Home Office

NON-TECHNICAL SUMMARY

Isolation and propagation of virus in eggs

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, Pandemic, Epidemic

Animal types	Life stages
Domestic fowl (Gallus gallus domesticus)	embryo

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 isolate and propagate influenza viruses in embryonated hens' eggs.

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.

The vast majority of influenza vaccines for humans are produced in hens' eggs and require the initial virus from which the vaccine is produced to have been isolated in embryonated hens' eggs and to have been exclusively propagated in hens' eggs. Hens' eggs are also the most appropriate for examining the properties of certain influenza viruses of animals (e.g. poultry) that infect humans, and so pose a threat to public health and pose a pandemic threat. This project primarily covers the isolation and propagation of human and animal influenza viruses in embryonated hens' eggs and the analysis of the properties of these viruses.

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

The work entails the isolation of influenza viruses in hens' eggs and their detailed characterisation. These isolates can be further developed for the generation of egg-propagated candidate vaccine viruses that can be used by vaccine manufacturers for the production of influenza vaccines. This will be done as an ongoing process throughout the period of the project.

Assessing the properties of new influenza viruses of animals that infect humans allows for an assessment of the impact such viruses can have on human health, this includes the susceptibility of such viruses to antiviral medicines. The results of these analyses will be shared with the World Health Organisation (WHO). This will also 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.

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

The isolation and propagation of egg isolates of human influenza viruses are the first steps on the path to the provision of the large majority of influenza vaccines. Selected viruses will be further developed as candidate vaccine viruses and, ultimately vaccine viruses for the production of human influenza vaccines.

Examining antiviral treatments and assessing the sensitivity of newly emerging viruses to antiviral treatment also benefits public health.

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

This work will continue throughout the project. It is critical to select viruses for isolation and characterisation in hens' eggs on a timely ongoing basis as the influenza viruses evolve. This ensures that the most appropriate viruses are available for seasonal influenza vaccines and for vaccines developed for pandemic preparedness purposes.

Virus isolates deemed suitable for further development will be shared with other laboratories that carry this out. Updates on progress will be shared with WHO and vaccine manufacturers.

Assessment of the susceptibility of newly emerging influenza viruses to antiviral medicines is one of the key factors in the assessment of the risk posed by zoonotic influenza viruses to human health and is part of a process conducted by WHO -The Influenza Pandemic Risk assessment (see https://apps.who.int/iris/handle/10665/250130).

Species and numbers of animals expected to be used

- Domestic fowl (Gallus gallus domesticus): 28,000

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.

At present the vast majority of influenza vaccines are produced in hens’ eggs and are based on viruses exclusively propagated in hens’ eggs. Alternative platforms, cell culture-based vaccines and recombinant vaccines, have been developed but these are not yet extensively used and the work here is to produce viruses for use in the egg-based vaccine platforms. Under these circumstances, it is not possible to develop an alternative to the isolation of viruses in hens’ eggs and have an adequate vaccine supply to meet public health needs.

Isolation of viruses following amniotic inoculation is needed for most human influenza viruses. The efficiency of this process is much higher following inoculation of 14-day old embryos rather than, for example 10-day old embryos. The use of the older aged embryos is not possible to avoid without compromising the number of isolates recovered.

The examination of antiviral treatment in ovo provides a mid-point between examining the effects in tissue culture and in small animal models. This would be done on 9-day old to 11-day old embyronated eggs, but is included in this application to cover an experiment that might be required to proceed into the 14th day of embryonic development.

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

Embryonated hens' eggs of typically 14 to 16 days of embryonic development are to be inoculated in the amniotic cavity with virus with clinical samples or virus having been propagated in eggs or in tissue culture. The eggs will be incubated at 34^{o} to 37^{o} for typically 48 to 72 hours and then chilled to 4o prior to harvesting the propagated virus.

Embryonated hens' eggs of typically 10 to 11 days of embryonic development are to be inoculated in the allantoic cavity with virus with clinical samples or virus having been propagated in eggs or in tissue culture. The eggs will be incubated at 34^{o} to 37^{o} for typically 48 to 72 hours and then chilled to 4^{o} prior to harvesting the propagated virus.

In antiviral experiments 9-day old to 11-day old embryos will be used and the effects of possible antiviral medicines on virus replication will be examined for up to four days and then chilled to 4^{o}.

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

Most of the influenza viruses used cause no obvious adverse effects on the embryo. For those that do, like highly pathogenic avian influenza viruses that can cause zoonotic infections and may have pandemic potential, pilot studies will be carried out on small numbers of eggs of younger age (typically 9- to 11-days of embryonic development) to determine the mean death time, usually between 24 and 48 hours following inoculation, and in subsequent work eggs will be chilled at least 2 hours prior to the mean death time to reduce severity.

Amniotic inoculation is a difficult procedure and a proportion of eggs can die as a result of damage to the embryonic membranes. Allantoic inoculation causes very little damage to the embryo.

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 obvious adverse effects on the embryo. Most highly pathogenic avian influenza viruses are likely to be inoculated into earlier aged embryos and so will fall outside of the scope of the act. Work on such viruses has represented less than 5% of the viruses with which we have worked in recent years. Inoculation with such viruses can kill the embryo however, this is prior to sentience (2-days prior to hatching) and so severity is deemed mild. Chilling at least two hours prior to death is used to reduce impact on the embryo.

Amniotic inoculation can result in the loss of up to 20% of the inoculated embryos. Allantoic inoculation results in the loss of approximately 5% of embryos.

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?

As indicated above, at present the vast majority of influenza vaccines are produced in hens' eggs and are based on viruses exclusively propagated in hens' eggs. Alternative platforms, cell culture-based vaccines and recombinant vaccines, have been developed but these are not yet extensively used. Under these circumstances it is not possible to develop an alternative to the isolation of viruses in hens' eggs and have an adequate vaccine supply to meet public health needs.

For studying antiviral intervention, embryonated hens' eggs can serve as an intermediate between studying antiviral activity in tissue and organ culture, and in small animals. Use of eggs of 9-days or 10-days of embryonic development for this would be usual but some experiments might need to be extended up to and including 14-day old embryos.

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

Egg-based influenza vaccines remain the mainstay for public health intervention against influenza. Under this current situation, there is no non-animal alternative for this type of activity for the production of such viruses to be developed for use in vaccines and we are committed to making such virus isolates for development into vaccines.

Tissue culture and organ culture are the first steps in analysing antiviral activities but eggs can serve as an intermediate between tissue and organ culture and small animal models. Thereby, eggs serve to reduce the use of animals, and will not usually be done at late stages of development (after 14-days of embryonic development).

Why were they not suitable?

There was no alternative available for producing viruses in hens' eggs for egg-based vaccines, and these remain the main type of influenza vaccine used globally.

In ovo assessment of antiviral treatments serves to reduce animal usage and will typically use eggs at earlier stages of development, and this work will build on work done in tissue and organ culture.

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 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 initiating the development into new vaccine candidates as the human influenza viruses continually evolve, causing yearly epidemics and occasional pandemics, and warranting new influenza vaccines on a very frequent basis. Also needed are isolates of zoonotic and potentially pandemic influenza viruses.

It is important that sufficient viruses are isolated in hens' eggs are available to screen for ones expected to be able to be developed into a suitable influenza vaccine. The isolation rate of viruses from suitable clinical samples is currently in the order of 50% to 60%. Typically up to around 100 human clinical samples are taken each year for attempted virus isolation in eggs.

Most antiviral experiments done *in ovo* will be initiated on early-stage embryos and very few eggs will progress to 14-days of embryonic development.

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

In most cases once a virus has been clearly established as an isolate we can use eggs of lower embryonic age for further propagation, and so not subject to the legislation of the Animal (Scientific Procedures) Act 1986. This reduces the number of animals used under the project licence.

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 that can be developed further. Not all selected viruses will prove to have the properties to take them forward for further development and some of those developed further might not be deemed to be suitable for use as a candidate vaccine virus.

In studying antiviral treatments, eggs of the youngest age (e.g. 9-day old to 11-day old) will be used to initiate the experiments. As above, it is not envisaged that many treatments would extend to 14-days of age.

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.

Embryonated hens' eggs are to be used and these will be inoculated with samples containing influenza viruses into the amniotic and allantoic cavities.

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

Success in the initial isolation and the early passage of virus, notably for influenza A(H3N2) viruses, one of the main causes of epidemic influenza, is best performed using older-aged embryos. This is based on our observations, along with those of others, that as the human influenza virus has evolved the ability to isolate viruses in hens' eggs has become markedly reduced. This decrease in virus isolation in eggs reduced the number of viruses that could be developed into viruses suitable for influenza vaccines, thus potentially compromising influenza vaccine availability. It was discovered that inoculation of embryos of 14 or 15 days of development, followed by incubation for 3 or 4 days, increased the probability of virus recovery and thus provided us with a reasonable number of candidate viruses that have the potential to be developed into vaccine viruses. Very few embryos, if any, would reach a stage of development when the embryo is deemed to be sentient (1 to 2 days prior to hatching).

Following an isolate being successfully propagated in 14-day old embryos it will be subsequently passaged in 10-day old embryos and incubated for 48 to 72 hours, an age not covered by the law.

Antiviral work will be initiated in 9-day old to 11-day old embryos, but the treatment might extend to or beyond 14-days of development.

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

Minimisation of any suffering will be done by using embryos less well developed whenever possible. For viruses that might cause the death of the embryo, pilot studies will be carried out to determine the mean time to death in younger aged embryos (typically 9 - to 11-days of embryonic development) and in subsequent work the embryos will be chilled to 4^{o} prior to this time.

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

The production of viruses in hens' eggs for the development of viruses for development into egg-based human influenza vaccines is an activity done by very few laboratories globally. We will discuss with the other laboratories that carry out similar work whether they discover that younger aged embryos can be used for virus isolation without a significant reduction in the successful isolation of viruses and whether they have improved procedures.

Work on antiviral treatments will be done according to protocols developed to be robust.

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. We discuss our work with the small number of other laboratories doing similar work globally.