Non-technical summary: Understanding mechanisms for controlling picornavirus infection

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
Picornavirus, Conserved Epitopes, Protective Immune Responses, Antivirals, Disease Resistant Animals

Animal types	**Life stages**
Mice	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?
Viruses in the picornavirus family include important pathogens of animals and humans. The overall aim of this project is to use mouse models of infection to understand the replication of these viruses and to develop novel approaches to control the diseases they cause.

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 animal picornavirus relevant to this application is foot-and-mouth disease virus (FMDV), which is responsible for one of the most significant diseases of livestock, leading to large economic losses due to reduced productivity and trade embargoes for areas not certified as disease-free. The disease is endemic to many low-income countries, compromising economic and social development and contributing to poverty and poor nutrition in rural communities. Occasional outbreaks of FMD also have a significant impact in high-income regions in which the disease is not normally present, with the UK outbreak in 2001 leading to losses of ~£10 billion. Vaccination can be used to control FMD but current vaccines have notable shortcomings, such as short duration of immunity and poor protection against diverse circulating strains. In order to overcome these shortcomings, there is a requirement to understand how to induce more broadly protective and longer lasting immune responses. An alternative strategy to prevent FMD in livestock is to genetically modify the animals so that they are no longer susceptible to the virus. The concept of such genetic modification (GM) has been significantly refined in recent years by a process known as gene editing where advantageous traits can now be introduced with very subtle changes to the animal

genetics and without insertion of any foreign genetic material. The work in this project is required in order to assess the suitability of such technology for generating FMD resistant animals.

Human pathogens in the picornavirus family are exemplified by viruses in the enterovirus genus of the family which includes human rhinovirus (HRV). HRV is thought to infect humans more frequently than any other virus, being responsible for approximately 70% of all respiratory tract infections (the common cold) and was estimated in 2003 to cost $40 billion annually in healthcare and lost productivity in the US alone. In the last two decades it has been recognised that HRV infection is also associated with more serious clinical outcomes such as severe lower respiratory tract infections of infants and exacerbations of chronic lung diseases (asthma, cystic fibrosis and chronic obstructive pulmonary disease). HRV therefore creates an enormous direct and indirect social and economic global burden. There are no vaccines or drugs available to control or treat HRV. HRV is the main target enterovirus for this work but the approaches proposed would also be of relevance to a number of other important and closely related viruses in the family. For example, a number of enteroviruses have emerged in the last decade to also become significant concerns for regional and global public health, such as enterovirus 71 (EV71), enterovirus 68 (EV68) and Coxsackieviruses which are responsible for large epidemics in China, South East Asia and the US and which have featured severe disease including neurological complications and child deaths. The work in this project will contribute to novel antiviral or vaccine approaches to control these viruses.

What outputs do you think you will see at the end of this project?
At the end of this project we will have gained new understanding of i) the relationship between virus replication, pathogenesis and immune responses, ii) the anti-viral effect of antibodies capable of recognising all variants of a virus, iii) antigen persistence and its role in enhancing antibody responses and iv) novel antiviral approaches and host genetic targets for engineering disease resistant animals. All the outputs will be communicated by publication in high quality peer reviewed, open access scientific journals.

Who or what will benefit from these outputs, and how?
In the short-medium term, the new knowledge derived from this work will have academic impact from improved understanding of virus replication and antigenicity. Information relating to antibody responses will be translated to further studies in the natural target species for FMDV, in order to develop improved vaccines to control FMD. Gene targets found to be suitable for editing for engineering disease resistant animals will be translated to studies to make gene edited pigs resistant to FMD. Novel approaches for vaccines and antivirals against HRV or other human viruses identified in this work may lead to human clinical trials. In the long term this work could contribute to improved vaccines against FMDV, the development of FMD resistant pigs and other livestock, novel vaccines and antivirals for HRV and other human picornaviruses.

How will you look to maximise the outputs of this work?
The outputs of this work will be instrumental in guiding i) strategic research to improve FMD control within the establishment, ii) existing collaborative studies aimed at generating disease resistant animals, iii) existing collaborative studies to investigate novel vaccines for HRV. Knowledge will be further disseminated through scientific meetings and networks such as the Global foot-and-mouth disease Research Alliance (GFRA) the Food and Agriculture Organisation (FAO) of the United Nations, the European Study Group for the Molecular Biology of Picornaviruses (EUROPIC) and medical networks via clinical collaborators.

Species and numbers of animals expected to be used
Mice: 1000

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 will be used for these studies because the use of adult mice as models for studies of immunogenicity and virus infection are well established, such that experiments can be designed with predictable outcomes that minimize pain, suffering, or distress. Inbred mice will be used to ensure uniformity between all animals, increasing the predictability of experiments and reducing the number of animals required. The use of mice also provides access to existing collections of gene knock out animals which are readily available from UK and global repositories.

In addition, measures for refinement of experimental conditions such as enhanced environment can be implemented effectively.

Typically, what will be done to an animal used in your project?
A typical animal might experience up to four injections of immunizing antigen over several weeks and/or injection to inoculate with virus, which may result in infection lasting up to one week. Small volumes of blood sampled periodically throughout.

What are the expected impacts and/or adverse effects for the animals during your project?
The majority of effects will be mild. For example, animals will experience mild and transient pain associated with an injection or blood sample being taken. Some infected animals may experience discomfort and distress, weight loss or abnormal behaviour due to the onset of disease which may reach a moderate level for a short period.

After intranasal inoculation of adult mice with HRV, viral replication is restricted to relatively low viral loads in the airways, causing mild signs of infection, typically transient weight loss, ruffled fur and lethargy, with recovery within 7 days.

Intranasal inoculation with enterovirus-68 (EV-68) in mice also produces an acute disease with signs of infection including transient weight loss and recovery within 7 days.

FMDV causes an acute infection in adult mice after intraperitoneal injection. Disease severity can range from subclinical to lethal and is determined by the virus strain used. Strains of FMDV which reproducibly induce either mild or severe disease have been previously characterised. Lowpathogenicity strains of virus result in mild, transient clinical signs such as weight loss, ruffled fur and lethargy (also measurable virus in the blood) which usually resolve within 7 days. Pathogenic strains of virus can lead to systemic infection with virus replicating in multiple organs leading to weight loss, respiratory distress, neurological signs and death. Where possible, virus strains leading to only mild disease will be used but in some studies more virulent strains may be required. However, in all cases, severity will be limited by careful monitoring and humanely killing any animals reaching moderate severity limits.

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)?
The majority of animals (80%) will experience only mild effects. The remainder (20%) are predicted to experience a moderate severity.

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?
We carry out research to understand and control viral diseases of animals and people. After many years of in vitro research (not using animals) we have developed several new strategies that we believe will help us understand how to make better vaccines or allow us to produce livestock animals that are resistant to disease. The only way for us to confirm if these strategies will work is by doing experiments with animals.

Which non-animal alternatives did you consider for use in this project?
In vitro methods such as cell culture have been used extensively in the research leading to this application. Primary cell cultures, organ on a chip and organoid approaches have been considered for this project. The use of antibody-like reagents generated by recombinant methods may be used instead of generating monoclonal antibodies from animals.

Why were they not suitable?
Cell culture methods will continue to be used wherever possible (e.g. to provide preliminary characterization of attenuated viruses) but no in vitro approach is able to provide assessment of disease pathogenesis or the role of complex immune responses in protecting against 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?
The number of animals to be used in these studies has been determined with the help of statisticians to ensure the data generated is scientifically robust, reproducible and uses the fewest animals possible. Pilot experiments with small numbers of animal numbers will inform and refine the experimental design of subsequent comparable studies.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?
In addition to online tools, experimental design has incorporated advice from local statistician, NVS and local researchers experienced in use of mouse models.

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 conducted where appropriate.

The use of mice as models for immunization and for picornavirus infection are well established and outcomes are predictable.

The design of experiments will continue to be scrutinized as the project progresses to optimise the numbers of animals being used.

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.
The use of adult mice as models for studies of immunogenicity and virus infection is well established, such that experiments can be designed with predictable outcomes that minimize pain, suffering, or distress.

In addition, mice are thought to be the least sentient mammalian laboratory animal, such that measures for refinement of experimental conditions such as enhanced environment can be implemented effectively.

Why can’t you use animals that are less sentient?
Immature mice or non-mammalian species would not have a relevant immune system response and/or would not be susceptible to infection.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Animals will be acclimatized to their accommodation for 7 days prior to the start of any experiment and during this time will be familiarised to handling.

HRV and EV-68 cause mild signs of infection in mice after intranasal challenge, typically transient weight loss, with recovery within 7 days. FMDV causes an acute infection in mice after intraperitoneal injection with disease severity determined by the virus strain and ranging from subclinical to lethal. Signs of mild disease usually resolve within the first 7 days post challenge and any clinical signs are expected to occur within this period. Therefore, in order to monitor the occurrence of signs and limit the severity, mice infected with pathogenic strains of virus will be observed at least 3 times a day during the first 7 days post challenge time period and weighed daily at the same time each day over this period. If any of the severity limits defined in the protocol are reached, the animal will be humanely culled. Models of infection will be chosen so that the least severe option will always be selected, for example using infection with less pathogenic strains of FMDV where appropriate and using noninfectious antigen in studies of persistence which will avoid animals being infected.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
Refinement best practice specific for mice will follow guidelines issued by LASA and NC3Rs.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
Up to date information will be checked via online resources from LASA and NC3Rs and via regular discussion with the local NACWO with special expertise in small animals.