

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 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. 51-F-0001
CUSTOMER NO. 432

FORM APPROVED
OMB NO. 0579-0036

ANNUAL REPORT OF RESEARCH FACILITY
(TYPE OR PRINT)

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

UNIFORMED SERVICES
UNIV. OF THE HEALTH SCIENCES
4301 JONES BRIDGE RD.
BETHESDA, MD 20814

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)

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

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 in these animals and the reasons such drugs were not used must be attached to this report)	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
4. Dogs					
5. Cats					
6. Guinea Pigs				6	6
7. Hamsters					
8. Rabbits		15	12		27
9. Non-Human Primates					
10. Sheep					
11. Pigs			338		338
12. Other Farm Animals					
Goat			34		34
13. Other Animals					
Ferret			82	44	126
Sand Rat		9	87		96
Cotton Rat		42			42

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 the 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 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)

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

SIGNATURE OF C.E.O. OR INSTITUTIONAL OFFICIAL

NAME & TITLE OF C.E.O. OR INSTITUTIONAL OFFICIAL (Type or Print)

DATE SIGNED

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

12/06/2005

Raw

PART 1 - HEADQUARTERS

(AUG 91)

APHIS Form 7023 Column E Explanation

This form is intended as an aid to completing the APHIS Form 7023 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: 51-F-0001

2/3. Species (common name) & Number of animals used in this study:

Guinea Pigs (6)

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

The Sereny test is the standard small animal model for assaying the virulence (invasiveness) and cell to cell spread of *Shigella* spp. Shigellosis is caused by invasion and multiplication of *Shigella* within intestinal epithelial cells resulting in inflammation and destruction of these cells. The eye of a guinea pig represents an epithelial cell surface that undergoes the same infectious process observed in the human intestine. The technique involves inoculating a drop of a concentrated bacterial suspension onto the eye of a guinea pig. Alternatively, a sterile cotton swab may be used to smear a concentrated paste of bacterial growth from a petri dish onto the surface of the eye and under the upper eyelid. The uninoculated eye of the animal serves as the negative control and therefore fewer animals are needed. Bacteria that are invasive and capable of intercellular spread provoke a keratoconjunctivitis within 48-96 hours and the virulent bacteria can subsequently be recovered from the conjunctival exudate. The infection is self-limiting and the conjunctivitis gradually clears up and the eye regains its normal appearance within 2 weeks. Since this assay is used to evaluate virulence, it will be used throughout the project period.

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)

1. Use of analgesics is not a currently accepted modification of the Sereny test. Researchers in the field of *Shigella* pathogenesis continue to use the Sereny test without the use of pain alleviating measures.

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):

Agency:

CFR:

1. Registration Number: 51-F-0001 / 432

2/3. Species (common name) & Number of animals used in this study:

Guinea Pigs (6)

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

The Sereny test is the standard small animal model for assaying the virulence (invasiveness) and cell to cell spread of *Shigella* spp. Shigellosis is caused by invasion and multiplication of *Shigella* within intestinal epithelial cells resulting in inflammation and destruction of these cells. The eye of a guinea pig represents an epithelial cell surface that undergoes the same infectious process observed in the human intestine. The technique involves inoculating a drop of a concentrated bacterial suspension onto the eye of a guinea pig. Alternatively, a sterile cotton swab may be used to smear a concentrated paste of bacterial growth from a petri dish onto the surface of the eye and under the upper eyelid. The uninoculated eye of the animal serves as the negative control and therefore fewer animals are needed. Bacteria that are invasive and capable of intercellular spread provoke a keratoconjunctivitis within 48-96 hours and the virulent bacteria can subsequently be recovered from the conjunctival exudate. The infection is self-limiting and the conjunctivitis gradually clears up and the eye regains its normal appearance within 2 weeks. Since this assay is used to evaluate virulence, it will be used throughout the project period.

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)

1. Use of analgesics is not a currently accepted modification of the Sereny test. Researchers in the field of *Shigella* pathogenesis continue to use the Sereny test without the use of pain alleviating measures. a. The PI solicited expert scientific opinion on the use of analgesics in the Sereny test from nine investigators who use, or have used, the assay. Responses were received from seven of these investigators. Each of these investigators stated that they had never used analgesics in the Sereny test. b. Since pain alleviation agents are not a part of the generally accepted procedure for the Sereny test, there is concern that if the current protocol was modified to include pain alleviating agents, the validity of any tests using such agents may be called into question by study section reviewers and journal reviewers. 2. A report by Swearngen et al. in 1993 (1) suggested that analgesics can be used in the Sereny test without compromising the results. a. A literature search (PubMed) was performed to examine whether other investigators have incorporated the use of analgesics into the Sereny test since the publication of the Swearngen et al. paper (i.e. since 1993). The search results showed that general acceptance of the findings of the Swearngen et al. study is lacking and that investigators continue to use the Sereny test without employing drugs to alleviate pain. In no case was an analgesic used in the Sereny test to alleviate pain in any of these studies. Some of the most recent publications that employ the Sereny test are listed in the bibliography (ref. 2-5) for your reference. b. The Swearngen et al. study (1) addressed the use of buprenorphine in a particular experimental protocol and its effect on the outcome of the particular protocol. Its conclusions cannot be broadly applied to all protocols that use the Sereny test. Specifically, the study design used by Swearngen et al. tested only one wild type strain of *Shigella flexneri* and did not include mutant strains of *Shigella*. There are no data that demonstrate the effect of analgesics on the Sereny test when other wild type strains or mutant strains of *Shigella* are employed. It remains possible that the outcome of the Sereny test for a mutant strain would differ when buprenorphine is used. This point is of particular importance to the proposed protocol since the Sereny test will be used to test the virulence of mutant strains of *Shigella* as well as to examine the potential protective effects of chemical compounds. 3. Use of analgesic agents actually has an adverse effect on the health of the animals as indicated by lethargy, suppression of the self-grooming behavior and reduced weight gain. a. Guinea pigs treated with buprenorphine in the Sereny test showed reduced grooming behavior and excessive purulent buildup around the eye. This effect was somewhat reduced at the lower doses of drug (1). However, the low dose group showed as much exudate buildup around the eyes as the untreated controls. b. A recent study by Hanson et al. confirmed these observations and pointed out that buprenorphine-treated animals showed a significant increase in mucopurulent ocular discharge as compared to the saline control group. Moreover, the treated animals showed a significant reduction in body weight (6). c. In both studies the excessive exudates buildup in the buprenorphine-treated animals made it more difficult for investigators to score the degree of inflammation in the Sereny test. In light of these reports from the scientific literature, it is far from clear that use of buprenorphine as a pain alleviating measure in the Sereny test is totally without adverse consequences on the experimental outcome. Thus, a requirement to include buprenorphine as a pain alleviating agent in this protocol would not appear to be warranted.

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):

Agency:

CFR:

Approval Status:

Approved/Disapproved By:

Date:

Disapproved Reason:

APHIS Form 7023 Column E Explanation

This form is intended as an aid to completing the APHIS Form 7023 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: 51-F-0001

2/3. Species (common name) & Number of animals used in this study:

Ferret (44)

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

Two types of therapeutic agents will be tested in the ferret model of Shiga toxin (Stx)-producing Escherichia coli (STEC) associated systemic disease. The first type of compound will target the inflammatory prodrome period of 3+ days between bloody diarrhea and the onset of acute renal disease. We believe this period of inflammation is essential for the development of Hemolytic uremic syndrome (HUS) and provides an opportunity for clinical therapeutic intervention. Secondly, humanized monoclonal antibodies are to be tested for neutralization of systemic Stx2. Thirdly, due to their different modes of action, combinations of these agents will be examined in the animal model.

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)

Our justification for the use of the ferret model to study STEC disease is that tissue culture systems cannot be used to evaluate potential therapeutics to treat STEC-mediated disease. Lastly, the str-treated ferret model is one of two small animal models available for studying the systemic delivery of Stx from the intestinal tract and for identifying methods to prevent or neutralize the effect of that toxin and/or STEC-mediated disease. The str-treated mouse is the other model, and that model, unfortunately does not exhibit the glomerular damage that is seen in humans. No analgesics will be given to STEC-infected animals for the following two reasons. First, inflammation and/or the inflammatory response are key components of both STEC-mediated hemorrhagic colitis (inflammation of the colon and neutrophilic infiltrate) and STEC-mediated HUS (pro-inflammatory cytokines are believed to play a role in HUS potentially by up-regulating toxin receptor expression or by exacerbating Stx-mediated damage). Because we will be using these animal models to study both the pathogenesis of disease and possible treatment therapies, we believe that the use of non-steroidal anti-inflammatory drugs (NSAIDs) could confound or possibly mask the extent of STEC-mediated damage that we will be evaluating by histological examination or toxicity. Secondly, the use of opioids as analgesics have the potential to exacerbate symptoms rather than eliminate pain since peristalsis would be reduced. Opium compounds have been used in several animal models for gastroenteritis to increase the likelihood of establishing and/or maintaining bacterial infection. Additionally, anti-motility agents are not recommended for patients with suspected STEC-mediated diarrhea because this treatment may increase the risk of progression to HUS.

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):

Agency:

CFR: