Non-technical summary: Understanding the replication of and immune responses to coronaviruses in pigs

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
(iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes

Key words
coronavirus, respiratory disease, virus replication, immune responses, PRCV

Animal types	Life stages
Pigs	juvenile, 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?
The aim of this project is to establish a model of porcine respiratory coronavirus (PRCV) infection in pigs to investigate protective immune responses to infection and vaccination. This will include two pilot studies which will inform a subsequent follow up studies with selected PRCV strains. This model will help understand protective immune responses to coronaviruses, with relevance to SARS-CoV-2 infection of humans.

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?
The coronavirus responsible for the COVID-19 pandemic, SARS-CoV-2, is a new virus about which very little is known. Animal models are being developed which show susceptibility to infection, however the virus is not known to naturally occur in any of them. Investigating the immune responses to both infection and vaccination of a naturally occurring respiratory coronavirus in pigs, would allow detailed host responses to be defined and help understand the biology of respiratory coronaviruses.

What outputs do you think you will see at the end of this project?
The output from this project will be development of a porcine respiratory coronavirus model which mirrors the pathology seen in COVID-19 / SARS-CoV-2 infection of humans. This model would then be available to study various aspects of vaccinology, immunogenicity and therapeutic interventions as a tool to assist COVID-19 research. Increasing the duration of

the licence and number of animals will allow us to define the best targets for second generation vaccine and whether or not local T cell responses are essential to obtain the best protection. This cannot all be achieved in 2 years.

Who or what will benefit from these outputs, and how?
Novel therapeutic and prophylactic anti-viral solutions are urgently required to control the spread of emerging coronaviruses including SARS-CoV-2. An understanding of coronavirus replication and the host responses to coronavirus infection will inform future strategies to protect the human and livestock populations from potential new incursions of novel coronaviruses.

This project aims to develop porcine respiratory coronavirus as an animal model to understand more about how coronaviruses cause disease and the immune responses that are raised against coronaviruses and vaccines in the natural host.

Our studies will benefit researchers working in the fields of molecular virology, virus evolution and particularly, coronavirus research. However, it will also be important to medical researchers investigating antiviral therapies.

How will you look to maximise the outputs of this work?
The data will be shared in open-access data repositories a soon as practicable to allow other researchers to benefit from these results. Knowledge generated by this project will be widely disseminated to the research community as soon as practicable through open-access peer-reviewed journals and presentations at national and international virology conferences, collaborative discussions and interactions with members of the scientific community. Conference attendance will allow the dissemination of results and facilitate collaborative discussions, enhancing potential outputs from the proposed project.

Species and numbers of animals expected to be used
Pigs: 180

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.
Pigs are being used as they are the natural host for porcine respiratory coronavirus (PRCV), and this infection has previously been shown to produce very similar lung disease as SARS (and by extension COVID-19). The ages of pigs being used are in line with previously published literature.

Typically, what will be done to an animal used in your project?
The duration of the pilot experiments is likely to be 14 days , with the first study only lasting seven days. Follow up studies to investigate local and systemic immune responses to PRCV may last up to 25 days after infection, while the typical duration of vaccine evaluation studies is 21 to 49 days long. Studies will involve taking swabs and blood samples from the pigs. The pigs will be infected by virus administration either i) directly into the nose; ii) into the nose and into the trachea via the mouth or iii) by aerosol using a close-fitting face mask following sedation. Animals will be monitored closely at least 2 times a day. Further swabs and blood samples will be taken from the animal between being inoculated and being culled at the end of the study. At the end of the study, the pigs will be killed by administering an overdose of anaesthetic.

What are the expected impacts and/or adverse effects for the animals during your project?
Animals will experience mild and transient pain associated with a blood sample being taken, which includes restraint and insertion of a needle through the skin. The same level of discomfort will be experienced by those pigs which are sedated. The effects of PRCV in pigs are mainly either subclinical or mild. The mild signs will be intermittent coughing or a mild elevation in body temperature. Relatively rarely, animals infected with PRCV will have difficulty breathing, become lethargic or anorexic. In this project, any animals which show these more severe signs will be killed if the duration exceeded the endpoints in this licence.

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)? According to the cumulative literature, 95% of animals are expected to show either no or mild clinical signs. Less than 5% are expected to show moderate clinical signs but one of the objectives in the pilot is to develop the PRCV model and evaluate the clinical signs in pigs. The two pilot studies showed that no animals developed moderate or severe clinical signs, with some animals developing mild clinical signs which did not last for more than 1-3 days.

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

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?
Animals are required to study the immune responses to virus infections.

Which non-animal alternatives did you consider for use in this project?
Cell culture and molecular biology techniques will be used to characterise PRCV in the laboratory before infecting pigs. We will use laboratory techniques to understand the responses of the pigs to infection with PRCV using blood samples and tissue samples taken post mortem. We shall also use a porcine respiratory epithelial cell model and tracheal organ cultures to study virus replication. We have also established in vitro porcine lymph node and spleen organ cultures to evaluate antibody and T cell responses.

Why were they not suitable?
It is not possible to analyse pathogenicity and protective immune responses to virus infection without using a host animal because the immune system is very complex.

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?

The number of pigs in the first pilot study is based on the available literature and involves assessing viral kinetics in the first pilot, to determine breed and virus specific effects. Once determined, the second pilot will involve using data from the first pilot study and undertaking staged post mortems to assess progress of pathology of the virus in the host determined in the first pilot study. After these two pilot studies have been completed the data generated will be used to determine optimum animal numbers per experimental group. Based on the data from the two pilot studies we have identified PRCV strains inducing different lung pathology. In order to understand the mechanisms of pathogenicity we will compare the early and late immune responses induced by the pathogenic and attenuated PRCV strains by performing staged post-mortems at different time points from day 1 to 25 after infection on 5 animals at each time and for each virus. 4 time points x 5 animals x 2 viruses = 40 animals. Differences in response between virulent and avirulent porcine coronavirus strains can be detected with 79% power and 95% confidence using groups of five pigs.

We will perform experiments to identify exactly what parts of the virus are recognised by white blood cells called CD4 and CD8 T cells, which are important for protection against viruses. We will first use inbred Babraham pigs for this because individual outbred animals may recognise different parts of the virus. When we have identified how many different parts of the virus are recognised by T cells of Babraham pigs we will test how many parts are recognised by outbred pigs and whether, if we incorporate these parts into vaccines, they will induce protective immunity to the virus. Although such regions of the virus may be identified by sequence analysis these have to be tested experimentally to confirm that they induce immune responses and are protective. The animal numbers for the vaccine studies will be determined in consultation with a statistician.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?
As these are pilot studies, the design of the experiment is based on the published literature on this subject. The third study to evaluate the pathogenicity and immune responses to virulent and attenuated PRCV strains is based on data for viral load, lung pathology and antibody and T cell responses from the two pilot studies. Some studies will use inbred Babraham pigs to define the regions of the virus recognised by CD4 and CD8 T cells. Inbred pigs have identical molecules called SLA which are important for allowing T cells to recognise parts of the virus. This may allow for reduced number of animals to be used.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?
Pilot studies will be used to optimise the number of pigs required for future experiments. During the pilot studies we have collected various tissues from the respiratory and gastrointestinal tract to assess viral load and immune responses. We also established in vitro cultures using trachea, lymph nodes and spleen from infected animals to further characterise viral replication and immune responses in vitro. These studies will validate the finding from in vitro culture with in vivo responses.

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.
We will take blood samples from pigs, sedate them to avoid causing distress and infect them with a porcine coronavirus. According to the available literature, most of the pigs will experience mild clinical signs (Jung et al, J Virol 2007, DOI: 10.1128/JVI.01702-07; Charley B et al Ann N Y Acad Sci, 2006 DOI: 10.1196/annals.1373.014). They will be closely monitored by experienced staff throughout the study. Blood samples and swabs will be taken at regular intervals and the pigs will be killed at the end of the study to evaluate the pathology caused by the virus and assess the immune responses in the pigs. The blood samples and swabs will not cause lasting distress or harm to the pigs.

Why can't you use animals that are less sentient?
Pigs are a good model for human respiratory disease due to their size and the shape of their lungs. They are also a natural host of a respiratory coronavirus.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
These are two pilot studies and the pigs will be monitored closely by experienced staff throughout the experiment. The results of these studies will be used to inform future experimental design.

We have included the trans-tracheal method for administering PRCV to the pigs as a refinement. The trans-tracheal method involves reduced time under anaesthesia and less potential injury to the larynx than the intra-tracheal method more commonly used in PRCV studies in the literature. We have developed a purpose built mask for aerosol administration which allows maximum exposure in the minimum time.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
We will follow guidelines from the NC3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
We will consult the AWERB for advice about the 3Rs and participate in the annual 3Rs workshops organised onsite.