam in 2005 as part of the TCMC train-up prior to my deployment with a level 1 battalion aide station group. Skills that I learned in this course, specifically, intubation, cricotomy, and chest tube placement were extremely helpful. I was in an environment that required me to use these skills on many occasions and know for a fact that my animal lab training helped me save lives. I strongly support this training for FNPs

[redacted]

I am the sole health care provider on a FOB that is mortared regularly. This type of training would have been great.

[redacted]

This was the most important training I've ever undergone. I can't overstate how important it is and how much confidence it builds when any practitioner has the opportunity to use live tissue. Actually seeing the femoral artery lacerated, then applying pressure and a tourniquet, then introducing Quickclot into the wound and actually feeling it burn your own hand through the Kerlix, then removing the Kerlix and viewing the clot that has formed, is the best training anyone could ever have. Insertion of a chest tube was likewise memorable. In Afghanistan, I'm absolutely sure the live tissue training enabled me to save lives that would otherwise have been lost.

[redacted]

I had to suture parts of the body that I'd never had experience suturing e.g. a flap on the nose, fingers, hands, feet etc..., I had patients that I put F.A.S.T. 1's in because I had never done a femoral line (of course it worked and I was able to evac right away, so all was well both times). These are some of the most important areas that we would benefit from having an animal lab. I was able to do an animal lab before I deployed

(b)(6) [redacted]

I am currently deployed and about a month before deployment I went to the animal skills lab and cadaver lab at Fort Sam and I thought the animal lab was great. I thought the animal lab was more beneficial than the cadaver lab because you could feel the time pressure you have as a provider in a trauma. Also you witness the benefit of the different clotting products and decided ahead which one you prefer.

[redacted]

I served as the Battalion Surgeon for 3rd BDE 505 PIR 82D ABN DIV during a 15 month deployment to Iraq. Prior to my deployment, I went to Ft Sam for the TCMC course which included a live tissue lab. I found this training to be absolutely invaluable in terms of preparing me for combat trauma. I was properly trained and ready should I need to perform those advanced life-saving techniques. It really helped lower my stress level. Thankfully, I only had one traumatic amputation that required those skills but at least I was ready and saved this soldier's life due to the training I received. I also taught my medics the skills I had learned and they also found them extremely re-assuring since they were often "outside the wire" without my direct support.

(b)(6) [redacted]

During my deployment to Iraq in 2006-2007, I deployed with the 10th MTN DV as an augmentee filler. During that deployment , I was in a Strongpoint position in a far forward area in the Sunni triangle (outside of Baghdad) . I believe that my confidence to treat the war injuries from gunshots, shrapnel, burns from explosive, control bleeding and do ACLS with intubation (intubated 2 casualties) was due to the animal trauma skills lab training that we received before deployment . The clinical experience and skills refresher or simply introducing a new skill set and putting into practice made the deployment experience more rewarding. In addition, DNBI casualties and routine minor surgical procedures (I&D, foreign body protrusion in limbs) were part of the mix of patients that I treated.

(b)(6)

Currently I am working in a Level II facility in Iraq. We have seen GSW's, delivered a baby, several full codes to include running all of the ACLS protocols, open fractures and such.

Conclusion

The words of the above officers speak for themselves. Army FNPs are seeing and should plan to see trauma in the theater of operations. The mission of the University and the Graduate School of Nursing is to prepare its students for the operational demands that they are likely to meet executing their war-time missions. By adding an animal live-tissue experience to the curriculum, we will improve on our mission to prepare our students to care for those in harm's way.

The responses of all the Army Family Nurse Practitioners who contributed to this proposal were supportive of live-tissue training. Although many are provided the opportunity to attend TCMC, some did not and unfortunately many FNPs from sister services are either unaware of the training or are unable to attend.

As the major provider of Family Nurse Practitioners, Perioperative Clinical Nurse Specialists and Psychiatric Mental Health Nurse Practitioners for the Department of Defense, the Uniformed Services University has the opportunity to heighten the operation readiness of these future providers by ensuring that they all will have at least one opportunity to practice ATLS-type skills on a live tissue model. This can be done without sacrificing additional animals or incurring additional cost by reinstituting an addendum to the animal protocol used by the Department of Surgery for the medical students.

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

October 21, 2009

MEMORANDUM FOR DRS. [redacted] DEPARTMENT OF SURGERY

SUBJECT: IACUC Approval of Protocol - Major Modification

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) on October 21, 2009:

Animal Protocol Title: "MS-III Surgical Skills and Review Laboratory (Swine Model)"

USUHS Protocol Number: SUR-07-521

Expiration Date: December 18, 2010

Supporting Grant(s) Number: 302100

Name of Principal Investigator: Dr. [redacted]

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

[redacted]
(b)(6)
Chair, Institutional Animal
Care and Use Committee

cc:
Office of Research

SEP 25 AM11:56

USUHS FORM 3206B
ANIMAL STUDY PROTOCOL
(modification/addendum)

Animal Protocol Number: SUR-07-521

Grant Number:

Principal Investigator: [redacted] MD

Department: SURGERY

Phone:

MS-III Surgical Skills and Review Laboratory (Swine Model).

Animal Protocol Title:
(If animal species not identified in title, list parenthetically after title, i.e., rat, mice, etc.)

Grant Title (if different from above):

Description of the proposed modification:
Due to the retirement o[redacted] we would like to request a change of PI to this protocol SUR-07-521
We are replacing [redacted]
Dr Ritter has over 30 years of experience in human surgery; over 20 years with swine model and a member of IACUC. He is also a Coinvestigator with this protocol.

(b)(6) [redacted]

Principal Investigator Signature 8/11/08 **Date**

(b)(6) [redacted]

Department Chairperson or Activity Head 8/11/08 **Date**

5

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

Institutional Animal Care and Use Committee

August 6, 2008

MEMORANDUM FOR DR. [redacted] DEPARTMENT OF PEDIATRICS

SUBJECT: IACUC Approval of Protocol - Triennial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) via Designated Review on August 6, 2008:

Animal Protocol Title: "Pediatric Intubation (Ferret) Training"

USUHS Protocol Number: PED-08-373

Expiration Date: August 5, 2011

Supporting Grant(s) Number: N/A

Name of Principal Investigator: Dr. [redacted]

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

[redacted]
(b)(6)
Chair, Institutional Animal
Care and Use Committee

training protocol deactivated & teaching procedures were stopped Sept 2008

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

JUN 6 AM 8:08

PROTOCOL NUMBER: PED-~~05~~08-373

PROTOCOL TITLE: Pediatric Intubation (Ferret) Training

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER:

FUNDING AGENCY: USUHS MDL

EARLIEST ANTICIPATED FUNDING START DATE: July 2008

PRINCIPAL INVESTIGATOR: [redacted]

(b)(6) [redacted]

PED
Department

[redacted]
Office/Lab Telephone

3 June 08
Date

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

(b)(6) [redacted]

Chair
Title

[redacted]
Telephone

04 June 08
Date

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

Statistician Signature
Typed Name:

Department

Telephone

Date

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

Attending/Consulting Veterinarian Signature
Typed Name:

LAM
Department

[redacted]
Telephone

Date

Revised July 11, 2008

USUHS FORM 3206
ANIMAL STUDY PROPOSAL
PROTOCOL COVER SHEET
IACUC Date Stamp

PROTOCOL NUMBER: PED-~~05~~ 06-373

PROTOCOL TITLE: Pediatric Intubation (Ferret) Training

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER:

FUNDING AGENCY: USUHS MDL

EARLIEST ANTICIPATED FUNDING START DATE: July 2008

PRINCIPAL INVESTIGATOR: [redacted]

Principal Investigator Signature	PED Department	[redacted] Office/Lab Telephone	3 June 08 Date

SCIENTIFIC REVIEW: This animal use proposal received appropriate peer scientific review and is consistent with good scientific research practice.

Research Unit Chief / Dept. Head Signature Typed Name:	Title	Telephone	Date

STATISTICAL REVIEW: A person knowledgeable in biostatistics reviewed this proposal to ensure that the number of animals used is appropriate to obtain sufficient data and/or is not excessive, and the statistical design is appropriate for the intent of the study.

Statistician Signature Typed Name:	Department	Telephone	Date

ATTENDING VETERINARIAN: In accordance with the Animal Welfare Regulations, the Attending Veterinarian was consulted in the planning of procedures and manipulations that may cause more than slight or momentary pain or distress, even if relieved by anesthetics or analgesics.

Attending/Consulting Veterinarian Signature Typed Name:	LAM Department	[redacted] Telephone	Date

ANIMAL PROTOCOL NUMBER: PED-05-373

PRINCIPAL INVESTIGATOR:

ANIMAL PROTOCOL TITLE: Pediatric Intubation (Ferret) Training

GRANT TITLE (if different from above):

USUHS PROJECT NUMBER:

CO-INVESTIGATOR(S):

TECHNICIANS(S):

I. NON-TECHNICAL SYNOPSIS:

Inadequate resuscitation of an ill newborn in the delivery room can have disastrous consequences. With the emphasis on primary care in medicine it is likely that most graduates from the USUHS School of Medicine will be involved in the care of newborn infants. It is therefore important that they receive training in this important skill early in their careers. This training laboratory is part of a course where both medical students beginning their clinical rotations and Pediatric resident physicians learn to perform delivery room resuscitation of newborn infants. The ferret model is used to provide hands-on experience with endotracheal intubation. It supplements lectures and additional practical experience with mannequins.

II. BACKGROUND:

II.1. Background:

Many infants fail to adequately make the transition to extrauterine life and require resuscitation in the delivery room. With simple airway management and attention to thermoregulation nearly all of these infants do well. However, the consequences of delayed efforts at resuscitation can be disastrous. The American Academy of Pediatrics recommends that at least one person skilled in neonatal resuscitation to include endotracheal intubation be present at every delivery. With the current emphasis on primary care in military medicine it is likely that most graduates from the USUHS School of Medicine will be involved in care of the newborn infant. It is therefore desirable that they receive training in this important skill early in their careers. The USU medical students are not going to receive this training in the brief time they spend in the nursery during their third-year clerkship and the other intubation training they receive focuses on the adult patient.

Pediatric resident physicians are required to attend the deliveries of high-risk neonates. They must become proficient in newborn resuscitation to include endotracheal intubation during their training. However, new regulatory requirements limit resident work hours and the amount of time residents can spend in Newborn medicine. Pediatric residency programs must look beyond the traditional bedside teaching to provide adequate education for the residents. The intubation lab, in conjunction with specific training courses of the National Capital Consortium and the

American Heart Association/American Academy of Pediatrics allows the resident physicians to gain valuable intubation experience.

The present protocol uses animal models to bridge the gap between classroom instruction and clinical medicine. The ferret model is used to provide hands on experience in endotracheal intubation. The ferret is well suited for this purpose due to the similarity between the upper airway of the ferret and that of the human newborn. Over the nearly 20 years the course has been in existence, student feedback has demonstrated this experience to be well received and successful in providing the background required for more advanced study. Feedback from Neonatology staff physicians has also demonstrated the utility of the experience.

II.2. Literature Search for Duplication:

II.2.1. Literature Source(s) Searched:
BRD and CRISP

II.2.2. Date of Search:
3 June 2008

II.2.3. Period of Search:
1998-present

II.2.4. Key Words and Search Strategy:
Endotracheal and intubation and training
Endotracheal and intubation and training and animal
Endotracheal and intubation and training and ferret

II.2.5. Results of Search:
Twenty-three results were returned from the BRD search. All were similar projects and all were from military institutions. The animals utilized were ferrets and one older protocol listed cats. No new techniques were described. Only one result was returned from the CRISP search. It was a study of the effect of out-of-hospital endotracheal intubation errors from the University of Pittsburgh. No animals were used in this protocol.

III. OBJECTIVE\HYPOTHESIS:

The objective of this protocol is to provide third year medical students and Pediatric Residents in the National Capital Consortium Pediatric Residency with training in neonatal resuscitation. This training is given on two parts: 1) didactic session on neonatal resuscitation with practice in endotracheal intubation using mannequins. 2) endotracheal intubation using the ferret model

IV. MILITARY RELEVANCE:

As noted in the background section, many infants require resuscitation in the perinatal period. The current emphasis on primary care in military medicine makes it likely that most graduates from the USUHS School of Medicine will be involved in care

of the newborn infant. It is therefore desirable that they receive training in this important skill early in their careers.

V. MATERIALS AND METHODS:

V.1. Experimental Design and General Procedures:

V.1.1. Experiment 1:

Anesthesia and positioning of the ferrets on a warming blanket will be accomplished by LAM personnel. The lab instructor will demonstrate endotracheal intubation of the ferret after the didactic session is completed. Each student will then be allowed to attempt intubation with a laryngoscope and endotracheal tube. The laryngoscope blades (Miller 0 and 1) and endotracheal tubes (2.5 mm uncuffed) used in this lab are identical to the equipment used in hospital delivery rooms. No more than five attempts at intubation will be made on each ferret during the lab session. An attempt is considered to be the placement of an endotracheal tube into the ferret whether or not it is successfully positioned in the airway. **Successful intubation is verified by noting the presence of condensation in the endotracheal tube during spontaneous respirations, or with direct visualization by the instructor. The number of successful intubations per ferret varies, but will be no more than five in a single class. Each ferret receives five attempts at intubation per class session, but not all the student attempts will be successful.**

Sufficient animals are anesthetized to allow each student three attempts at intubation. Each lab session has between eight and 12 students. After the lab session, the ferret's upper airway is examined for signs of trauma by LAM personnel and the animal is taken to LAM for recovery. The ferrets are reused for additional lab sessions in this protocol. No more than 10 lab sessions are spaced throughout the year so that breaks of at least a month occur between each lab. The ultimate decision as to the reuse of an individual ferret rests with the LAM staff and the attending veterinarian.

V.1.2. Experiment 2:

V.2. Data Analysis:

The only data obtained from this training protocol will be feedback from the students and instructors. These data are reviewed by the course director.

No published data is available on which to base an animal number calculation. The number of intubation attempts allowed per ferret (five) was provided by a LAM attending veterinarian at the onset of this protocol. The number of intubation attempts allowed per student (three) was based on consensus of the lab instructors over the years. We feel that three attempts allows for adequate familiarization with the equipment and procedure.

V.3. Laboratory Animals Required and Justification:

V.3.1. Non-animal Alternatives Considered:

The use of mannequins and human neonates were considered in the design of this protocol. While mannequins are used in this protocol, they can only supplement experience with living models. Initial experience with intubation using human neonates

is unsafe and it is unavailable to medical students during their Pediatric clerkship. The American Academy of Pediatrics guidelines say that intubation attempts in the neonate must be limited to 20 seconds. This does not leave adequate time for medical students to become familiar with the procedure. The animal models are used to bridge the gap between these two extremes.

V.3.2. Animal Model and Species Justification:
Ferrets are used for the intubation labs. The ferret is well suited for this purpose due to the similarity between the upper airway of the ferret and that of the human newborn. The size of the upper airway approximates that of an infant at 35-36 weeks gestational age. **This statement is based on the PI's 25-year experience in intubating human neonates and 15-year experience in intubating ferrets.** The ferrets tolerate the procedure well and can be used for multiple laboratory sessions. The ferret is the lowest animal on the phylogenetic scale that can be used for these laboratories. While some institutions may have used cats in similar labs, their status as a companion animal makes their continued use impractical.

V.3.3. Laboratory Animals

		Species #1	Species #2
V.3.3.1.	**Genus & Species:**	Mustela Putorius furo	
V.3.3.2.	**Strain/Stock:**	outbred	
V.3.3.3.	**Source/Vendor:**	Animals obtained from the in-house ferret colony	
V.3.3.4.	**Age:**	Adult **1-6 years**	
V.3.3.5.	**Weight:**	**750-1200 grams**	
V.3.3.6.	**Sex:**	either	
V.3.3.7.	**Special Considerations:**	NA	

V.3.4. Number of Animals Required (by Species):

13 ferrets are required for this protocol. Approximately 30 labs will be performed over the period covered by this protocol. Each lab will utilize six or seven animals depending on the number of students enrolled. The ferrets are used for multiple labs, but many need to be replaced at some point over the three years of this protocol. Therefore the number of animals requested allows for a replacement for each ferret used.

V.3.5. Refinement, Reduction, Replacement (3 Rs):

V.3.5.1. Refinement:

Xylocaine **2%** is sprayed into the upper airway prior to the intubation attempts to minimize discomfort during recovery.

V.3.5.2. Reduction:
This protocol reduces the number of ferrets required by utilizing each ferret for multiple labs.

V.3.5.3. Replacement:
This protocol replaces older protocols utilizing cats with an animal lower on the phylogenetic scale.

V.4. Technical Methods:

V.4.1. Pain / Distress Assessment:

V.4.1.1. APHIS Form 7023 Information:

V.4.1.1.1. Number of Animals:

	Species #1	Species #2
V.4.1.1.1.1. Column C:	0	
V.4.1.1.1.2. Column D:	13	
V.4.1.1.1.3. Column E:	0	

V.4.1.2. Pain Relief / Prevention:

V.4.1.2.1. Anesthesia/Analgesia/Tranquilization:
Anesthesia will be accomplished by LAM personnel using one of three different regimens: 1)Ketamine (18-25 mg/kg of 100 mg/mL solution) and xylazine (1-2 mg/kg of 20 mg/mL solution) or 2)Ketamine (25-35 mg/kg of a 100 mg/mL solution) and diazepam (2-3 mg/kg of a 5 mg/mL solution), or 3) Telazol (12-22 mg/kg of a 100 mg/mL solution) IM into caudal thigh muscle with a 21-25g needle. The lowest doses are administered initially and the ferrets are closely monitored for gagging and purposeful movement as indicators of the need for more anesthetic agent. An additional dose of anesthetic can be administered if necessary with the total dose not to exceed the maximum dosage listed in the protocol. The volume of anesthetic injected depends on the weight of the ferret, but no more than 1 mL is injected into any single site. Yohimbine (0.5-1 mg/kg of a 2 mg/mL solution) IM may be used to reverse xylazine if needed. A single spray of **xylocaine** (2%) may be administered into the upper airway prior to the intubation attempts as needed for laryngospasm.

V.4.1.2.2. Pre- and Post-procedural (not surgery) Provisions:
Pre and post procedural care is provided by LAM personnel. The ferrets are part of a larger colony that receives care and enrichment as prescribed by the attending veterinarians. The ferrets are kept **Nil Per Os** (NPO) for three hours prior to the lab. After the procedure the ferrets are returned to LAM and maintained in a warm location

with continuous monitoring by LAM technicians until they have recovered from anesthesia. The animals are then returned to their home cage and monitored during routine room checks.

V.4.1.2.3. Paralytics: NA

V.4.1.3. Literature Search for Alternatives to Painful or Distressful Procedures:

V.4.1.3.1. Sources Searched:
AWIC and Altweb

V.4.1.3.2. Date of Search:
3 June 2008

V.4.1.3.3. Period of Search:
2000-present

V.4.1.3.4. Key Words of Search:
Endotracheal and intubation
Endotracheal and intubation and pain
Endotracheal and intubation and ferret
Endotracheal and intubation and alternatives

V.4.1.3.5. Results of Search:
No alternatives to intubation were found except for the use of mannequins. Mannequins are already utilized in this course. The search for endotracheal and intubation was the only query that produced any meaningful results. The citations included uses of endotracheal intubation and methods of intubation in many different animal species.

V.4.1.4. Unalleviated Painful or Distressful Procedure Justification:
NA

V.4.2. Prolonged Restraint:
NA

V.4.3. Surgery:

V.4.3.1. Pre-surgical Provisions:
NA

V.4.3.2. Procedure:
NA

V.4.3.3. Post-surgical Provisions:
NA

V.4.3.4. Location:

NA

V.4.3.5. Surgeon:
NA

V.4.3.6. Multiple Major Survival Operative Procedures:

V.4.3.6.1. Procedures:
NA

V.4.3.6.2. Scientific Justification:
NA

V.4.4. Animal Manipulations:

V.4.4.1. Injections:
See details in section V.4.1.2.1 Anesthesia/Analgesia/Tranquilization.

V.4.4.2. Biosamples:
NA

V.4.4.3. Adjuvants:
NA

V.4.4.4. Monoclonal Antibody (MAbs) Production:
NA

V.4.4.5. Animal Identification:
Animals are identified by cage cards and possibly by ear tag or tattoo.

V.4.4.6. Behavioral Studies:
NA

V.4.4.7. Other Procedures:
NA

V.4.4.8. Tissue Sharing:
NA

V.4.5. Study Endpoint:
The animals are recovered and returned to the colony after each lab. The number of years the ferrets will be kept is determined by the attending veterinarian based on the health of the animals.

V.4.6. Euthanasia:
Any ferrets that become ill or are no longer suited for use on this protocol will be euthanized by Center for LAM personnel in accordance with the most recent Report of the **American Veterinary Medical Association (AVMA)** panel on Euthanasia.

Generally ferrets will be anesthetized with ketamine and xylazine as previously described in this protocol. Another agent may be used at the direction of the attending veterinarian. When deeply anesthetized (no response to toe pinch) an intracardiac injection of euthanasia solution (≥ 1mL/10 lb) will be administered using a 20-21g, 1-1 ½ inch needle. Death will be verified by lack of respirations and auscultated heart beat. An additional method to confirm death (e.g., bilateral thoracotomy) may be performed.

V.5. Veterinary Care:

V.5.1. Husbandry Considerations: Except as noted below, routine animal husbandry will be provided in accordance with LAM Husbandry SOPs for each species in this protocol.

V.5.1.1. Study Room:

Building(s) A Room Number(s) MDL Labs

V.5.1.2. Special Husbandry Provisions:

Food Restriction: Yes X No

Fluid Restriction: Yes X No

V.5.1.3. Exceptions:
NA

V.5.2. Veterinary Medical Care:

V.5.2.1. Routine Veterinary Medical Care:
Routine veterinary care will be provided by LAM, IAW published SOPs.

V.5.2.2. Emergency Veterinary Medical Care: All emergency, weekend, and holiday care is provided by two animal husbandry technicians, one or more veterinary technicians, and an on-call veterinarian. Essential husbandry procedures and health rounds are conducted by LAM personnel once daily during weekend and holidays.

All emergency, weekend and holiday care is provided by LAM personnel overseeing the ferret colony.

V.5.3. Environmental Enrichment:

V.5.3.1. Enrichment Strategy: Except as indicated below, all animals on this protocol will be provided with routine environmental enrichment in accordance with LAM SOPs and IACUC Policies. Examples include nestlets and tunnels for rodents; balls, toys and food enrichment treats for large animal species.

V.5.3.2. Enrichment Restrictions:
NA

VI. STUDY PERSONNEL QUALIFICATIONS AND TRAINING:

STUDY PERSONNEL QUALIFICATIONS/TRAINING

Protocol activity or procedure (e.g., tail vein injections, euthanasia)	Name of person performing activity	Qualifications of person performing activity (e.g., research technician, 2 yrs experience)	Specific training in this activity or procedure (e.g., rodent handling class, 1999)
Injections, euthanasia	LAM technicians	Training and supervision from experienced technicians and veterinarians	Investigator training
intubation	(b)(6)	17 years experience	Investigator training
intubation		16 years experience	Investigator training

The students participating in teaching labs using animals have traditionally been exempt from inclusion in section VI. of the protocol and there has been no IACUC policy guidance to indicate that this has changed. By virtue of the fact that this is a training laboratory, the students are reasonably expected to be untrained on the procedure being conducted and only "handle" the ferrets under the supervision of the course instructors who are covered under section VI. of the protocol.

VII. BIOHAZARDS/SAFETY:

NA

VIII. ENCLOSURES:

NA

IX. ASSURANCES:

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a modification is specifically approved by the IACUC prior to its implementation.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an individual who is qualified to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration and made the proper coordinations regarding all applicable rules and regulations concerning radiation protection, biosafety, recombinant issues, and so forth, in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures / manipulations / observations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures / manipulations.

F. Training: I verify that I have attended the USUHS Investigator/Animal User Training Course.

(b)(6) [redacted]

3 June 2008
Date

G. Training: The following personnel will attend the next USUHS Investigator/Animal User Training Course:

_____ _____

_____ _____

H. Responsibility: I acknowledge the inherent moral, ethical and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" that the DOD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible and conducting humane and lawful research.

(b)(6) [redacted]

3 June 2008
Date

I. Painful Procedure(s):

I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals. This potential pain and/or distress **WILL or WILL NOT** be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures; however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(b)(6)

3 June 2008
Date

X. PROTOCOL ABSTRACT:

A. Animal Protocol Number: PED-05-373

B. Animal Protocol Title: Pediatric Intubation (Ferret) Training

C. Principal Investigator: [redacted]

D. Performing Organization: Department of Pediatrics USUHS

E. Funding: MDL

F. Objective and Approach:

Many infants fail to adequately make the transition to extrauterine life and require resuscitation in the delivery room. The consequences of delayed or improper efforts at resuscitation can be disastrous. With the current emphasis on primary care in military medicine most military physicians will be involved in the care of newborn infants. This training protocol uses animal models to supplement the teaching of neonatal resuscitation. The trainees are third year medical students and Pediatric residents. Formal lectures in neonatal resuscitation are given which include the use of mannequins. The ferret model is used to provide hands on experience in endotracheal intubation. The ferrets are recovered and can be used for multiple labs under department of laboratory animal medicine guidelines. This protocol reduces the number of animals that might otherwise be required by using the ferrets for multiple laboratory sessions.

G. Indexing Terms (Descriptors): animals, ferrets, endotracheal intubation, neonatal resuscitation