Non-technical summary: Immunity to influenza viruses 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
(c) Development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs and feedstuffs or any other substances or products, with one of the following aims mentioned in paragraph (b)

Key words
influenza virus, pig, influenza vaccine, immune response, mucosal immunity

Animal types
Pigs

Life stages
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 understand protective immunity to influenza viruses in pigs. We will also determine how best to induce protective immunity by vaccines and evaluate novel therapeutics and monoclonal antibodies to prevent disease and virus transmission.

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?
Globally approximately 1.5 billion pigs are produced annually for pork production, which accounts for more than one-fourth of total protein consumed worldwide. The demand for pork has led to intensification of production, with farms often housing thousands of animals leading to rapid pathogen transmission. Influenza virus infection in pigs is a major farming problem, causing morbidity, mortality and loss of productivity. Economic losses due to swine influenza (SI) are among the top three health challenges in the swine industry.

Furthermore, pigs are natural hosts for the same subtypes of influenza A viruses as humans and are integrally involved in virus evolution with frequent interspecies transmissions in both directions. The emergence of the 2009 pandemic H1N1 virus (H1N1pdm09) illustrated the importance of pigs in the evolution of zoonotic strains.

What outputs do you think you will see at the end of this project?
1) Characterisation of the specificity, magnitude, and duration of mucosal and systemic immunity to current and emerging influenza viruses.
2) Identification of early key events in the innate immune response that determine the outcome of infection and severity of disease.
3) Evaluation of the capacity of influenza vaccines and therapeutics to prevent clinical signs and limit virus transmission. The role of local and systemic immunity and how best to deliver vaccines and therapeutics to induce universal protection against different strains will be established. The part of the respiratory tract that should be targeted by vaccines or monoclonal antibodies for optimal protection will be identified.
4) Characterisation of the turnover of tissue resident memory cells (TRM) and their distribution in the respiratory tract.

The outputs of the project will be new knowledge about the nature of the disease caused by different influenza viruses and what are the protective mechanisms of immunity to influenza in pigs. In the short term we will also obtain information about the efficacy of novel influenza vaccines and therapeutics in preventing clinical signs of disease and limiting virus transmission. In the medium and long term these outputs will enable improvements to be made in the prevention of influenza virus infection in pigs and in humans. The outputs of the project will be disseminated primarily via scientific publications and conference presentations.

Who or what will benefit from these outputs, and how?
Pig farming industry: A key deliverable of this proposal is to determine how to make better and more efficient vaccines and control measures to limit the spread of disease. The beneficiary will be the pig farming industry. Both productivity and animal health and welfare will be improved, and secondary infections and severe disease reduced.

General public and human health: A more efficient immunisation strategy against influenza virus infection will also reduce transmission and the zoonotic threat posed by swine influenza viruses. Increased productivity in the pig industry will lower costs for the consumer.

As both pigs and humans are readily infected with influenza A viruses of similar subtype, the pig is a robust and appropriate model for investigating both swine and human disease. Like humans, pigs are outbred, and physiologically, anatomically, and immunologically similar to humans. The porcine lung also resembles the human in terms of its lung structure, physiology, morphology, and distribution of receptors bound by influenza viruses. Any knowledge gained from the pig model could be easily translated to humans for development of better immunisation strategies and treatments.

Food security and environment: Consequences of improved pathogen control include reduction in antibiotic treatment for secondary bacterial infections, the risk of contamination of the food chain and the environment, as well as the risk of developing antibiotic resistance.

Benefits to the commercial private sector: The knowledge gained in this project can be applied to other respiratory tract infections in pigs and also to other livestock species and humans. Our close relationships with industry will allow us to fast track the new immunisation strategies into field use. We will identify new correlates of protection that evaluate pulmonary as well as systemic immunity to respiratory tract infections and this will accelerate vaccine development in pigs, other livestock and humans.

Benefits to policy makers; International development: Development of control measures and better vaccines for animals and humans will have an enormous impact on health policy and quality of life throughout the world. Diagnostic and consultancy services are commissioned by the UK Department for Environment, Food and Rural Affairs (DEFRA) and equivalent organizations worldwide (EC DGSANCO, the World Organisation for Animal Health and the Food and Agriculture Organisation), who will therefore also be primary beneficiaries.

How will you look to maximise the outputs of this work?
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 conferences, collaborative discussions and interactions with members of the scientific community. Outputs of this work will also be distributed to other stakeholders and the general public through press releases and social media channels.

Species and numbers of animals expected to be used
Pigs: 480

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.
We propose to use a mammalian host (i.e. pig) appropriate to the influenza viruses we are studying, such that key information, e.g. clinical/pathological data can be used by the farming industry, veterinarians, animal keepers and national veterinary authorities responsible for disease surveillance, disease outbreak management and policy formulation.

Pigs are a natural host for influenza viruses and their immune and respiratory systems are very similar to humans. Pigs are susceptible to some strains of human influenza viruses, especially H1N1pdm09, and human viruses or human-origin gene segments frequently adapt to transmit efficiently in pigs. The similarity of clinical disease and pathogenesis of influenza infection in the two species, make pigs an excellent animal model to evaluate novel vaccines. In our experience vaccines which are protective in mice and ferrets are not always protective in pigs, further emphasizing the difference between small and large animals.

The pig is therefore the most suitable animal to study immunity to influenza virus infection and to evaluate the effectiveness of vaccines and therapeutics. Weaned piglets will be used as they are most reproducibly infected with influenza viruses.

Typically, what will be done to an animal used in your project?
Typically, animals used in this project will be immunised by an injection of influenza vaccine candidate into the muscle or by administration into the nose or by aerosols into the lungs, for which the animals may be sedated. The immunisation typically will be conducted twice at an interval of 1 to 8 weeks. Blood samples will be taken to characterise the immune responses. Immunised and unimmunised animals will typically be inoculated once with influenza virus into the nose. Nasal swabs will be taken daily in the first 7 days to evaluate the shedding of challenge virus. Blood samples will be taken weekly to characterise the immune responses. Animals will then be culled humanely to assess lung pathology and tissues will be collected to assess virus load and for further analysis of immune responses. The typical duration of an experiment is 21-56 days.

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. Intranasal or aerosol administration of vaccines or therapeutics will be done using mucosal atomisation device or by aerosolisation, to reach the deep lung, using close fitting mask following sedation.

Our extensive experience using H1N1pdm09 and published data indicates that clinical signs of disease are consistently mild in pigs under experimental conditions, moderate being the highest severity ever observed, dependent on the virus strain used. The maximum severity recorded with H1N1pdm09strains is moderate, but in our extensive experience with these viruses the maximum severity is mild. The mild signs consist of intermittent coughing or a mild elevation in body temperature for 1 to 2 days. Rarely, animals infected with influenza viruses may develop moderate clinical sign consisting of laboured breathing, becoming lethargic or anorexic for 1 to 3 days. In this project, any animals which show these more severe signs will be killed if the duration reaches the endpoints in this licence.

In the case of unknown viruses (with unknown severity), pilot experiments will be performed, and challenge virus will be administered at times to ensure, wherever possible, that the peak phase of clinical impact falls within normal working hours, enabling regular observation. However there are provisions for out of hours checks. To allow severity to be monitored and minimised, clinical signs will be serially observed and recorded against systems-based clinical scoring sheets that include clearly defined end-points and intervention criteria. Pigs will be required to be anaesthetised transiently for some procedures and very rarely vomiting has been observed after anaesthesia. Pigs will be monitored throughout the procedure and recovery to avoid any adverse effects associated with the depression of body systems during anaesthesia and / or accidents during recovery.

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 and our extensive experience, 85% of animals are expected to show either no or mild clinical signs. Less than 15% are expected to show moderate clinical signs. However, all animals will be clinically monitored both post-vaccination and post-infection. Assessments and interventions as appropriate will be performed at predefined frequencies in the experimental protocol, including euthanasia on welfare grounds if required.

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 immunisation and infection and to determine whether they are protective without the use of animals. It is also impossible to study the lifespan of lymphocytes in vitro because we wish to understand the effect of the local lung environment

on their survival and cell division. In vitro experiments will tell us how long lymphocyte can survive in tissue culture conditions but has no bearing on their survival in the lung environment. Therefore in vitro experiments cannot tell us how long immunity to influenza viruses could persist in the lung.

Which non-animal alternatives did you consider for use in this project?
Cell culture and molecular biology techniques will be used to characterise influenza virus in the laboratory before infecting pigs. We will use laboratory techniques to understand the responses of the pigs to infection with influenza viruses using blood samples and tissue samples taken post mortem. Protection against respiratory diseases is carried out by white blood cells in the lung, called tissue resident memory cells (TRM). After establishing how best to induce TRM we shall perform a series of in vitro experiments to determine what factors influence their maintenance and survival.

We shall also use a primary porcine airway epithelial cell model and tracheal organ cultures to study virus replication, anti-viral therapeutics and vaccines for the control of influenza. We have established for the first time in vitro porcine tonsil, lymph node and spleen organ cultures to dissect antibody and T cell responses. The parallel use of in vitro models and in vivo studies, will validate the predictive value of these in vitro models.

Why were they not suitable?
In line with the objectives of the programme of work a complete biological system is required to study transmission and protective immune responses to influenza virus infection. Therefore, in vivo studies have to be performed to provide critical data and biological materials that correspond to the outcomes and responses to viral infections in the animal itself. Given the nature and localisation of the TRM it is not possible to use an in vitro system to study how these cells are induced following immunisation, nor is it possible to measure immune responses to or protective efficacy of a vaccine without the use of animals.

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 and 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?
The experiments are designed to ensure the appropriate number of animals are used - numbers are selected that enable robust experimental design compatible with obtaining reliable and meaningful results. The advice of an experienced biostatistician from our establishment will be sought, as well as from other collaborators as appropriate. The animal studies are designed to maximise collection of biological materials/data from each study and enhance the development and use of in vitro and ex vivo methods where appropriate.

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

• Using inbred large white pigs (Babraham pigs) in some studies may enable us to obtain significant results with smaller groups of animals since these pigs are 85% identical as assessed by genome-wide SNP analysis and are matched for MHC type I and type II molecules.
• When investigating novel antigens, tissue culture analyses in the laboratory are conducted where possible to refine the number of candidate antigens for evaluation. This allows for a reduction in the number of in vivo studies required.

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.
Animal experiments are designed with close consideration of the likely overall severity and the period of peak severity caused by administration of a defined and consistent dose of virus (for example between 1 x 106 to 5 x 106 pfu per animal for H1N1pmd09) based on previous experience and published information.

We have developed a purpose built mask for aerosol administration which allows maximum exposure in the minimum time.

Sample collection will be carried out within clearly defined limits specified in individual experimental protocols, and repeated sampling will be done at frequencies such that the method of sampling causes no more than momentary pain and suffering and no lasting harm.

Substances administered to animals by injection or intranasal inoculation will be made in the smallest volume commensurate with the aims of the procedure according to good practice guidelines.

Because of the rapid air changes in the rooms in the high containment facilities at our establishment, in order to best mimic natural room air, a purpose built Perspex pen (246 cm x 246 cm x 140cm) high is used for the contact challenge which allows the animals to exhibit their natural behaviour.

The severity level after influenza infection is mild in most cases. However, some pigs may develop moderate signs of disease which will not exceed the specified humane endpoints. Symptomatic treatment as agreed with the veterinary surgeon will be provided to alleviate suffering whenever possible.

Pigs housed in the high containment facilities at our establishment are provided with various enrichment items, including a straw bed, hanging toys and a rotation of ground objects to interact with. We have developed an enrichment monitoring program to help define which enrichment items are useful, and how frequently they should be rotated etc.

Why can’t you use animals that are less sentient?
Pigs are natural hosts for influenza viruses and a source for novel zoonotic strains, which may cause epidemics or pandemics in humans. Mice cannot be infected with most strains of

the influenza virus and do not recapitulate signs of illness observed in pigs and humans; guinea pigs do not exhibit overt signs of illness; and ferrets may have different drug pharmacokinetics to pigs and humans. Only in a natural host such as pigs, is it possible to dissect the pathogenesis of disease and identify how to control the virus.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Pigs are intelligent animals and so enrichment is particularly important to create a stimulating environment. They are given straw beds which also enables them to express their species-specific behaviour of rooting and investigating. They are supplied with a variety of fruit, vegetables and toys which are frequently rotated.

Where possible, pigs are trained with positive reinforcement to reward desired behaviour e.g. cooperating with procedures which could include swabbing without restraint. This is a refinement in animal handling methods to improve animal welfare and the value of animals in research. Animals are also housed in pairs or groups to allow for normal social interaction.

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.

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?
I will attend appropriate 3Rs conferences, read the relevant scientific literature including the veterinary literature on pain relief, and undertake regular project licence holder training and refresher courses. I will take advantage of news and information provided by the NTCO. I will also use other sources of information such as:
The NC3Rs
(AALAS) American Association for Laboratory Animals Science
(FELASA) Federation of European
Laboratory Animal Science Associations (ICLAS) International Council for Laboratory Animal Sciences