

## 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. 268**

**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.**

Infection with *Clostridium perfringens*. Chickens were selected because the main objectives of the proposed studies are to investigate host-pathogen interactions using immunological and genomics technology to develop antibiotic-free alternative strategies to prevent poultry necrotic enteritis. These infections are species specific so another species could not be used.

**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 sedatives is not appropriate for this protocol for multiple reasons. Anesthetics and sedatives affect blood and tissue samples. The experiments are short term so sacrifice is typically done a few days after infection. Some of the trials rely on behavioral observations which can be altered by the use of the sedatives/anesthetics/analgesics. Also, birds may become moribund and die acutely before treatment can be instituted.

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

**2. Number of animals used under Column E conditions in this study. 80**

**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 objectives of the studies were to investigate host-pathogen interactions using immunological and genomics technology to develop antibiotic-free alternative strategies to prevent poultry necrotic enteritis. With increasing regulation on the use of antibiotics in the poultry farming, there has been increasing incidence of necrotic enteritis in the US and other countries. We used the *Clostridium perfringens* (CP) infection model to evaluate the pathogenesis capability of bacterial CP strains responsible for necrotic enteritis (NE). In addition, *E. maxima* infection is one of the predisposing factor identified. We also used this co-infection as well to study host-pathogen immunobiology using genomics tools. We used oral infections of *Eimeria maxima* (4 days) followed by oral infection with *C. perfringens*. At two days post bacterial infections, birds were sacrificed for intestine content collection for genomics study, lesion score analysis, and histological studies. We aimed to use NE disease model to evaluate various antibiotic alternative methods such as recombinant vaccines, phytochemicals and 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 sedatives is not appropriate for this protocol for multiple reasons. Anesthetics and sedatives affect blood and tissue samples. The experiments are executed for a short term so sacrificing is typically done a few days after infection. Some of the trials rely on behavioral observations which can be altered by the use of the sedatives / anesthetics. The single infection with *C. perfringens* may not cause any lesion, while dual infections with both *Eimeria* and CP infections for 2 days may cause transient lesions in intestines, and birds can recover later quickly.

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1. **Registration number: 51-G-0001**
2. **Number of animals used under Column E conditions in this study.** 1501
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. 144**

**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 infection in chickens. The animals cannot be treated for the coccidiosis because we could not determine the effect 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.