Column E" Explanation

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1.Registration Number: **7 4-R-0012**

2.Number of animals used in this study: **1 4**

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

4.What is the purpose of this 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: Guinea pigs are placed in a cotton mesh restraint device that allows for postural adjustment but prevents the animals from turning around. The triatomine vectors are allowed to feed on the animals for one hour per week.

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:Because we are studying the behavior of triatomine vectors during and after the time of bloodfeeding, chemical sedation will not be used because this could impact the vector's behavior

7. What, if any, federal regulations require this procedure? Cite the agency, the code 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: **13**

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

4. What is the purpose of this study?

   Coxiella burentii, the etiologic agent of Q fever, is a highly transmissible pathogen with significant risk 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 countries 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.

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

   Guinea pigs infected with Coxiella burentii can develop clinical disease and will experience fever, respiratory difficulty, and weight loss. Clinical disease is necessary to determine the effectiveness of vaccine candidates. All vaccination preps will be tested in mice prior to testing in guinea pigs. Guinea pigs will be monitored daily after infection for fever. Once animals have fever they will be monitored daily for fever, weight loss, and respiratory difficulties and scored using Karnofsky's scale designed for disease progression and stress in Guinea pigs infected with C. burnetii.

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:

   The aims of the study are to analyze immune responses elicited from vaccination and the protection afforded after challenge. Administration of analgesics would compromise the experimental design by altering the immune response in these animals. Guinea pigs develop clinical disease and will be monitored daily after infection for fever. At the first signs of fever the animals will be classified as ill and will then have respiratory rates and weight taken on a daily basis. Animals will be removed from the study if their total Karnofsky score is equal/greater than 6 or if they have a score of 4 in any one category.

7. What, if any, federal regulations require this procedure? Cite the agency, the code 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: **70**

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

4. What is the purpose of this study?

   C. *difficile* infections (CDI) most commonly occur after antibiotic treatment. Antibiotics are thought to disrupt the normal colonic flora, thereby providing a niche for C. *difficile* to colonize. Treatment of CDI burdens the US healthcare system with between $750 million and $3.2 billion in annual treatment associated costs. Thus there is an urgent need to understand the underlying biology of C. *difficile* and to produce new, targeted therapies against C. *difficile.*

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:
   The Syrian hamster has been used for approximately 30 years to study Clostridium *difficile* pathogenesis. The Syrian hamster is exquisitely sensitive to C. *difficile* infection and disease progression is similar to the disease in humans, with eventual development of pseudomembranous colitis. Hamsters are gavaged with C. *difficile* spores and monitored for signs of disease (weight loss, lethargy, poor fur coat and wet tail). Hamsters showing signs of disease are immediately euthanized, however because disease is present, hamsters are listed as Category E.

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: (*For Federally mandated testing, see Item 6 below)*
   We are examining the effects of certain bile acids on C. *difficile* virulence. Virulence involves the infection of the animal and measuring disease (see above for symptoms). As in humans, disease symptoms could be relieved with antibiotic treatment. However, this would prevent the onset of disease and would obscure the results. During the experiment, animals will be weighed daily and visually monitored 4 times daily (7:30-9am, 12:30-2pm, 5-7pm and 9-11pm) including weekends and holidays for the signs of C. *difficile* disease until the end of the experiment. Pain/distress will be monitored and if the animal appears to be in distress (i.e. anorexic, dehydrated, or moribund), they will be euthanized.

7. What, if any, federal regulations require this procedure? Cite the agency, the code 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: **7 4-R-0012**

2. Number of animals used in this study: 8

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

4. What is the purpose of this study?
   To investigate the efficacy of new drug candidates for the treatment of an intestinal parasite (Cryptosporidium) that inhibits a protein called AccD6 within the parasite. Cryptosporidium is commonly found in contaminated water sources worldwide in both developing and highly developed countries. Cryptosporidium causes diarrhea in both humans and livestock and is the leading cause of waterborne disease among humans in the United States. It can be lethal in individuals with weakened or compromised immune system such as children, elderly, and AIDS patients. Currently, there are no fully effective drugs to treat this parasitic infection so if our novel drugs prove effective, they may be able to save lives.

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

   Animals challenged with cryptosporidium may exhibit self-limiting diarrhea and fever.

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 looking for signals of efficacy and those include the length of time the animal exhibits signs of clinical illness, fecal inconsistency, and oocyte shedding.

7. What, if any, federal regulations require this procedure? Cite the agency, the code 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: **10**

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

4. What is the purpose of this study?

   To feed ticks for purposes of (1) maintain tick colonies, (2) generating tick tissue, protein, DNA, and RNA specimens, and (3) testing the anti-tick vaccine efficacy of candidate recombinant tick vaccine antigens.

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

   During tick infestation, we place an Elizabethan collar to prevent rabbits from scratching off the tick containment apparatus. Repeatedly infested rabbits can develop immunity to tick saliva proteins. In severe cases this can cause skin irritation and itching. We limit the formation of these reactions by limiting tick feeding periods to two per animal.

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: (*For Federally mandated testing, see Item 6 below)*

   The central goal of the research is to understand how ticks evade the host defense mechanism to allow the ticks to feed. By determining these mechanisms we will be able to find important tick proteins that can be used to develop anti-tick vaccines. Artificial feeding of the ticks would not be a suitable alternative since we need an intact host immune system to determine the role of the tick proteins. The potential pain and/ or distress associated with this study is due to the rabbits developing an immunological (allergic) response to the tick proteins which results in skin irritation and itching. This normally occurs after repeated tick feeding episodes. We attempt to minimize these reactions by limiting the tick feeding to two periods. Although this process eliminates most of the allergy development, we can't guarantee it will eliminate all of the reactions to the tick proteins. We could prevent animals from developing resistance to tick feeding by injecting them with immune-suppressants; however this would compromise our results since we are trying to elucidate how the tick proteins affect the host immune system. We place an Elizabethan collar on the rabbits to prevent them from scratching their ears excessively and removing the protective stockinette. In addition, the rabbits are monitored twice daily to observe any unusual or excessive reactions to the ticks or the Elizabethan collar.

7. What, if any, federal regulations require this procedure? Cite the agency, the code 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: **16**

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

4. What is the purpose of this study?
   Effect of sepsis on protein metabolism. 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

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress:
   Surgery: implantation of multiple catheters and stoma: (under general anesthesia)
   Post-surgery recovery: (pain relief is given for 4 days)
   Sepsis is induced by IV infusion of Pseudomonas aeroginosa 10-14 days after surgery
   During induction of sepsis (1st 6 hrs.), pigs will develop a fever and may show clinical signs (chills, malaise, lethargic.)
   Sepsis recovery begins after 6 hours by giving antibiotics and pain relief to mimic the human clinical condition.

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: (*For Federally mandated testing, see Item 6 below)*
   This sepsis/recovery model needs to be clinical relevant in order to translate the metabolic changes that we are studying to human situations. In most human clinical sepsis situations, treatment (including pain relief) is started after 6 hours of the start of the septic condition (when typical symptoms are diagnosed). Therefore, we do not give pain relief in the first 6 hours of the sepsis.

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