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According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0579-0036. The time required to complete this information collection is estimated to average 2 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

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-0065

Customer Number: 628

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

Tufts University
200 Westboro Road (b)(2)High, (b)(7)f
North Grafton, MA 01536

Telephone: (508) 839 7992

NOV 2 5 2009

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	23	0	23
5. Cats	0	0	10	0	10
6. Guinea Pigs	0	0	0	2	2
7. Hamsters	0	3	0	7	10
8. Rabbits	0	9	209	0	218
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	83	53	0	136
11. Pigs	0	1606	19	107	1732
12. Other Farm Animals Cattle	0	44	12	4	60
Goat	0	0	33	0	33
13. Other Animals peromyscus	0	105	0	47	152
Gerbils	0	6	0	0	6
Horse	0	0	11	0	11
Llama	0	0	4	0	4

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.))
I certify that the above is true, correct, and complete (7 U.S.C. Section 2143).

SIGNATURE OF C.E.O. OR I.O.

NAME AND TITLE OF C.E.O. OR I.O. *(Type or Print)*

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

DATE SIGNED 11/23/09

APHIS AUG 2009

EG 12-3-09

TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University School of Veterinary Medicine
North Grafton, Massachusetts
2008-2009 Annual Report of Research Facility
Registration Number: 14-R-0065

Explanation for Column E:

Animals are trapped in box traps and may remain within the traps for up to 12 hours. The trapped animals may be within traps overnight, but no longer than 12 hours due to our setting traps in the evening and checking them 2 hours after dusk and then in the morning 2 hours after dawn. Although nutritive bait is used, water is not provided because it would foul the live traps and wet animals would suffer hypothermia. Animals are evaluated immediately on discovery within the trap. Snap traps efficiently kill small mammals (mice, voles, shrews), but we can not exclude the possibility that an occasional capture will not be killed outright and will suffer overnight, caught by a tail or hind limb. In 20 years of the PI's experience, no more than 1 in 100 rodents have been caught and not immediately killed in a snap trap. Such animals are immediately euthanized when discovered. Shrews have a very high metabolic rate and require a readily accessible food source. Unfortunately, they cannot subsist on the food used as bait for live traps (peanut butter, oats, raisins) and may die before traps are checked, depending on when they are captured. The PI carries canned cat food and provides this within the trap when a live shrew is found. We cannot, however, routinely add cat food to our bait because of issues with fouling the traps as well as the likelihood of repelling rodents and attracting raccoons. Accordingly, there is some trap mortality with shrews that cannot be eliminated.
Species: Wild Mice Number: 47

Ticks naturally feed on rodents, and the infectious we study are maintained in nature by rodents. In this study, ticks (no more than 50 nymphs, or 200-500 larvae per animal) are brushed onto the anesthetized and/or restrained animal. In an endemic site, an average of 30-40 larvae may be counted on an animal. In most instances, a typical host-parasite relationship has been developed, and pathology occurs mainly as a function of non-natural dose or infection in a non-natural hose (or immune-compromised host). We keep the number of ticks infesting animals to what might be expected to occur in nature, and tick delivered infections run a natural course. Ticks detach between days 4-7. We anticipate that our experimental hosts do not disproportionately experience distress and pain due to tick-feeding or infection; thus drugs are not administered to relieve pain or distress unless there is clear evidence on an individual basis that there is significant distress (ticks might accumulate around eyes, nostrils, or within the ear canal proper) in which case animals are euthanized immediately. We cannot administer antibiotics or antipyretics because we seek to maintain the infections in as natural a manner as possible. All animals are checked daily. In the case of

virus and tularemia, all animals are monitored every 12 hours for the first 2 days and then every 4 hours for signs of illness such as neurologic signs, ruffled fur and lassitude for tularemia and euthanized upon detection of such signs. Animals that have been infested may be (1) euthanized after serving as tick hosts; (2) kept in standard cages to be reused every 4 weeks for tick feeding; or (3) if infected during the course of feeding, maintained for weeks or months to determine the course of the infection or serve as hosts to produce infected ticks.
Species: Hamster Number: 7 Species: Guinea pig Number: 2

Cattle are used to passage *Cryptosporidium* sp. within calves in an effort to propagate the infectious form of this parasite, namely the oocyst, for laboratory-based studies. Cell culture techniques do not produce significant numbers of organisms necessitating the *in vivo* model for propagation. Calves are inoculated orally with *Cryptosporidium* sp. oocysts. Calves usually begin to develop diarrhea and shed oocysts 3-5 days following inoculation. In addition, calves may develop anorexia, dehydration, and/or general weakness. The animals are monitored carefully a minimum of 2-6 times/day. Calves are maintained up to 6 weeks at which time they are euthanized. Calves that develop signs of extreme weakness, and fail to respond to oral and/or subcutaneous rehydration within a 48 hour period are euthanized. Although infection with *Cryptosporidium* sp. is responsive to the antibiotic, paromomycin, treatment of calves with this drug would eliminate the infection and subsequent oocyst shedding. Administration of anti-diarrheal drugs would also be expected to eliminate the diarrhea that occurs as a result of *Cryptosporidium* sp. infection. However, elimination of the diarrhea would similarly reduce fecal oocyst shedding. Administration of analgesics would be expected alleviate the gastrointestinal and abdominal discomfort associated with cryptosporidial diarrhea. However, the analgesic may also affect gastronintestinal motility and/or oocyst production and shedding.
Species: Cattle Number: 4

Gnotobiotic piglets are orally challenged with oocysts of *Cryptosporidium sp.* Following the inoculation, the piglets are monitored for the onset, quantity and duration of oocyst excretion in feces until oocysts are no longer excreted. Because cryptosporodiosis is a gastrointestinal illness, piglets are expected to develop diarrhea coincident with oocyst shedding. Animals may develop anorexia, diarrhea and/or general weakness. The piglets will be monitored 3 times/day for development of clinical signs. Animals that exhibit signs of severe dehydration, wasting, unresponsive to handling, or inability to ambulate properly will be euthanized immediately. Experiments are terminated 2-3 weeks following challenge.

The goals of these studies are to characterize immune response against *Cryptosporidium sp.*, determine the extent of cross-protection among them, use gnotobiotic piglet as a model for cryptosporidosis and produce *Cryptoporidium sp.* oocytes. Gnotobiotic piglet model is used to study these parameters because the clinical symptoms are similar or identical to those observed in humans. It is therefore important to perform these studies in an unaltered environment in order to clearly understand the immune response against *Cryptosporidium* sp. Therefore, treatment of the infection with antibiotics, anti-diarrheals and analgesics will not be attempted as they may adversely affect the natural picture of the immune response.
Species: Swine Number: 14

The gnotobiotic piglet used in this study will be orally infected with strains of *Shigella dysenteriae.* Such strains are expected to induce diarrhea. As a result, these piglets may also become weak and dehydrated. All of the potential adverse effects that may be seen are related to *Shigella dysenteriae* infection. Piglets will be monitored a minimum of four times/day. Piglets that exhibit signs of severe dehydration or wasting, are unresponsive to handling or exhibit neurological signs will be euthanized immediately. The only means by which to abrogate development of the expected adverse effects is to administer antibiotics to which these *Shigella dysenteria* strains are susceptible. Unfortunately, this will eliminate the infection. Given that the basis of this study to develop a gnotobiotic piglet model of shigellosis, relies on in vivo infection with these strains, administration of such antibiotics would obviate the purpose of the study.
Species: Swine Number: 62

The gnotobiotic piglets used in this study are orally infected with *Clostridium difficile.* Such strains are expected to induce diarrhea. As a result, these piglets may also become weak and dehydrated. Piglets will

be monitored a minimum of 4 times/day. Piglets that exhibit signs of severe dehydration or wasting, are unresponsive to handling or become moribund will be immediately euthanized. Piglets will be maintained for up to 10 days following derivation. All of the potential adverse effects that may be seen are related to *Clostridium difficile* infection. The only means by which to abrogate development of the expected adverse effects is to administer antibiotics to which *Clostridium difficile* is susceptible. Unfortunately, this will eliminate the infection. The basis of this study is to use gnotobiotic piglets to model the effects of *Clostridium difficle*-mediated colitis, to determine how Toxins A and B affect the disease development, to identify and characterize the role of other factors which may be involved in disease development and to determine whether there are specific substances involved in germination of *Clostridium difficile* spores. These goals rely on in vivo infection to evaluate the effect of *Clostridium difficle*-associated virulence factors and to assess the establishment of this model, reduction or elimination of these clinical signs by administration of such antibiotics would obviate the purpose of the study.
Species: Swine Number: 27

TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University School of Veterinary Medicine
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Death as an endpoint:

No studies to report for 2009.

TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University
Cummings School of Veterinary Medicine
North Grafton, Massachusetts
2009 Annual Report of Research Facility
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November 4, 2009

Summary of Exceptions to the regulations and standards:

#G767-06 Production of Cryptosporidium sp. Oocysts in Calves
#G2009-17 Production of Cryptosporidium sp. Oocysts in Calves

1) Exception from *The Guide* housing size recommendation. Initially calves will be housed in a large pen containing wood shavings. Within 24 hours of arrival at TCSVM, calves will be inoculated orally with Cryptosporidium sp. oocysts. Following inoculation, calves will be monitored daily for fecal shedding of oocysts. Once calves begin shedding oocysts (usually 3-5 days following inoculation), they will be transferred to a free-standing stanchion. These stanchions are 5'x 2' with a raised grate flooring to facilitate placement of pans for collection of feces containing the oocysts. Calves typically shed Cryptosporidium sp. oocyts for up to 2-6 weeks following inoculation. The adverse effect of the stanchion is that the calf is unable to ambulate freely, but generally, the stanchion is well-tolerated. Non-standard housing (indoors within a free-standing stanchion) is required to facilitate collection of oocysts present in feces and reduce risk of infection of personnel.
Species: Cattle Number: 4

#G755-05 Gnotobiotic Piglet Model of *Shigella dysenteriae* Infection
#G2009-08 Gnotobiotic Piglet Model of *Shigella dysenteriae* Infection
#G768-06 Gnotobiotic Piglet Model of Cryptosporidiosis
#G2009-16 Gnotobiotic Piglet Model of Cryptosporidiosis
#G861-06 Hamster and Gnotobiotic Piglet Models of *Clostridium difficile*

2) Exception from *The Guide* housing size recommendations. Piglets are housed for up to 1-8 weeks in gnotobiotic isolators in sectioned pens or individual cages to allow manipulation and monitoring of individual piglets during the study. Piglets are able to move freely and assume normal postures within the limited space. Non-standard housing (in pens/cages within isolators) is required to maintain gnotobiotic status, facilitate collection of oocysts present in feces, and reduce the risk of infection of personnel.
Species: Swine Number: 107

#G789-06 Ecology of Tick-Maintained Zoonoses

3) Exception to standard housing. Live traps are not standard housing. Animals are held for periods of 1-12 hours, depending on the time of capture. Because the traps are baited only with oats (nonaromatic bait is required for this study) no source of water is available between the time of capture and when the trap is checked in the morning. Animals are examined and released as soon as possible after capture.
Species: Peromyscus Number:47

#G895-07 Laboratory Maintenance of the Life Cycles of Ticks and the Pathogens They Transmit

4) Exception to standard housing. The exceptions approved are (1) cage limits (2) cage changing schedule (3) separation of litters from the general cage population. One male and several female white footed mice (4-6 mice total) are housed in a shoe box cage or 12-18 mice of mixed sex allows for sufficient breeding with intrinsic regulation of the numbers of offspring produced. In this manner, only enough mice are produced for adequate turnover of the colony, but do not experience breeding that needs frequent culling. In addition, cleaning the cages less frequently enhances breeding, probably because of pheromone marking of the cage. Finally, although gravid females could be separated, we have found that such litters survive less frequently than do those allowed to remain with the other mice.
Species: Peromyscus Number: 105