Home Office

NON-TECHNICAL SUMMARY

Investigation of Cryptosporidium Host-Pathogen Interactions

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words

infectious parasitic disease, immunity, disease prevention, parasite biology, vaccination

Animal types	Life stages
Mice	juvenile, adult, neonate, pregnant, embryo

Retrospective assessment

The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the projects objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

To understand how the host immune system recognizes and responds to a Cryptosporidium infection, and how the parasite has evolved to evade this process.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

Why is it important to undertake this work?

Cryptosporidium is a major cause of diarrheal disease and causes significant mortality, morbidity, and developmental stunting in children around the world. Annually, there are an estimated 200,000 deaths and over 10 million disability adjusted life years attributable to Cryptosporidium infection. Despite this significant impact of public health, there are no fully effective drugs or vaccines available and the basic knowledge to drive their development is scant.

What outputs do you think you will see at the end of this project?

The goal of this project is to develop a better understanding of the host-pathogen interactions of the Cryptosporidium parasite. From the perspective of the host, we hope to identify the genetic factors that play a role in resistance and immune response. From the perspective of the parasite, we hope to identify the virulence factors that the parasite uses to evade host immunity and cause infection. Through these studies we will also determine whether vaccination with certain parasite antigens can protect the host from infection and subsequent disease. Overall, we hope to broaden the foundation of knowledge that we can use to develop better therapies to treat and prevent infection. We will communicate our research as frequently as possible through public engagement and presentation, scientific conferences, and publications.

Who or what will benefit from these outputs, and how?

The Cryptosporidium research community will greatly benefit from the research we will perform. The scientific community at large will also benefit, to a lesser extent, as we hope that our research will uncover immunity and virulence mechanisms relevant to other fields. We will regularly publish our findings and communicate our research to the public. Cryptosporidiosis is a serious public health concern and we will do our best to advance the science, train new researchers, and educate our community about the disease.

How will you look to maximise the outputs of this work?

During the project we will encourage visiting scientists and students to come and learn techniques that we use and develop. We will also make these methods and protocols available through publications

whenever possible. Data will be published in a timely manner and presented often to the wider scientific community during various conferences.

Species and numbers of animals expected to be used

- Mice: 5000

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

Explain why you are using these types of animals and your choice of life stages.

We use a mouse model of cryptosporidiosis for three important reasons:

1) To mimic human disease, 2) investigate the immune response to natural infection and vaccination, and 3) to propagate Cryptosporidium parasites.

The ability to mimic human disease in a rodent model is an invaluable tool that will help us to understand disease pathology, immune response, and develop better therapeutics. Human disease typically occurs in the first 10 years of life and thus neonates, juvenile and adult mice are infected for this research (1 week to 2 months of age).

There is no cell culture system to propagate Cryptosporidium, therefore, to maintain the parasite lines that we use for both in vivo and in vitro research we must use mice. Some species of Cryptosporidium, especially those that naturally infect humans, do not reproduce well in healthy mice, thus immunocompromised mice are required for propagation.

Typically, what will be done to an animal used in your project?

Mice are required for comparative infections and propagation of Cryptosporidium parasite strains.

For comparative infections, mice will be inoculated with Cryptosporidium parasites by oral gavage. Infection will then be monitored indirectly via collection of faecal material or directly via whole animal imaging.

For propagation of parasites, mice will also be inoculated with Cryptosporidium through an oral gavage. Faecal material will then be collected for 2-3 weeks during the peak parasite shedding period of infection.

What are the expected impacts and/or adverse effects for the animals during your project?

Most mice do not develop overt symptoms and recover from infection in 2-3 weeks. Some will experience bloating, gastrointestinal discomfort, and loose stools.

Expected severity categories and the proportion of animals in each category, per species.

What are the expected severities and the proportion of animals in each category (per animal type)?

Highly immunocompromised mice occasionally show more severe illness and symptoms such as bloating and gastrointestinal discomfort; if any of those symptoms appears animal will be humanely killed.

What will happen to animals at the end of this project?

- Killed

Replacement

State what non-animal alternatives are available in this field, which alternatives you have considered and why they cannot be used for this purpose.

Why do you need to use animals to achieve the aim of your project?

The use of animals for project is required for two main reasons:

1) Mice serve as a naturally infected host in which we can study both parasite virulence and host susceptibility. To develop new therapeutics for this organism we require a better understanding of parasite biology and host immunity. Mouse models of infection allow for both.

2) There are no reliable and reproducible methods to propagate Cryptosporidium strains in vitro. Therefore, mice are required to maintain wild-type and transgenic strains required for all research.

Which non-animal alternatives did you consider for use in this project?

There are no non-animal alternatives for the propagation of Cryptosporidium. However, intestinal organoids were considered to replace mouse models to study the infection biology of epithelial cells.

Why were they not suitable?

Until there are reliable and reproducible methods to propagate Cryptosporidium in vitro, mice will be required to maintain and produce the parasite strains required for this research. Intestinal organoids, in contrast, are a suitable replacement for animals for the study of epithelial cell biology during infection.

Reduction

Explain how the numbers of animals for this project were determined. Describe steps that have been taken to reduce animal numbers, and principles used to design studies. Describe practices

that are used throughout the project to minimise numbers consistent with scientific objectives, if any. These may include e.g. pilot studies, computer modelling, sharing of tissue and reuse.

How have you estimated the numbers of animals you will use?

For genetic screens, sample sizes have been determined using power calculations based on a pilot screen of the founder populations of mice. The number of mice required for confirmatory follow up screening has been estimated based on previous comparative infection studies. For mice required for propagation of Cryptosporidium parasite lines, the number of mice has been estimated based on the number of lab members and the annual usage rate of labs that perform similar research.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?

Pilot screens were performed to determine the means and standard deviation of the parasite burden in the founder strains of mice. Sample sizes were then determined using power calculations to minimize the number of mice for each experimental group.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?

We will continue to perform pilot studies to determine samples sizes for mouse experiments with quantitative data. And for large experiments we will collect as much information (including faecal and tissue samples) as time allows to avoid repeat experiments performed to look at new parameters from a previous study.

Refinement

Give examples of the specific measures (e.g., increased monitoring, post-operative care, pain management, training of animals) to be taken, in relation to the procedures, to minimise welfare costs (harms) to the animals. Describe the mechanisms in place to take up emerging refinement techniques during the lifetime of the project.

Which animal models and methods will you use during this project? Explain why these models and methods cause the least pain, suffering, distress, or lasting harm to the animals.

In this protocol mice will be used to model Cryptosporidium infection and to propagate parasite strains. Each step has been optimized to reduce stress and suffering and mice are monitored closely during infection.

Why can’t you use animals that are less sentient?

The mouse model allows for control over host genetic factors, which is crucial to studying the pathogenesis of the disease. With this model we can investigate how the immune system recognizes

and responds to infection, which can lead to new avenues of treatment and prevention.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?

We will work closely with the veterinary staff to ensure that we are always refining our protocols to minimize harms for the animals we work with. Where applicable we may minimise use of wire mesh cage floors replacing them with any suitable bedding.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?

We will stay up to date with the best practice guidelines developed by the National Centre for the Replacement, Refinement, & Reduction of Animals in Research, and the scientific literature for estimation of sample sizes based on power calculations.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?

We will keep up to date on the latest Cryptosporidium scientific literature. Should there be an advancement that allows for us to improve our protocols, in respect to the 3Rs, we will gladly do so.