Home Office

NON-TECHNICAL SUMMARY

Dissemination and immune subversion of Toxoplasma gondii

Project duration

5 years 0 months

Project purpose

- (a) Basic research

Key words

Toxoplasma gondii, infection, parasitic

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures
- Required at inspector’s discretion

Objectives and benefits

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

What's the aim of this project?

The parasite *Toxoplasma gondii* infects a broad range of warm-blooded animals, including humans, and is arguably the most common parasitic infection in man. Toxoplasma has developed various strategies to escape the immune response of the host. The parasite injects proteins, so called virulence factors, into the host cell that divert cellular processes in favour of the parasite.

The acute phase of infection generally causes no symptoms in healthy individuals and proceeds silently to the chronic phase of infection with the development of cysts in tissues like the brain and heart muscle. *Toxoplasma* infection is incurable and tissue cysts of the parasite reside in those tissues for the rest of the lifespan of an infected individual. People with a non-functional immune system, such as HIV patients or recipients of organ transplant, are at high risk of suffering from re-infection due to reactivation of the dormant tissue cysts in the brain leading to the development of often deadly inflammation of the brain. Healthy individuals on the other side can lose their eyesight when infected with specific strains of *Toxoplasma* and an unborn child can have birth defects if the mother becomes infected during pregnancy. Currently there is no cure or vaccine available.

In the proposed work we aim to identify yet unknown virulence factors used by the parasite to tamper with the hosts' immune defence. We have invented a method to test 200 parasite proteins at the same time and to further analyse how essential they are for the parasite to establish (acute phase) or maintain (chronic phase) the infection. This analysis will allow us to identify and access new possible drug targets.

A retrospective assessment of these aims will be due by 29 July 2024

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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.

What are the potential benefits that will derive from this project?

This project will firstly increase our knowledge about the mechanisms the parasite uses to modulate the immune response to its benefit. This will ultimately lead to the discovery of new pathways that might equally be targeted by other pathogens, such as bacteria, or viruses, causing diseases such as malaria or tuberculosis. We will make our results publicly available to the scientific community and beyond using appropriate outreach media. Next to providing insights into the molecular mechanisms regulating the immune response to Toxoplasma and how the parasite is able to escape or modify the immune response, this study will pave the way for follow-up studies in humans to design counter-measures or a vaccine strategy.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

We will use mice in our study as they are the natural host of the parasite. We determined 5000 animals to be sufficient in the 5-year period of this project to re-sult in reliable results. We will make sure to use the minimum amount of animals per experiment to achieve results reliable results.

Predicted harms

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

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

We will infect mice with Toxoplasma and monitor their immune status during the infection. For some experiments we can use parasites engineered to emit light, enabling us to image the infection without harming the mice, comparable to imaging methods used for humans in the hospital. Nonetheless, infection with Toxoplasma can cause severe symptoms of illness and result in the death of an animal. To avoid unnecessary suffering we will treat animals with antibiotics where appropriate and closely monitor their health status. We will euthanize animals that show severe signs of infections in accordance with established humane endpoints. As Toxoplasma infections are incurable, all animals used in this study will at the end of an experiment be euthanized and tissue samples will be taken for further analysis.

A retrospective assessment of these predicted harms will be due by 29 July 2024

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

Once infected with *Toxoplasma*, all hosts including humans establish a chronic infection that is kept under control by the host's immune system. If the immune system fails, *Toxoplasma* can kill the host. We are not able to recreate the complex interplay between different arms of the immune system with state-of the art laboratory techniques, as it relies on the structural integrity and connectedness of organs and tissues. Therefore, we still need to use animals to answer our specific research questions.

Toxoplasma does infect warm blooded animals, rendering the use of alternative models, such as fruit fly and wax worm difficult. Zebra fish larvae can be used as alternative if studying the early phase of infection, but will be limited to only one part of the immune system present in humans. Also, fish are not a natural host of Toxoplasma and the results obtained by its study may not reflect the biology of host-pathogen interaction.

A retrospective assessment of replacement will be due by 29 July 2024

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

Reduction

Explain how you will assure the use of minimum numbers of animals.

We established a method that allows us to investigate 200 different Toxoplasma virulence factors at the same time in one mouse. Before, investigated virulence factors were analysed one by one instead. Our method substantially reduces the number of mice needed. This is true for our laboratory, and once we made our method available to the scientific community, laboratories worldwide. Moreover, the results of this study will inform others which virulence factors are worthwhile to study in mice and which ones not.

Our second advantage is that we can often use *Toxoplasma gondii* engineered to emit light, thus being able to image the parasite in living animals. Using this method, we can follow the infection in each animal in real time and do not need to sacrifice animals to analyse each time point individually.

To ensure we obtain meaningful scientific results with the minimum amount of mice, we calculate hwo many mice are required before the onset of an experiment. We routinely review our breeding strategies and cryopreserve any strains of mice not being actively investigated.

A retrospective assessment of reduction will be due by by 29 July 2024

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

The mouse model is currently the only extensively refined animal model of *Toxoplasma* infection. As we are studying how the immune system restricts the parasite in both the acute and chronic phase of the infection, we are reliant on a whole organism to understand the complex interplay of different cellular players of host defence.

Using the *in vivo* parasite imaging approach, we are able to administer a minimal dose of the parasite and still be able to assess differences in parasite load between wild-type and genetically altered mice. We have developed mouse body condition scoring sheet aiming for robust monitoring of any adverse effects of infection and prompt intervention where necessary.

A retrospective assessment of refinement will be due by by 29 July 2024

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?