Home Office

NON-TECHNICAL SUMMARY

Influenza in ferrets

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

Influenza, Vaccine, Epidemic, Pandemic, Antigenicity

Animal types	Life stages
Ferrets	adult

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is required, and should be submitted within 6 months of the licence's revocation date.

Reason for retrospective assessment

This may include reasons from previous versions of this licence.

- Contains severe procedures

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 infect ferrets with human and animal influenza viruses for the generation of post-infection antisera for the antigenic analysis of influenza viruses.

To produce ferret hyperimmune antisera also for the antigenic analysis of influenza viruses.

To characterise the replication of influenza viruses in the ferret in vivo model of influenza.

A retrospective assessment of these aims will be due by 16 August 2027

The PPL holder will be required to disclose:

- Is there a plan for this work to continue under another licence?
- Did the project achieve it's aims and if not, why not?

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?

Influenza viruses continue to threaten human and animal health. They are best controlled by vaccines and antiviral medicines. Our aim is to define the antigenic properties of new and emerging influenza viruses and to understand their replication in a small animal model.

This work leads to the generation of information about the antigenic properties of circulating and emerging viruses to guide the selection of influenza vaccines, how vaccination might be effective against these viruses, and to generate information that can be used to assess the risks associated with influenza viruses of animals infecting humans (zoonotic influenza viruses) in terms of replication and spread in mammalian models and their sensitivity to antiviral treatments.

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

Antigenic characterisation of circulating and emerging influenza viruses is key to the development of seasonal influenza vaccines, vaccines prepared for pandemic preparedness purposes, and for vaccines to be used during an influenza pandemic. The data generated as an outcome of this project, on how post-infection antisera recognise new influenza viruses are used for the biannual recommendations drawn up by the World Health Organisation (WHO) on the composition of influenza vaccines for seasonal influenza. Similarly, recommendations on the development of vaccines made for

pandemic preparedness purposes include the antigenic characterisation of influenza viruses of animals (e.g. birds, pigs, dogs and horses) that might pose a risk to humans.

Data generated on how viruses replicate in mammals and cause disease, and how they spread between infected and uninfected animals will be used in risk assessments of newly emerging influenza viruses (done, for example, using the WHO The Influenza Pandemic Risk Assessment tool, see https://apps.who.int/iris/handle/10665/250130).

Assessing how vaccines or antiviral medicines protect against influenza infection in animal models is one of the starting points for assessing the likely benefits of vaccination by established and new methods, or antiviral use.

The results of these analyses will be shared with the WHO. This will be done as an ongoing process throughout the period of the project. Work will be published in peer-reviewed journals where appropriate.

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

Public health will benefit from the work.

Antigenic characterisation of circulating viruses, newly emerging and zoonotic viruses and candidate vaccine viruses for seasonal influenza vaccines and vaccines made for pandemic preparedness purposes, is essential for the development of influenza vaccine recommendations. Antisera recovered following infection and the use of these for determining the antigenic properties of new and circulating viruses and candidate vaccine viruses are the linchpin for the global influenza surveillance activity conducted through the WHO Global Influenza Surveillance and Response System (GISRS).

The results from examining the properties of viruses of animals that infect humans, zoonotic influenza viruses, will be shared with WHO for risk assessments of the pandemic potential of such viruses. These assessments lead to the subsequent prioritization of viruses for the generation of vaccines for pandemic preparedness purposes.

The efficacy of vaccines and antiviral medicines will, in the long term, we hope lead to improvements to medical practice.

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

It is critical to characterise antigenically and genetically any newly emerging influenza virus. WHO GISRS provides a framework to ensure that viruses from around the world are analysed antigenically and genetically to build up a global picture of the changing characteristics of influenza viruses. Results are shared with other centres to develop recommendations on the composition of seasonal influenza vaccines and vaccines made for pandemic preparedness purposes. Therefore, from these analyses with ferret antisera new vaccines are developed.

Assessment of the properties of influenza viruses of animals causing zoonotic infection is a key step in influenza pandemic preparedness and this will be done, in conjunction with WHO, as new viruses

emerge and their properties are examined. These risk assessments are carried out to mitigate the impact of influenza.

This work will continue throughout the project. Results from new vaccines and new antiviral medicines will be shared with WHO and other public health agencies in a timely fashion, and published in Open Access peer-reviewed journals.

Species and numbers of animals expected to be used

- Ferrets: 450

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.

Adult ferrets are to be used in this work. Ferrets have been used in research on human influenza since the human influenza virus was first isolated in ferrets in 1933. They are recognised to be the best animal model for human influenza since they are susceptible to human influenza viruses without virus adaptation, following infection ferrets show a similar course of disease to humans, and post-infection ferret antiserum reveals a similar immune response to that seen in humans.

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

Adult ferrets will be infected with influenza viruses by intranasal instillation of virus and monitored for disease signs. If disease signs are severe then antiviral treatment will be given. In addition, in some cases, treatment with antiviral medicines will be given over the course of infection to assess their potential in an aniaml model to control infection by by standard virus or a newly emerged influenza virus. Prior vaccination of ferrets may be undertaken to assess the efficacy of a vaccine in a small animal model. Ferrets may be sampled for virus or serum antibody titres over the course of infection, or following secondary infection, or following boosting with virus antigen or vaccine. At the end of the procedure ferrets will be killed by a schedule 1 method, or exsanguinated under terminal anaesthesia, completed by killing using a Schedule 1 method.

Most experiments will last for a period of two weeks.

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

Most of the influenza viruses used only cause mild adverse effects on the ferret, exhibited for only a few days – mild adverse effects include sneezing and nasal discharge, some lethargy. For those influenza viruses that cause more severe disease, like highly pathogenic avian influenza viruses that can cause zoonotic infections and may have pandemic potential, antiviral treatment is an option to moderate the

disease signs. Severe disease signs include a sustained lack of movement, a failure to eat food, a loss of response to stimuli, diarrhoea, and laboured breathing.

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)?

As indicated above, most of the viruses used cause no serious adverse effects on the ferret, ferrets might be lethargic and show mild disease signs (e.g. sneezing, nasal discharge). Some influenza viruses, e.g. H5N1 and H7N9 avian influenza viruses, can show severe disease signs. Over the past five years, such viruses have represented the very small minority of viruses used, less than 5%. The large majority of viruses (95%) cause only relatively mild infection, resulting in sneezing and nasal discharge and some loss of interest in the environment.

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

- Killed

A retrospective assessment of these predicted harms will be due by 16 August 2027

The PPL holder will be required to disclose:

- What harms were caused to the animals, how severe were those harms and how many animals were affected?

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?

It is essential to analyse the antigenic characteristics of new and emerging human influenza viruses in order for recommendations for seasonal influenza vaccines and for vaccines made for pandemic preparedness purposes to be developed. This is done using antisera generated to reference viruses, and newly emerging influenza virus variants. The antigenic characteristics of influenza vaccines and candidate vaccine viruses are also required to be established prior to their use in humans. The generation of antisera (usually post-infection, sometimes hyperimmune) in a timely fashion is a necessary step in these processes. These processes to determine how the immune response of an animal to a virus recognises different stains of the same virus cannot be done without the use of animals to generate antisera.

The growth properties of newly emerging animal influenza viruses able to infect humans is a necessary component of the risk assessment of such viruses. The best animal model for understanding the disease, virus tissue tropism and virus transmission is the ferret. These results from such work done in

animal models complements work done in vitro and so no alternative to this in vivo work is feasible and so cannot be done without the use of animals. The work done in animals characterising new viruses complements those done with biochemical and biophysical methods on the virus done in vitro or ex vivo or in other systems.

New influenza vaccines and new influenza antivirals need to be tested in animals prior to human trials. Notably for the development of antivirals extensive work in tissue culture, in organ culture and in less sentient animal systems (e.g. embryonated eggs) is carried out prior to the work done on animals. Because the ferret is the best animal model for influenza, examining the efficacy of new influenza vaccines and antiviral treatments in ferrets is an important step in their development prior to testing in humans.

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

Human monoclonal antibody (h-mAb) panels have been considered as an alternative to using ferret antisera for tracking the antigenic characteristics of influenza viruses. These mAbs are made from white blood cells after usually after vaccination although they can also be made after infection. The window for taking the human blood sample is small, two to four weeks after vaccination or infection.

Why were they not suitable?

Panels of human monoclonal antibodies (h-mAb) have not been able to replace the use of post-infection ferret antisera for the antigenic analysis of newly emerging influenza viruses. As stated above, such h-mAb panels would usually be made following vaccination. However, it is essential to assess the antigenic property of any newly emerging influenza virus as soon as it is recognised because it is the antigenic properties of newly emerging viruses that are the main factors that lead to new influenza vaccines. Only after making a vaccine can a panel of human monoclonal antibodies usually be made – perhaps 18 months or more after the emergence of an antigenically distinct strain that might have gone on to cause an influenza epidemic. An alternative of making monoclonal antibodies following influenza infection is very difficult to set up currently due to the unpredictable nature of the infection and influenza epidemics, making access to relevant human plasma cells and B-cells in a timely manner highly problematic.

A retrospective assessment of replacement will be due by 16 August 2027

The PPL holder will be required to disclose:

- What, if any, non-animal alternatives were used or explored after the project started, and is there anything others can learn from your experience?

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?

We need to have produced panels of antisera against a number of influenza viruses of each influenza A sub-type (H1N1, H3N2) and influenza B lineage (B/Victoria lineage and B/Yamagata lineage) for generating antigenic data for developing recommendations for influenza vaccines for use globally. We use in the order of 10 to 12 antisera in the tests carried out throughout the year on viruses from many countries around the world. The panel of antisera is updated on a regular basis as new viruses emerge and others are displaced from circulation. In our experience 40 to 50 ferrets each year can provide suitable panels for seasonal influenza viruses in circulation. More antisera are needed to confirm the antigenic characteristics of candidate vaccine viruses (viruses with the potential to become vaccine viruses) and vaccine viruses, and antisera are also needed to characterise antigenically animal influenza viruses that infect humans for risk assessment purposes.

Those ferrets infected with animal influenza viruses that infect humans are also used to determine the replication characteristics of these new viruses, and transmission studies might be needed. These studies have been a minor component of our work over the last five years, but should such new viruses emerge we will need to study them in considerable detail for an assessment of the risk that these viruses might have pandemic potential. The numbers used will be dependent on the frequency of the emergence of unpredictable influenza zoonoses.

Ferrets used in assessing the potential of new vaccines and new antiviral medicines will be used in relatively small numbers, but reproducibility of treatments will be required to be established before further study is done.

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

We use small numbers of animals for each virus to be examined. Quite often single ferrets will be infected with single strain of viruses; here, reproducibility can be established by raising antisera against genetically similar viruses. Antisera raised against some viruses, for example vaccine viruses, are used in more tests, and can be shared with other centres for their parallel analyses. Therefore, antisera from more than one ferret are needed for some of these viruses that serve as candidate vaccine viruses and vaccine viruses, and those that serve as reference viruses for longer periods of time.

Studies on newly emerging viruses and on new vaccines or antivirals will be done on only small numbers of animals, enough to ensure reproducibility. The design of the experiments will be done by applying the NC3R’s Experimental Design Assistant, or other similar design assistants.

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

We estimate the numbers from our experience over the last decade. For some studies, for example on new animal influenza viruses that infect humans, several animals will be infected to ensure reproducible and robust results.

A retrospective assessment of reduction will be due by 16 August 2027

The PPL holder will be required to disclose:

- How did you minimise the numbers of animals used on your project and is there anything others can learn from your experience?

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 ferret model of influenza is to be used. The ferret antibody response is the mainstay of the global surveillance of emerging human influenza viruses. The ferret is chosen because human influenza viruses are usually able to replicate in ferrets without prior adaptation, the disease signs of ferrets are usually similar to those observed in humans, and the immune response to infection is similar to a primary influenza infection of children.

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

Mature, usually adult, ferrets are used for this work. It is not possible to do this type of work with less sentient animals for the reasons outlined above: the disease signs of ferrets are usually similar to those observed in humans, and the immune response to infection is similar to a primary influenza infection of children. For example, human influenza viruses often adapt when mice are infected, and so the outcome of infection or the immunological response is caused by, and directed towards, the mouse-adapted variant and not the human virus under study. Similar problems can be seen in other animal models.

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

Infected ferrets will be monitored closely and should a ferret show disease signs it is possible to prescribe an antiviral medicine, e.g. oseltamivir, to reduce disease severity, given with food and water or by direct oral delivery or in food supplements like custard.

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

Methods of infection of ferrets and following infection are very well established and we will follow this best practice. Examples of good practise have been described in Besler et al., Am. J. Pathology 190, p11-24 (2020)

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

The host institution provides regular updates on advances in the 3Rs and news from the NC3Rs is regularly provided.

A retrospective assessment of refinement will be due by 16 August 2027

The PPL holder will be required to disclose:

- With the knowledge you have now, could the choice of animals or model(s) used be improved for future work of this kind? During the project, how did you minimise harm to the animals?