Column E Explanation

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1. Registration Number: 2022-0079

2. Number of animals used in this study: 70

3. Species (common name) of animals used in the study: Mesocricetus auratus (Syrian hamster)

4. What is the purpose of the study? To understand how Clostridium difficile causes disease and colonizes a host. Also, to design novel drugs to treat the infection.

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

Infection by Clostridium difficile may cause unrelieved pain or distress to the animals. We mitigate this by closely monitoring infected animals for signs of disease. Disease in hamsters is severe and progresses rapidly. Hamsters are immediately euthanized when signs of disease are evident.

Because the animals will become infected by C. difficile, normal disease progression will occur. Animals have the possibility experience fatigue or diarrhea. Signs of disease include wet-tail, lethargy, poor fur coat and weight loss and typically present 2-3 days after the inoculation with C. difficile.

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 hamsters infected with wildtype or mutant C. difficile (USDA Category E animals – infected with C. difficile) will allow us to define important stages in the lifecycle of this organism. Infected hamsters are categorized as USDA Category E animals because they have the potential to experience more than momentary pain or distress due to the infection. Anesthetics, analgesic or tranquilizing drugs are not appropriate as they may influence the outcome of the infection (as published by Maseda & Aronoff 2019) or prevent us from accurately assessing the infection status. However, non-drug-based comfort will be provided by moving nests during cage changes. This will allow the animal to have a pre-made nest in the event it is unwilling or unable to build a new nest.

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):

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1. Registration Number: 74-R-0012

2. Number of animals used in this study: 12 cattle and 30 rabbits

3. Species (common name) of animals used in the study: Bovine and Rabbit

4. What is the purpose of the study? Elucidation of acquired tick immunity mechanism in cattle and evaluation of anti-tick vaccines

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress: During the tick infestation on cattle and rabbits, there was itchiness and 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: Our study investigates the immune response of cattle and rabbits to tick feeding. One of the important parts is studying the innate immune response. The innate immune response triggers infiltration of inflammatory and immune cells at the feeding site causing pain and inflammation. Using pain/inflammation inhibition drugs would clear up the reaction and interfere with our result.

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1. Registration Number: 74-R-0012

2. Number of animals used in this study: 22

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

4. What is the purpose of the study? 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 muscle function and have no adverse physiological effects.

5. Describe what pain and/or distress occurred; and explain the procedure producing pain and/or distress: In 25 kg pigs, catheters (to allow painless infusion of medicine and test fluids and blood sampling) will be implanted in blood vessels. After a post-surgery recovery period of approx.18 days, sepsis is induced (day 0) by infusion of live bacteria for 9 hours. The controlled bacteremia induced sepsis is associated with clinical symptoms of systemic (system-wide) illness such as fever, chills, malaise, and a general feeling of sickness, but as we know from clinical situations, pain is not a primary symptom.

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: Sepsis is associated with clinical symptoms of systemic (body-wide) illness such as fever, chills, malaise and a general feeling of sickness. No analgesics are given during the 9 hours of sepsis and 6 hours of sepsis recovery because it will influence the proposed metabolic study in a strong way. During the induction of acute sepsis (first 9 hours), it has a direct effect on a key metabolic organ, the liver. Indirect effects of analgesics can occur via the cardiovascular system that can cause unexpected downregulation of the blood pressure on top of the hyper-dynamics in the cardiovascular system related to the induction of sepsis. Because the animals are lethargic and sleepy this is not preferable and can lead to less control of this model, potential leading to unexpected death. In the first 6 hours of the recovery, when lethargy and sleepiness are disappearing, an analgesic can lead quickly to anxiety/agitation, which is characterized by elevated heart rate on top of a higher heart rate associated with the septic condition. Without analgesic in the first 6 hours of recovery, there is also a very low chance of anxiety/agitation. However, as we know from clinical situations, pain is not a primary symptom. Therefore, it is acceptable to measure metabolism in the conscious state as long as the animal is not in shock, which is characterized by multiple organ failures and the necessity of ventilation with sedation {Reith, 2016, Dtsch Med Wochenschr. 2016 Jul;141(15):1082-90 ; Soerensen, 2012, APMIS 120: 909–921}.

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): n/a

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