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OMB APPROVED
0579-0036

This report is required by law (7 U.S.C. 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

Interagency Report Control No. 0180-DOA-AN

Fiscal Year: 2009

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

REGISTRATION NUMBER: 14-R-0082

Customer Number: 140

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include ZIP Code)*

Tufts- New England Medical Center, Inc.
171 Harrison Avenue
Boston, MA 02111

(b)(2)High, (b)(7)f

Telephone: (617) 636 5615

3. REPORTING FACILITY *(List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)*

FACILITY LOCATIONS *(Sites)* *See Attached Listing*

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress on these animals and the reasons such drugs were not used must be attached to this report.)*	F. TOTAL NUMBER OF ANIMALS (Cols. C + D + E)
4. Dogs	0	0	8	0	8
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	0	10	0	10
7. Hamsters	0	271	47	90	408
8. Rabbits	0	0	14	97	111
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	0	0	0	0
11. Pigs	0	0	84	0	84
12. Other Farm Animals	0	0	0	0	0
13. Other Animals Ferret	0	2	0	0	2

ASSURANCE STATEMENTS

1.) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2.) Each principal investigator has considered alternatives to painful procedures.

3.) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all such exceptions is attached to this annual report.** In addition to identifying the IACUC approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4.) The attending veterinarian for this research facility has appropriate authority to ensure the provisions of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer (C.E.O.) or Legally Responsible Institutional Official (I.O.))

SI

(b)(6), (b)(7)c

ATE SIGNED

10-21-09

AF
AL

12-3-09

OCT 28 2009

BY:

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: **14-R-0082**

2. Number **90** of animals used in this study.

3. Species (common name) **hamster** of animals used in this study.

4. Explain the procedure producing pain and/or distress.

Female Syrian hamsters (80 – 120g) will be treated orally with Clindamycin (30 mg/kg), using a 20-gauge 1.5 inch gavage-needle attached to a 1 ml syringe, 24 hr before inoculation with C. difficile to induce susceptibility to C. difficile infection. Hamsters will be orally inoculated with C. difficile across a range of infectious doses. Since the Syrian hamster is sensitive to C. difficile infection, normal progression of the disease will occur. Disease symptoms included watery diarrhea, hunched posture, lethargy, weight loss, distended abdomen and wet tail (proliferative ileitis). Following inoculation, hamsters will be monitored for signs of disease until animals appear to be in a moribund state, at which point will be euthanized, or have recovered from the challenge.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below).

The specific objectives of this project are to test the virulence of a strain of *Clostridium difficile* that cannot sporulate and to test the role of sporulation in the spread of *C. difficile* between hosts. Sporulation is a developmental process that members of the *Bacillus* and *Clostridia* family of bacteria use when growth conditions become unfavorable. They transform themselves from actively growing, or vegetative, bacteria into dormant spores. The dormant spore is highly resistant to heat, radiation, chemicals, and antibiotics and is believed to be the infectious form of *C. difficile*. Our hypothesis is that vegetative *C. difficile* is capable of causing disease. Our specific objective is to introduce mutations into *C. difficile* that prevent the bacteria from sporulating (Spo$^-$). We will then test the difference between a wild-type strain of *C. difficile* and the Spo$^-$ mutant in an animal model of *C. difficile* disease. This simple experiment will either prove the importance of the spore as an infectious agent for *C. difficile* or will show that vegetative bacteria are also capable of causing disease. We also hypothesize that sporulation is essential for *C. difficile* to spread between hosts. To analyze this we will place uninfected animals and animals infected with either spore-forming *C. difficile* or a non-sporulating mutant strain in the same cage. The Syrian hamster is exquisitely sensitive to *C. difficile* infection and disease progression is similar to the most severe disease progression in humans, with eventual development of pseudomembranous colitis. For this reason hamsters need to be infected without any therapeutic intervention in order to assess data accurately. Criteria used to determine if the animals are in a moribund state include: loss of 20% of their starting weight, inactivity, diarrhea and poor fur coat. When the animals meet these four criteria, they will be euthanized by CO2 asphyxia followed by thoracotomy as a physical method of euthanasia.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113:102):

N/A

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: **14-R-0082**

2. Number **97** of animals used in this study.

3. Species (common name) **rabbits** of animals used in this study.

4. Explain the procedure producing pain and/or distress.

Infant rabbits are fed Shiga toxin, ricin, or a control intragastrically, which will cause inflammation of the intestines and diarrhea. Experimental groups will be given doses of MAPKinase inhibitors or inhibitor vehicle. All animals are housed with their mother and will be sacrificed between 48-72 hours after inoculation. Animals will be monitored for diarrhea three times per day until sacrifice. At these times, animals will be assessed for: diarrhea, shallow breathing, scruffy fur, poor color, lethargy, decreased muscle tone, and failure to nurse. Diarrhea will be graded according to I: no diarrhea, II: mild to moderate diarrhea (feces stuck to perineum and/or legs), or III: severe diarrhea (feces stuck to hind legs, wet tail, and prolapse of rectum). Severe, bloody diarrhea will constitute immediate euthanasia.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below).

The infant rabbit is the only species that responds to Shiga toxins in the same way as humans do. Feeding Shiga toxin 2 (Stx2) to rabbits that are 2-3 days old results in the kind of damage that is observed in humans exposed to Shiga toxins. We aim to determine if the gene activating effects of Shiga toxin that we observe in human intestinal epithelial cells *in vitro* are also occurring in the infant rabbit model. Because we are studying how these toxins affect the whole intestine, we must utilize a live animal model and the effects of the inoculation in the *in vivo* system. We will be assessing how certain infection-fighting cells called "neutrophils" are obtained from the blood circulating through the intestinal blood vessels in response to these toxins and how they migrate into the deeper layers of the organ. We will analyze these effects in two ways, by clinical observations of the rabbits and the amount of diarrhea caused and by histopathological evaluations of neutrophil infiltration, edema/swelling of the tissue, and any amount of hemorrhage. Therefore, we need the animals to be infected without any therapeutic intervention to be able to assess our data accurately.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g. APHIS, 9 CFR 113:102):

N/A