

16 NOV 2017

## **Addendum to the FY2017 APHIS Form 7023 Submission for North Carolina State University USDA Registration #55-R-005/842-Category E Report**

### **Category E Report for the Reporting Period of FY2017**

#### **Category E Study #1**

- 1. Registration Number: 55-R-005**
- 2. Number of animals used under Column E conditions in this study: 6**
- 3. Species (common name): Dog**
- 4. Explain the procedure producing pain and/or distress, including reason(s) for species selected:**

Six dogs were inoculated intradermally (two sites over the pelvis; 0.5mL/site) with  $5 \times 10^5$  TCID<sub>50</sub> of *Rickettsia rickettsii*. This inoculum was expected to produce a moderately severe infection in dogs. Fever, lethargy, scleral injection, oral pustules were noted as possible clinical effects from the normal health and behavior of an untreated animal. The goal of the study was to enhance the acute phase diagnosis of RMSF in dogs. In order to initiate diagnostic test development, it is necessary to determine the dog's immunogenic proteins by Mass spectrometry.

- 5. 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):**

Due to the fact that NSAIDS inhibit platelet function, we do not administer NSAIDS to dogs with RMSF in the clinical setting. In addition, fever is a protective response that enhances leukocyte activity and bacterial killing.

Note: All 6 dogs were infected with a low dose of *Rickettsia rickettsii* and experienced very short duration of febrile illness with spontaneous recovery within a few days.

## Category E Study #2

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1. **Registration Number:** 55-R-005

2. **Number of animals used under Column E conditions in this study:** 1

3. **Species (common name):** Dog

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected:**

Dogs of approximately 35-40 pounds are initially bled and determined to be free of infection and anti-canine *Babesia sp.* antibodies. Following at least 1 week of conditioning, the dogs are splenectomized so that the animals are unable to mount a protective immune response against these protozoans. Two weeks are allowed for recovery from splenectomy during which time the animals are monitored twice daily for temperature, as well as general body condition, and the surgical site observed for proper healing. Every 2<sup>nd</sup> day 0.5-2 mls of blood are drawn via venipuncture and monitored for PCV, differential and presence of other blood pathogens.

Approximately 2 weeks post-splenectomy, the animal is inoculated IV with a cryostabitate of the desired *Babesia* species. Monitoring of thin blood films are done at the beginning and end of each day and additionally as warranted.

When ~5- 10% its erythrocytes are infected with *Babesia* organisms, the dog is sedated and 50 – 100 mls of anti-coagulated whole blood is collected by venipuncture then the animal is euthanized. In past procedures infection has resulted in only mildly elevated temperatures (103.5) and occasional inappetence. Dogs will not be allowed to become seriously ill. Typically the time from inoculation to euthanasia is 5-10 days.

Animals will be monitored (temperature, responsiveness to human interaction, alertness) at least twice daily, more often during the final 2-3 days of infection.

Dogs are the only species that may be infected by these *Babesia* species.

5. **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):**

Past experience indicates that a parasitemia of ~ 10% is needed to prepare diagnostic antigen. Parasitemias that are significantly lower or higher are not as satisfactory.

In the unexpected event that an animal experiences severe illness such as severe depression, complete anorexia, or recumbency, the animal will be euthanized. We monitor animals very closely once parasites are seen in the blood (usually every 6-8 h) and will euthanize any animal within 12-24 h of exhibition of depression, anorexia, etc.