Non-technical summary: Novel vaccine development for porcine reproductive and respiratory syndrome virus

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
Porcine reproductive and respiratory syndrome virus, Pig, Immunology, Virulence factors, Vaccines A

Animal types
Pigs

Life stages
juvenile Mice 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?
To develop safer and more efficacious vaccines to aid control of porcine reproductive and respiratory syndrome viruses.

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?
Porcine reproductive and respiratory syndrome (PRRS) remains one of the most economically important infectious diseases affecting the global pig industry, with an estimated cost of over €4 billion each year. Vaccination is a key component of PRRS control. However, current vaccines have safety concerns and provide limited protection, which drives the evolution of an ever-expanding diversity of PRRS virus (PRRSV) variants. More effective vaccines are required to better control PRRSV and alleviate the significant animal welfare and economic burden they cause.

What outputs do you think you will see at the end of this project?
New information on the viral components that determine the severity of disease caused by PRRSV will enable the design of new vaccines that can be safely used to protect pigs.

New information on how arteriviruses dysregulate the immune system by studying lactate dehydrogenase elevating virus (LDV) infection in mice, which could inform the development of improved PRRS vaccines.

Proof-of-concept that engineering live attenuated PRRSV to express immunomodulators can improve their potency.

Proof-of-concept that RNA vectors expressing PRRSV glycoproteins can induce protective virus neutralising antibodies.

Proof-of-concept that viral vectors expressing PRRSV glycoproteins or derived components can induce protective virus neutralising antibodies.

Proof-of-concept that immunisation with hypervariable epitope libraries of PRRSV glycoproteins induces more broadly reactive and protective virus neutralising antibodies.

Proof-of-concept that a live attenuated PRRSV expressing consensus sequence glycoproteins is safe and effective.

Providing vaccine candidates for further development as products.

All results from the project will be published in Open Access scientific journals once intellectual property has been protected.

Who or what will benefit from these outputs, and how?
The scientific community will benefit from the improved knowledge of PRRSV virulence factors, immune evasion by arteriviruses, and the performance of new vaccine approaches. This could lead to the development of safer and more efficacious vaccines that result in enhanced PRRSV control and consequently improved animal welfare and productivity in the pig industry. This would bring benefits to policy makers involved in livestock disease control, the pharmaceutical and veterinary sector, and the general public through improved food security.

How will you look to maximise the outputs of this work?
All outputs from this project will be published in Open Access scientific journals; this will include unsuccessful PRRS vaccine approaches. Outputs of this work will also be disseminated to other stakeholders and the general public through press releases, presentations at meetings/congresses and social media channels.

Species and numbers of animals expected to be used
Pigs: 220 Mice: 170

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.
The only species known to be susceptible to infection with PRRS virus are pigs and wild boar. The pig is therefore the most suitable animal to study PRRSV infection and to evaluate the effectiveness of vaccine approaches. Weaned piglets will be used as they are most reproducibly infected with PRRSV. We may use mice as a preliminary screen to assess novel vaccine candidates and guide their selection for further evaluation in pigs.

LDV, which naturally infects mice, is closely related to PRRSV. We hypothesise that the principal mechanisms that underlie impaired antibody responses are shared between PRRSV and LDV. We can exploit the wealth of tools available in the mouse, including

transgenic models, to better dissect and understand these mechanisms. Findings from the LDV-mouse system, will direct studies with PRRSV in pigs.

Typically, what will be done to an animal used in your project?
Typically, pigs used in this project will be immunised by injection of PRRSV vaccine candidate into the muscle. This will typically be conducted once or twice. Blood and nasal swab samples will be taken at intervals to characterise the immune response and to assess shedding of the vaccine. Vaccinated and unvaccinated animals will typically be challenged once by administration of PRRSV into the nose. Blood samples and nasal swabs will again be taken at intervals to quantify levels of challenge virus and immune responses. This will typically be done twice weekly. Animals will then be culled humanely to assess lung pathology and tissues will be collected to assess PRRSV loads and for further analysis of immune responses. The typical duration of an experiment is 21-56 days.

Typically, mice used in this project will be infected with LDV by injection once into the peritoneal cavity. The typical duration of an experiment is 14 days. At the end of a study, animals will then be culled humanely to assess virus loads and immune responses. Alternatively, mice used in this project will be immunised by injection of PRRSV vaccine candidates under the skin, into the skin or the muscle. This will typically be conducted once or twice. The typical duration of an experiment is 56 days. At the end of a study, animals will be culled humanely to assess immune responses in blood and tissues.

What are the expected impacts and/or adverse effects for the animals during your project?
Mild to moderate clinical signs for a few days' duration may be observed following inoculation with PRRSV. This will most commonly present as an elevated temperature and lethargy. Sneezing, nasal discharge, coughing and lack of appetite may be observed. No adverse effects are expected following immunisation with PRRSV vaccine candidates. However, all animals will be clinically monitored both post-vaccination and -challenge. Assessments and interventions as appropriate will be performed at predefined frequencies in the experimental protocol, including euthanasia on welfare grounds if required. The impact of blood sampling, swabbing and inoculation of vaccine or virus will be both mild and transient.

No clinical signs of disease are expected for mice inoculated with LDV or immunised with vaccine candidates, and any adverse effects due to procedures are mild and transient.

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 maximum expected severity for pigs that are vaccinated and challenged is moderate. Unvaccinated control pigs that are also expected to experience a mild to moderate severity depending upon the virulence of the PRRSV challenge strain.

To map virulence factors, pigs are expected to experience mild-moderate clinical signs of disease depending on the PRRSV strain used.

It is estimated that 76% of pigs will be in the mild severity category and 24% of pigs in the moderate severity category.

The maximum expected severity for mice that are infected with LDV or immunised with vaccine candidates is mild.

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?
Due to the complex nature of the immune system, it is not currently possible to study immune responses to vaccination/infection and to determine whether they are protective without the use of animals.

It is necessary to use animals to assess the effects of identified virulence factors on PRRSV pathogenicity and immunogenicity.

Which non-animal alternatives did you consider for use in this project?
Cell culture-based systems will be used to generate and characterise vaccine candidates including viral vectors, cultivate vaccine and challenge virus strains and to evaluate virus-neutralising antibody characteristics. In vitro cell-based assay systems will first be used to map and study PRRSV virulence factors before confirmatory studies are conducted in pigs. Expression of immunomodulators by recombinant PRRSV, and their properties, will be analysed in vitro prior to evaluating these as vaccines in pigs. PRRSV glycoprotein expression by recombinant viral and nucleic acid vectors will similarly be confirmed in cell culture prior to their evaluation in animals.

We will explore murine macrophage cell lines and primary macrophages as an in vitro system for propagating and titrating LDV.

Why were they not suitable?
No replacement options are available to replace the whole animal at this time as an entire organism, including the immune system, need to be present.

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?
Animal numbers to be used have been estimated using data previously collected from similar studies or from relevant published literature in consultation with a statistician.

Pilot studies using small numbers of animals will be performed for new investigations.

Small animal numbers may be used to propagate and titrate LDV.

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

Statistical analysis of data collected from previous related studies. Samples will be stored in a biobank, and we will maximise collection of samples post-mortem to facilitate further investigations without the requirement for additional animal experiments.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?
Use of in vitro models to map and study PRRSV virulence factors and to characterise novel PRRSV vaccine strains. Basing study design on recently conducted relevant studies.

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 only species known to be susceptible to infection with PRRS virus are pigs and wild boar. The pig is therefore the most suitable animal to study PRRSV infection and to evaluate the effectiveness of PRRSV vaccine candidates. Mice may be used as a preliminary screen to assess novel vaccine candidates and guide their selection for further evaluation in pigs.

A well characterised live attenuated PRRSV strain will be selected to be engineered to express immunomodulators. These peptides have previously been evaluated as vaccine adjuvants by our collaborators in mouse models with no adverse effects. Vaccinated and unvaccinated control pigs will be challenged with a previously characterised low virulence PRRSV strain. This enables us to assess protection, by reduction in virus infection, without the animals having to suffer clinical disease.

Previously characterised PRRSV strains of known low and moderate virulence will be used to map virulence factors. In vitro cell based assays have been established that have shown to correlate with PRRSV virulence in vivo. The exchange of virulence factors between these strains is not expected to increase the virulence beyond that of either parental strain.

Mice are natural hosts of LDV, a close relative of PRRSV. LDV infection in mice provides a natural virus-host system to provide insights into how arteriviruses modulate the immune system. This could then be explored in the context of PRRSV in pigs and inform PRRS vaccine development. Characterised strains of LDV will be used that are not expected to cause clinical disease.

Animals will be inoculated with vaccine or challenge virus in the smallest volume commensurate with the aims of the procedure.

Why can’t you use animals that are less sentient?
The only species known to be susceptible to infection with PRRSV are pigs and wild boar. The only species known to be susceptible to infection with LDV are mice.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Animals will be housed together with bedding and other items of enrichment. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are

comfortable and to maximise their welfare status. We have 24/7 CCTV surveillance which can be used to monitor the animals' behaviour over time.

Pre-study meetings involving the NVS, NACWO and animal services staff will be held to discuss any advances in animal care. Meticulous records will be kept of behavioural, physiological, immunological, and virological measures in order to identify predictive markers and refine humane endpoints. All experiments will be followed by a wash-up meeting to discuss all aspects of the study.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
Adherence to the ARRIVE guidelines for reporting these studies, as well as reference to the FELASA guidelines for pig health monitoring to help ensure the most robust health assurance for animals used in this study. FELASA guidelines for administration of substances has been used to limit the maximum volumes for each of the routes.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
Through continued CPD and frequent review of the CAAT (Center for Alternatives to Animal Testing) and NC3Rs websites, I will keep informed about advances in the 3Rs. Included in CPD will be annual attendance at relevant science conferences