NP 1/15/18

"Column E" Explanation

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

2. Number of animals used in this study: **51**

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

4. What is the purpose of this study?

   Brucellosis is an infectious disease caused by bacteria of the genus *Brucella,* that affect many animals like, sheep, goats, cattle, deer, elk, pigs and dogs. Humans become infected by coming in contact with animals or animal products that are contaminated with Brucella. Brucellosis can cause on humans a range of symptoms similar to the flu (fever, sweets, headache, back pains, and physical weakness), infections of the central nervous systems and long-lasting or chronic symptoms that include recurrent fevers, joint pain, and fatigue. There are vaccines available for animals that have safety issues and no vaccine available for humans.

   The purpose of these studies is to evaluate new vaccine strains for the disease brucellosis in mice and guinea pigs via enhanced delivery method, microencapsulation. Specifically, we will compare microencapsulated to non-encapsulated Brucella vaccines candidates to determine the degree to which the animals are protected against different challenge routes with wild type organism, as well as to determine the optimal capsule formulation in these animal species. From these studies, we hope to gain insight for animal and human vaccine formulation.

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

   Guinea pigs are one of the most susceptible laboratory animal species to Brucella infection. Guinea pigs infected with Brucella can develop febrile response and develop bacteremia for 6 weeks after inoculation. It has also been described that infected animals might develop granulomatous lesions in the spleen and liver. We do not anticipate seeing this in the majority of our animals, since our vaccine candidates have been proven to be safe in other animal species. Only control animals that do not receive the vaccine strains, but are challenged could potentially develop the disease. To minimize the number of guinea pigs affected by these overt clinical signs, mice are being used as a first tier model to optimize vaccination deliver and components. Further, infected guinea pigs were evaluated daily for fever and weight loss and euthanized if their weight drops below 80% of their original weight.

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 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. Animals appearing depressed, anorexic or exhibiting abnormal behavior were monitored for these pain and distress criteria; if the condition persisted through a second health check, they were 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: **74-R-0012**

2. Number of animals used in this study: **16**

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: **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: **7**

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**

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

2. Number of animals used in this study: **301**

3. Species (common name) of animals used in the study: **Fox (1), Shrew (6), Squirrel (1), Raccoon (11), Field Mice (I89), Wood Rat (69), Opossum (23)**

4. What is the purpose of this study?

   Lyme Disease (LD) is caused by the spirochetal bacterium *Borrelia burgdorferi* and is the most prevalent arthropod-borne infection in the United States. In late stages of the disease, this bacterium can cause cardiovascular disorders (10% of untreated adults), as well as arthritis (60% of the cases). Almost all LD ecological studies completed to date are focused in the Northeast and Midwest regions of the US where this disease is more prevalent, with no major studies done in Southern US. There is a critical need to determine the ecological factors promoting the differential incidence of LD even though the agent, vector and mammalian hosts are present in both geographic regions. In addition, there is limited information on the genetic diversity of *Ixodes scapularis*, the tick vector for this disease, and on the role that the vector's genetic diversity has in explaining the risk of contracting LD. Host availability, diversity, and abundance vary across both spatial and temporal scales. Therefore, individuals of a given vector species may be subjected to distinct selective pressures in different geographic portions of its distribution. This may result in vector populations being structured by geography, host species, or a combination of both. Consequently, we will approach this issue by proposing two specific aims. Specific Aim 1: Quantify local host use patterns and *B. burgdorferi* infection in deer ticks in two contrasting environments in Texas within the geographic distribution of this tick species. We will also test for differences of vertebrate host communities, tick prevalence, and *B. burgdorferi* infection between anthropogenically degraded and pristine habitats at the selected Texas eco-regions. Specific Aim 2: We will test for genetic population structure and host associated genetic differentiation of the tick vector across the different eco-regions outlined above. At the completion of these studies, it is our expectation that we will have identified the local host use patterns of the tick vector, as well as the host associated genetic differences. We also expect to have delineated the distribution of *B. burgdorferi* in the studied vertebrate hosts and evaluated their competence as reservoirs for this pathogen in the state of Texas.

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

   In order to collect the ticks, we need to capture the various animals from the identified ecosystems using humane live traps and house them to collect the ticks as they feed and fall off of the animals. Although this does not cause any pain to the animals, they could be in distress since they are wild caught, and for this reason they are categorized as 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)*

No painful procedures will be performed. The animals are listed in Category E due to the potential of distress since they are wild caught. The animals will be captured using humane live traps which will be baited with food treats, set out at sunset and checked at sunup. Due to capture and caging we could see lack of appetite, although previous experiences have shown that the captive animals eat well. Shelters were provided to the animals to find a comfortable place to hide as to cope with the stress. This strategy has proven very efficient and animals tend to eat and drink fluids after caging. We provided plenty of food and water, limited contact with humans and maintain optimal sanitation and controlled light/dark periods. Since these are wild animals, tranquilization for the sake of keeping animals calm in and of itself may be deleterious and alter the animal's normal behavior, appetite and water consumption, due to extra handling of the animals.

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**