This report is required by law (7 USC 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 21! NOV 2 8 2008 See attached form for additional information. Interagency Report Control no.

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. CERTIFICATE NUMBER: 14-R-0065
CUSTOMER NUMBER: 628

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

Tufts University
School Of Veterinary Medicine
200 Westboro Road Bldg. 17
North Grafton, MA 01536

Telephone: (508) -839-7992

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

FACILITY LOCATIONS (Sites) - See Atached 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 animal being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not ye used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use o 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 an 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 wh the use of appropriate anesthetic, analgesic, or tranquiliz drugs would have adversely affected the procedures, res or interpretation of the teaching, research, experiments, surgery, or tests. (An explanation of the procedures producing pain or distress in these animals and the reasc such drugs were not used must be attached to this report	F. TOTAL NUMBER OF ANIMALS (COLUMNS C + D + E)
4. Dogs	0	0	23	0	23
5. Cats	0	0	0	0	0
6. Guinea Pigs	0	4	0	0	4
7. Hamsters	0	2	12	0	14
8. Rabbits	0	2	295	8	305
9. Non-human Primates	0	0	0	0	0
10. Sheep	0	78	87	0	165
11. Pigs	0	1287	24	172	1483
12. Other Farm Animals Llamas	0	0	4	0	4
Minipigs	0	3	0	0	3
13. Other Animals Goats	0	0	36	0	36
Wild Mice	0	0	0	93	93
Horse	0	0	26	0	26
Cattle	0	73	13	4	90

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesteic, analgesic, and tranquilizing drugs, prior to, during, and following actual rese 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 ap 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 inc 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 provision 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 or Legally Responsible Institutional Official)

L OFFICIAL (Type or Print) | DATE SIGNED 11/25/08

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(AUG 91)

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TUFTS UNIVERSITY
School of Veterinary Medicine

Division of Teaching and Research Resources

Tufts University
Cummings School of Veterinary Medicine
North Grafton, Massachusetts
2008 Annual Report of Research Facility
Registration Number: 14-R-0065
REVISED: February 19, 2009

Summary of Exceptions to the regulations and standards:

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

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 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
#G768-06 Gnotobiotic Piglet Model of Cryptosporidiosis
#G861-06 Hamster and Gnotobiotic Piglet Models of *Clostridium difficile*
#G875-07 Gnotobiotic Piglet Model of *Norovirus* Infection
#G817-06 Studies on *Cryptosporidium* Genotypes in Piglet Model of *Cryptosporidiosis*
#G681-05 Effects of Antibiotics on Stx Production

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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: 172

#G952-08 Ali/Ali Rabbit Model of E. coli, Norovirus and Shigella Infection

Exception to standard housing. The rabbits will be housed in microbiological isolators. Rabbits 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 protect the surrounding environment and reduce the risk of infection of personnel.
Species: Rabbit Number: 2

#G789-06 Ecology of Tick-Maintained Zoonoses

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:48

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

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: 45

#G857-06 Evaluation of a SC Glucose Monitoring Sensor

Exception to standard restraint. The Lomir jacket will be used to cover the devices attached to the pig. The jacket should not restrict movement in any way but will protect the device from damage and maintain cleanliness. The Panepinto sling will be used to restrain the pig for attachment of devices, blood sampling, and examination of the

devices, if necessary. The pig will be acclimated to the jacket and sling before the study begins. Typically, swine adapt well to sling restraint and exhibit no adverse effects. Positive reinforcement (food treat) is used following sling restraint and wearing the jacket.
Species: Swine Number: 1

#G905-07 InVitro Testing Donor Pig Protocol

Exception to standard restraint. The Panepinto sling will be used to restrain the pigs. Pigs will be acclimated to the Panepinto sling. Pigs will be placed in or trained to walk into the sling for 5-30 minute periods 1-2 times/day with food treat positive reinforcement.
Species: Swine Number: 1

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Explanation for Column E:

Rabbits will be used to study the exact time course for infection of E. coli, Norovirus and Shigella Infection. Rabbits may develop clinical signs of infection including lethargy, diarrhea, inability to move and dehydration. The animals are closely monitored 3-6 times per day as clinical signs are exhibited. Rabbits which become moribund as evidence by lack of responsiveness to handling, severe dehydration, or severe depression will be euthanized immediately. Given that the basis of this study relies on in vivo infection to determine the exact time course for the infection, administration of anti-viral agents, antibiotics or analgesics would obviate the purpose of the study.
Species: Rabbit Number: 8

Wild mice are used to study the ecology of tick-maintained zoonoses. It is possible that animals may not die immediately when snap trapped or may be captured by a limb or tail. Accordingly, such animal may suffer during the 8 overnight hours before traps are checked. Drugs could not be administered during this time. Any animal that is not dead when snap traps are checked will be immediately killed by halothane exposure.
Species: Wild Mice Number: 58

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 restrained and anesthetized animal. In an endemic site, an average of 30-40 larvae may be counted on a mouse. 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: Wild Mice Number 35

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

Enterohemorrhagic E. coli (EHEC) are pathogens that cause bloody diarrhea and in susceptible individuals, hemolytic-uremic syndrome (HUS). Stx are toxins produced by EHEC. Swine are used in this study to determine if antibiotic administration affects Stx release/production and if so, how. Within 24 hours following derivation, gnotobiotic piglets are orally infected with Stx. Within 24 hours following infection, piglets will be given antibiotics or a placebo for up to 10 days post-infection. The animals are maintained for up to 2 weeks following derivation. Piglets are monitored three to six times daily throughout the duration of the experiment for diarrhea, neurological signs and death due to Stx activity. Piglets will be euthanized at the end of the study or if they develop severe dehydration, wasting, or are unresponsive. In addition, if a piglet exhibits one or more neurological sign, it will be monitored every 15 minutes for up to two hours at which time, if it is still exhibiting one or more neurological sign, it will be euthanized.

EHEC strains are expected to induce diarrhea. As a result these piglets may also become weak and dehydrated and develop CNS signs which may include seizures, ataxia, and/or paralysis. All of the potential adverse effects that may be seen are related to the EHEC infection. Administration of antibiotics and/or analgesics and/or anti-diarrheal drugs would be expected to eliminate the EHEC infection, adverse effects and clinical signs. Given that the goals of this study is to evaluate whether antibiotic administration enhances Stx release/production by EHEC, elimination of these clinical signs and/or infection would likely affect the ability to evaluate the effect of antibiotic administration.
Species: Swine Number: 9

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 3-6 times/day for development of clinical signs. Animals that exhibit signs of severe dehydration, weakness, wasting, white diarrhea for more than 2 days 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 included by *C. parvum* in pigs 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 *C. parvum* 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: 74

Piglets will be involved in Stx administration to determine the in vivo localization o f Stx1 and Stx2. Within one hour following the toxin administration, piglets will be euthanized and organs collected for evaluation. A second group of animals will receive Hu-MAb or a control in an effort to determine the in vivo localization of Stx-Hu-MAb immune complexes. Four hours following Hu-MAb administration or control, the piglets will be given Stx1 or Stx2. Blood samples will be obtained for evaluation during the 15 minutes following the administration of Stx. Then the animals will be humanely euthanized and organs collected for evaluation. The piglets will be monitored continuously following Stx administration. If any animals become unresponsive to handling or exhibit neurological signs, they will be euthanized immediately.

The goals of this protocol include the use of gnotobiotic piglets to determine the fate of Stx-Hu-MAb immune complexes. Based on the studies, it is known that Stx-induced toxicosis is responsive to administration of specific antiserum or antibodies against Stx, if administered early enough following toxin exposures. Piglets in the experimental groups will be receiving antibodies which have indeed shown protection. However, animals in the control groups will not be receiving such antibodies. Administration of such antibodies to the control groups would negate their function as controls for Hu-MAb administration.
Species: Swine Number: 6

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: 51

The gnotobiotic piglets used in this study will be orally challenged with Norovirus particles. Following inoculation, piglets will be monitored daily for development of diarrhea and fecal shedding of Norovirus. Piglets will be maintained for up to 3 weeks following derivation or euthanized once fecal shedding tapers off. Piglets will be monitored 3-6 times/day for development of clinical signs. Piglets which exhibit signs of severe dehydration, weakness, wasting, or inability to ambulate properly will be euthanized immediately.

The goal of this study is to develop a non-primate animal model for human noroviruses, a major cause of gastroenteritis (diarrhea, vomiting) in humans. Because Norovirus is a gastrointestinal illness, piglets are expected to develop diarrhea coincident with Norovirus shedding. In addition, piglets may develop anorexia, diarrhea, and/or general weakness. The only means by which to abrogate development of the expected adverse effects is to administer anti-viral agents to which Norovirus is susceptible. Unfortunately, this will eliminate the infection. Given that the basis of this study to develop a non-primate animal model for human noroviruses relies on in vivo infection with Norovirus, administration of such agents would obviate the purpose of this study.
Species: Swine Number: 7

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: 25