Protocol: 2001KLIFERC  
Species (common name): Ferret  
Number: 2  
Explanation of procedure producing pain and/or distress:  
The potential pain and discomfort will come from the actual progression of the highly pathogenic avian influenza virus in only a few animals.  
Justification why pain and/or distress could not be relieved:  
Most ferrets exhibit a modest rise in temperature, minimal weight loss and little to no decrease in activity when infected with the Influenza virus. Many viruses that are highly pathogenic in domestic poultry will also cause only moderate disease in ferrets. In a few animals, progression of the disease may cause more severe respiratory and systemic symptoms. When retained through the second week of infection, these animals may also develop neurological symptoms. Onset of neurological symptoms may occur as early as day 7 or as late as 13 days post-infection. Neurological signs include torticollis, ataxia and hind-limb paresis. When these neurological signs are observed, the animal is immediately euthanized. Since animals are observed twice a day, ferrets would exhibit signs for less than 1 day before euthanasia. The onset of these symptoms and progression of the disease is rapid, often measured in less than 24 hours, leaving little time to effectively treat the ferrets. The stress of receiving the treatment can also hasten the death of the ferret. For this reason, at the first sign of any neurological symptom, the animal is euthanized. In rare cases, animals may die without exhibiting respiratory or neurological symptoms and with disease apparently no more severe than those that survive.

Protocol: 2077DONFERC  
Species (common name): Ferret  
Number: 5  
Explanation of procedure producing pain and/or distress:  
Infection of ferrets with highly pathogenic H5N1 or H7N7 virus may cause severe morbidity, including neurological symptoms and death. Therefore, we believe that a Category E pain class is warranted in cases where animals exhibit severe respiratory symptoms as well as multiple organ dysfunction.  
Justification why pain and/or distress could not be relieved:  
Two kinds of drugs were considered: 1) Non-steroidal anti-inflammatory drugs (NSAIDs, COX inhibitors) and related analgesics. This class of drugs can not be used because they may alter viral replication levels, body temperature and body weight loss; all of which are important parameters in our studies. NSAID therapy is not considered because of its potentially detrimental impact on the interpretation of the effects of the vaccine. 2) Opioid drugs. These agents cause depression of respiratory control centers in the CNS. Highly pathogenic influenza virus infection in ferrets can cause an acute respiratory distress syndrome which may be aggravated by opioid drugs. Consequently, opioid therapy is not considered because of its potentially detrimental impact on the interpretation of the effects of the vaccine. Pain will be minimized by observation of the animals as frequently as 4-hour intervals to evaluate the severity of the symptoms and make the earliest possible decision (according to predetermined criteria) on humane termination by euthanasia.

Protocol: 2081BIRHAMC  
Species (common name): Hamster  
Number: 35  
Explanation of procedure producing pain and/or distress:
The focus of this study is two-fold; first, to establish a Syrian hamster model of Andes virus infection; second, to use this robust model to evaluate the efficacy of promising therapeutic drugs. The nature of the study therefore requires the animals to be infected with lethal Andes virus. Pain and distress will be minimized as much as practically possible by close adherence to the post-infection monitoring and euthanasia algorithms.

**Justification why pain and/or distress could not be relieved:**
Pain and distress will be minimized as much as practically possible by close adherence to the post-infection monitoring and euthanasia algorithms described in the protocol description section. All efforts will be taken to ensure that the hamsters experience the least amount of pain and distress as is absolutely necessary to accomplish the goals of the experiment. Analgesics have been proven to interfere with immunologic responses and can exacerbate liver damage and cause catabolism of plasma. It is also known that inflammation and inflammatory mediators play a major role in the pathogenesis of flaviviral diseases. Based on these factors, analgesics should not be used since they could affect the clinical outcome of the disease and alter the theoretical basis of the experiment.

References include:

**Protocol:** 2097MAIFER
**Species (common name):** Ferret
**Number:** 1

**Explanation of procedure producing pain and/or distress:**
Ferrets are used to model influenza virus infection because they respond in similar ways as humans when infected and they have a similar distribution of receptors in their respiratory tracts as humans. Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanatized if they reach 10 points on the 10-point euthanasia scale detailed in the Animal Use Protocol.

**Justification why pain and/or distress could not be relieved:**
Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanatized if they reach 10 points on the 10-point euthanasia scale detailed in the Protocol Description section. The focus of our study is to understand the mechanisms of transmission of influenza virus. After animals are infected with influenza virus, an initial inflammatory response occurs that is important to disease progression and virus shedding which directly affects the ability of a virus to transmit from one animal to another (1,2). Any manipulation of the inflammatory response with analgesics will affect the overall immune response and will preclude an accurate assessment of disease and transmissibility. For example, Type I and II interferon and NK cell responses are important components of the immune response to influenza. Manipulation of these responses with anti-
inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, fever, activity levels and additional disease parameters that can play a role in the frequency of virus transmission (3-6). Our preliminary studies suggest that a majority of viruses to be evaluated in this study will actually be attenuated for ferrets. Therefore, we estimate that only 25% of animals at most may experience symptoms that require euthanasia.


Protocol: 2115DAMPRAC
Species (common name): Prairie Dog
Number: 16
Explanation of procedure producing pain and/or distress:
Animals will be anesthetized during sampling to avoid pain and discomfort. However, pain associated with infection will not be treated with anesthetics or analgesics such as metacam because metacam is shown to interfere with the Nuclear Factor Kappa B (inflammatory pathway) which is believed to be an important process for pox virus infections.

Justification why pain and/or distress could not be relieved:
The nuclear factor ?B (NF-?B) transcription factor is involved in the transcription of many proinflammatory as well as antiapoptotic genes and therefore is an important component in the progression of inflammatory diseases, including poxviruses such as monkeypox (Barnes et al 1997). Orthopoxviruses appear to have acquired mechanisms to inhibit antiviral effects of NF-?B activation; encoding multiple proteins that act in various ways to prevent NF-?B activation (Reviewed by Moss and Shisler 2001; Seet et al 2003). The ability of orthopoxviruses to modulate NF-?B activation likely plays an important role in the ability of these viruses to cause disease, and therefore the utilization of substances that inhibit NF-?B activation would greatly change the normal virus lifecycle. Numerous drugs have been shown to inhibit NF-?B and therefore have anti-inflammatory results in experimental models (Reviewed by D'Acquisto, May and Ghosh 2002). These include salicylates, NSAIDs, and glucocorticoids to name a few.
Metacam is commonly prescribed by veterinarians and falls into the NSAID category. Because this study is aimed at better understanding the pathogenesis of the two monkeypox strains, including through the use of recombinant viruses, it is important that the life cycle of the two virus strains as well as wild-type and recombinant viruses not be altered by the use of NSAIDs or other anti-inflammatory agents.

References:

Protocol: 2103MAIFERC
Species (common name): Ferret
Number: 6
Explaination of procedure producing pain and/or distress:
Ferrets are used to model influenza virus infection because they respond in similar ways as humans when infected and they have similar distribution of receptors in their respiratory tracts as humans. Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanized if they reach 10 points on the 10-point euthanasia scale.

Justification why pain and/or distress could not be relieved:
The crux of our study is to understand the mechanisms of disease caused by influenza virus infection. Disease is assessed based on clinical signs observed in infected animals such as lethargy, fever, weight loss, etc... that would be obscured by pain relieving drugs. After influenza virus infection, the initial inflammatory response, as well as the subsequent adaptive T and B cell responses, are crucial to the progression of disease and directly affects the clinical signs observed in the animals (1,2). Any manipulation of the inflammatory response with analgesics will affect the overall immune response and will prevent an accurate assessment of disease. For example, Type I and II interferon and NK cell responses are important components of the immune response to influenza. Manipulation of these responses with anti-inflammatory drugs such as NSAIDS or opioid drugs directly affect virus shedding, incidence of fever and additional disease parameters (3-6). Our preliminary studies suggest that a majority of viruses to be evaluated in this study will actually be attenuated for ferrets. Therefore, we estimate that only 25% of animals at most may experience symptoms that require euthanasia.

Protocol: 2123FRAHMC
Species (common name): Hamster
Number: 10
Explanation of procedure producing pain and/or distress:
The development of clinical rabies infection is expected to occur in a subset of the infected animals. Based on prior experience with hamsters under similar experimental protocols we might expect up to 10% of animals that develop signs of rabies that progress to a terminal state before euthanasia can be administered.

Justification why pain and/or distress could not be relieved:
All animals infected with rabies virus will be euthanized at first onset of clinical signs of rabies. Beginning 7 days post infection animals will be examined at least twice daily by rabies staff and ad hoc by animal care staff during routine husbandry so that euthanasia can be promptly administered. However, occasionally animals progress rapidly from apparently healthy to a terminal state. This can occasionally occur overnight or between routine check periods. The majority of animals will be euthanized at first onset and we do not expect a significant number of animals to rapidly progress as described above.

**Protocol:** 2137RUPFOX1  
**Species (common name):** Fox  
**Number:** 2  
**Explanation of procedure producing pain and/or distress:**  
The development of clinical rabies infection is expected to occur in a subset of the infected animals. There are occasionally animals that progress to a terminal state before we are able to humanely euthanize them. However, it is critical to understand infection dynamics in this important rabies reservoir.

**Justification why pain and/or distress could not be relieved:**  
Every attempt will be made to euthanized animals at first onset of clinical signs of rabies. Beginning 7 days post infection animals will be examined at least daily by rabies staff and ad hoc by ARB staff during routine husbandry so that euthanasia can be promptly administered. However, occasionally animals progress rapidly from apparently healthy to a terminal state. This can occasionally occur overnight or between routine check periods. The majority of animals will be euthanized at first onset and we do not expect a significant number of animals to rapidly progress as described above. We would expect no more than 10% of animals developing rabies to progress and become moribund before euthanasia could be administered.

**Protocol:** 2197TUMFERC  
**Species (common name):** Ferret  
**Number:** 1  
**Explanation of procedure producing pain and/or distress:**  
Ferrets are used to model influenza virus infection because they are susceptible to virus infection and have a course of disease that is similar to humans. It is generally believed that some pain and/or distress occurs when ferrets are infected with influenza strains that are considered highly pathogenic. During FY 2010, under this protocol, one ferret was infected with highly pathogenic H5N1 influenza strain which caused significant morbidity. The animal was monitored twice daily and euthanatized when it reached 10 points on the 10-point euthanasia scale detailed in the Protocol Description section.

**Justification why pain and/or distress could not be relieved:**  
It has been demonstrated that analgesic drugs or anti-inflammatory agents affect the respiratory inflammation during influenza infections (references on form). We have chosen not to use pain relieving drugs because they can have serious consequences towards the outcome of virus infection. The main purpose of this animal protocol is to better understand the mechanisms of disease cause by highly pathogenic influenza virus infection. Disease is assessed based on clinical signs observed among infected animals such as lethargy, fever, weight loss, leukopenia and cytokine production, many of which would be obscured by pain relieving drugs. After influenza virus infection, the initial acute inflammatory response, as well as subsequent adaptive T and B cell responses are crucial to the progression of disease and directly affects the clinical signs observed in the animals. Any manipulation of the inflammatory responses with analgesics...
will affect the acute assessment of the disease. Moreover, manipulation of these responses with 
anti-inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, incidence of 
fever, and additional disease parameters as indicated in references. It is also important to note 
that this animal model is being used to model human influenza virus infection where in most 
cases; infected individuals will not be taking NSAIDS or opioids. Thus, the data obtained from 
treated animals would not model the real-life situation. Our ultimate goal is to understand the 
unmanipulated disease process so we can more precisely target strategies of treatment.
1. Immunol Rev. Pathogenesis of emerging avian influenza viruses in mammals and the host 
morbidity in ferrets. 2009 Apr;83(8):3843-51.

Protocol: 2195TUMFERC
Species (common name): Ferret
Number: 22

Explanation of procedure producing pain and/or distress:
Ferrets are used to model influenza infection because they are susceptible to virus infection and 
have a course of disease that is similar to humans. It is generally believed that some pain and/or 
distress will occur when ferrets are infected with influenza strains that are considered highly 
pathogenic. During FY 2010, under this protocol, some ferrets were infected with highly 
pathogenic influenza strains which caused severe morbidity and even death in some cases. Death 
was not an endpoint and every attempt was made to euthanize the animal prior to the 
development of severe disease. Animals were monitored twice daily and euthanatized when they 
reached 10 points on the 10-point euthanasia scale detailed in the Protocol Description section.

Justification why pain and/or distress could not be relieved:
Analgesic drugs or anti-inflammatory agents affect the respiratory inflammation during influenza 
infections. We are choosing not to use pain relieving drugs because they can have serious 
consequences towards the outcome of the infection. The main purpose of this animal protocol is 
to better understand the mechanisms of disease caused by highly pathogenic influenza viruses. 
Disease is assessed based on clinical signs observed among infected animals such as lethargy, 
fever, weight loss, leucopenia, and cytokine production, many of which would be obscured by 
pain relieving drugs (1, 2). After infection, the initial acute inflammatory response, as well as the 
subsequent adaptive T and B cell responses, are crucial to the progression of disease and directly 
affects the clinical signs observed in the animals (1, 2). Any manipulation of the inflammatory 
response with analgesics will affect the acute and adaptive immune response and will preclude an 
accurate assessment of disease. For example, Type I and II interferon and NK cell responses are 
important components of the immune response to influenza. Manipulation of these responses with 
anti-inflammatory drugs such as NSAIDS or opioids directly affect virus shedding, incidence of 
fever and additional disease parameters as indicated in the following references (3-6). It is also 
important to note that this animal model is being used to model co-infection of human influenza 
virus infection and bacteria where in most cases; infected individuals will not be taking NSAIDS 
or opioids. Thus, the data obtained from treated animals would not model the real-life situation.
Our ultimate goal is to understand the unmanipulated disease process so we can more precisely target strategies of treatment.


Protocol: 2173MAIFERC
Species (common name): Ferret
Number: 10

Explanation of procedure producing pain and/or distress:
Ferrets are used to model influenza virus infection because they respond in similar ways as humans when infected and they have similar distribution of receptors in their respiratory tracts as humans. Infection of ferrets with highly pathogenic H5N1 viruses may cause severe morbidity and even death in some cases. Every attempt will be made to euthanize the animal prior to it reaching a moribund state. Animals will be monitored daily and will be euthanatized if they reach 10 points on the 10-point euthanasia scale.

Justification why pain and/or distress could not be relieved:
The crux of our study is to understand the mechanisms of disease caused by influenza virus infection. Disease is assessed based on clinical signs observed in infected animals such as lethargy, fever, weight loss, etc... that would be obscured by pain relieving drugs. After influenza virus infection, the initial inflammatory response, as well as the subsequent adaptive T and B cell responses, are crucial to the progression of disease and directly affects the clinical signs observed in the animals (1, 2). Any manipulation of the inflammatory response with analgesics will affect the overall immune response and will prevent an accurate assessment of disease. For example, Type I and II interferon and NK cell responses are important components of the immune response to influenza. Manipulation of these responses with anti-inflammatory drugs such as NSAIDs or opioids directly affect virus shedding, incidence of fever and additional disease parameters (3-6). Our preliminary studies suggest that a majority of viruses to be evaluated in this study will actually be attenuated for ferrets. Therefore, we estimate that only 25% of animals at most may experience symptoms that require euthanasia.

Protocol: 08-011
Species (common name): Grasshopper mouse
Number: 83

Explanation of procedure producing pain and/or distress:
The goal of the study are to compare susceptibility of Y. pestis infection in two subpopulations of grasshopper mice (one potentially resistant and one susceptible). Exposure to Y.pestis could result in fatal infection.

Justification why pain and/or distress could not be relieved:
The drugs approved for use as analgesics in mouse studies are opiates, thiazine derivatives, non-steroidal anti-inflammatory drugs and anti-pyretics. Data available in the literature provide clear evidence that measurements taken in this study (cytokine expression levels, bacterial burden, and febrile response) would be altered by the administration of analgesics. Increased monitoring (at least every 6 hours) during the expected infection window and defined clinical endpoints will minimize pain and distress and are described in detail in the protocol folder.