Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study: 8 hamsters, 9 guinea pigs

3. Species (common name) of animals used in this study:
   - Cavia porcellus (Hartley guinea pig)
   - Mesocricetus auratus (Syrian golden hamster)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

After the adaptation processes, Marburg virus (MARV) causes lethal disease in guinea pigs and hamsters which closely mimics the hemorrhagic fever syndrome observed in humans infected with MARV. A mouse model is also available; however, the disease in the mouse differs in several aspects from human disease. Therefore, additional lethal small rodent models would be extremely beneficial to study pathogenesis and concepts for vaccination and therapies. This will further our understanding and help to reduce the use of nonhuman primates the ultimate disease 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. (from ASP, Section F)

The development of small animal disease models for MARV is essential for studying pathogenesis as well as the development of vaccines and anti-virals. The potential illness experienced by the some of the animals exposed to MARV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanisms responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS. During the passage experiments for obtaining lethal variants of MARV, it is impossible to predict the outcomes of these studies and especially the severity of disease associated with individual agents in the guinea pigs and hamsters. Animals will be scored daily according to an approved scoring sheet and will be euthanized when they reach a point where recovery seems unlikely to reduce suffering. There will be a conscious effort by all investigators and the animal care personnel to provide as much additional consideration for the comfort and wellbeing of the animals as is consistent with the scientific integrity of the protocol.

Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 12

3. Species (common name) of animals used in this study:
   Mesocricetus auratus (Syrian golden hamster)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

   It is unknown whether Zaire Ebola Virus (ZEBOV) will cause disease in T cell-depleted hamsters. In order to develop and characterize the immune response in animal models mimicking VHF's in humans, hamsters will be used.

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. (from ASP, Section F)

   The illness experienced by the animals exposed to the VHF viruses indicated below must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.
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1. Registration Number: 51-F-0016
2. Number of animals used under Column E conditions in this study: 4
3. Species (common name) of animals used in this study: *Cavia porcellus* (Guinea pigs, Hartley & Strain 13)
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

Experimental manipulations will be done on anesthetized animals. Guinea pigs are susceptible to infection with Lujo virus; however only mild signs of infection are apparent following infection with wild-type virus (Salmonetz, Feldmann unpublished data). The majority of animals in this study will be euthanized prior to the onset of terminal signs of disease (as a part of the serial passaging process). During serial passage of Lujo virus through Guinea pigs it is expected that the virus will acquire mutations allowing it to evade the host immune responses and replicate more efficiently in a variety of tissues. As these mutations accumulate we expect to observe clinical signs of disease that may include lethargy, increased weight loss, hemorrhage, respiratory distress and neurological disorders, which ultimately might be fatal. Also, guinea pigs have already been successfully used to develop a lethal disease model of Lassa virus, a close relative of Lujo virus.

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. (from ASP Section F)

Since the aim of these experiments is to lethally adapt Lujo virus to inbred and outbred Guinea pigs, we are unable to alleviate these potential signs of disease because treatment will interfere with the adaption process / disease manifestations and ultimate outcomes of infection. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Pierpont et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.


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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 14

3. Species (common name) of animals used in this study: Guinea pigs (Cavia porcellus)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section)

Currently Guinea pigs are the only small animal model described for Lassa fever. In these studies we will be using two Lassa virus strains, one which has been adapted to outbred Guinea pigs and the parental Lassa virus strain which infects Guinea pigs but is not uniformly lethal. Infected animals demonstrate signs of disease which can include weight loss, ruffled fur, labored breathing and hemorrhagic manifestations which are ultimately lethal in 30% (for wild-type Lassa virus Josiah) or 100% (for Guinea-pig adapted Lassa virus Josiah) of Guinea pigs. The purpose of this work is to characterize the outbred Guinea pig model for Lassa virus infection.

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. (from ASP, Section F)

In these experiments Guinea pigs will be infected with a challenge dose of Lassa virus which has previously been determined to cause lethal disease in 30-100% of animals (dependent on the strain of Lassa virus utilized). Following challenge, infected animals will appear normal until around 5-7 days, after which they may demonstrate signs of disease including weight loss, ruffled fur and lethargy. The purpose of these studies is to compare the disease progression associated with infection of two Lassa virus strains in Guinea-pigs. Animals will be euthanized at scheduled time points or when signs of advanced disease are apparent. Health status of individual animals will be assessed according to a numerical scoring index as follows: 0 = no signs; 1 = ruffled fur; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss >5%; 4 = ruffled fur, hunched posture & weight loss >10%; 5 = ruffled fur, hunched posture, weight loss >15% or paralysis of limbs or hemorrhagic manifestations or dyspnea; 6 = ruffled fur, hunched posture, weight loss >20% or paralysis of limbs or hemorrhagic manifestations or dyspnea; 7 = death. Animals will be euthanized if they reach a score >5, or at 45 days post infection. We are unable to alleviate signs of disease in these animals since the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process, which is hypothesized to be important in HPS disease progression. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.


Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 4

3. **Species (common name) of animals used in this study:** Cynomolgus macaques (*Macaca fascicularis*)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** *(from ASP Section F)*

The challenge dose of Lassa virus which will be administered in these studies has previously been shown to result in severe infection with a lethal outcome in naive nonhuman primates. Cynomolgus macaques are susceptible to Lassa virus infections therefore are the most appropriate species for these studies. The veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, unable to move from the ground of the cage, and severe weight loss (>20%). The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversible (based on the clinical evaluation by the veterinarian).

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.** *(from ASP, Section F)*

We have established a scoring system that will assist us in determining the humane endpoint for euthanasia. Animals challenged with Lassa virus may experience pain and distress and the infection may even be lethal, however the results of literature searches suggest treatment of pain / distress will interfere with the goals of this study. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthases, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. The illness experienced by the Lassa infected animals must not be treated because treatment will interfere with studying the pathogenesis of the disease and identifying potential correlates of immunity. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Persman et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modifying the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study: 4

3. Species (common name) of animals used in this study: Cynomolgus macaques (Macaca fascicularis)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

Marburg virus causes significant disease, which is associated with distress in nonhuman primates. Causing infection in nonhuman primates is necessary in order to evaluate the efficacy of a vaccine. The investigator will notify the facility staff when animals begin the Column E study. The veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, unable to move from the ground of the cage, and significant weight loss (>15%). The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversible based on the clinical evaluation by the veterinarian in consultation with PI.

Animals infected with Marburg virus will experience pain and distress and the infection will be lethal in non-protected animals. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target organ systems that are being evaluated in this study. Opiates are not indicated since they have depressant effects on the cardiovascular and respiratory systems and could alter the parameters to be measured and even accelerate the pathology and death. Instead we have established a scoring system that will allow us to determine the humane end point for euthanasia. The illness experienced by the animals exposed to Marburg virus must not be treated with analgesics because such treatment will interfere with studying the pathogenesis of the disease. More importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to cause respiratory and cardiovascular depression. They also interfere with the mechanism(s) responsible for interferon production (1,2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics, including buprenorphine, can cause an histamine release and respiratory depression (4-6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process, which has to be considered as a critical component in the pathogenesis of Marburg virus. Studies by Pierzma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids, fentanyl and buprenorphine, directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following the administration of LPS.

References:

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. (from ASP, Section F)

Control and vaccinated/depleted animals challenged with Marburg virus may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we have established a scoring system that will allow us to determine the humane end point for euthanasia.
Column F Explanation Form For Regulated Species

This form is intended as an aid to completing the Column F explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column F explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 51-F-0016

2. Number of animals used under Column F conditions in this study: 10

3. Species (common name) of animals used in this study: Sus scrofa domestica (domestic pig)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

Following inoculation of pigs with PRRSV, animals may develop signs of disease that could include lethargy, inappetence, labored breathing or swelling of joints. Recreating disease, and possibly serious disease, in pigs is necessary to understand pathogenesis of this virus and for the development of vaccines and antiviral treatments. To minimize pain and distress, the pigs will be checked twice daily beginning on day 1 of the study and any animals exhibiting clear signs of distress/pain will be euthanized after evaluation by the attending veterinarian and in consultation with the PI. All euthanasia procedures will be done by trained personnel.

Swine are the appropriate model to use in these experiments, since they are the natural host of PRRSV.

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. (from ASP, Section F)

The refinement of the swine model for PRRSV is essential for studying pathogenesis as well as the development of vaccines and antivirals. The potential illness experienced by some of the animals exposed to PRRSV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study.
Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 28 hamster and 30 guinea pigs

3. Species (common name) of animals used in this study: Guinea pigs and hamsters

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

After the adaptation processes, Marburg virus (MARV) will cause lethal disease in guinea pigs and hamsters which closely mimics the hemorrhagic fever syndrome observed in humans infected with MARV. A mouse model is also available; however, the disease in the mouse differs in several aspects from human disease. Therefore, additional lethal small rodent models would be extremely beneficial to study pathogenesis and concepts for vaccination and therapies. This will further our understanding and help to reduce the use of nonhuman primates in the ultimate disease model.

The study endpoint is euthanasia at different time points for each experiment in this ASP as outlined in the corresponding paragraph in section F or at a time point when animals appear to be in an advanced stage of disease as determined in previous experiments with EBOV (weight loss >20%, dyspnea, and/or neurological signs).

The health of animals will be assessed daily according to the following criteria:

0 = no signs of disease; 1 = ruffled fur; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss >5%; 4 = ruffled fur, hunched posture & weight loss >10%; 5 = ruffled fur, hunched posture, weight loss >15%; 6 = ruffled fur, hunched posture, weight loss >20% or encephalitic signs or hemorrhagic signs or paralytic signs or respiratory distress (dyspnea); 7 = death. Euthanasia will occur at a score of 6.

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. (from ASP, Section F)

The development of small animal disease models for MARV is essential for studying pathogenesis as well as the development of vaccines and antivirals. The potential illness experienced by some of the animals exposed to MARV must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

During the passage experiments for obtaining lethal variants of MARV, it is impossible to predict the outcomes of these studies and especially the severity of disease associated with individual agents in the guinea pigs and hamsters. Animals will be scored daily as outlined above and will be euthanized when they reach a point where recovery seems unlikely to reduce suffering. There will be a conscious effort by all investigators and the animal care personnel to provide as much additional consideration for the comfort and well-being of the animals as is consistent with the scientific integrity of the protocol.

Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 25

3. Species (common name) of animals used in this study:
   Ferret (*Mustela putorius furo*)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

Pandemic H1N1 isolates may cause severe or lethal disease in ferrets which partially mimics the respiratory disease observed in humans infected with the virus. The seasonal and 2009 pandemic influenza A virus strain will likely cause limited morbidity or mortality compared to the 1918 H1N1 influenza A virus.

In general, different animal models are used to study pathogenesis, transmission and immune response to influenza virus infection including nonhuman primates, ferrets and mice. At present no alternatives are available to study these complex virus-host interactions.

The ferret is currently the best characterized and accepted small animal model of influenza pathogenicity studies.

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. (from ASP, Section F)

The illness experienced by the animals exposed to the human influenza A virus must not be treated with analgesics because treatment will interfere with the disease manifestation and study parameters such as innate immune responses, immunology and virology. In order to minimize pain and distress, animals will be clinically evaluated at least daily and will be euthanized if they reach a point of severe disease, or at 14 days post exposure.
Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 114

3. Species (common name) of animals used in this study: Syrian hamsters (Mesocricetus auratus)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):
   Andes virus causes lethal hantavirus pulmonary syndrome (HPS)-like disease in Syrian hamsters. Currently the Andes virus / hamster model of HPS is the only small animal model available for the study of hantavirus pathogenesis and potential therapeutics, therefore at this time it is the only model with which we can study the effect of preventing SIP receptor signaling on HPS development. Animals receiving SIP inhibitors will be euthanized if they appear to have entered the terminal stages of disease (i.e. respiratory distress). Control (vehicle treated) animals will be euthanized when respiratory distress become apparent.

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 (from ASP Section F):
   In these experiments hamsters will be infected with a challenge dose of Andes virus which has previously been determined to cause lethal disease in 100% of animals. Following challenge, infected hamsters will appear normal until around 11 or 12 days post infection, after which they will demonstrate signs of disease including respiratory insufficiencies and death within approximately 24 hours. It is the goal of these studies to determine if blocking SIP receptor signaling can prevent or reduce mortality associated with lethal HPS disease in this animal model. As such, animals receiving SIP inhibitors will be euthanized if they appear to have entered the terminal stages of disease (i.e. respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics in treated hamsters could directly interfere with the disease progression of the virus and/or the immune mediated protection, thereby making the data collected impossible to interpret.
Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 1 hamster

3. **Species (common name) of animals used in this study:**
   Syrian hamsters (Mesocricetus auratus)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**
   Currently no animal models are available for the novel human coronavirus. Although no data is currently available on the novel coronavirus it can be anticipated that inoculation with the novel coronavirus will be associated with distress in hamsters and non-human primates. Hamsters inoculated with the novel coronavirus will be euthanized if they appear to have entered the terminal stages of disease (i.e. respiratory distress).

   For the non-human primates, the veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, unable to move from the ground of the cage, and significant weight loss (>15%). The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversible (based on the clinical evaluation by the veterinarian in consultation with PI).

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 (from ASP, Section F):**
   Animals inoculated with the novel coronavirus may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems.

   We have established a scoring sheet that will allow us to determine the humane end point for euthanasia for the non-human primates.
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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 12

3. **Species (common name) of animals used in this study:**
   Syrian hamsters (Mesocricetus auratus)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**
   Andes virus causes lethal hantavirus pulmonary syndrome (HPS)-like disease in Syrian hamsters. Currently the Andes virus / hamster model of HPS is the only small animal model available for the study of hantavirus pathogenesis and potential therapeutics, therefore at this time it is the only model with which we can study the effect of inhibiting specific host responses on HPS development. Animals receiving inhibitors will be euthanized if they appear to have entered the terminal stages of disease (i.e. respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent.

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 (from ASP, Section F):**
   In these experiments hamsters will be infected with a challenge dose of Andes virus which has previously been determined to cause lethal disease in 100% of animals. Following challenge, infected hamsters will appear normal until around 11 or 12 days post infection, after which they will demonstrate signs of disease including respiratory insufficiencies and death within approximately 24 hours. It is the goal of these studies to determine if blocking specific host responses can prevent or reduce mortality associated with lethal HPS disease in this animal model. As such, animals receiving inhibitors will be euthanized if they appear to have entered the terminal stages of disease (i.e. respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent. The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics in treated hamsters could directly interfere with the disease progression of the virus and/or the immune mediated protection, thereby making the data collected impossible to interpret.
Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the USDA Annual Report of NIAID Research Facilities Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study: 24

3. Species (common name) of animals used in this study: Syrian hamster

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):

Infection of hamsters with MA-ZEBOV could cause distress in immunocompetent animals. As not all aspects of EBOV hemorrhagic fever, most notably coagulation disorders, are fully recapitulated in the mouse model, we propose to use the hamster model here instead. The hamster model does more accurately portray the disease syndrome.

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 (from ASP, Section F):

Hamsters infected with MA-ZEBOV may experience pain and distress and the infection may be lethal. NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to reduce VSV replication – which could be extrapolated to affect ZEBOV replication. These affected systems are target systems being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use daily clinical evaluation that will allow us to determine the humane endpoint for euthanasia.

Recreating disease, and possibly serious disease, in these animals is necessary in order to test the efficacy of the treatments proposed. The investigator will notify the facility staff when animals begin the Column E study. The veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are observed: dyspnea, anorexia, weight loss greater than 15% or colitis. The animal will be euthanized at the specified time points or when clinical disease is considered non-reversible, #5 on scoring evaluation (See below).

SCORING:
0 = no signs
1 = ruffled fur,
2 = ruffled fur & weight loss <5%
3 = ruffled fur, hunched posture & weight loss > 5%
4 = ruffled fur, hunched posture & weight loss > 10%
5 = ruffled fur, hunched posture & weight loss > 15% OR paralysis of limb(s) OR colitis OR respiratory distress
6 = ruffled fur, hunched posture & weight loss ≥ 20% OR paralysis of limb(s) OR respiratory distress
7 = death
Euthanasia will occur at a score of 5
Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 17

3. **Species (common name) of animals used in this study:** Mesocricetus auratus (Syrian golden hamster)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**

   Nipah virus infection causes lethal disease in hamsters which closely mimics human disease (acute respiratory distress, encephalitis). In order to develop and characterize the immune response and vaccine efficacy we propose to use an established small rodent disease model, the Syrian hamster.

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 (from ASP, Section F):**

   The utilization of an animal disease model is essential for studying pathogenesis as well as the efficacy testing of vaccine candidates and anti-virals. The potential illness experienced by some of the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Search of the literature (PubMed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to alter the replication of viruses. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use clinical evaluation that will allow us to determine the humane endpoint for euthanasia.
Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 63

3. Species (common name) of animals used in this study: Syrian hamster (Mesocricetus auratus)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):

   Andes virus causes lethal hantavirus pulmonary syndrome (HPS)-like disease in Syrian hamsters. Currently the Andes virus/hamster model of HPS is the only small animal model available for the study of hantavirus pathogenesis and potential therapeutics, therefore at this time it is the only model with which we can study the protective efficacy of effect of HPS vaccines.

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 (from ASP, Section F):

   In these experiments hamsters will be infected with a challenge dose of Andes virus which has previously been determined to cause lethal disease in 100% of animals. Following challenge, infected hamsters will appear normal until around 8 or 9 days post infection, after which they will demonstrate signs of disease including respiratory insufficiencies and death within approximately 24 hours. It is the goal of these studies to test second generation vaccine vectors for their protective efficacy following administration prior to or after ANDV challenge in the HPS hamster model in order to define the most potent candidate vaccine for prophylactic vaccination and post-exposure treatment. Animals receiving the experimental hantavirus vaccines may experience mild to moderate signs of illness and still recover, therefore, they will be euthanized if they appear to have entered into the terminal stages of disease (i.e., respiratory distress). Control animals will be euthanized when breathing insufficiencies first become apparent. The use of analgesics could alter the pathogenic and immunologic response to infection or post-exposure immunization, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics in immunized hamsters could directly interfere with the disease progression of the virus, thereby making the data collected impossible to interpret.
Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 11

3. **Species (common name) of animals used in this study:** Mesocricetus auratus (Syrian golden hamster)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**

   Nipah virus infection causes lethal disease in hamsters which closely mimics human disease (acute respiratory distress, encephalitis). In order to develop and characterize the immune response and vaccine efficacy we propose to use an established small rodent disease model, the Syrian hamster.

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 (from ASP, Section F):**

   The utilization of an animal disease model is essential for studying pathogenesis as well as the efficacy testing of vaccine candidates and anti-virals. The potential illness experienced by some of the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the disease manifestation, thus rendering the data collected unreliable. Search of the literature (PubMed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as well as stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to alter the replication of viruses. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use clinical evaluation that will allow us to determine the humane endpoint for euthanasia.
Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 19

3. Species (common name) of animals used in this study: Cavia porcellus (Guinea pigs)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):

Lujo virus infection in adult strain 13 Guinea pigs results in a systemic infection with internal hemorrhage and multi-organ failure leading to death. Currently there is no other disease model described for Lujo virus, therefore strain 13 Guinea pigs are the only in vivo option to evaluate medical countermeasures against this highly pathogenic arenavirus.

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 (from ASP, Section F):

Experimental manipulations will be done while the animals are anaesthetized. However since we are assessing the therapeutic benefit of treating Lujo virus infection with ribavirin, we are unable to alleviate the disease progression in animals. Ribavirin has several hypothesized modes of action, one of which is modulating the host's immune response. Upon injection with Lujo virus Guinea pigs are expected to develop signs disease which will include lethargy, weight loss and breathing distress, which ultimately leads to death. The illness experienced by the animals exposed to Lujo virus must not be treated because treatment will potentially interfere the pathogenesis of the disease and/or the effect of ribavirin treatments. Importantly, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production (1, 2). Moreover, opioids can suppress NK cell activity (3). Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release (4, 5) and respiratory depression (6). Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages (7), inhibit interferon-alpha release from dendritic cells (8), and increase the synthesis and release of IL-10 from human macrophages (9). Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process, which is hypothesized to be important in HPS disease progression. Studies by Piersma et al. provide a final example of how analgesics may modify the expression of the disease (10). These investigators, using an established murine model of endotoxemia, showed that the opioids fentanyl and buprenorphine directly altered the outcome of their experiments by modulating the immune response. In this case, both of the opioids caused significant decreases in circulating levels of tumor necrosis factor-alpha following administration of LPS.

Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 4

3. **Species (common name) of animals used in this study:** Cynomolgus macaques (Macaca fascicularis)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**

Macques are the model of choice for Ebola viruses and the best surrogate model for human disease. Therefore, we would like to conduct this study in the nonhuman primate model. We propose to use Cynomolgus macaques since earlier studies with the 1989/90 strains have revealed that Cynomolgus macaques are most susceptible among tested nonhuman primate species. In this study we want to compare the pathogenic potential of both Reston ebolaviruses (REBOVs), the swine-derived REBOV strain, REBOV-08, and the REBOV-89/90 strain in NHPs. NHPs are generally valued as the best surrogate model for human disease in Ebola virus research. Although so far there are no human cases known for REBOV, this virus is closely related to the other human-pathogenic species like Zaire ebolavirus (ZEOBV), which cause case fatality rates up to 90% in humans. ZEOBV has been shown to cause disease in pigs and can be transmitted from pigs to NHPs [11, 12]. For REBOV, as a closely related pathogen to ZEOBV, the potential to spread from infected pigs to humans remains (see World Health Organization Report, page #14). This study will advance work previously done in rodents and give more insight into REBOV pathogenicity in NHPs.

We have previously performed a comparative study in knockout mice with REBOV-89/90 and REBOV-08. This study determined a higher virulence for the macaque-derived isolate from 1989/90 indicating distinct pathogenic potentials for certain REBOV strains. Macaques are the gold standard animal disease model for Ebola viruses and the best surrogate model for human disease. Therefore, we would like to move into the nonhuman primate model. We propose to use Cynomolgus macaques since earlier studies with the 1989/90 strains have revealed that Cynomolgus macaques are most susceptible among tested nonhuman primate species.

Reston ebolavirus (REBOV) causes significant disease, which is associated with distress in nonhuman primates. Causing infection in nonhuman primates is unavoidable in order to study virulence and disease progression. The investigator will notify the facility and RMVB staff when animals begin the Column E study. Staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, unable to move from the ground of the cage, and significant weight loss (>15%). The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversible (based on the clinical evaluation by the veterinarian in consultation with PI).

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 (from ASP, Section F):**

Animals infected with Reston ebolavirus (REBOV) will experience pain and distress and the infection can be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotene synthesis, and stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target organ systems that are being evaluated in this study. Opiates are not indicated since they have depressant effects on the cardiovascular and respiratory systems and could alter the parameters to be measured and even accelerate the pathology and death. Instead we have established a scoring sheet that will allow us to determine the humane end point for euthanasia. The illness experienced by the animals exposed to REBOV must not be treated with analgesics because such treatment will interfere with studying the pathogenesis of the disease.
Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 8

3. **Species (common name) of animals used in this study:** cynomolgus macaque (macaca fascicularis)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):**

Currently the Cynomolgus macaque is the best surrogate model for human influenza disease. Available data indicate that Cynomolgus macaques will develop different degrees of respiratory disease following influenza A virus infection by intrabronchial installation. Veterinary staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are seen: dyspnea, anorexia, paralysis, unable to move from the ground of the cage, and significant weight loss (>15%). The animals will be euthanized at the specified time points or when clinical disease progression is considered irreversable (based on the clinical evaluation by the veterinarian in consultation with PI).

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 (from ASP, Section F):**

Animals inoculated with influenza A H7N9 and HPAIV H7N7 viruses may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. We have established a scoring sheet that will allow us to determine the humane end point for euthanasia for the non-human primates.
Column E Explanation Form For Regulated Species

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 3

3. Species (common name) of animals used in this study: rhesus macaque (Macaca mulatta)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected (from ASP Section F):

Infection of rhesus macaques with MERS-CoV results in fever, increased respiration rate, cough, piloerection and hunched posture, as such disease progression will be closely monitored using scoring systems established for previous respiratory disease models. Currently rhesus macaques are the only known animal model for MERS-CoV. Available data indicate that rhesus macaques can be infected with MERS-CoV and develop respiratory disease.

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 (from ASP, Section F):

Animals inoculated with the novel coronavirus may experience pain and distress and the infection may even be lethal. NSAIDS cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that are being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise, which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. We have established a scoring sheet that will allow us to determine the humane end point for euthanasia for the non-human primates.
Column E Explanation Form For Regulated Species

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1. **Registration Number:** 51-F-0016

2. **Number of animals used under Column E conditions in this study:** 18

3. **Species (common name) of animals used in this study:** Syrian hamsters (*Mesocestoides auratus*)

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.** (from ASP Section F)

   Andes virus causes lethal hantavirus pulmonary syndrome (HPS)-like disease in Syrian hamsters. Currently the Andes virus / hamster model of HPS is the only small animal model available for the study of HPS disease and potential therapeutics or vaccines, therefore at this time it is the only model with which we can study the protective efficacy of T-705 therapy. Animals receiving T-705 will be euthanized if they appear to have entered the terminal stages of disease (i.e. ruffled fur, hunched posture, weight loss ≥ 15%, and/or respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent.

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.** (from ASP, Section F)

   In these experiments hamsters will be infected with a challenge dose of Andes virus which has previously been determined to cause lethal disease in 100% of animals. Following challenge, infected hamsters will appear normal until around 9 or 10 days post infection, after which they will demonstrate signs of disease including respiratory insufficiencies and death within approximately 24 hours. It is the goal of these studies to determine if the administration of T-705 can prevent or reduce mortality associated with lethal HPS disease in this animal model. As such, animals receiving T-705 will be euthanized if they appear to have entered the terminal stages of disease (i.e. ruffled fur, hunched posture, weight loss ≥ 15%, and/or respiratory distress). Control (vehicle treated) animals will be euthanized when breathing insufficiencies become apparent.

   The use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production. Moreover, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics in treated hamsters could directly interfere with the disease progression of the virus and/or the immune mediated protection, thereby making the data collected impossible to interpret.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

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1. Registration Number: **51-F-0016**

2. Number of animals used under Column E conditions in this study: **2**

3. Species (common name) of animals used in this study: **Grammomys surdator (African thicket rats)**

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

   Infection of the African thicket rat with the murine malaria parasite *P. berghei* (originally isolated from this species of rodent) is not supposed to be lethal; however, some of the infected animals showed some signs of sickness, such as ruffled fur, hunched posture, and/or reluctance to move (lethargy). In order for us to properly characterize this novel system, we needed to let the malarial parasite infection progress to a point of definitive sickness before we intervene with curative therapeutics.

   The state described above (ruffled fur, hunched posture, and/or lethargy) appeared in some of the thicket rats, and at anywhere from four days to up to two weeks following infection with the malaria parasite. Experience showed that most animals infected with the *P. yoelii* 17X (non-lethal) malaria parasite progressed to this state and then mounted a sufficiently effective immune response for spontaneous and complete recovery within a few (3 to 5) days.

   Two of our African thicket rats infected with malaria parasites did show signs of sickness, including ruffled fur and hunched posture.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

   Analgesics would not have relieved the modest distress due to the infection. Once we saw far the clinical signs progressed (nothing more than piloerection, hunched posture, and lethargy – this parasite (*P. berghei*) **did not cause significant** parasitemia and anemia in the thicket rat host), we cured the affected animals with anti-malarial drugs or euthanized them for sample collection.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

   Animals were given extra food treats on cage floor and extra bedding for nesting.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

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1. Registration Number: **51-F-0016**

2. Number of animals used under Column E conditions in this study: **14**

3. Species (common name) of animals used in this study: *Graphiurus kelleni* (Kellen's African dormouse)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Dormice were infected intranasally with a vaccinia virus expressing firefly luciferase and imaged daily. Two different volumes of inocula were used in order to determine the route and extent of virus spread. Dormice are exquisitely sensitive to poxvirus infection and are one of only a few models available for studying monkeypox virus pathogenesis.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

Analgesics were not used because they would cause profound effects on the immune system and thus would impair proper evaluation of immune responses. Full evaluation of disease progression would be compromised by use of pain relief. Animals were observed and weighed daily; if they exhibited clinical signs of extreme lethargy or weight loss, they were humanely euthanized.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Animals infected with vaccinia virus were provided moistened feed or other food supplementation on the cage floor.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 50

3. Species (common name) of animals used in this study: Mustela putorius furo (common ferret)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

This project is to develop vaccines to protect humans against respiratory viruses, namely highly pathogenic avian influenza viruses. Viral infection and the induction of an immune response can only be studied in living animals. We are limited in our ability to study these virus infections and vaccine responses in the natural human host or in permissive primate models because of limited availability, limited genetic tools, and ethical considerations. Ferrets are good mammalian models to study influenza disease and to evaluate potential vaccine candidates. Avian influenza viruses are not uniformly virulent for ferrets. Infection of ferrets with some highly pathogenic avian influenza viruses can result in clinical signs of disease that can range from very mild disease up to pneumonia and even, if untreated, death. In this regard, it resembles the rare avian influenza infections reported in humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

For the attenuation studies, we conducted studies to evaluate the level of attenuation of live vaccine candidate viruses compared to the wild-type viruses that cause the disease in nature. H5N1 wild-type influenza viruses have been shown to cause severe clinical signs in ferrets (Zitzow et al. 2002). Since the attenuation studies measure the ability of the virus to replicate in the animal, and some influenza virus subtypes cause clinical signs in ferrets, we did not administer antivirals or antipyretics/analgesics to animals that showed clinical signs. There are two reasons why nonsteroidal anti-inflammatory drugs (NSAIDs) were not administered to attenuation-study ferrets that exhibit fever. One reason is that understanding the fever response to these infectious agents is an important endpoint of validating this model and these viruses. Secondly, anti-inflammatory properties of the NSAID will affect the immune response to the viruses, which may affect the course of the disease. However, the clinical signs observed were not severe in the time period of the studies (up to 5 days post-infection).

6. Indicate the supportive care and humane measures provided to the animals on these studies.

For generation of antisera, viral replication is necessary to generate the antibody response in the ferrets, so antivirals were not administered to ferrets inoculated with wild-type viruses. Antisera-generation ferrets that showed signs of significant illness, for example, high fever (>105°F for more than 24 hours), pronounced lethargy, respiratory distress, or dehydration, were given fluids and supportive care including approved antipyretics and/or analgesics at the discretion of the facility veterinarian.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

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1. Registration Number: 51-F-0016

2. Number of animals used under Column E conditions in this study: 6

3. Species (common name) of animals used in this study: *Macaca mulatta* (Rhesus macaque)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Severe malaria disease resulting from *P. coatneyi* infection is a possibility in our study. Rhesus macaques were chosen because they present with similar clinical signs and disease pathology when infected with *P. coatneyi* as seen in *P. falciparum*-infected humans.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

Systemic analgesics and pain-relieving measures were not used because they would have interfered with the experimental results by altering immune and/or inflammatory responses as well as the animal's compensatory physiological responses. Treatment of *P. coatneyi* infection with anti-malarials would have interfered with the diagnosis of clinical endpoints used in this study. These clinical criteria are necessary for the diagnosis of severe malaria and for comparisons with the clinical signs of disease seen in *P. falciparum*-infected humans.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Palliative measures were taken to keep the animals comfortable. A variety of fruits and treats were offered to animals that were not eating normally. For animals that became severely anorexic, i.e., not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry was performed. Those animals were offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications. Animals that became dehydrated from not drinking or excessive fluid loss through diarrhea were administered fluids IV, IP or SC.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

This form is intended as an aid to completing the USDA Annual Report of Research Facilities Column E explanation. 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-E-0016

2. Number of animals used under Column E conditions in this study: 2

3. Species (common name) of animals used in this study: Macaca mulatta (Rhesus macaque)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Animals that developed immunodeficiency as a result of SHIV or SIV infection frequently experienced anorexia, weight loss and/or diarrhea. In previous experiments, most animals were euthanized before clinical signs became evident, and evidence of disease only became apparent post-mortem. For example, Pneumocystis-induced disease, giant cell pneumonia, and meningoencephalitis have been demonstrated histopathologically at the time of necropsy, but were not clinically evident prior to euthanasia. Vital signs in these animals have remained within normal limits.

Some SHIV- or SIV-infected animals also exhibited neurological signs or signs of respiratory distress. From previous experiments using a specific neurotropic viral strain, the animals developed tremor, balance issues, head tilt, difficulty perching and ataxia/poor motor coordination.

Diagnostics were performed at the discretion of the attending veterinarian. The diagnostics included but were not limited to: rectal culture with sensitivity, radiographs, and CBC/Differential with Serum Chemistry.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

Animals inoculated with neurovirulent SIV were allowed to progress to clinical signs of SIV disease, including neurological signs such as tremor, head tilt, and ataxia, in order to allow us to distinguish by the PET and MRI scans (and post-euthanasia pathology) any alterations in the dopaminergic and/or serotonergic systems similar to those observed in AIDS patients. Anti-retroviral therapies were not used, because they would subvert the purpose of the experiments.

NSAIDS such as ibuprofen and ketoprofen cannot be used because they would interfere with the immune response of the animals on study, which might be highly pertinent to the development of neuropathy. Study animals with signs of discomfort received non-NSAID analgesics (i.e., huprenorphine) at the discretion of the attending veterinarian.

Palliative measures were taken to keep the animals comfortable. A variety of fruits and treats were offered to animals that were not eating normally. For animals that became severely anorexic, i.e., not eating for 24 or more hours, oro gastric tube feeding a nutritional supplement or biscuit slurry was performed. Those animals were offered highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications.

Animals that became dehydrated from not drinking or excessive fluid loss through diarrhea, were administered fluids IV, IP or SC.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

Supportive care was administered at the discretion of the attending veterinarian. The care included, but was not limited to fluid therapy, the use of antibiotics and anti-diarrhea medications, oro gastric tube feeding under sedation, offering highly palatable food items such as Ensure, Pediasure, primatreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

This form is intended as an aid to completing the USDA Annual Report of Research Facilities Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study:
   - 3 African green monkeys
   - 6 macaques
   - 3 Squirrel monkeys.

3. Species (common name) of animals used in this study:
   - Chlorocebus aethiops (African green monkey)
   - Macaca mulatta (Rhesus macaque)
   - Saimiri sciureus (Squirrel monkey).

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

   Influenza A virus infection can cause pneumonia associated with distress in non-human primates. Also, co-infection with Streptococcus pneumoniae causes distress in humans. Recreating disease, and possibly serious disease, in non-human primates is necessary in order to develop and evaluate animal models to study pathogenesis and vaccine development.
   The investigator notified the facility staff when animals began the Column E study. The veterinary staff monitored the animals, and the investigator was notified when the animals became clinically ill. The animals were euthanized at the specified time points or when clinical end-point was reached, based on the clinical evaluation by the veterinarian.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

   Animals infected with the 2009 pandemic human influenza A virus (H1N1) will likely experience pain and distress, but the viral infection is typically non-lethal. However, infection with the 1918 H1N1 virus or co-infection with Streptococcus pneumoniae may increase severity of influenza infection and may be lethal.
   NSAIDS were not used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis, stabilization of lysosomal membranes that may reduce the release of cytokines. These affected systems are target systems that were being evaluated in this study.
   Opiates were not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we established a scoring sheet that helped us to determine the humane endpoint for euthanasia.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

   Palliative measures were taken to keep the animals comfortable. A variety of fruits and treats were offered to animals that were not eating normally. For animals that became severely anorexic, not eating for 24 or more hours, orogastric tube feeding a nutritional supplement or biscuit slurry was performed. Those animals were offered highly palatable food items such as Ensure, Pediasure, primetreats, Gatorade, banana mash, pudding, peanut butter sandwiches, and other diet modifications.
   Animals that became dehydrated from not drinking or excessive fluid loss through diarrhea, were administered fluids IV, IP or SC.
COLUMN E Explanation Form

This form is intended as an aid to completing the Column E explanation. 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-0016

2 Number of animals used under Column E conditions in this study: 6

3 Species (common name) of animals used in this study: Common marmoset

4 Explain the procedure producing pain and/or distress, including reason (s) for species selected:
The marmosets in this protocol will be used for the animal model for multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE) and another animal model of demyelination, Cuprizone. EAE is induced by subcutaneous injections of human white matter homogenate in an adjuvant containing Mycobacteria tuberculosis, to incite an immune response. A major hallmark of MS is demyelination, a process in which neurons lose the myelin sheath insulating the axon. Understanding how demyelination can be measured with MR imaging is a major goal of this work. Cuprizone, a well-known copper-chelating agent, has been shown to induce highly-reproducible, reversible demyelination in mice (and to a lesser extent in rats) following oral ingestion. Cuprizone-induced demyelination is characterized by degeneration of the myelin-producing cells in the brain. These diseases may result in the development of various neurological deficits, including ataxia and paralysis, which while not being painful to the animals, it will impair their ability to move around their environment. This species was selected because marmosets are well-established systems of EAE. It is increasingly apparent that marmoset EAE (relative to rodent EAE) has superior translational applicability, which is ideal for a drug study. This is due to the fact that marmoset EAE shares highly relevant similarities with MS such as CD8 T-cell involvement, the presence of brain and spinal cord lesions and importantly, the ability for MRI analysis of lesions. Moreover, marmosets are particularly appropriate for studies involving MRI monitoring because their white matter/grey matter ratio resembles that of humans, which is relevant to both the EAE and Cuprizone studies.

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.
As EAE is a relapsing, remitting disease, we expect the extent and duration of neurological symptoms to differ for each animal and anticipate that some marmosets may recover. While we do not expect the marmosets to be in pain, restriction of movement may cause distress to the animals. Marmosets will be allowed to progress clinically to the point of hind limb paralysis and to remain in this state for up to 48 hours, to allow for recovery before euthanasia. To mitigate distress to the animals during this time, we plan to provide access to food and water on multiple levels of the cages, provide heating discs and express bladders as needed. Marmosets unable to ambulate around the cage will be housed individually in a padded kennel with easy access to food and water.
Column E Explanation Form for Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study. 24

3. Species (common name) of animals used in this study. Nonhuman Primates

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.
   To date, infection of nonhuman primates with orthopoxviruses has produced disease which most closely resembles the sequelae of human infection. The requirements for proof of protection in NHPs by vaccines and therapeutics intended for use in humans demand that the pathogenesis of the disease and correlates of immunity be understood in NHPs. Many immunological assays developed for humans can be performed on NHPs due to the phylogenetic relatedness of these two species. These considerations make macaques the most appropriate animal models to study human poxvirus infections.

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.
   The infection will likely result in serious disease that must not be treated with analgesics because treatment will interfere with the pathogenesis of the disease, and thus prevent our ability to examine normal viral infection and host response to the infection. Analgesics, both non-steroidal anti-inflammatory drugs (NSAIDS) like aspirin and ibuprofen, and acetaminophen, and opioids (narcotics) can have a profound effect on the immune system which would alter the pathogenic and immunologic response to infection, thus making it not feasible to interpret the data obtained in the study. We need to measure the number of immunological parameters in the study to elucidate and further characterize the mechanisms of poxvirus pathogenesis and to attempt to identify correlates of protection.
EXPLANATION FOR COLUMN E LISTING FOR REGULATED SPECIES

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1. Registration Number: **51-F-0016**

2. Number of animals used under Column E conditions in this study: **214**

3. Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian Hamster)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

   Leishmanial diseases are major parasitic diseases of man. The stage of the parasite that grows in the vertebrate host and causes disease cannot be generated *in vitro*. It can only be obtained from *in vivo* sources. In nature, most leishmanial species are maintained within animal reservoirs, usually rodents. The hamster is the only laboratory animal that develops visceral leishmaniasis. There is no way to test the action of vaccines *in vitro*. The whole animal is required to study experimental vaccines, protective immune responses and the outcome of infection of vaccinated animals. Information derived from the immune system responses being examined cannot be gathered by using cell culture or computer models.

   Visceral leishmaniasis in hamsters is manifested as hepatomegaly and anemia. The progression of visceral infection in hamsters is not associated with any overt pathology or changes in behavior until infection is severe, at which time hamsters begin to move slowly and lose their appetite.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine (personal experience or literature search) that pain and/or distress relief would interfere with test results.

   The point of onset of morbidity was variable, but generally occurred in the period 12 to 16 weeks post infection (when parasite inoculum was low and parasites were injected intradermally). Without intervention, over several weeks, the affected hamsters would have become cachectic, moribund, and eventually die.

   The only means for pain or distress relief was euthanasia. Analgesia was not be used during the two-day period after morbidity was observed, because this intervention would have affected the infected organs that were evaluated for size, histology, and parasite load as an endpoint to compare vaccinated and non-vaccinated animals.

6. Indicate the supportive care and humane measures provided to the animals on these studies.

   Infected hamsters were euthanized before any major signs of distress developed due to visceral leishmaniasis.
Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016
2. Number of animals used under Column E conditions in this study: 6
3. Species (common name) of animals used in this study: Syrian Golden Hamster (Mesocricetus auratus)
4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP, Section F)

Following infection with orthobunyaviruses, some hamster may demonstrate signs of disease which could include weight loss, encephalitis or other neurological and/or hemorrhagic signs. Infection with some of these viruses may also result in a lethal outcome. To the best of our knowledge, hamsters have not been rigorously assessed as potential animal model for the viruses being studied; although limited evidence suggests that they may serve as a lethal disease model for Bunyamwera virus. Further, they have been shown to be highly susceptible to several other Bunyaviruses, as well as being excellent models for other hemorrhagic fever diseases. Animal health will be evaluated twice daily during the acute phase of disease (see experimental end point for scoring criteria) and will be euthanized when they reach a point of advanced disease according to scoring criteria.

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. (from ASP, Section F)

It is the goal of this study to evaluate the ability of hamsters to serve as an infection/disease model reflecting human virulence among orthobunyaviruses. To this end, in these experiments hamsters will be infected with potentially lethal challenge doses of Bunyamwera virus, Batai virus and Ngari virus. While we cannot rule out the possibility that some animals will develop signs of terminal disease, infected animals will be euthanized if they enter an advanced stage of disease as determined by scoring criteria. The signs of disease cannot be relieved as treatment may interfere with immunopathological events associated with disease progression, as judged by an extensive review of the scientific literature. In particular, the use of analgesics could alter the pathogenic and immunologic response to infection, thus making it impossible to interpret the data obtained in this study. Narcotic analgesics have been shown to interfere with mechanism(s) responsible for interferon production, which are known to be important in resistance to bunyavirus infection. Similarly, opioids can suppress NK cell activity. Of particular importance in this study is the fact that analgesics including buprenorphine can cause histamine release and respiratory depression. Histamine is a well-known inflammatory mediator and plays a central role in the pathogenesis of allergic and inflammatory diseases by modulating vascular and airway responses. Histamine has been shown to induce activation of human macrophages, inhibit interferon-alpha release from dendritic cells, and increase the synthesis and release of IL-10 from human macrophages. Clearly, the analgesic-induced release of histamine would directly interfere with the inflammatory process. In summary, alleviating the pain or discomfort with analgesics could directly interfere with the disease progression of the virus and/or the immune mediated protection, thereby making the data collected impossible to interpret.
Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study: 28

3. Species (common name) of animals used in this study: Mesocricetus auratus (Syrian golden hamster)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. (from ASP Section F)

   Nipah virus challenge causes lethal disease in hamsters which closely mimics human disease (acute respiratory distress, encephalitis). In order to develop and characterize the immune response and vaccine efficacy we propose to use an established small rodent disease model, the Syrian hamster.

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. (from ASP Section F)

   The utilization of an animal disease model is essential for studying pathogenesis as well as the efficacy testing of vaccine candidates and anti-virals. The potential illness experienced by some of the animals exposed to Nipah virus must not be treated with analgesics because treatment will interfere with the disease manifestation thus rendering the data collected unreliable. Search of the literature (Pubmed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to alter the replication of viruses. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use clinical evaluation that will allow us to determine the humane endpoint for euthanasia. Therefore, animals will be assessed/scored when presenting with signs of disease according to the following criteria: 0 = no signs of disease; 1 = ruffled fur; 2 = ruffled fur & weight loss <5%; 3 = ruffled fur, hunched posture & weight loss > 5%; 4 = ruffled fur, hunched posture & weight loss > 10%; 5 = ruffled fur, hunched posture, weight loss > 15%, or encephalitic signs, or hemorrhagic signs, or paralytic signs or dyspnea; 6 = ruffled fur, hunched posture, weight loss > 20%, or encephalitic signs, or hemorrhagic signs, or paralytic signs, or dyspnea; 7 = death. Euthanasia will occur at a score of 5.
Column E Explanation Form For Regulated Species

This form is intended as an aid to completing the Column E explanation. 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-0016

2. Number of animals used under Column E conditions in this study: 22

3. Species (common name) of animals used in this study: *Mesocricetus auratus* (Syrian hamster)

4. Explain the procedure producing pain and/or distress, including reason(s) for species selected. *(from ASP Section F)*

Infection of hamsters with MA-ZEBOV, hamsters are susceptible to mouse adapted Ebola, could cause distress in immunocompetent animals. Recreating disease, and possibly serious disease, in these animals is necessary in order to test the efficacy of the treatments proposed. The investigator will notify the facility staff when animals begin the Column E study. The veterinary and/or program staff will monitor the animals and the investigator will be notified when the animals are clinically ill or the following signs of morbidity are observed: dyspnea, anorexia, weight loss greater than 15%. The animal will be euthanized at the specified time points or when clinical disease is considered non-reversible. Hamsters are used other small animals do not appear to recapitulate all aspects of disease caused by Ebola virus to the extent that hamsters do.

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. *(from ASP, Section F)*

Hamsters infected with MA-ZEBOV may experience pain and distress and the infection may be lethal. Search of the literature (PubMed) indicates that NSAIDs cannot be used because these drugs produce profound effects on the immune system, such as inhibition of prostaglandin and leukotriene synthesis as was stabilization of lysosomal membranes that may reduce the release of cytokines. In addition, certain classes of NSAIDs have been documented to reduce VSV replication – which could be extrapolated to affect ZEBOV replication. These affected systems are target systems being evaluated in this study. Opiates are not indicated since the pain produced consists of a non-specific malaise which would likely not be affected by opioids. Many opioids could also increase mortality due to effects on the cardiovascular or respiratory systems. Instead we will use daily clinical evaluation that will allow us to determine the humane endpoint for euthanasia.