Non-technical summary: IBV: attenuation and vaccine development

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

Key words
chicken, IBV, Coronavirus, pathogenicity, immunogenicity

Animal types	**Life stages**
Domestic fowl (Gallus gallus domesticus)	adult, neonate, juvenile

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?
Infectious bronchitis virus (IBV) causes a respiratory disease in chickens. It causes economic losses and is a risk to food security as infected chickens do not gain as much weight or produce as many good quality eggs as uninfected chickens. IBV is prevalent throughout the world and there are many different strains of the virus circulating. Currently available live attenuated vaccines are not able to protect chickens against all of these strains and have the potential to revert to a more virulent form.

The aim of this project is to produce a rationally attenuated IBV vaccine vector that can be modified for the production of effective vaccines against a variety of IBV strains belonging to different serotypes. In order to achieve this aim, we have three objectives:
1. To identify pathogenic determinants present in the IBV genome.
2. To identify immunogenic determinants present in the IBV genome.
3. To determine the protective efficacy of IBV vaccine candidates.

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 avian coronavirus, infectious bronchitis virus (IBV), is a highly contagious poultry pathogen prevalent in all types of poultry flocks worldwide and is responsible for economic losses, welfare problems in chickens and a potential risk to food security. IBV preferentially causes respiratory disease, but can also infect other organs such as the kidneys (resulting in

kidney disease) or the reproductive tract (resulting in loss of egg production and/or egg quality).

IBV is the aetiological agent of infectious bronchitis; an economically important infectious disease affecting chickens in the UK. It is estimated that IBV affects 22 million chickens and costs the UK poultry industry £23 million every year. Poultry is an important food source worldwide; with an estimated 55 billion chickens produced worldwide per annum, including 5 billion for egg production.

What outputs do you think you will see at the end of this project?
Outputs of this work will include new information regarding pathogenic and immunogenic determinants within the IBV genome. Outputs may include the generation of genetically modified IBVs that have potential to act as live attenuated vaccines against multiple serotypes. We always aims to publish our work in scientific journals in a time appropriate manner therefore outputs will also include publications. This ensures the scientific community remains up to date with the results that we produce. We will also file patents for any results that may have commercial potential/impact.

Who or what will benefit from these outputs, and how?
Our work uses the host species that IBV affects and therefore is of direct benefit for the development of IBV vaccines to protect chickens against this disease. All commercial chickens are vaccinated against IBV. Vaccine breakdown has a major effect on the UK poultry industry, not only financially, costing £23 million every year, but also affecting bird welfare and risking food security. This work will establish whether our novel approach to vaccine design through rational attenuation and modification of vaccine serotype is capable of producing safe and efficacious vaccines for the control of IBV that are less likely to revert to virulence. The development of safer vaccines will reduce the amount of antibiotics used to treat secondary bacterial infections associated with infectious bronchitis, which would have positive environmental impact. Results of these studies may reveal correlates of protection against IBV, informing the design of future studies.

In a shorter time scale, this project will allow for the identification of pathogenic and immunogenic determinants within the IBV genome. This is not only specific for the IBV field of research but will also prove information for the wider coronavirus field, and therefore may aid in vaccine research and design for other coronaviruses. The coronavirus family consists of a large number of viruses that infect a diverse range of species including swine, bovine and humans. The outputs of this project will therefore feed into the One Health initiative. The results of this project will be of direct benefit to the poultry industry and vaccine developers.

How will you look to maximise the outputs of this work?
This study will inform the wider academic community including other researchers working in the fields of molecular virology, livestock health and coronavirus research, particularly IBV, and will inform approaches to development of other veterinary and human vaccines. Knowledge generated by this project will be widely disseminated to the research community through peer-reviewed publications and presentations at national and international virology conferences and interactions with members of the poultry industry and veterinarians. We will aim to set up collaborations with both academic partners and industry partner when appropriate. We will openly discuss and aim to publish “negative” data which in the regard of this project involves the identification of genome factors which are not pathogenic or immunogenic determinants.

Species and numbers of animals expected to be used
Domestic fowl (Gallus gallus domesticus): 1750

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 are using the natural host of IBV, the domestic chicken for which we wish to contribute to better IB disease control. Only the chicken can be used for propagating most strains of IBV, assessment of pathogenicity and analysing potential vaccines; we are developing vaccines for protecting chickens against IBV.

Typically, what will be done to an animal used in your project?
This project will include two main types of experiments. The first, to determine pathogenicity will involve birds being inoculated/vaccinated via the intraocular (eye drop) and/or intranasal route with IBV. Chickens will be monitored twice daily. Clinical signs including snicking (sneeze) and rales (tracheal vibrations) will be assessed in a non invasive way for up to 10 days post infection. Swabs of the oral cavity, oesophagus and trachea maybe taken daily. Typically, at either 4 or 6 days post inoculation randomly selected birds will be euthanised. The second type of experiment to assess immunogenicity or vaccine efficacy will begin as described for the first experiment. This experiment will differ as chickens will typically be challenged three weeks post vaccination. The challenge virus will be a pathogenic IBV and will also be administered via the intraocular (eye drop) and/or intranasal route. Chickens will be monitored both post vaccination and post challenge for up to 10 days. Blood samples will typically be taken from a superficial vein (e.g. wing vein) two days pre vaccination and two days pre challenge. Typically, at either 4 or 6 days post challenge randomly selected birds will be euthanised. All birds will be culled at the end of both experiments as described; the end of the experiment will be 14 days post the last inoculation.

What are the expected impacts and/or adverse effects for the animals during your project?
All our experiments using chickens involve the development of vaccines ultimately for the benefit and protection of chickens against disease and are carried out in environmentally controlled experimental animal facilities. We keep animals under regular observation and use non-invasive measurement of clinical signs of infection. IBV mainly causes clinical signs very similar to the common cold in humans; a few days of snicking (akin to sneezing), watery eyes and wheezing. Chickens are expected to recover from respiratory disease within ten days and will experience a maximum of moderate disease severity, most will only experience mild disease. As we need to analyse virus growth and disease pathology in different organs, all chickens will be humanely euthanised at the end of the experiment.

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)? Chickens are expected to recover from respiratory disease within ten days and will experience a maximum of moderate disease severity, most will only experience mild disease. It is estimated that 60% of chickens will experience mild severity and 40% moderate.

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?

Pathogenicity and vaccine efficacy can only be assessed using the natural host of IBV, chickens; there is no in vitro or ex vivo alternative model. Embryos cannot be used as alternatives to chickens as even strains non-pathogenic for hatched birds can cause morbidity in embryos. Also, although the nonpathogenic Beaudette strain does cause ciliostasis when inoculated onto ex vivo tracheal organ cultures (TOCs) in the laboratory, it does not cause tracheal ciliostasis following eyedrop/nasal inoculation of chickens. In vitro assessment of immunogenicity, for example virus neutralization assays will be carried out where ever possible, however the identification of virus neutralizing antibodies in vitro has been shown not to correlate with protection *in vivo*.

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

Tissue culture, including primary chicken kidney cells *and ex vivo* tracheal organ cultures (TOCs) will be used whenever possible. The growth and titration of IBV isolates that cannot be grown in cell culture or TOCs will be carried out using embryonated eggs.

Why were they not suitable?

Pathogenicity, immunogenicity and vaccine efficacy can only be assessed using the natural host of IBV, chickens; there is no in vitro or ex vivo alternative model. As stated above, although the nonpathogenic Beaudette strain does cause ciliostasis when inoculated onto ex vivo TOCs in the laboratory suggesting that it may be pathogenic, it does not cause tracheal ciliostasis following intraocular (eye drop) and intranasal inoculation of chickens. Additionally the H120 and Beaudette strains of IBV which are both attenuated in vivo, are pathogenic in embryos.

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?

A statistician is consulted prior to each study to ensure that an appropriate number of chickens is used to generate meaningful results. The number of birds per group at each time point is selected to guarantee statistically relevant results for the assessment of protection and pathogenicity based on many years of experimental work on IBV and the recently published meta-analysis of the required sample size in vaccination-challenge experiments with IBV.

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

We have consulted the publication on the meta-analysis of the required sample size in vaccinationchallenge experiments with IBV and we have taken into account the results from the many years of IBV in vivo studies that have been carried out.

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

We will continually review the published literature with a statistician to ensure optimal number of chickens are being used. We will also keep up to date with the standards set for IBV vaccination by the European Pharmacopoeia. Post-mortem tissues will be shared with other researchers.

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.

We are using the natural host of IBV, the domestic chicken for which we wish to contribute to better IB disease control. Only the chicken can be used for the assessment of pathogenicity and analysing potential vaccines; we are developing vaccines for protecting chickens against IBV. Most of the chicks that we infect develop mild respiratory disease from which they recover within a few days. Chickens will be observed twice daily following inoculation with IBV to ensure that clinical signs and welfare of the chickens are closely monitored. Inoculation of the chickens via the intraocular (eye drop) and intranasal route mimics both spray vaccination practices and natural infection via the aerosol route. Chickens inoculated in this manner only experience mild momentarily discomfort.

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

Embryonated eggs will be used for the growth of IBV isolates that cannot be grown in cell culture. Cell culture and TOCs will be used where possible to minimise the use of embryonated eggs.

Pathogenicity and vaccine efficacy can only be assessed using the natural host of IBV, chickens; there is no in vitro or ex vivo alternative model. Embryos cannot be used as alternatives to chickens as even strains non-pathogenic for hatched birds including Beaudette and H120, can cause morbidity in embryos. Tracheal ciliary activity in ex vivo tracheal organ cultures is also not an appropriate model for pathogenesis in vivo as IBV strains such as Beaudette can cause ciliostasis when inoculated onto ex vivo TOCs in the laboratory but cannot not cause tracheal ciliostasis following intraocular (eyedrop) and intranasal inoculation of chickens.

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

Our procedure for determining pathogenicity and vaccine efficacy has been used for many years by us and others, and has been published. We inoculate the chickens via the intraocular (eye drop) and intranasal route, mimicking spray vaccination and aerosol transmission but in a more controlled way. Chickens will be monitored twice daily following inoculation with IBV to ensure that clinical signs and welfare of the chickens are closely observed. The chickens used in this research will be housed in open raised floor pens with solid floors which were specifically designed in consultation with our Animal Technicians and NACWOs. Flexibility was an important feature of the design to ensure pen sizes can be increased as the birds grow. Chickens will be provided with enrichment including perches, pecking blocks and live feed. Foraging, scratching and pecking are all important behaviours to birds and so enrichment provided will enable them to express their species specific behaviour.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
We will adhere to the ARRIVE guidelines for reporting of in vivo studies.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
We will keep updated with published literature regarding animal experimentation in poultry and will regularly consult the NC3Rs website. We will maintain an open dialogue with the animal technicians and NACWOs in relation to enrichment that can be provided.