Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY18)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: D
Species (common name): Ferret
Number: 2
Explanation of procedure producing pain and/or distress:
Ferrets are naturally susceptible to influenza viruses, including many non-seasonal subtypes of animal-origin. Because influenza virus infection in the ferret model is known to mirror that of a human infection, high titers of strain-specific antibody in serum is generally produced. The ferret blood volume allows for relatively large volumes of serum to be collected per animal reducing the overall number of animals required to meet research needs. Although not all strains of highly pathogenic avian influenza A (H5N1) virus will cause illness in ferrets, some of the influenza viruses studied under this protocol will be virulent for ferrets and may cause more severe morbidity and potentially mortality.

Justification why pain and/or distress could not be relieved:
Every attempt will be made to euthanize the animal prior to it reaching severe illness. Steps will be taken to reduce pain by using infectious doses that are not predicted to cause significant weight loss, clinical illness and death. Any animal that loses greater than 20% body weight loss and/or accrues a total score of 10 on the clinical scale will be humanely euthanized. Twice daily animal visits by investigators and/or animal care staff will help to monitor animals for disease symptoms. The purpose of these studies is to generate antibody in serum of ferrets infected with influenza virus. We are concerned that ferrets may experience pain or distress as a result of these experiments. However, it could be counterproductive to treat them with analgesics, which may alter the immune response to infection causing results that are not reproducible. Specifically, the use of non-steroidal anti-inflammatory drugs (COX inhibitors) has been shown to alter the immune response during viral infection, notably during influenza A virus infection. Although studies on the use of opiates during influenza infection have not been performed in ferrets, it is believed that in the ferret model (as with most mammals) opiates are immunosuppressive, suggesting that their use will affect the outcome of infection with influenza virus and negatively impact the production of antibody. This would lead to unnecessary repetition of infections and an increase in the number of animals required.

Protocol: E
Species (common name): Ferret
Number: 3
Explanation of procedure producing pain and/or distress:
This study involves the use of animals (ferrets) to generate strain-specific antisera to influenza viruses for use in evaluation of current vaccines as well as developing new vaccines and diagnostics for seasonal influenza viruses as well as viruses with pandemic potential. In vitro systems cannot generate polyclonal and strain-specific antibodies to influenza viruses from clinical isolates. The production of monoclonal antibodies via the development of hybridomas will not guarantee that the scientific objectives to be met, therefore, an animal is the first choice for raising strain-specific polyclonal antisera. On rare occasions, a clinical isolate my cause more severe morbidity and potentially mortality.

Justification why pain and/or distress could not be relieved:
Every attempt will be made to euthanize the animal prior to it reaching severe illness. Analgesia may interfere with virus replication cycle and affect the immune responses generated by the ferret which are critical for production of antisera for identification of influenza virus variants. Analgesics have been shown to blunt antibody responses to vaccines in mice and humans; therefore, use of analgesia is not recommended for this study. Animals will be humanely euthanized if weight loss exceeds 25% of pre-infection weight or animals are moribund, show signs of neurological effects (torticollis/ataxia), or dyspnea, and/or accrue a total score of 10 on the clinical scale.

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Protocol: A
Species (common name): Guinea Pig
Number: 4
Explanation of procedure producing pain and/or distress:

This study investigates the use of particles that resemble Lassa virus, as a vaccine candidate in the guinea pig model of Lassa fever. The guinea pig is a well-described model of disease and has been used in previous therapeutic screening studies of arenavirus infection. A subset of animals inoculated with Lassa virus are expected to develop severe clinical disease.

Justification why pain and/or distress could not be relieved:

Because the functions of these studies are to characterize disease course and compare clinical parameters in treated and untreated animals, animals must be followed even after first clinical signs of disease are observed. However, every effort will be made to euthanize ill animals at a humane endpoint based on rigid euthanasia criteria. Analgesics cannot be used in these studies as they have been shown to interfere with immunological responses and can exacerbate liver damage. It is also known that inflammation and inflammatory mediators play an important role in arenavirus disease. Based on these factors, analgesics should not be used because they could affect clinical outcome of the disease and alter the intended study investigation.

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Protocol: B
Species (common name): Hamster
Number: 13
Explanation of procedure producing pain and/or distress:

The aim of this study is to develop a thermostable rabies vaccine and evaluate its stability, potency and immunogenicity in the Syrian hamster model. A thermostable vaccine will resolve major cold chain storage challenges in developing countries and allow for more cost-efficient vaccination. The Syrian hamster is a well-established animal model frequently used in the study of rabies pre- and post-exposure prophylaxis. It is well standardized and widely accepted by the experts in the field as the best model to study novel rabies virus vaccines and other biologics. The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before they can be humanely euthanized.

Justification why pain and/or distress could not be relieved:

Numerous drugs have been shown to have anti-inflammatory affects in experimental models such as salicylates, NSAIDs, and glucocorticoids. Metacam is commonly prescribed by veterinarians and falls into the NSAID category. Because this study is aimed at better understanding the vaccine immunogenicity and efficacy under the close to natural condition of viral infection, it is important that the life cycle of the challenge viruses is not being altered by the use of NSAID's or other anti-inflammatory agents. All animals infected with rabies virus will be euthanized at the onset of clinical signs of rabies according to the listed euthanasia criteria. Based on euthanasia score, any more than transient or momentary pain will be alleviated by euthanasia. However, occasionally animals may progress rapidly from an apparently healthy status to a terminal state. This can occur occasionally overnight or between routine check periods. The majority of animals will be euthanized at first onset and a significant number of animals are not expected to rapidly progress as described above. Based on prior experience, 3-10% of animals that develop signs of rabies are expected to progress to death before euthanasia can be administered. These animals will subsequently be categorized as pain category E. In addition, if animals have general signs of disease (e.g. ruffled fur, hunching, etc.) but do not have specific signs of rabies (such that they do not meet the euthanasia criteria) but are later found moribund or paralyzed, then these animals will also be reported in category E at the time of annual review.
Using the clinical signs of rabies as the experimental endpoint instead of death prevents four to five days of suffering for animals. Approximately 7 days following inoculation of rabies virus, control animals may start to show signs of rabies. After day 7 post infection (day 7 through day 21 post-infection), all experimentally infected animals will be checked twice daily by investigators in addition to routine husbandry checks performed by animal care staff.

Protocol: C
Species (common name): Hamster
Number: 19
Explanation of procedure producing pain and/or distress:

Currently no antiviral compounds have been shown to work specifically against rabies. Scientific evidence is needed to support antiviral treatment of people diagnosed with rabies. Hamsters are commonly used as model systems to study the effects of antiviral compounds on the progression of rabies virus infection. Syrian hamsters are well established in the laboratory and in the literature for rabies pathogenesis studies and the evaluation of new biologics. The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before they can be humanely euthanized.

Justification why pain and/or distress could not be relieved:

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Analgesia cannot be used because animals may progress rapidly from an apparently healthy status to a terminal state. This can occur occasionally overnight or between routine check periods. In addition, use of analgesia could mask clinical signs. Analgesia can be used to alleviate any pain/discomfort not related to rabies (e.g. injury due to fighting with cage mates). Anesthesia will be used as needed to minimize distress/discomfort due to antiviral treatment. The majority of animals will be euthanized at first onset of rabies virus infection and a significant number of animals are not expected to rapidly progress as described above. Based on prior experience, 3-10% of animals that develop signs of rabies are expected to progress to death before euthanasia can be administered. These animals will subsequently be categorized as pain category E. In addition, if animals have general signs of disease (e.g. ruffled fur, hunching, etc.) but do not have specific signs of rabies (such that they do not meet the euthanasia criteria) but are later euthanized, then these animals will also be reported in category E at the time of annual review. Using the clinical signs of rabies as the experimental endpoint instead of death prevents four to five days of suffering in animals. Approximately 7 days following inoculation of rabies virus, control animals may start to show signs of rabies. Starting day 7 post-infection through day 21 post-infection, all experimentally infected animals will be checked twice daily by investigators in addition to routine husbandry checks performed by animal care staff.

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Protocol: F
Species (common name): Mouse, white-footed (lab bred)
Number: 20
Explanation of procedure producing pain and/or distress:
Pain Class E is necessary during the 4-5 day duration of nymphal tick feeding. Mice must be housed on wire grates over approximately ½" of water to collect ticks as they detach from the mice. After the ticks have attached (~3 hours) mice will be provided with a 9 cm petri dish lid as a platform on which to rest during the tick feed. During the last 24 hours of the tick feed, when the replete ticks will be dropping off the mice, the platform will be removed and the mice will not have a resting place.

Justification why pain and/or distress could not be relieved:
Wire grate housing is necessary for the safety of individuals handling the mice during tick feeding and to prevent mice from ingesting the fed ticks. The removal of the resting platform during the last 24 hours of the tick feed is necessary to prevent the platform from collecting the replete ticks and thus allowing for ingestion of the ticks by the mice.