Column E Explanation

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration number: 51-G-0001**

2. **Number of animals used under Column E conditions in this study. 973**

3. **Species (common name) of animals used in this study. Chicken**

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

   The Eimeria challenge could cause distress because a course of natural infection may occur. Chickens were chosen because avian *Eimeria* spp. are very host specific and the *Eimeria* species of interest will only infect chickens.

5. **Provide a scientific justification for why pain and/or distress could not be relieved by use of anesthetics, analgesics or tranquilizers.**

   The chickens infected with *Eimeria* oocysts may experience transient reduced weight gain and inappetence between days 4-7 post-inoculation due to multiplication of the parasite in intestinal tissue. Our goal is to elicit protective immunity in chickens so that they can withstand *Eimeria* infection and thereby not experience clinical signs of disease. Anesthetics, analgesics, sedatives, and tranquilizers are contraindicated in this protocol because they would impact primary and secondary immune responses that would otherwise limit multiplication of the parasite. Treatment with anesthetics, analgesics, sedatives, and tranquilizers may have different effects on immunized and non-immunized groups, making it impossible to test the protective effects of any vaccine.

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1. **Registration number: 51-G-0001**

2. **Number of animals used under Column E conditions in this study. 22**

3. **Species (common name) of animals used in this study. Mouse**

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

   Mice were orally inoculated with a single dose of 200, Stage 3 Ostertagia ostertagi larvae.

5. **Provide a scientific justification for why pain and/or distress could not be relieved by use of anesthetics, analgesics or tranquilizers.**

   Currently, cattle must be used to propagate cattle-specific gastrointestinal (GI) nematode parasites (e.g. Ostertagia ostertagi, a bovine stomach worm). Nematode propagation in a host species is to allow the parasite to complete the parasitic stage, developing from the infective stage 3 larvae to adult stage. To reduce the use of cattle in research the gene knockout (KO) mice were tested to determine if they were susceptible to the cattle nematode and could be used in routine nematode propagation.

   The KO mice have a lowered immune system allowing for infection by the GI nematode. The infected animals will not be treated with anthelmintics so that the animals may be observed for disease and nematode development and reproduction. In the cases of severe clinical symptoms, the animals are either treated with anthelmintics or euthanized.

   Preliminary experimentation showed that animals infected with O. ostertagi had no obvious clinical symptoms over a 45-60 day post infection. Slight to mild lymphocytic infiltration was observed in the stomach of the infected animals. The preliminary results suggest that the cattle nematode may not establish infection under current experimental conditions explaining why there were no clinical signs observed.

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1. **Registration number: 51-G-0001**

2. **Number of animals used under Column E conditions in this study. 378**

3. **Species (common name) of animals used in this study. Chicken**

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

Chickens are infected with *Eimeria*, a protozoan parasite which can cause gastrointestinal problems, such as diarrhea, decrease in feed consumption, and decreased weight gain. The species of *Eimeria* that is investigated infects only chickens. In poultry farms *Eimeria* causes economic losses and it is ubiquitous in nature and in production. To study the nature of *Eimeria* infection and test substances which ameliorate infection only chickens can be used in our studies.

5. **Provide a scientific justification for why pain and/or distress could not be relieved by use of anesthetics, analgesics or tranquilizers.**

The purpose of our studies is to determine the possible benefits of adding short chain fatty acids into poultry diets on the effects of *Eimeria i*nfection in chickens. The animals cannot be treated for the coccidiosis because that would affect impact of adding the short chain fatty acids. Therefore, clinical coccidiosis needs to be induced which is then treated with experimental diets to test their possible effects.

Another group of experiments was to determine the effect of *Eimeria* infection on the microbiome composition as well as the health of the gut. For these studies a clinical infection has to be induced and ameliorating effects of the infection would not be appropriate since we are studying the effect of the infection.

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1. **Registration number: 51-G0001**

2. **Number of animals used under Column E conditions in this study. 425**

3. **Species (common name) of animals used in this study. Chickens**

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

The main study objectives are to investigate host-pathogen interactions and vaccine protection efficacy using immunological and genomic technologies to develop antibiotic-free alternative strategies including vaccines to prevent poultry necrotic enteritis (NE). With increasing regulation on the use of antibiotics in poultry farming, there has been increasing incidence of NE in the US and other countries. *Clostridium perfringens (CP)* strains are the commensal bacteria in normal healthy chickens, and even a single infection with pathogenic CP may not cause any lesions. We used the CP model for multiple infections to evaluate the vaccine protection efficacy of bacterial CP strains responsible for NE in vaccinated chickens. In addition, *E. maxima* infection is one of the most common predisposing factors identified. We also used this co-infection model as well to study the vaccine protection efficacy. We immunized chicks with recombinant protein vaccine candidates twice, and then challenged vaccinated birds with single oral infections of *Eimeria maxima* followed by single oral infection with *C. perfringens* at 4 days post Eimerial infection. At two days post bacterial infection, birds were sacrificed for lesion score analysis and sera collection for antibody titer determination. We aimed to use NE disease models to evaluate the vaccine protection efficacy of recombinant protein vaccines, of phytochemicals and of other dietary immunomodulation strategies to reduce the negative consequences of NE.

5. **Provide a scientific justification for why pain and/or distress could not be relieved by use of anesthetics, analgesics or tranquilizers.**

The use of anesthetics or analgesics is not appropriate for this protocol for several reasons. Anesthetics/analgesics affect blood and tissue samples. The experiments are executed for a short term and sacrificing is typically done within a few days post-infection. Some of the trials rely on behavioral observations and vaccine protection efficacy can be altered by administering anesthetics/analgesics.

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1. **Registration number: 51-G-0001**

2. **Number of animals used under Column E conditions in this study. 86**

3. **Species (common name) of animals used in this study. Mouse**

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

   Citrobacter rodentium infection is an *E. coli* like organism that naturally infects mice causing disease in mice similar to pathogenic *E. coli* infections in humans. Thus, this is a useful model for gaining insight into the pathogenesis and immune response to an important class of pathogens.

5. **Provide a scientific justification for why pain and/or distress could not be relieved by use of anesthetics, analgesics or tranquilizers.**

The use of analgesics, anesthetics, sedatives or tranquilizers are contraindicated in this protocol for several reasons. First, some of these compounds can alter immune function and thus could alter the inflammatory response of the host to infection. Since we are looking for pro- or anti-inflammatory dietary effects, any agent that could potentially interfere with the normal inflammatory response would completely void any results obtained. Administration of analgesics can alter normal immune (1*) or physiological responses (2*), and can affect the activity of enzymes (e.g., prostaglandin synthesis, myeloperoxidases (3*). Furthermore, to minimize pain and suffering we carefully monitor animal behavior, especially looking for a decrease in activity, one of the clearest signs of illness. If the animals are sedated in any way, it will be difficult to assess changes in activity levels, thus making it more difficult to accurately gauge if or when the mice have become ill and potentially prolonging the time before euthanasia would occur.

1*-Graham NM, Burrell CJ, Douglas RM, Debelle P, Davies L. Adverse effects of aspirin, acetaminophen, and ibuprofen on immune function, viral shedding, and clinical status in rhinovirus-infected volunteers. J Infect Dis. 1990 Dec;162(6):1277-82.
2*-Sanchez S, Martin MJ, Ortiz P, Motilva V, Alarcon de la Lastra C. Effects of dipyrone on inflammatory infiltration and oxidative metabolism in gastric mucosa: comparison with acetaminophen and diclofenac. Dig Dis Sci. 2002 Jun;47(6):1389-98.
3*-Chou TM, Greenspan P. Effect of acetaminophen on the myeloperoxidase-hydrogen peroxide-nitrite mediated oxidation of LDL. Biochim Biophys Acta. 2002 Mar 15;1581(1-2):57-63.