Home Office

NON-TECHNICAL SUMMARY

Control of C. difficile Infection

Project duration

5 years 0 months

Project purpose

- (c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words

No answer provided

Animal types	Life stages
Mice	adult
Hamsters	adult

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?

Our long-term aim is to develop a solution to the control of *C. difficile* infection. In the first instance this would be a prophylactic solution that prevents or reduces susceptibility to infection.

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?

C. difficile is a disease afflicting humans worldwide and in the UK almost 28,000 people are infected each year with over 1,000 deaths. Indeed, it is the predominant, antibiotic-induced hospital-acquired infection that is found in industrialised countries. This disease mostly occurs in the elderly and for those in hospital so it is important that this disease is controlled whether this be by vaccination or by the use of other interventions. No vaccine is currently available while existing treatment options are not 100% effective and often entail the use of antibiotics for which there is a globally recognised need to find alternatives.

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

This project should lead to significant improvements in our understanding of *C. difficile* infection, an important hospital-acquired infection that leads to more than 1,000 deaths annually. This work will be disseminated to the scientific community through publications and, it is hoped, to the wider community through press-releases etc. Most importantly, our work will support the development of new treatments for this disease either to prevent infection or for therapy. After 5-years, if not sooner, it is possible that we are ready to enter evaluation in humans.

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

This project should lead to the introduction of our prototype product into clinical evaluation and we anticipate applying for human studies (phase 1) within the tenure of this project.

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

We would like to publicise our results and raise awareness. This would occur through publications, conferences and participation with advocacy groups with whom we sponsor.

Species and numbers of animals expected to be used

- Mice: 4,500
- Hamsters: 1,300

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.

Mice and hamsters will be used to evaluate the ability of vaccines or other novel interventions to prevent C. difficile infection. The scientific community working on this disease numbers several hundred groups worldwide and all use mice and hamsters to evaluate the efficacy of emerging treatments. It is important that data generated here can be compared to related work that has been published. This is an important and critical step in developing a treatment for an unmet clinical need.

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

Animals will be given test vaccines or biological interventions by the oral route. This will typically range from 3-12 separate dosings. Controls may include animals injected (3-4 times) with the same or similar substance for comparison. Animals will then be given a course of antibiotics (in drinking water or by oral dosing) after which they will be administered an infective dose of C. difficile. This will be given orally and sufficient to cause infection. Animals will typically be housed in groups for 4-8 weeks but during the period of infection they will be housed individually in cages. In all cases animals are re-housed to 1 animal/cage 12-24h before the first administration of antibiotic in Protocols 2 and 3. Two types of infection will be assessed, a) colonisation, that is the ability of the pathogen (C. difficile) to grow and survive within the animal and b) virulence, which is the manifestation of symptoms of disease in the animal.

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

For animals where colonisation is assessed animals will experience no symptoms nor discomfort. For animals where the virulence of disease is to be assessed the animals will experience some level of discomfort including lethargy, lack of appetite and mild diarrhoea. The period of this discomfort is likely to be at most 12h.

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)?

Protocol 1: 100% of animals the severity is MILD

Protocol 2: Mice - 70% of infected animals will show symptoms (MODERATE) unless protection is observed. For hamsters, 100% of infected animals will show symptoms (MODERATE) unless protected.

Protocol 3: 100% of animals the severity is MILD

Protocol 4: Mice - 70% of infected animals will show symptoms (MODERATE) unless protection is observed. For hamsters, 100% of infected animals will show symptoms (MODERATE) unless protected.

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?

With current scientific product development a biologic with prophylactic or therapeutic properties (that is to be assessed in humans) it is required that efficacy is first demonstrated in one and preferably two animal models should they exist. In the case of C. difficile infection both mice and hamsters provide determination of whether a treatment option can prevent infection. Use of both animal systems is recognised by the worldwide scientific community as is evident from the plethora of international publications relating to this disease. Accordingly, regulators who facilitate evaluation of a biologic in humans will demand evidence that a product demonstrates efficacy using established animal models of infection.

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

Currently, there are no non-animal alternatives available. It is worth emphasising that the decision to enter animal testing is itself determined by a number of in vitro tests that taken together support evaluation in animals. While in vitro tests are not confirmatory of efficacy they play an important role in the decision to evaluate in animals and the likelihood of success.

Why were they not suitable?

A disease caused by a bacterium is nearly always multi-factorial, that is, the manifestations of disease result from a number of independent events. For example, production of toxins, attachment to a substrate, induction of innate immunity etc. Each of these events can be assessed individually using in vitro tests and taken together provide an indication that a test product may work but only in an animal model can this be categorically defined as positive or negative.

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?

Previous studies will have used power calculations to determine group sizes and the validity of these calculations demonstrated from previous studies. As a rule, numbers of animals used per group should be sufficient to enable statistical significance between groups. Since the studies contained here have the same primary objectives we can use the same group sizes. If the primary objective will be different a new power calculation will be performed to determine animal numbers.

We plan for between 3-6 studies per year for clients. A typical study in mice would be 50-70 animals and for hamsters 30 (total of ~2100 mice and 450 hamsters over 5 years). We estimate that SporeGen would use the same number of animals making an approximate total of ~4200 mice and ~900 hamsters over 5 years.

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

In the long-term animal numbers are reduced by careful project design reducing the need for further experiments. A core tenant of the design of animal experiments is the correct use of statistics (for interpretation of significance) and power calculations (to determine group size). The level of variability in some primary endpoints necessitates larger numbers to achieve statistically and biologically meaningful data. The most appropriate statistical tests Mann-Whitney (or the students t-test) for significance and ANOVA for analysis of variance between groups.

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

In our experience for studies are to be undertaken where large number of animals are to be used then small pilot studies using 2-3 animals/group are preferable. This is particularly important where a challenge dose must be determined or a dosing regimen established. Although statistical significance is not determined, in the long term, this avoids a negative outcome involving large numbers of animals.

Additional factors are to ensure the use of the same animal supplier (where possible) and the use of similar aged animals.

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.

Mice and hamsters (Golden Syrian) will be used to evaluate C. difficile infection using two basic approaches:

a) Asymptomatic colonisation. This model uses mice only and shows no symptoms of disease.

This model is informative for understanding whether a test product prevents proliferation of the pathogen (C. difficile) in the GI-tract. It can also provide data on the presence or absence of toxins.

b) Virulence models. In mice and hamsters using these models of infection animals will develop symptoms of disease. Using an accurate assessment of symptoms animals confirmed to be infected are killed before more acute symptoms develop ensuring minimal discomfort to the infected animal/s. To ensure accurate assessment frequent and then continuous monitoring of animals is undertaken and only using trained staff entirely familiar with the infection process and its associated symptoms in animals.

In general, vaccines and biological interventions are always tested first in mice using the asymptomatic models. Demonstration of efficacy in these models is then followed by evaluation in the asymptomatic models (mice preferred) if required and deemed necessary.

The least invasive route of substance administration and using flexible gavage needles will be used where possible. Negative control groups (baseline groups) will be minimised whenever statistically feasible.

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

Mice and hamsters are used since these are the lowest vertebrate group that can be used for in vivo evaluation of C. difficile interventions or vaccines.

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

Improvements in any process can always be made and we are receptive to any suggestions as well as our own assessments. We are keen to consider and if possible implement non-aversive handling of animals as well as practices for enrichment of quality of life for animals housed under solitary conditions. We will implement these where possible using only suitably trained staff and discussion with the RVC unit and NACWO representative as necessary.

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

NC3R guidelines. We will follow the NC3Rs guidelines on the "Responsibility in the use of animals in bioscience research" and consult all the relevant references listed therein.

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

By interaction with the Head of Animal unit at RVC and the NACWO Representative. Continuous monitoring of publications and the NC3Rs website for new and alternative models that could be implemented as part of this project and relevant information is circulated by AWERB. Whenever possible we will implement these refinements into our studies.