Georgia State University
Registration Number: 57-R-0012
Customer ID Number: 907

Explanation of and reason why pain and distress could not be relieved for **Category E Guinea Pigs**:

1. Anti-inflammatory and analgesics are known to inhibit certain virus replication and modulate the immune response. Administration of pain-relieving substances will significantly interfere with the scientific objective of the study of the pathogenesis of SARS-COV-2. The animals will be very closely observed animals for any severe clinical signs.
2. The purpose of the project is to develop universal influenza vaccines which will induce broadly protective immunity against influenza viruses from different subtypes by skin vaccination. Vaccine candidates must be tested in animals to confirm if the candidate vaccines induce broad cross protection before clinical trials in humans. The studies will include challenge studies which require immunized guinea pigs to be infected with different influenza live viruses to test if animals are protected from infection (nasal virus shedding). Although influenza virus is nonpathogenic to guinea pigs, sometimes infection may result in a weak disease state. There are no alternative approaches so far. Usually infected animals recover from the infection in two weeks because the infection can trigger protective immune responses. The infected guinea pigs will be monitored closely, and those animals approaching their humane endpoints will be euthanized.
   Species: Guinea Pigs
   Number of Animals Affected: 12

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Explanation of and reason why pain and distress could not be relieved for **Category E Hamsters**:

1. One study will involve brief exposure to aggressive conspecifics to induce conditioned defeat. The purpose of the work is to determine how social defeat alters the brain and subsequent behavior. Giving a drug to alleviate the stress would alter the natural responses to this social stressor and thus defeat the purpose of the research. This behavior is naturally produced and of a brief duration.
2. One study will involve brief exposure to aggressive conspecifics and restraint stress. The purpose of the work is to determine how aggression and stress alters the brain and subsequent behavior. Giving a drug to alleviate the stress would alter the natural responses to this social stressor and thus defeat the purpose of the research. The researcher will study behavior that these animals naturally produce and that it is relatively mild and of a short duration.
   Species: Syrian Hamsters
   Number of Animals Affected: 1152

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Explanation of and reason why pain and distress could not be relieved for **Category E Cotton Rats**:

1. Some of control (unvaccinated or mock control) animals that are infected with live RSV are expected to involve mild symptoms of illness (10-15% body weight loss) but they are expected to show normal activity in the behavior of eating and movement. The goal of this project is to assess the protective efficacy after RSV vaccination of cotton rat animals compared to that of unvaccinated control animals. Mild symptoms of illness will not be treated with anesthetic, analgesic, or tranquilizing drugs. Treatment with anti-inflammatory analgesics that may interfere with the RSV disease progress in unvaccinated animals would affect the vaccine efficacy results of comparing the different vaccine groups compared to the unvaccinated group.
   Species: Cotton Rats
   Number of Animals Affected: 78

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Explanation of and reason why pain and distress could not be relieved for **Category E Ferrets**:

1. In the ferret model, Influenza infection results in airway mucus production, lethargia, loss of bodyweight, increase of body temperature, and may even result in temporary limb paralyses. Providing pharmacologic agents for pain relieve will confound the model due to interference with the host immune response. Therefore, interventions need to be kept to an absolute minimum for a rigorous assessment of the benefit resulting from treatment with the drug candidates compared to vehicle-only treatment.
2. In the ferret model, CDV infection results in lethargia, loss of bodyweight, fever, vomiting, paralyses, diarrhea, rash and possibly seizures. Providing pharmacologic agents for pain relieve will confound the model due to interference with the host immune response (https://www.ncbi.nlm.nih.gov/pubmed/16764216). Although, buprenorphine has been shown to exhibit less immunosuppressive effects on host immune response in comparison to other opioids, it has still been shown to be capable of modulating the humoral immune response (https://www.ncbi.nlm.nih.gov/pubmed/29197801) and should not be used as an analgesic. Therefore, interventions need to be kept to an absolute minimum for a rigorous assessment of the benefit resulting from treatment with the drug candidates compared to vehicle-only treatment.
3. In the ferret model, the project is to develop universal influenza vaccines which will induce broadly protective immunity against influenza viruses from different subtypes. Designed vaccine candidates must be tested in animals to confirm if the candidate vaccines induce broad cross protection before clinical trials in humans. The studies will include challenge studies which require immunized ferrets to be infected with different influenza live viruses to test if animals are protected from infection or showing decreased sickness/symptoms. The influenza virus is pathogenic to ferrets. For some weakly protected ferret groups or negative control groups, influenza virus infection may cause sickness/death including pain or distress to the ferrets. There is no alternative approach so far. Usually infected animals recover from infection in two weeks because the infection triggers protective immune responses. The infected ferrets are monitored closely and euthanized when approaching their humane endpoints.
   Species: Domestic Ferret
   Number of Animals Affected: 185