Column E" Explanation

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1. Registration Number: **74-R-0012**

2. Number of animals used in this study: **8**

3. Species (common name) of animals used in the study: **Dairy Cattle**

4. What is the purpose of the study?

Bovine anaplasmosis is a tick-borne disease that results in estimated losses of over 300 million USD per year in the US. Bovine anaplasmosis is caused by the pathogen *Anaplasma marginale* that infects red blood cells and can result in anemia, fever, weight loss, decreased milk production, and spontaneous abortions. In naïve herds, this pathogen can cause up to 30% mortality. Treatment costs can be over 400 USD per animal in the US, imposing a financial burden on cattle producers. Recent studies indicate that current treatments may not be effective, and no commercial vaccine is available in the US.

The development of an effective vaccine is needed, but it is hindered by several factors: 1) the ability of the pathogen to escape cattle's immune responses, 2) the capacity of tick saliva to hamper the cow's immune response, and 3) the bacteria's ability to enhance detrimental effects in the immune system of the animal caused by tick saliva. This project aims to understand how *A. marginale* affects bovine skin immune responses by altering tick salivary secretions. By furthering our understanding of the tick-pathogen-host interactions at the time of initial infection, we can identify additional antigens and mechanisms that could be leveraged in the development of a vaccine that protects against *A. marginale* infection.

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:

Tick bites cause distress and discomfort.

6. 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:

We are studying the effect of tick bites and the transmission of pathogens on the immune responses. Relieve of the bite would interfere with the immune responses.

7. What, if any, federal regulations require this procedure? Cute the agency, the cods of Federal regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): **None**

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1. Registration Number: **74-R-0012**

2. Number of animals used in this study: **55**

3. Species (common name) of animals used in the study: **Guinea Pigs**

4. What is the purpose of the study?

Coxiella burentii, the etiologic agent of Q fever, is a highly transmissible pathogen with significant rick for use as a biological agent of terrorism. Currently, there are no vaccines available for widespread use in the US, and those that are available in other coutnries may cause severe adverse reactions and require time-consuming testing prior to vaccination. A new vaccine is therefore needed that is both effective in preventing Q fever and safe for routine use. Recombinant proteins and soluble fractions from C. burnetii strain will be produced and used to immunize animals. Animals will subsequently be challenged with virulent C. burnetii and the safety and protectiveness of vaccine candidates will be determined.

COVID-19 is currently a pandemic were there are no FDA approved treatments or vaccines. This study will investigate the immunogenicity of potential vaccine candidate to move into Phase I and II clinical trials. The antigens include viral spike proteins, viral core proteins, and viral envelope proteins that will be mixed with TLR agonist as adjuvants. The proteins will be produced in tobacco plants at iBio through technology that has been used before to produce recombinant proteins for use in animals. The adjuvants produced by IDRI which are TLR agonist have been tested in animals and some in humans.

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:

Guinea pigs will develop clinical disease and will experience fever, respiratory difficulty, and weight loss.

6. 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:

Clinical disease is necessary to determine the effectiveness of vaccine candidates.

7. What, if any, federal regulations require this procedure? Cute the agency, the cods of Federal regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): **None**

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1. Registration Number: **74-R-0012**

2. Number of animals used in this study: **14**

3. Species (common name) of animals used in the study: **Guinea Pigs**

4. What is the purpose of the study?

Chagas disease, caused by infection with the parasite *Trypanosoma cruzi,* is a lifelong, life-threatening infection affecting over eight million people worldwide. Blood-feeding insects of the family Triatominae, also called kissing bugs, transmit *T. cruzi* during or shorty after a blood meal when infected bug feces enters the bite wound or a mucous membrane. The speed and location of kissing bug defecation is a key determinant of the likelihood of parasite transmission to a vertebrate animal—the more quickly the triatomine defecates while taking a blood meal, the more likely transmission is to occur. These measures will not only provide insight into behaviors of understudied triatomines relevant to parasite transmission in the United States, but will also feed into a model of the efficiency of disease transmission, measuring the risk presented by insect transmitters of human and domestic animal parasites. The development of this analytical framework is critical to predicting parasite transmission and targeting the insect and vertebrate species most likely to transmit *T. cruzi*.

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:

Animals will be restrained for 1 hour in the experimental enclosure potentially causing distress.

6. 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:

Inhalant or chemical anesthesia could interfere with the behavior of the triatomine insects that will feed on the guinea pigs, and insect behavior is the key data that will be collected in the study.

7. What, if any, federal regulations require this procedure? Cute the agency, the cods of Federal regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): **None**

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1. Registration Number: **74-R-0012**

2. Number of animals used in this study: **4**

3. Species (common name) of animals used in the study: **Swine**

4. What is the purpose of the study?

Sepsis is a complex disease state characterized by both cardiovascular and metabolic responses. The metabolic state is characterized by "cachexia", which is the rapid wasting of muscle mass. Treatment of sepsis is multi factorial, and includes nutritional support aimed at minimizing the damaging aspects of the metabolic response. Commonly sepsis occurs in the intensive care unit. Mortality varies between 20-50% in the first month at the ICU and increases thereafter. Not only is high mortality associated with sepsis, but the weakening effects are long-lasting. Treatment options for sepsis are limited and often consist primarily of physiological support. Survival in sepsis is related to loss of muscle mass. Loss of more than 25% of muscle mass is incompatible with survival.

Overall Goal: The primary significance of this project is the development of a new approach to nutritional support in sepsis that will promote and preserve muscle mass and have no adverse physiological effects.

Specific Aim: To test a series of hypotheses related to responses to specific formulations of amino acids (the building blocks of protein/muscle mass) and other potential muscle mass preserving nutrients in a pigs with sepsis. Because the gut and muscle metabolism of the pig are comparable in human, the results of the study will be used to develop scientifically-based nutritional therapies for humans with sepsis.

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:

After induction of sepsis, pigs will get fever and will be feel sick (chills, malaise, lethargic)

6. 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 we know from clinical situations the animals will be distressed. No analgesics are given during the 6 hours of sepsis and 6 hours of sepsis recovery, because it will influence the metabolic study between 6-12 hours in a strong way.

7. What, if any, federal regulations require this procedure? Cute the agency, the cods of Federal regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): **None**

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1. Registration Number: **74-R-0012**

2. Number of animals used in this study: **12**

3. Species (common name) of animals used in the study: **New Zealand White Rabbits**

4. What is the purpose of the study?

Rabbits: **The rationale for choosing rabbits is that there are validated experimental animals for laboratory feeding of ticks. The use of rabbits in RNAi silencing and anti-tick vaccine efficacy assessment experiments will allow us to replicate our treatments in our proposed experiments. The alternative to rabbits is cattle. Because of space requirement in case of cattle, replication of treatments may be not feasible.**

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:

Repeated tick infestation of rabbIts could induce a very strong host immune response, which might cause strong inflammation response accompanied by itching and attempts to scratch. This may result in self - mutilation

6. 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:

Host immunity to tick feeding could be suppressed by injecting rabbits with immunosuppressant's or other anti-inflammatory agents. However this may predispose animals to infections, and most importantly, the goals of this research will not be achieved as the host's response to tick feeding will not be documented.

7. What, if any, federal regulations require this procedure? Cute the agency, the cods of Federal regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102): **None**