Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: L
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.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: D
Species (common name): Fruit Bat
Number: 11
Explanation of procedure producing pain and/or distress:
R. aegyptiacus are the only known natural reservoir for Marburg virus. This finding has led to the use of this animal as the only appropriate natural reservoir animal model for the study of Marburg virus. The objective of this study is to investigate immune responses and pathogenesis in the context of Marburg virus infection using chemically immunosuppressed bats. In non-immune compromised bats, Marburg virus causes little to no disease. However, due to the immune suppressive treatment of these animals, it is unknown what disease may be caused, but immunosuppressed bats infected by virus could potentially develop symptomatic, even severe illness that would require euthanasia.

Justification why pain and/or distress could not be relieved:
Because the suppression of specific arms of the bat immune system have never been done before, it is difficult to know the subsequent health outcome. As the objective is to study immune responses and pathogenesis of the virus, analgesia cannot be used, as it may alter the immune response to viral infection and could mask clinical signs necessary to study the pathogenesis of the disease. All efforts will be made to ensure that the animals experience the least amount of pain and distress as is absolutely necessary to accomplish the goals of the experiment. All investigators are properly trained to observe the monitoring protocol and algorithm concerning the health status and euthanasia criteria of each individual animal. A euthanasia and body condition score algorithm, adapted from a previously CDC IACUC approved protocol for bats, will be utilized to determine appropriate endpoints to prevent unnecessary pain and suffering. All animals will be monitored daily by an experienced animal care technician, the PI or the Co-PI for signs of clinical illness. If the animal has attained a total score of 6 points or greater the animal will be observed twice daily to ensure that any animal suffering or discomfort is limited to as short a time period as practically possible. If any animal reaches the designated total score of eight (8) or more points or a loss of body weight of >20%, the animal will be humanely euthanized.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: A
Species (common name): Big Brown Bat
Number: 3
Explanation of procedure producing pain and/or distress:
Multiple variants of the Rabies virus persist within the bat populations of the western hemisphere, and these variants have been responsible for human rabies cases. Although there are oral rabies vaccines for use in various terrestrial carnivores, no commercially available rabies vaccine exists for bats. This species is a highly synanthropic rabies reservoir in the United States. Big brown bats are the most frequently submitted bat for rabies testing, suggesting high likelihood for human exposure. Furthermore, this species is common, locally abundant, and can survive well in captive conditions. The objective of the study is to evaluate the interactions between immunity and rabies infection within reservoir hosts. Painful/distressful procedures will include the inoculation of animals with Rabies virus. Every attempt will be made to euthanize all animals infected with rabies virus at first onset of clinical signs of rabies.

Justification why pain and/or distress could not be relieved:
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. Every attempt will be made to euthanize all animals infected with rabies virus at first onset of clinical signs of rabies. Beginning 5 days post infection, animals will be examined and scored for a pain index at least daily during the week and at least once over the weekend by rabies staff and animal care staff during routine husbandry so that euthanasia can be promptly administered. A significant number of animals are not expected to progress to Category E. Any animals that expired before euthanasia were added to Category E at the time of annual review.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: B
Species (common name): Ferret
Number: 1
Explanation of procedure producing pain and/or distress:
The ferret is the gold standard model for influenza pathogenesis and transmission studies for several reasons. They are naturally susceptible to influenza infection and they display many clinical signs similar to humans. The receptors in the upper and lower respiratory tracts of ferrets are also similar to humans resulting in comparable tropism characteristics and the general transmissibility phenotypes exhibited by influenza viruses in humans are also demonstrated in laboratory ferrets. The majority of influenza viruses, including most strains of highly pathogenic avian influenza viruses, cause a mild to moderate respiratory disease in ferrets. Our laboratory team has studied influenza viruses using the ferret model for many years and only a fraction of the strains tested are capable of causing severe disease and death in this species. Nevertheless, over the years we have collected enough data to recognize when an animal is likely to experience severe disease and we euthanize the animal before they reach this point. During the last year, a single animal (out of 177 used for experimentation) passed suddenly during the night. This animal did not show signs of pain or distress during observation on that day. This leads us to speculate that the ferret may have had an underlying condition that contributed to his rapid decline.

Justification why pain and/or distress could not be relieved:
Every attempt will be made to euthanize animals before they experience severe disease or reach a moribund state. Analgesia is not an option for this study because we need to record clinical signs of infection (such as fever, weight loss, and lethargy), which are masked by treatment. Clinical signs are monitored daily to document the severity of disease and to quickly identify any cases in which veterinary intervention is needed. Any animal that loses greater than 25% body weight and/or accrues a total score of 10 on the clinical scale will be humanely euthanized; animals will be monitored twice daily if they reach a 3 on the clinical scale. The animal that passed suddenly this year showed no signs of pain or distress during observations prior to being found dead.

Protocol: C
Species (common name): Ferret
Number: 3
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 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.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: F
Species (common name): Hamster
Number: 18
Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat Nipah virus disease in humans. Griffithsin (GRFT), a natural compound isolated from red algae, has recently been shown to have antiviral activity against Nipah virus. The anti-Nipah virus activity of GRFT needs to be further evaluated in a disease model of Nipah virus. Hamsters are an established disease model of Nipah viruses and show similar pathogenesis and disease as humans. Nipah virus causes severe encephalitis and respiratory disease in humans and similar symptoms may present in hamsters.

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 Nipah virus infection diseases. Based on these factors, analgesics should 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 will be made to ensure that the animals experience the least amount of pain and distress as is absolutely necessary to accomplish the goals of the experiment. All animals will be 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 will be utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points will be monitored two times per day. Animals scored at 10 total points or above will be humanely euthanized, and euthanasia will be performed prior to endpoint criteria being reached whenever possible.

Protocol: G
Species (common name): Hamster
Number: 17
Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat Nipah virus disease in humans. The aim of these studies is to investigate the use of defective interfering particles (DIs; non-spreading, non-replicating particles) as therapeutics for Nipah virus disease. Hamsters are an established disease model of Nipah viruses and show similar pathogenesis and disease as humans. Nipah virus causes severe encephalitis and respiratory disease in humans and similar symptoms may present in hamsters.

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 Nipah virus infection diseases. Based on these factors, analgesics should 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 will be made to ensure that the animals experience the least amount of pain and distress as is absolutely necessary to accomplish the goals of the experiment. All animals will be 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 will be utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points will be monitored two times per day. Animals scored at 10 total points or above will be humanely euthanized, and euthanasia will be performed prior to endpoint criteria being reached whenever possible.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: H
Species (common name): Hamster
Number: 3
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 can be used during the study to treat any non-rabies related condition. Animals infected with rabies virus 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 we do not expect a significant number of animals to rapidly progress as described above. Based on our prior experience we might expect 3-10% of animals that develop signs of rabies to progress to death before euthanasia can be administered; however, we have developed a rabies specific pain scale to help us euthanize those animals that show specific neurological rabies signs before they succumb to the disease.

In the rare case that we find an animal dead during the study, these animals will subsequently be categorized as pain category E. All animals infected with rabies virus will be euthanized at the onset of clinical signs of rabies according to the listed euthanasia criteria. 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 mice (Hartinger, et al. 2001). 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 veterinary or program staff. Additionally, program associates are on call at all times during an infection.

Protocol: I
Species (common name): Hamster
Number: 2
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:
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 veterinary or program staff in addition to checks by routine husbandry staff. Additionally, program associates are on call at all times during an infection.

Protocol: J
Species (common name): Hamster
Number: 2
Explanation of procedure producing pain and/or distress:
This study aims to examine the effectiveness of Griffithsin to inhibit rabies virus when used as a part of a pre or post-exposure prophylaxis in hamsters. Syrian hamsters are well established in the literature for rabies pathogenesis studies and the evaluation of 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:
All animals infected with rabies virus will be euthanized at the onset of clinical signs of rabies according to the listed euthanasia criteria. The majority of animals will be humanely euthanized, and we do not expect a significant number of animals to experience pain or distress. Analgesia can be used during the study to treat any non-rabies related condition. However, animals infected with rabies virus may progress rapidly from an apparently healthy status to a terminal state. This can occur occasionally overnight or between routine check periods. Based on our prior experience we might expect 3-10% of animals that develop signs of rabies to progress to death before euthanasia can be administered. To minimize these deaths, we have developed a rabies specific pain scale to help us euthanize those animals that show specific neurological rabies signs before they succumb to the disease. Using the clinical signs of rabies as the experimental endpoint instead of death prevents four to five days of suffering in mice (Hartinger, et al. 2001). 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 veterinary and/or program staff. Additionally, program associates are on call at all times during an infection. In the rare case that we find any animal dead during the study, these animals will subsequently be categorized as pain category E.

Protocol: K
Species (common name): Hamster
Number: 6
Explanation of procedure producing pain and/or distress:
Currently there are no approved antiviral therapies available to treat Nipah virus disease in humans. Recently a novel single-dose, non-infectious, messenger RNA (mRNA)-based vaccine platform was shown to be effective in both small animal and non-human primate models of Zika virus infection. Since this platform does not utilize any live replicating virus vector, it is less likely to cause adverse effects. In this vaccination + virus challenge study,

we are investigating the use of this mRNA vaccine against Nipah virus using a hamster model. Hamsters are an established disease model of Nipah viruses and show similar pathogenesis and disease as humans. Nipah virus causes severe encephalitis and respiratory disease in humans and similar symptoms may present in hamsters.

Justification why pain and/or distress could not be relieved:
All efforts will be made to ensure that the animals experience the least amount of pain and distress as is absolutely necessary to accomplish the goals of the experiment. However, in these studies animals that demonstrate clinical signs but do not meet end-point criteria will be followed and not euthanized at first sign of disease, as recovery may occur. In addition, some of the viruses may cause sudden onset and rapid progression in a proportion of animals which may prevent euthanasia. 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 Nipah virus infection diseases. Based on these factors, analgesics should 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 animals will be 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 will be utilized to determine appropriate endpoints to prevent unnecessary suffering and pain. Animals scored at 8-9 points will be monitored two times per day. Animals scored at 10 total points or above will be humanely euthanized, and euthanasia will be performed prior to endpoint criteria being reached whenever possible.

Attachment A: Category E Explanations, USDA, APHIS Form 7023 (FY19)
Centers for Disease Control and Prevention
Registration Number: 57-F-0004

Protocol: E
Species (common name): Guinea Pig
Number: 12
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 will be used to alleviate discomfort associated with ear biopsies. Analgesia cannot be used for alleviation of pain or distress due to rickettsial infection 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 interfere with immune responses to rickettsial infection and mask clinical signs necessary to study pathogenesis. All infected animals are being monitored at least twice per day and any animal whose condition is likely to worsen to the point of suffering prior to the next health check is euthanized. All investigators and staff are trained to evaluate and assess animals according to the clinical scale, and any animal showing >25% weight loss or a total score of 10 on the clinical scale is humanely euthanized. However, some of the infected animals may progress to a terminal state very abruptly, within 2 to 4 hours, and there is a realistic chance that a small percentage of these animals could die of their disease progress and they will be categorized as Category E.