Home Office

NON-TECHNICAL SUMMARY

Investigating the niche of mucosal parasitic nematodes and their interactions with their hosts

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

Soil-transmitted helminths, Infection, Host-parasite interactions, Immunology, Mucosa

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

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?

The aim of this project is to understand how mucosal parasitic nematodes, specifically whipworms and hookworms, invade, colonise and persist in their hosts, how the hosts respond to infection, and how the damage they cause to the host tissues (gut and lung) is repaired.

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?

Whipworms and hookworms are parasites that live inside the gut of hundreds of millions of people worldwide causing whipworm (also known as Trichuriasis) and hookworm disease. These parasitic worms can remain in their hosts for years by interacting with the lung and gut lining and surrounding cells to manipulate these organs structure and immune responses. How these parasites mediate these interactions is not understood. There are no vaccines available and current drugs are ineffective to cure these diseases. Knowledge generated from this work will open new avenues to eradicate whipworm and hookworm infections and control immune-mediated diseases.

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

By the end of this project, we will have new information on the host-whipworm relationship, including (1) how the parasite efficiently invades, colonises and persists in the gut, (2) how the host responds to this infection, and (3) how the gut lining adapts and repairs over chronic infections.

Moreover, we will have novel information on the host-hookworm relationship, including (1) how the parasite invades the lung and gut during its life cycle, (2) how the host responds to this infection, and (3) how the infection affects tissue repair responses leading to the development of chronic disease.

These findings will be published in peer-reviewed scientific journals following the ARRIVE 2.0 guidelines (a checklist of recommendations to improve the reporting of research involving animals). Moreover, these results will be presented at local, national and international scientific meetings.

Finally, the data obtained in this project will support future funding applications.

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

A better understanding of: 1) how whipworms invade, colonise and persist in the gut, how the host responds to infection, and how the gut repairs during chronic infection; and 2) how hookworms invade the lung and gut, how the host responds to infection, and how this infection affects tissue repair responses leading to the development of chronic disease, will be of considerable value to the wider helminthology and mucosal immunology research community within the time-frame of this five year project.

Findings on how whipworms and hookworms invade and colonise the mucosal tissues of their hosts will guide future studies on how these parasites sense the lung (in the case of hookworms) and gut lining cells and on the pathways they use to invade them. In the long term, this knowledge will help to develop vaccines and to discover drugs that are desperately needed to effectively and efficiently control whipworm and hookworm infections at endemic areas in the tropics and subtropics. Whipworms infect 500 million people worldwide, particularly affecting children 5-15 years of age. Hookworms infect 450 million people around the globe, and can cause iron deficiency anaemia, especially in high-risk populations (children and women of childbearing age). Whipworm and hookworm infections contribute to the vicious cycle of poverty and neglected tropical infectious diseases that prevent the economical development of endemic areas. Eradicating parasitic worm infections will help to break this cycle and positively impact the health and economy of those regions.

Data on how the gut lining adapts and repairs over chronic infections will lead to investigations on how these adaptations affect: 1) the establishment of subsequent whipworm infections; and 2) predispose to gut cancer. Data on how hookworms impact lung lining repair after their transit through the lung and how this leads to chronic pulmonary disease will lead to research on the mechanisms involved in lung regeneration upon other injury caused by similar inflammation. Moreover, these findings will yield broader insights into the mechanisms of mucosal repair upon damage that could be used to better understand other lung and gut inflammatory diseases and provide new avenues towards controlling them. Thus, in the long term, this work could result in the development of new treatments for diseases such as Inflammatory Bowel Disease and asthma, which have an incidence of 6.8 million and 262 million cases globally, respectively.

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

To maximise the outputs of this work, I will:

1) Collaborate with researchers in national and international institutions, who will provide materials and know-how that will complement this research project.

2) Disseminate openly and promptly the data, methods and results (including both successful and unsuccessful approaches) of this project to the scientific community via: 1) sharing data in open access online databases; 2) publications in leading international peer-reviewed scientific journals; 3) presentations in local, national and international scientific conferences. Participation on conferences will lead to the development of new collaborations in which the know-how and knowledge generated in this project will be shared with others to tackle important questions focused on the interactions other parasitic worms with their hosts and their impact on tissue repair and immunomodulation.

Species and numbers of animals expected to be used

- Mice: 11230

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.

Currently, there is no laboratory model to study the human whipworm (*Trichuris trichiura*). Therefore, for our studies we use a mouse model of infection with the natural rodent-infecting species *T. muris.*

While there is a laboratory model for human hookworms (*Necator americanus*) by infecting immuno-supressed hamsters, the data from this model may be misleading when applied to human hookworm host-parasite biology. Therefore, for our studies we will use the rodent parasite *Nippostrongylus brasiliensis* to infect mice*,* as it is the most commonly employed laboratory model of experimental hookworm infection.

These are good and widely accepted models for this research as they closely mirrors infections in humans.

We infect/stimulate with parasite products adult mice (6-8 weeks old), both wild type and genetically altered animals, where the immune system has completely developed.

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

Genetically altered mice will be bred in order to study the function of intestinal and alveolar epithelial, immune and stromal (connective tissue) cells and to label and visualise these populations in the tissue. These mice may require a fresh tissue sample taken for confirmation of their genetic make-up (genotype) by ear punching (commonly 2 mm in diameter). This is not expected to cause lasting harm.

Wild type and genetically altered mice will be infected via ingestion (oral gavage, OG) of mouse whipworm (*T. muris)* eggs or larvae. A high dose (200-3000 eggs/larvae) recreates an acute infection, where mice expel the worms after 2 weeks, and a low dose (20 eggs) recreates a chronic infection, where mice remain infected for months. Mice will be infected for different times (up to 10 weeks) depending on the experimental question.

Wild type and genetically altered mice will be infected via injection under the skin (subcutaneously (SC)) of 200-500 rodent hookworm (*N. brasiliensis*) larvae or stimulated with hookworm products via the nose intranasally (IN). Mice expel the worms around day 7 post infection (p.i) but will be studied up to 300 days p.i. depending on the experimental question.

In some experiments mice will be administered orally (OG) or injected into the abdomen, nose, trachea, muscle, vein or under the skin (intraperitoneally (IP), intranasally (IN), intratracheally (IT), intramuscular (IM), intravenously (IV) or subcutaneously (SC)) with molecules, drugs or cells that activate or suppress the immune response, to evaluate the role of specific mediators during infection.

In some experiments, mice will be administered in diet or drinking water, orally (OG), or injected into the abdomen, nose, trachea, or under the skin (intraperitoneally (IP), intranasally (IN), intratracheally (IT), or subcutaneously (SC)) with substances to induce or delete genes in genetically altered mice, to label and visualise cells in the tissue and analyse the fate of their daughter cells.

In some experiments mice will be given drugs to kill the whipworms via ingestion (OG).

In other experiments, mice will be exposed to radiation (irradiated) to deplete their immune system and replace it with immune cells from another mouse strain, to understand the importance on genes in different cellular populations during infection.

In some cases blood and/or faeces will be sampled to measure immune response in the blood over the course of the infection and changes in the faecal microbiota, respectively. Faeces will be collected by scruffing the mouse or placing it on an empty shallow container for sufficient time (maximum 5 minutes) to allow the mouse to defecate.

Mice will be subjected to non-recovery anaesthesia, under which the mice remain deeply unconscious, in experiments where blood will be collected from the heart (via cardial puncture). Mice will be humanely killed immediately following the cardiac puncture procedure.

Mouse lung and gut lining, immune, stromal cells and whipworm/hookworm responses during infection will be assessed by doing an extensive study of mouse organs after the animals are killed at the end of each experiment.

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

Expected adverse effects for the mice during this project would include:

1) Wasting disease with hunched body posture, ruffled fur, weight loss and diarrhoea that occurs after 19 days of whipworm infection. However, these symptoms (phenotypes) are rare and in cases where this is observed, mice will be closely monitored to ensure that the animal shows no signs of pain or distress and does not become dehydrated. In case the condition of the mice does not improve, and if signs reach predetermined humane endpoints such as weight loss greater than 15% plus clinical signs of disease, the animals will be humanely killed.

2) Labouring breathing on days 2-4 of hookworm infection, which resolves in 24 hours. Mice will be closely monitored during this time and in case and animal shows signs of pain or distress and signs reach predetermined humane endpoints such as weight loss greater than 15% plus clinical signs of disease, the animals will be humanely killed.

3) Upon injections, one could observe bleeding at the site of injection or inflammation at the injection site. Animals that display any of these signs will immediately be humanely killed.

4) Toxicity and/or allergic reaction to anti-parasitic (helminth) drugs. However, these drugs have been extensively employed in mouse (murine) models of trichuriasis, and rarely induce toxicity and/or allergic reactions at the doses to be employed in the current project.

5) Suppression or over-activation of immune pathways due to administration of immunomodulatory substances that could lead to signs of sickness behaviour or transient weight loss. We expect in the majority of cases adverse effects will resolve within 48 hours of the treatment being administered, or treatment being ceased.

6) Administration of substances to induce or delete genes could lead to transient weight loss. We expect in the majority of cases that this will resolve within 2-7 days of the treatment being administered.

7) Irradiation of mice could result in infection. However, the implantation of donor cells prevents adverse effects of irradiation, and therefore, when used in this way, irradiation is not expected to result in any adverse effects. In the result of an infection, antibiotics may be supplied in the drinking water. However, in rare occasions irradiated animals can develop acute radiation toxicity and clinically present with one of the following signs: weight loss, lethargy, poor coat condition, goosebumps (piloerection), hunched body posture and diarrhoea. In the event of at least one sign of acute radiation toxicity, mice will be immediately culled. Moreover, all mice will be closely monitored and daily weighed, following all aspects of the procedure as well as at days 7-10 post procedure for unexpected graft rejection causing signs of pain and distress displayed by adverse clinical signs such as hunching, reduced movement and piloerection. In the event of an animal showing up to three of these clinical signs, i.e. from 1 to 3 signs, it will be killed immediately.

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)?

For whipworm infection:

Mouse Sub-threshold: approximately 30%.

Mouse: Mild 60%.

Mouse: Moderate 10%.

For hookworm infection and stimulation with hookworm products:

Mouse Sub-threshold: approximately 30%.

Mouse: Mild 60%.

Mouse: Moderate 10%.

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?

Currently, there is no model to study human whipworms *(T. trichiura)* and the disease they cause, Trichuriasis. The use of mice is essential to study the overall gut lining and immune responses to whipworm infections. This is because the gut lining and immune response to whipworms is very complex and cannot be replicated in cells in a laboratory *(in vitro)*.

N. brasiliensis infection of mice is the most commonly used experimental model of human hookworm (*N. americanus*) infection as they have a very similar life cycle. The parasites infect through the skin, migrate through the lung from where they are coughed up and swallowed to finally reach the intestine. This model is essential to study the overall lung and gut lining and immune responses to hookworm infections. This is because the lung and gut lining and immune response to hookworms is very complex and cannot be replicated in cells in a laboratory *(in vitro)*.

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

In this project, I will use *in vitro* models of whipworm and hookworm infection that uses mini-lungs and mini-guts (organoids), which I recently developed. Currently, these models use mouse organoids and the rodent hookworm (*N. brasiliensis*) and whipworm *(T. muris)*, but I am actively working to translate these systems to the human hookworm (*N. americanus*) and whipworm *(T. trichiura)* using human organoids.

Why were they not suitable?

Unfortunately, these *in vitro* models does not fully recapitulate the complexity of the lung and gut or the life cycle of the parasites. Thus, to study complex hookworm and whipworm interactions with the lung and gut lining and immune cells during infection and to validate the *in vitro* observations, infections of live animals are essential.

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?

My extensive previous experience, and that of collaborators at the University of Manchester, of infecting mice with whipworms and hookworms, enables me to make good estimates of how many animals will be required for each experiment.

To estimate the numbers of mice I will use in this project I have first identified the experimental unit and calculated the group size by using statistic methods (power calculations) based on previous experiments. Then, for each experiment, I have calculated the numbers of groups required and considered each experiment needs to be performed independently up to three times.

Moreover, I have estimated the numbers of animals I need to breed from the different strains I will use in this project, in order to obtain the mice required for experiments.

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

Extensive review of the scientific literature has been conducted to enable predictions for likely outcomes of experiments planned, so the minimum number of mice can be used.

Then, for designing my experiments, I run power calculations based on pilot or previous experiments and use the NC3Rs Experimental Design Assistant (EDA) and the PREPARE guidelines. I randomise mice on experimental groups, and when possible, I blind the experiments. Control animals, either uninfected or wild type (depending on the experiment) are always included. I use age and sex matched cohorts to reduce variability. These measurements ensure animal numbers are at a minimum and welfare is maximised.

I will conduct my experiments in such a way that I will be able to publish according to the ARRIVE guidelines.

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

To optimise the number of animals I plan to use in this project, I will:

1) Optimise genetically altered mice colony breeding and management, and track it using a software to manage the mouse colonies. This will ensure there is as little over-breeding as possible. This project will aim to keep “surplus” animals to a minimum. In order to reduce the numbers of breeding pairs the mice will be kept as purebred lines (when appropriate), provided that they do not show any signs of disease.

2) Perform pilot studies for experiments I will run for the first time. Data from these experiments and from previous projects will feed into power calculations.

3) Participate in animal sharing schemes when possible to make others aware of available tissue of uninfected mice used as controls of my experiments.

4) Some of the samples collected from mice as part of experiments planned will be stored long term at -20C or fixed for histology. This will make the samples available for future analysis by scientists working on this project, and also for any collaborators.

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.

During this project, I will use the mouse model of whipworm infection with the natural rodent-infecting species *T. muris.* This model has been selected for this project as it is the best way to study human gut lining and immune responses to whipworms.

In addition, in this project, I will use the mouse model of hookworm infection with the rodent parasite *N. brasiliensis.* This model is the most commonly used experimental model of human hookworm (*N. americanus*) infection as they have a very similar life cycle and will allow me to study the overall lung and gut lining and immune responses to hookworm infections.

The mouse also benefits from well-established and robust technologies for modifying their genes. Where possible we will use experiments of the shortest duration so long as to do so will yield satisfactory data.

In this project mice will be infected with whipworms or hookworms or stimulating them with hookworms products, then their lung and gut lining and immune responses will be studied by collection and analysis of different organs after death. In order to understand the function of host genes on the response to these parasites and visualise different cells, we will need to: 1) infect with whipworms or hookworms genetically altered mice lacking those genes or where cells are labelled; 2) treat mice with substances (including, but not restricted to, products of the parasites, antibodies, inhibitors, drugs); or 3) replace their immune system with the one from other mice. These procedures can cause clinical signs, but it is essential that we follow the outcome of infection to fully investigate how worms invade, colonise and persist in their hosts and how the tissue repairs after damage caused by the infection. Previous experience has shown that weight loss can correlate closely with the disease caused by whipworms and hookworms. Monitoring weight loss is therefore an effective way of determining infection, and is a widely used and accepted measure. However, weight loss is not the only measure of infection and we will use a comprehensive monitoring and scoring system to assess the animals throughout experiments and ensure they do not undergo pain and suffering.

Procedures will be performed by competent personal licensees and we will endeavour to minimise animal suffering. We have access to a state-of-the-art animal facility, staffed by highly trained and dedicated animal technicians and managers that have access to a sophisticated database to track the health status of every animal and alert when there is a loss of condition.

Infection is achieved by ingestion of whipworm eggs via feeding (oral gavage) or injection of hookworm larvae under the skin. These route of infection replicates infection in nature.

In some experiments, mice will be stimulated with hookworm molecules via the nose using the minimal volumes required.

In some experiments, during the infection, mice will be injected with substances in the abdomen, veins, nose, muscle or under the skin using the minimal volumes required and adequate needle gauge to cause the least pain.

In other experiments, mice will be given substances in their diet or drinking water.

In some experiments, mice will be given with drugs via feeding (oral gavage) to ensure the animals are given the correct dose.

In other experiments, mice will be irradiated to deplete their immune system and injected with immune cells from another mouse strain (intravenously) using the minimal volumes required and adequate needle gauge to cause the least pain. After these procedures, mice will be closely monitored to early identify any animals showing signs of rejection of the replacement immune cells so that appropriate intervention can be taken promptly to avoid unnecessary suffering. In the event of infection, irradiated mice may be treated with antibiotics in their drinking water.

Mice will be subject to non-recovery anaesthesia in experiments where blood will be collected from the heart.

Finally, this project aims to use non-surgical embryo transfer for breeding genetically modified mice. This method is a refinement to the existing surgical method of embryo transfer.

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

Mice are the least sentient animals available that share the anatomy and functions of this disease as humans. Most of the mice in this license will display mild symptoms upon infection with whipworms and hookworms.

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

In this project, to minimise the harms for the animals the following husbandry/care measures will be taken:

- Monitoring will be specifically tailored for the procedure in question and take into consideration the strain of mice, the stage or phase of the disease development and the rate of change of the animal’s condition. Thus, mice will be checked at least daily and weighed at least weekly from the point of infection to carefully monitor for adverse clinical signs and general welfare of the animal. A record of weights and animal condition will be kept on an electronic database.

- Refinement of housing and care will be assured, e.g. use of soft, non-tangling nesting material, provide effortless access to easy-to-eat food and water.

- Before all procedures, mice will be acclimatised to handling.

- For any procedures requiring anaesthesia, mice will be monitored closely for the duration of their recovery.

- Carefully consider needle gauge, and keeping volumes and doses to the minimum necessary, for injection of substances.

- The duration of experiments will be reduced, provided this is compatible with the study aims, i.e. all the necessary data can be obtained within the study time.

- The risk of aggression will be reduced by establishing groups early, using littermates, ensuring that animals are not subsequently mixed where possible, and selecting appropriately designed refuges in the cage.

- Assure that all those responsible for assessing animals should receive adequate training in recognising indicators of suffering associated with murine trichuriasis such as diahrrea, dehydratation, piloerection and hunching and murine hookworm disease such as laboured breathing, piloerection and hunching .

- Mice will be monitored in the next hour after they undergo any regulated procedure.

- Ensure that appropriate welfare assessment protocols are defined and regularly reviewed.

- Keep up with development in animal welfare monitoring technology.

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

In this project the following guidance will be followed to ensure experiments are conducted in the most refined way:

1) Laboratory Animal Science Association (LASA) guidelines to make sure all experiments are conducted appropriately. In particular we will follow the 'Guiding principles on good practice for Animal Welfare and Ethical Review Bodies'.

2) PREPARE guidelines for planning experiments, and ARRIVE guidelines for thorough, responsible reporting of results.

3) NC3Rs recommendations on non-aversive mouse handling, genetically altered mice, welfare assessments, euthanasia and anaesthesia, and administration of substances to mice.

4) LASA and NC3Rs guidance on dose level selection for regulatory general toxicology studies for pharmaceuticals.

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

To stay informed on advances in the 3Rs, the duration of this project the following websites will be regularly checked:

NC3Rs website: http://www.nc3rs.org.uk.

RSPCA website: http://science.rspca.org.uk/sciencegroup/researchanimals

Moreover, we will register for the regular NC3Rs e-mails and newsletter updates. Regular reference to guidance documents provided by Laboratory Animal Science Association (LASA) and the RSPCA will be made.

Researchers working under this project will also engage regularly with the organisational teams in the facilities in which our mouse work is conducted, including NVS, NACWO and Named Information Officer, to discuss on how to implement 3Rs advances. Any new recommendations will be incorporated into our experimental plan wherever possible.