Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY20)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: A
Species (common name): Guinea Pig
Number: 6
Explanation of procedure producing pain and/or distress:
Guinea pigs are known to be susceptible or immunogenic to many human pathogens; for that reason, this species has been used for identification, isolation, and even differentiation of rickettsiae. Our studies have demonstrated that Guinea pigs have been found susceptible to *Rickettsia rickettsii*, *R. parkeri*, *R. conorii*, and *R. slovaca* with noticeable variations between pathogens in severity and dynamics of clinical signs as well as in necropsy results. Infestation of guinea pigs with infected ticks of these species reproducibly resulted in typical clinical signs of infection. The more virulent isolates of *R. rickettsii* can cause rapidly progressive illness in some of the guinea pigs. There may occasionally be animals that progress to a terminal state before they can be humanely euthanized.

Justification why pain and/or distress could not be relieved:
Lidocaine or prilocaine topical analgesic cream was used to alleviate discomfort associated with ear biopsies. Analgesia could not be used for alleviation of pain or distress due to rickettsial infection because use of analgesia could interfere with immune responses to rickettsial infection and mask clinical signs necessary to study pathogenesis. In addition, some animals progressed rapidly from an apparently healthy status to a terminal state overnight or between routine check periods. . All infected animals were monitored at least twice per day and any animal whose condition was likely to worsen to the point of suffering prior to the next health check was euthanized according to humane endpoints. All investigators and staff were trained to evaluate and assess animals according to the humane endpoints scale, and any animal showing >25% weight loss or a total score of 10 on the scale was humanely euthanized. However, a small percentage of infected animals progressed to a terminal state very abruptly, within 2 to 4 hours. Any animals that died prior to reaching humane euthanasia endpoint criteria were reported in category E.

Protocol: B
Species (common name): Guinea Pig
Number: 1
Explanation of procedure producing pain and/or distress:

This study will investigate the use of particles that resemble (b) (7)(F) as a vaccine candidate in the guinea pig model of (b) (7)(F). The guinea pig is a well-described model of disease and has been used in previous therapeutic screening studies of (b) (7)(F) infection. To determine the efficacy of the virus vaccine candidate, animals will be inoculated with (b) (7)(F) Some animals may develop clinical signs of disease. All animals will be monitored daily, and a euthanasia and body condition score algorithm that considers the total health parameters of each individual animal will be utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Occasionally, disease may progress rapidly, and any animals that succumb prior to reaching humane euthanasia endpoint criteria will be reported in category E.

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 are observed. However, every effort was made to euthanize ill animals at a humane endpoint based on rigid euthanasia criteria. Analgesics could not 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 (b) (7)(F) disease. Based on these factors, analgesics could not be used because they could affect clinical outcome of the disease and alter the intended study investigation.

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Protocol: C
Species (common name): Hamster
Number: 28
Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat (b) (7)(F) in humans. The aim of these studies is to investigate the use of (b) (7)(F) therapeutics for (b) (7)(F). Hamsters are an established disease model of (b) (7)(F) and show similar pathogenesis and disease as humans. (b) (7)(F) infection in hamsters can produce a respiratory disease phenotype that can rapidly progress from absent or mild clinical signs to severe disease and death with very little time for intervention in certain individuals.

Justification why pain and/or distress could not be relieved:
Analgesics have been proven to interfere with immunologic responses and can exacerbate liver damage. It is also known that inflammation and inflammatory mediators may play a major role in the pathogenesis of (b) (7)(F) infection diseases. Based on these factors, analgesics could not be used since they could affect the clinical outcome of the disease and interfere with assessment of therapeutics, and thus alter the theoretical basis of the experiment. All efforts were made to ensure that the animals experienced the least amount of pain and distress necessary to accomplish the goals of the experiment. All animals were monitored daily by an experienced animal care technician or the PI for signs of clinical illness; a pain/euthanasia scale that takes into account the total health parameters of each individual animal was utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points were monitored two times per day. Animals scored at 10 total points or above were humanely euthanized, and euthanasia was performed prior to endpoint criteria being reached whenever possible. In this case, despite all attempts made to prevent it, a subset of animals was not able to be euthanized prior to succumbing to infection.

Protocol: D
Species (common name): Hamster
Number: 2
Explanation of procedure producing pain and/or distress:
In recent years, no major paradigm shifts have occurred in the utilization of new products for the prevention and control of rabies. The development of a more thermostable and cost-effective rabies treatment than is currently available is critical in continuing to prevent and reduce disease. Hamsters are commonly used as model systems to study the effects of antiviral compounds on the progression of rabies virus infection. Those antiviral compounds that inhibit rabies virus *in vitro* need to be tested in an animal model to assess their efficacy *in vivo*. 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:
Analgesia was used during the study to treat any non-rabies related condition. A small number of animals infected with rabies virus progressed rapidly from an apparently healthy status to a terminal state. This occurred overnight or between routine check periods. We did not expect a significant number of animals to rapidly progress as described above, and the majority of animals were euthanized at first onset of clinical signs. We have developed a rabies-specific pain scale to help us euthanize these animals before they succumb to disease. All animals infected with rabies virus that met the listed euthanasia criteria were euthanized at the onset of clinical signs of rabies. Using the clinical signs of rabies as the experimental endpoint instead of death can prevent four to five days of suffering in mice. Any animals found dead during the study were categorized as pain category E.

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Protocol: E
Species (common name): Hamster
Number: 3
Explanation of procedure producing pain and/or distress:
In the United States, human rabies cases are attributed to bat rabies strain variants at least 70% of the time. To accurately study this disease and potential treatments, the morbidity and mortality of canine variant rabies strains (from foxes and coyotes) and bat variant rabies strains need to be validated in a live animal model. The hamster has been used and is considered the gold standard model for studying the pathogenesis of rabies since the 1950's due to its predictability and susceptibility of disease. The development of clinical rabies infection is expected to occur in the majority of the infected animals. Despite stringent monitoring and the use of humane euthanasia endpoint criteria, 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:
Analgesia was used during the study to treat any non-rabies related condition. A small number of animals infected with rabies virus progressed rapidly from an apparently healthy status to a terminal state. This occurred overnight or between routine check periods. We did not expect a significant number of animals to rapidly progress as described above, and the majority of animals were euthanized at first onset of clinical signs. We have developed a rabies-specific pain scale to help us euthanize these animals before they succumb to disease. All animals infected with rabies virus that met the listed euthanasia criteria were euthanized at the onset of clinical signs of rabies. Using the clinical signs of rabies as the experimental endpoint instead of death can prevent four to five days of suffering in mice. Any animals found dead during the study were categorized as pain category E.