East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

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October 9, 2012

C. Jeffrey Smith, Ph.D.
Department of Micro/Immuno
Brody 5E-106
ECU Brody School of Medicine

Dear Dr. Smith:

Your Animal Use Protocol entitled, "Role of B. Fragilis Oxygen Stress Response in Infection" (AUP #K155a) was reviewed by this institution's Animal Care and Use Committee on 10/9/12. The following action was taken by the Committee:

"Approved as submitted"

Please contact Dale Aycock at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

enclosure

EAST CAROLINA UNIVERSITY
ANIMAL USE PROTOCOL (AUP) FORM
LATEST REVISION JULY, 2012

Project Title:

Click here to enter text.
Role of B. Fragilis Oxygen Stress Response in Infection

	Principal Investigator	Secondary Contact
Name	C. Jeffrey Smith	Edson R. Rocha
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For IACUC Use Only

AUP # K155a			
New/Renewal Renewal 10/5/12			
Full Review/Date	DR/Date		
Approval Date 10/9/12			
Study Type B. fragilis			
Pain/Distress Category D			
Surgery ✓	Survival ✓	Multiple	abdominal OCM
Prolonged Restraint			
Food/Fluid Regulation			
Other			
Hazard Approval/Dates	Rad	IBC ✓ bacteroides fragilis	EHS
OHP Enrollment			
Mandatory Training			
Amendments Approved			

I. Personnel

A. Principal Investigator(s):
Charles Jeffrey Smith

B. Department(s):
Microbiology & Immunology

C. List all personnel (PI's, co-investigators, technicians, students) that will be working with live animals and describe their qualifications and experience with these specific procedures. If people are to be trained, indicate by whom:

Name	Role(s) and Responsibilities for this Project	Required ECU Training (Yes/No)	Other Relevant Animal Experience/Training
Charles Jeffrey Smith	PI	yes	8 years of animal research and completed the ECU animal welfare training
Edson R. Rocha	Co-PI	yes	8 years of experience and training in performing the procedures detailed in this application. The PI has developed the implanted tennis "ping pong" ball in the rat peritoneal cavity to study B. fragilis intra-abdominal infection. The PI is acquainted with animal care procedures. Completed ECU training.
Yanlu Cao	graduate student	yes	several months experience with this model. Has received training from Dr. Rocha and has completed the online animal welfare course.
Matthew Rosenbaum	Consultant/Collaborator	yes	Veterinarian, Assistant Professor of Comparative Medicine

II. Regulatory Compliance

A. Non-Technical Summary

Using language a non-scientist would understand, please provide a clear, concise, and sequential description of animal use. Additionally, explain the overall study objectives and benefits of proposed research or teaching activity to the advancement of knowledge, human or animal health, or good of society. (More detailed procedures are requested later in the AUP.)

Do not cut and paste the grant abstract.

Bacteroides fragilis is the most frequent pathogen isolated from anaerobic infections in humans such as intra-abdominal or pelvic abscesses. In the present study we propose experiments to identify and characterize the genes involved in the pathogenicity and virulence of B. fragilis. The animal experiments will specifically test the ability of B. fragilis mutants to survive in the rat peritoneal cavity. In addition, we will test the expression of virulence genes during the growth of these organisms inside the peritoneal cavity. To accomplish this, rats will be surgically implanted with an intra-abdominal tissue cage as a model for intra-abdominal infection. The cage will be inoculated with bacterial strains and then periodically sampled to determine viability of bacteria and gene expression. We also will perform "competition" studies in which both the wild-type and mutant strains are inoculated into the same 'ping pong ball. Then we will measure the differential survival over time. The idea here is that, in some cases differences in growth rate and final population level of the mutants may not be great enough to easily distinguish from the wild-type parent strain except in the case of very severe defects. Thus we will use mixed culture "competition" experiments plus the mono-culture experiments for a more complete picture of the role of virulence factors.

B. Duplication

Does this study duplicate existing research? No

If yes, why is it necessary? (note: teaching by definition is duplicative)

Click here to enter text.

C. Alternatives to the Use of Live Animals

Are there less invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation that can be used in place of the live vertebrate species proposed here? No

If yes, please explain why you cannot use these alternatives.

Click here to enter text.

D. Literature search to ensure that there are no alternatives to all potentially painful and/or distressful procedures

1. Please list the potentially painful or distressful procedures in the protocol:

surgery, implantation of tissue cage (ping pong ball), inoculation of tissue cage with bacteria, application of anesthesia and subsequent sampling of the tissue cage using a syringe and needle to aspirate material from inside the tissue cage, euthanasia of animals at the end of the experiment.

2. For the procedures listed above, provide the following information (please do not submit search results but retain them for your records):

Date Search was performed:	August 16, 2012
Database searched:	Medline via ECU OVID program
Period of years covered in the search:	1946-August 2012
Keywords used and strategy:	Bacteroides fragilis; experimental intra-abdominal infection, experimental model of anaerobic infections, animal models, and alternatives. I performed an individual search for each of the keywords above then combined each of the data sets with the connector "And" Also searched the database with the entire phrase without the punctuation.
Other sources consulted:	NCBI Pubmed and Google

3. Narrative indicating the results of the search (2-3 sentences) and explaining why there are no alternatives to your proposed procedures that have the potential to cause pain and/or distress:

There are no alternative procedures to in vivo experimental intra-abdominal infection. The procedure using tissue cage implanted in the rat abdominal cavity will reduce the number of animals required for this project by allowing multiple samples from the same animal. The use of culture cell models will not provide the complex immune system response that is essential for the recruitment of PMNs and macrophages in the initial stages of B. fragilis infection and abscess formation. The use of adult Sprague Dawley rats is appropriate for the tissue cage "pig-pong-tennis ball" model of intra-peritoneal infection. In addition, this model will allow us to obtain multiple sampling of intra-

abdominal exudates without the need to sacrifice the animal in each time point. This procedure will greatly reduce the number of animals to be used in this study. This model has advantages over direct inoculation of bacteria into the peritoneal cavity because the infection process is confined to inside of the encapsulated implanted ping-pong ball tissue cage. Our experience (Rocha and Smith combined) using this model with more than 75 rats is that we have observed six systemic infections due and have lost one animal to causes we could not directly attribute to the procedures. Generally the animals do not exhibit any signs of discomfort. Other methods of abscess formation do not allow multiple sampling at different time points and would require a large number of animals for each experiment.

E. Hazardous agents

1. Protocol related hazards (chemical, biological, or radiological): Please indicate if any of the following are used in animals and the status of review/approval by the referenced committees:

HAZARDS	Oversight Committee	Status (Approved, Pending, Submitted)/Date	AUP Appendix I Completed?
Radioisotopes	Radiation	NA	
Ionizing radiation	Radiation	NA	
Infectious agents (bacteria, viruses, richettsia, prions, etc.)	IBC	approved 11/2009	yes
Toxins of biological orgins (venoms, plant toxins, etc.)	IBC	NA	
Transgenic, Knock In, Knock Out Animals---breeding, cross breeding or any use of live animals or tissues	IBC	NA	
Human tissues, cells, body fluids, cell lines	IBC	NA	
Viral/Plasmid Vectors/Recombinant DNA or recombinant techniques	IBC	approved 11/2009	yes
Oncogenic/toxic/mutagenic chemical agents	EH&S	NA	
Nanoparticles	EH&S	NA	

Cell lines, tissues or other biological products injected or implanted in animals	**DCM**	NA	
Other agents		NA	

2. Incidental hazards

Will personnel be exposed to any incidental zoonotic diseases or hazards during the study (field studies, primate work, etc)? If so, please identify each and explain steps taken to mitigate risk:

Bacteroides fragilis is a normal gut flora bacterium. This is not a contagious agent and is classified at BSL-1. The only chance of infection is from direct injection of the organism and even in that case during my 30+ years of working with these organisms I do not know of any case of laboratory acquired infection. However, since these organisms have been genetically modified, the experiments will be conducted under ABSL-2 conditions to minimize chances of contamination. A Biological Safety Registration has been approved for this work.

III. Animals and Housing

A. Species and strains:

Sprague Dawley outbred rats.

B. Weight, sex and/or age:

>350g/11-13 weeks old male.

C. Animal numbers:

1. Please complete the following table:

Total number of animals in treatment and control groups	Additional animals (Breeders, substitute animals)	Total number of animals used for this project
198	+ 2	=200

2. Justify the species and number (use statistical justification when possible) of animals requested:

The species chosen for these experiments is the adult Sprague Dawley rat. The use of these animals is appropriate for the tissue cage "pig-pong-tennis ball" model of intra-abdominal abscess infection because of their size and historical use of rats in intra-abdominal infection studies. In addition, this model will allow us to obtain multiple sampling of intra-abdominal exudates without the need to sacrifice the animal in each time point. This procedure will reduce the number of animals to be used in this study. There is

no alternative in vitro model to study abscess formation or response to an abscess inducing organism such B. fragilis because no in vitro system has been able to duplicate the complex mammalian immune response. Also, this model has been used previously with a different species of bacteria (Bamberger et al. 2002, Antimicrob. Agents Chemother, 46:2878-2884).

There are two factors that must be considered to determine the number of animals needed for the study. The first factor is that we have a maximum of 8 candidate genes that we propose to test as virulence factors. For each candidate gene we will construct the corresponding mutant strain thus we will need to have 8 independent trials (including both competition assays and monoculture assay). In addition each trial will be composed of two identical experiments in order to demonstrate that the results are repeatable. The second factor is that we must determine the lowest number of animals needed in each experiment that can provide statistical significance for our results. Based on previous work with this model by Dr. Rocha and myself (see AUP #K146 and# K155) we can estimate the number of wild-type or mutant bacteria found per ml of fluid in the artificial abscess and use these numbers in power calculations to determine the sample size needed for statistical significance in each experiment.For these calculations we will be using a study design based on use of the unpaired t-test on group means. We have set P = .01 and power probability = 0.9. The bacterial numbers from previous work used for this analysis are:

wild type strain: 3.28 x 10exp9 (SD 2.07 x 10exp9) mutant strain: 6.43 x 10exp7 (SD 3.56 x 10exp7). Based on these numbers we have calculated that statistical significance can be achieved using groups of 5 animals and we do not see any difference in using the competition or monoculture approaches. In experimental design section (section IV.A) we present a table for the total number of animals to be used based on groups of 5 animals per experiment. Briefly, we propose to study 8 different mutants so that is 10 animals per mutant in monoculture experiments and 10 animals per mutant in competition assay experiment plus one uninoculated control per mutant for a total of 178 animals. The remaining animals are accounted for by the need to run two trials of monoculture experiments with the wild type parent strain to obtain gene expression data to use as baseline for when we perform the competition assays (20 animals).

3. Justify the number and use of any additional animals needed for this study:

a. For unforeseen outcomes/complications:

two additional animals should be ordered if there are any unexpected deaths that lead to invalid statistics.

b. For refining techniques:

n/a

c. For breeding situations, briefly justify breeding configurations and offspring expected:

n/a

d. Indicate if following IACUC tail snip guidelines: Choose an item.

(if no, describe and justify)

Click here to enter text.

4. Will the phenotype of mutant, transgenic or knockout animals predispose them to any health, behavioral, physical abnormalities, or cause debilitating effects in experimental manipulations? No **(if yes, describe)**

Click here to enter text.

5. Are there any deviations from standard husbandry practices?

Yes **If yes, then describe conditions and justify the exceptions to standard housing (temperature, light cycles, sterile cages, special feed, prolonged weaning times, wire-bottom cages, etc.):**

Animals will be housed individually on soft bedding. Tubes are not allowed due to surgery so alternative enrichments such as softer bedding, crinkle nesting will be provided.

6. Is it necessary for animals to be singly housed?

No **(If yes, describe housing and justify the need to singly house social species)**

Click here to enter text.

7. Are there experimental or scientific reasons why routine environmental enrichment should not be provided? No

(If yes, describe and justify the need to withhold enrichment)

Click here to enter text.

8. If wild animals will be captured or used, provide permissions (collection permit # or other required information):

NA

9. List all laboratories or locations outside the animal facility where animals will be used. Note that animals may not stay in areas outside the animal facilities for more than 12 hours without prior IACUC approval. For field studies, list location of work/study site.

none

IV. Animal Procedures

A. Outline the Experimental Design including all treatment and control groups and the number of animals in each. Tables or flow charts are particularly useful to communicate your design.

The objective is to determine if various mutant strains lacking various oxidative stress resistance genes can survive as well in the abscess as the wild type parent strain. We also need to

determine the overall gene expression patterns of the bacteria in the abscess and if the oxidative stress gene mutations modulate these patterns. For this work we have a bank of 8 mutant strains or proposed mutant strains that lack a variety of genes known to be important for in vitro resistance to oxidative stress. We now will test the role of these genes in vivo using the rat model. Briefly we will implant a sterile tissue cage into the peritoneal cavity of the rat. Then allow the animals to recover for 3-4 weeks to allow for encapsulation to occur according to Bamberger et al. 2002 (Antimicrob. Agents Chemother, 46:2878-2884). Next five rats will be inoculated with about 1,000,000 c.f.u./ml B. fragilis wild type strain in monoculture (Control) by injection into the implanted tissue cage. Samples will be aspirated from infected cages at 2, 4, 8, and 15 days post-infection. These control experiments will be repeated four times over the course of the entire project. For each mutant strain five rats will be used in monoculture experiments to determine survival and gene expression patterns. Also for each mutant strain 5 animals will be used in competition experiments in which mutant and wild-type strain are co-cultured in the tissue cage in order to determine if the mutant has a defect relative to the parent. The idea here is that, in the absence of competition, differences in growth rate and final population level may not be great enough to distinguish at a statistically significant level except in the case of very major defects. The competition assay is a tried and true method in bacterial pathogenesis studies. These experiments with the mutant will be repeated once. One uninoculated control will be used for each mutant strain and 2 for the wild type parent strain. Two animals may be needed to protect against adverse outcomes due to unforeseen complications (See Table Below).

Bacterial Strain/treatment	Animals per group	# experiments	# trials	total # animals
Wild-type monoculture	**5**	**2**	**2**	**20**
Mutant monoculture	5	2	8	80
Competition assay	**5**	**2**	**8**	**80**
Added for unforeseen complications	1	2	1	2
Uninoculated control	**1**	**1**	**18**	**18**
Totals				200

In sections IV.B-IV.S below, please respond to all items relating to your proposed animal procedures. If a section does not apply to your experimental plans, please leave it blank.

Please refer to DCM and IACUC websites for relevant guidelines and SOPs.

B. Anesthesia/Analgesia/Tranquilization/Pain/Distress Management For Procedures Other than Surgery:

Adequate records describing anesthetic monitoring and recovery must be maintained for all species.

If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

Click here to enter text.

1. **Describe the pre-procedural preparation of the animals:**
   a. **Food restricted for** Not needed **hours**
   b. **Food restriction is not recommended for rodents and rabbits and must be justified:**

   n/a
   c. **Water restricted for** not needed **hours**
   d. **Water restriction is not recommended in any species for routine pre-op prep and must be justified:**

   n/a

2. **Anesthesia/Analgesia *for Procedures Other than Surgery***

	Agent	Concentration	Dose (mg/kg)	Max Volume	Route	Frequency	Number of days administered
Pre-procedure analgesic							
Pre-anesthetic							
Anesthetic	isoflurane	2-4%			inhalation	6	<5 min
Post procedure analgesic							
Other							

3. Reason for administering agent(s):
To sedate rats for injection and aspiration.

4. For which procedure(s):
Sample injection into and aspiration from the implanted ping pong ball. In all cases aseptic technique will be used for these procedures. The skin is prepped prior to insertion of the needle by swabbing with alcohol.

5. Methods for monitoring anesthetic depth:
Foot withdraw

6. Methods of physiologic support during anesthesia and recovery:
n/a

7. Duration of recovery:
< 5 min

8. Frequency of recovering monitoring:
Click here to enter text.

9. Specifically what will be monitored?
respiration and mobility

10. When will animals be returned to their home environment?
when they are conscious and mobile

11. Describe any behavioral or husbandry manipulations that will be used to alleviate pain, distress, and/or discomfort:
Animals typically do not exhibit distress or discomfort following procedure

C. Use of Paralytics

1. Will paralyzing drugs be used? NO

2. For what purpose:
Click here to enter text.

3. Please provide scientific justification for paralytic use:
Click here to enter text.

4. Paralytic drug:
Click here to enter text.

5. Dose:
Click here to enter text.

6. Method of ensuring appropriate analgesia during paralysis:
Click here to enter text.

D. Blood or Body Fluid Collection

1. Please fill out appropriate sections of the chart below:

	Location on animal	Needle/catheter size	Volume collected	Frequency of procedure	Time interval between collections
Blood Collection					
Body Fluid Collection	Intra-abdominal from within the tissue cage	25-20 g	0.5-2 ml	once per sampling period	2, 4, 8, 15 days post infection
Other					

E. Injections, Gavage, & Other Substance Administration

1. Please fill out appropriate sections of the chart below:

	Compound	Location & Route of admin	Needle/catheter/gavage size	Max volume admin	Freq of admin (ie two times per day)	Number of days admin (ie for 5 days)	Max dosages (mg/kg)
Injection/ Infusion	Live Bacteroides fragilis. 4 ml at 10^6 to 10^8 cells/ml	Intra-abdominal injection into the tissue cage	25-20 g	4 ml	once to initiate the experiment	n/a	n/a
Gavage							
Other							

2. For all injections and infusions, PHARMACEUTICAL GRADE compounds should be used. If not available, refer to IACUC Guidelines for non-pharmaceutical grade compound use and provide required information below:
n/a

E. Prolonged restraint with mechanical devices

Prolonged restraint in this context means *beyond routine care and use procedures* for rodent and rabbit restrainers, and large animal stocks.

Prolonged restraint also includes *any* use of slings, tethers, metabolic crates, inhalation chambers, primate chairs and radiation exposure restraint devices.

1. **For what procedure(s):**

n/a

2. **Explain why non-restraint alternatives cannot be utilized:**

n/a

3. **Restraint device(s):**

n/a

4. **Duration of restraint:**

n/a

5. **Frequency of observations during restraint/person responsible:**

n/a

6. **Frequency and total number of restraints:**

n/a

7. **Conditioning procedures:**

n/a

8. **Steps to assure comfort and well-being:**

n/a

9. **Describe potential adverse effects of prolonged restraint and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

n/a

G. Tumor Studies, Disease Models, Toxicity Testing, Vaccine Studies, Trauma Studies, Pain Studies, Organ or System Failure Studies, Shock Models, etc.

1. **Describe methodology:**

The wild type and mutant bacteria will be injected into the sterile, artificial abscess and their ability to survive in the abscess will be determined. Samples will be taken on days 2,4,8, and 15 by aspiration using a sterile syringe and then plated on bacteriological growth media to determine the viable cell count. Some material also will be used for the extraction of RNA to be used in gene expression studies to determine which genes are expressed by the bacteria during the course of "infection".

2. **Expected model and/or clinical/pathological manifestations:**

It is expected that wild type bacteria will survive at a higher rate than mutant bacteria in the artificial abscess in the co-culture competition assays and we expect to see more rapid

clearing of the mutant strains in the monoculture assays. We do not expect any obvious tissue pathology.

3. Signs of pain/discomfort:

We have had several years of experience with this model and during this time seven animals became "sick" and displayed a hunched posture, depressed attitude and loss of appetite. These animals were provided analgesics but eventually had to be euthanized. This is a rare occurrence.

4. Frequency of observations:

daily

5. Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

If animals show signs of peritonitis, hunched posture, depressed attitude and loss of appetite, as described above we will first try to treat with analgesics but if no change after a day they will be euthanized.

H. Treadmills/Swimming/Forced Exercise

1. Describe aversive stimulus (if used):

n/a

2. Conditioning:

n/a

3. Safeguards to protect animal:

n/a

4. Duration:

n/a

5. Frequency:

n/a

6. Total number of sessions:

n/a

7. Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):

n/a

I. Projects Involving Food and Water Regulation or Dietary Manipulation

(Routine pre-surgical fasting not relevant for this section)

1. **Food Regulation**

   a. **Amount regulated and rationale:**

   n/a

   b. **Frequency and duration of regulation (hours for short term/weeks or months for long term):**

   n/a

   c. **Frequency of observation/parameters documented (i.e. recording body weight, body condition, etc.):**

   n/a

   d. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

   n/a

2. **Fluid Regulation**

   a. **Amount regulated and rationale:**

   n/a

   b. **Frequency and duration of regulation (hours for short term/weeks or months for long term):**

   n/a

   c. **Frequency of observation/parameters documented (body weight, hydration status, etc.):**

   n/a

   d. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

   n/a

3. **Dietary Manipulations**

   a. **Compound supplemented/deleted and amount:**

   n/a

   b. **Frequency and duration (hours for short term/week or month for long term):**

   n/a

c. **Frequency of observation/parameters documented:**
n/a

d. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
n/a

J. Endoscopy, Fluoroscopy, X-Ray, Ultrasound, MRI, CT, PET, Other Imaging

1. **Describe animal methodology:**
n/a
2. **Duration of procedure:**
n/a
3. **Frequency of observations during procedure:**
n/a
4. **Frequency/total number of procedures:**
n/a

5. **Method of transport to/from procedure area:**
n/a
6. **Describe potential adverse side effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
n/a
7. **Please provide or attach appropriate permissions/procedures for animal use on human equipment:**
n/a

K. Polyclonal Antibody Production

1. **Antigen/adjuvant used and justification for adjuvant choice:**
n/a
2. **Needle size:**
n/a
3. **Route of injection:**
n/a
4. **Site of injection:**

n/a

5. **Volume of injection:**
   n/a
6. **Total number of injection sites:**
   n/a
7. **Frequency and total number of boosts:**
   n/a
8. **What will be done to minimize pain/distress:**
   n/a
9. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
   n/a

L. Monoclonal Antibody Production

1. **Describe methodology:**
   n/a

2. **Is pristane used:** No
   Volume of pristane:
   n/a

3. **Will ascites be generated:** No
   i. **Criteria/signs that will dictate ascites harvest:**
      Click here to enter text.
   ii. **Size of needle for taps:**
      Click here to enter text.
   iii. **Total number of taps:**
      Click here to enter text.
   iv. **How will animals be monitored/cared for following taps:**
      Click here to enter text.
4. **What will be done to minimize pain/distress:**
   Click here to enter text.
5. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

Click here to enter text.

M. Temperature/Light/Environmental Manipulations

1. **Describe manipulation(s):**
n/a
2. **Duration:**
n/a
3. **Intensity:**
n/a
4. **Frequency:**
n/a
5. **Frequency of observations/parameters documented:**
n/a
6. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
n/a

N. Behavioral Studies

1. **Describe methodology/test(s) used:**
n/a
2. **Will conditioning occur? If so, describe:**
n/a
3. **If aversive stimulus used, frequency, intensity and duration:**
n/a
4. **Length of time in test apparatus/test situation:** ***(i.e., each test is ~10 mins)***
n/a
5. **Frequency of testing and duration of study:** ***(i.e., 5 tests/week for 6 months)***
n/a
6. **Frequency of observation/monitoring during test:**
n/a
7. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

n/a

O. Capture with Mechanical Devices/Traps/Nets

1. **Description of capture device/method:**
   n/a
2. **Maximum time animal will be in capture device:**
   n/a
3. **Frequency of checking capture device:**
   n/a
4. **Methods to ensure well-being of animals in capture device:**
   n/a
5. **Methods to avoid non-target species capture:**
   n/a
6. **Method of transport to laboratory/field station/processing site and duration of transport:**
   n/a
7. **Methods to ensure animal well-being during transport:**
   n/a
8. **Expected mortality rates:**
   n/a
9. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
   n/a

P. Manipulation of Wild-Caught Animals in the Field or Laboratory

1. **Parameters to be measured/collected:**
   n/a
2. **Approximate time required for data collection per animal:**
   n/a
3. **Method of restraint for data collection:**
   n/a
4. **Methods to ensure animal well-being during processing:**
   n/a
5. **Disposition of animals post-processing:**
   n/a

6. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

n/a

Q. Wildlife Telemetry/Other Marking Methods

1. **Describe methodology (including description of device):**

n/a

2. **Will telemetry device/tags/etc be removed?** n/a **If so, describe:**

n/a

3. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

n/a

R. Other Animal Manipulations

1. **Describe methodology:**

n/a

2. **Describe methods to ensure animal comfort and well-being:**

n/a

3. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**

n/a

S. Surgical Procedures

All survival surgical procedures must be done aseptically, regardless of species or location of surgery. Adequate records describing surgical procedures, anesthetic monitoring and postoperative care must be maintained for all species.

1. **Location of Surgery (Building & Room #):**

ECU/SOM Animal care facility operating room

2. Type of Surgery (check all that are appropriate):

☐ **Non-survival surgery (animals euthanized without regaining consciousness)**

X☐ **Major survival surgery (major surgery penetrates and exposes a body cavity or produces substantial impairment of physical or physiologic function)**

☐ **Minor survival surgery**

☐ **Multiple survival surgery**

If yes, provide scientific justification for multiple survival surgical procedures:
n/a

3. Describe the pre-op preparation of the animals:

a. **Food restricted for** 0 **hours**
b. **Food restricted is not recommended for rodents and rabbits and must be justified:**
   n/a
c. **Water restricted for** 0 **hours**
d. **Water restriction is not recommended in any species for routine pre-op prep and be justified:**
   n/a

4. Minimal sterile techniques will include (check all that apply):

Please refer to DCM Guidelines for Aseptic Surgery for specific information on what is required for each species and type of surgery (survival vs. non-survival).

X☐ **Sterile instruments**

How will instruments be sterilized?
Ethylene oxide for ping pong balls, autoclave for instruments

If serial surgeries are done, how will instruments be sterilized between surgeries:
Instruments will be sterilized by autoclave. For serial surgery, instruments will be sterilized by dry hot beads sterilizer.

x☐ **Sterile gloves**
x☐ **Mask**
x☐ **Cap**
☐ **Sterile gown**
x☐ **Sanitized operating area**
x☐ **Clipping or plucking of hair or feathers**
x☐ **Skin preparation with a sterilant such as betadine**
x☐ **Practices to maintain sterility of instruments during surgery**
x☐ **Non-survival (clean gloves, clean instruments, etc.)**

5. **Describe all surgical procedures:**

a. Skin incision size and site on the animal:

An abdominal incision of about 3 cm

b. Describe surgery in detail (include size of implant if applicable):

Click here to enter text.

c. Method of wound closure:

The rats will be given pre-emptive analgesia Buprenex prior to the procedure and then anesthetized with gas (isoflurane) anesthesia. Ketamine and xylazine (9:1) may be substituted for gas if instructed by veterinarian. Aseptic technique (sterile instruments, surgical gloves, masks and surgical prep) will be used. An abdominal incision of about 3 cm will be performed and a single sterile table-tennis ball with 300 1.5 mm-diameter holes will be implanted in the peritoneal cavity by sterile techniques, abdominal closure with at least two layers, 3-0 absorbable followed by 3-0 nylon skin. Animal will receive buprenex at 0.1mls/100 grams bw (0.03mg/ml conc) or an NSAID carprofen or meloxicam for post-op analgesia and every 8-12 hours post as determined by DCM veterinarian. Animal will be allowed to recover, for 5 weeks to allow for encapsulation to occur according to Bamberger et al. 2002 (Antimicrob. Agents Chemother, 46:2878-2884). Rats will be housed under standard laboratory housing conditions and receive food and drink at libitum and support care for anesthetized rodents at the animal housing facility of the Department of Comparative Medicine, East Carolina University, Greenville, North Carolina. The rats will be inoculated with about 108 c.f.u./ml B. fragilis strains into the implanted ball tissue cage. Samples will be aspirated from infected cages at 2, 4, 8, and 15 or 21 days post-infection.

i. Number of layers

Abdominal closure with at least two layers

ii. Type of wound closure and suture pattern:

Interrupted, 3-0 absorbable, and 3-0 nylon

iii. Suture type/size/wound clips/tissue glue:

Click here to enter text.

iv. Plan for removing of skin sutures/wound clip/etc:

Skin sutures removed after 7 – 10 days

6. Anesthetic Protocol:

a. If anesthesia/analgesia must be withheld for scientific reasons, please provide compelling scientific justification as to why this is necessary:

n/a

b. Anesthesia/Analgesia For Surgical Procedures

	Agent	Dose (mg/kg or %)	Volume	Route	Frequency	Number of days administered
Pre-operative analgesic	Buprenex	0.1 ml/100 g BW	1 ml depending on BW	SC	once	prior to anesthesia
Pre-anesthetic						
Anesthetic	isoflurane	2-4%		inhalation	once	6 times; at insertion of tissue cage, inoculation day, sample days 2, 4, 8, 15
Post-operative Analgesic	Buprenex	0.1 mg/100g BW	1 ml depending on BW	SC	once but more in indicated	8-12 h
Other	Alternative Anesthetic - Alternative- ketamine plus xylazine (9:1) Alternative Analgesic – meloxica	0.1 ml/100g BW of Ketamine (90mg/ml) + Xylazine (10mg/ml) 1.5 mg/kg	1 ml depending on BW 1 ml depending on BW	Ip PO	once SID, PRN	30-60 min 24 hr

c. Methods that will be used to monitor anesthetic depth (include extra measures employed when paralyzing agents are used):

Standard procedures for anesthetic monitoring, surgical plane of anesthesia. Animals will be adequately anesthetized prior to initiation of surgery and then maintained in a surgical plane throughout procedure the will be done by monitoring toe pinch and pedal reflexes to ensure adequate depth. Pulse oximetry will monitor HR and O2 saturation. Respiration Rate will be monitored by watching thoracic cavity as well.

d. Methods of physiologic support during anesthesia and immediate post-op period (fluids, warming, etc.):

On pad to conserve body heat (surgery takes about 20 min per rat). If needed warm 0.9% NaCl will be administered SQ (3-7mL) along with soft and caloric dense food being accessible on the cage floor.

e. List what parameters are monitored during immediate post-op period. Provide the frequency and duration:

Following surgery procedures in the "tissue cage model of infection", animals will be examined for distress and pain on an hourly basis for 4 hours on the day of surgery. The animals will be kept warm, given fluid and nutritional supplementation as required until recovery.

f. Describe any other manipulations that will be used to alleviate pain, distress, and/or discomfort during the immediate post-op period (soft bedding, long sipper tubes, food on floor, dough diet, etc.):

If necessary, food will be placed on cage bottom, and gel packs used for water (usually not required) will be provided.

g. List criteria used to determine when animals are adequately recovered from anesthesia and when the animals can be returned to their home environment:

monitor continuously until mobile and alert.

7. Recovery from Surgical Manipulations (after animal regains consciousness and is returned to its home environment)

a. What parameters (behavior, appetite, mobility, wound healing, etc.) will be monitored:

Animals will be assessed for hunched posture, depressed attitude, off feed. Animals also will be monitored for overt clinical signs of illness (distress, sick, scruffy appearance, immobile, anorectic, moribund.

b. How frequently (times per day) will animals be monitored:

once

c. How long post-operatively (days) will animals be monitored:

Animals will be monitored for eating, drinking, defecating, behavior and appearance (body condition scoring) for 4 hours post operatively that day until normal signs appear. Then daily for about 4 weeks until encapsulation of the implanted tissue cage.

8. Surgical Manipulations Affecting Animals

a. Describe any signs of pain/discomfort/functional deficits resulting from the surgical procedure:

Hunched posture, depressed attitude, off feed, distress, sick-looking, scruffy appearance, immobile, anorectic, moribund.

b. **What will be done to manage any signs of pain or discomfort (include pharmacologic and non-pharmacologic interventions):**
Analgesic will be provided as described above in analgesia/anesthesia table.

c. **Describe potential adverse effects of procedures and provide humane endpoints (criteria for either humanely euthanizing or otherwise removing from study):**
Abscesses are a self-contained "walled" off infectious process. Nonetheless, intra-abdominal abscesses can eventually rupture and release its purulent pus contents into the peritoneal cavity. This may lead to a disseminated systemic infection. No antibiotic can be administered since these will interfere with experimental infections. Thus, any animal showing clinical signs of illness (distress, sick, scruffy appearance, immobile, anorectic, moribund) will be euthanized prior to the end of the experiment. We have done this procedure on more than 75 rats and have lost six to sepsis and one to an unknown cause (likely intestinal strangulation).

V. Euthanasia

Please refer to the AVMA Guidelines on Euthanasia and DCM Guidelines to determine appropriate euthanasia methods.

A. **Euthanasia Procedure.** ***All investigators, even those conducting non-terminal studies, must complete this section in case euthanasia is required for humane reasons.***

1. Physical Method- If a physical method is used, the animal should be first sedated/anesthetized with CO_2 or other anesthetic agent. If prior sedation is not possible, a scientific justification must be provided:
n/a

2. Inhalant Method- Carbon Dioxide
(if other, describe the agent and delivery method)
Click here to enter text.

3. Non-Inhalant Pharmaceutical Method (injectables, MS-222, etc.)- Please provide the following:

a. **Agent:**
n/a
b. **Dose or concentration:**
n/a
c. **Route:**
n/a

B. Method of ensuring death (can be physical method, such as pneumothorax or decapitation for small species and assessment method such as auscultation for large animals):

check for signs of breathing and open the thoracic cavity

C. Describe disposition of carcass following euthanasia:

Each animal is placed in a disposal plastic bag and every three bags are wrapped up in another bag. Then all carcasses are put in a biohazard red bag, closed and they are put in the cold room container available at the animal house facility for disposal of carcasses.

I acknowledge that humane care and use of animals in research, teaching and testing is of paramount importance, and agree to conduct animal studies with professionalism, using ethical principles of sound animal stewardship. I further acknowledge that I will perform only those procedures that are described in this AUP and that my use of animals must conform to the standards described in the Animal Welfare Act, the Public Health Service Policy, The Guide For the Care and Use of Laboratory Animals, the Association for the Assessment and Accreditation of Laboratory Animal Care, and East Carolina University.

Please submit the completed animal use protocol form via e-mail attachment to iacuc@ecu.edu. You must also carbon copy your Department Chair.

PI Signature: C. Kelly Smith email **Date:** 10/5/12 ~~10/4/2012~~

Veterinarian: _____ **Date:** 10/9/12

IACUC Chair: S B McRae jh **Date:** 10/9/12

APPENDIX 1-HAZARDOUS AGENTS			
Principal Investigator: Charles Jeffrey Smith	Campus Phone: 744-2700		Home Phone: 252-756-8131
IACUC Protocol Number: K155	Department: Microbiology & Immunology		E-Mail: smithcha@ecu.edu
Secondary Contact: Department: Edson Rocha	Campus Phone: 744-9563	Home Phone: 756-8538	E-Mail: rochae@ecu.edu
Chemical Agents used: N/A		Radioisotopes used: N/A	
Biohazardous Agents used: Bacteroides fragilis	Animal Biosafety Level: ABSL2 based on use of recombinant DNA		Infectious to humans? Probably if large numbers are directly injected into the blood stream.
PERSONAL PROTECTIVE EQUIPMENT REQUIRED:			
Route of Excretion: There is no excretion of the organisms since they are confined to the artificial abscess			
Precautions for Handling Live or Dead Animals: No special precautions needed. Lab coats, eye protection, and gloves will be worn during injection of animals and sampling procedures. Bacteria closely related to B. fragilis are present in all rodent feces. .			
Animal Disposal: incinerate			
Bedding/Waste Disposal: normal procedures			
Cage Decontamination: normal procedures			
Additional Precautions to Protect Personnel, Adjacent Research Projects including Animals and the Environment: B. fragilis is a BSL-1 organism but due to the fact that we have genetically manipulated the organisms the experiments are elevated to ABSL-2 but risk of infection is minimal. Thus there are no special "Additional" precautions needed. Organisms closely related to B. fragilis are found in the feces of all rodents.			
Initial Approval Safety/Subject Matter Expert Signature & Date _____			

The Brody School of Medicine
Office of Prospective Health
East Carolina University
188 Warren Life Sciences Building • Greenville, NC 27834
252-744-2070 office • 252-744-2417 fax

Occupational Medicine
Employee Health
Radiation Safety
Infection Control
Biological Safety

TO: Dr. C. Jeffrey Smith
Department of Microbiology & Immunology

FROM: Eddie Johnson/John Williams
Biological Safety Officers

RE: Registration Approval

Date: December 10, 2009

Your Biological Safety Protocol J Smith, 09-01 "*Role of B. fragilis Oxygen Stress Response in Infection*" to use genetically modified micro organisms has received **approval** to be conducted under BSL-1 and ABSL-2 based on your registration submitted. This registration was approved by the ECU Biological Safety Committee with Dr. Greg Smith as acting chair.

This approval is effective for a period of 3 years and may be renewed with an updated registration if needed. Please notify the Animal Care staff before or if you begin work with Biohazard agents in animals. Also please keep in mind all individuals who will be exposed to or handle biohazardous agents in your work will be due for Blood Borne Pathogens refresher training annually.

Please do not hesitate to contact Biological Safety at 744-2070 if you have any questions, concerns, or need any additional information. Best wishes on your research.

cc: Dr. C. Jeffrey Smith, Chair, Biosafety Committee
Dr. Greg Smith, Community Member
Janine Davenport, IACUC
Dr. Robert Carroll, IACUC
Dale Aycock, IACUC