Non-technical summary: Infection and immunity of avian viruses

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
(ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
(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)
(d) Protection of the natural environment in the interests of the health or welfare of man or animals

Key words
Avian viral diseases, Zoonosis, Vaccines, Antivirals, Probiotics.

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

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?
The aim of this project is to improve disease control systems against several important avian viruses by defining how avian influenza viruses (AIVs) cause disease and persist in poultry. Additionally, determining the effects of co-infection with AIVs and other avian viruses on morbidity, mortality, and transmission, and by developing novel mitigation approaches (vaccines and antivirals) will help reduce production losses and zoonotic or pandemic threats from these avian viruses.

A retrospective assessment of these aims will be due by 18 October 2028

The PPL holder will be required to disclose:
Is there a plan for this work to continue under another licence?
Did the project achieve its 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?

Poultry production is a critical sector for food security, economic development and poverty reduction, but it faces significant challenges due to avian viruses such as AIVs. These viruses are posing a significant threat to poultry, and their spread is mainly due to migratory wild birds that can transmit the viruses to domestic poultry flocks. The current epidemic of high pathogenicity avian influenza H5N1 virus has led to the death or culling of over 200 million domestic poultry worldwide during 2022/2023. To control the spread of AIVs, pre-emptive measures such as mass culling of infected and potential contact flocks are taken, which can result in significant economic losses. We aim to develop a comprehensive knowledge base of AIVs circulating in wild birds and poultry to develop more effective disease control systems, including highly effective vaccines that provide full protection from AIVs together with other major viral diseases affecting poultry production.

Co-infection with multiple avian viruses is another significant threat to poultry, exacerbating the severity of the disease and reducing vaccine efficacy. We aim to study the mechanisms of co-infection and how they impact AIVs persistence in poultry.

Vaccines are essential tools for reducing the impact of viral diseases in poultry, but the administration of multiple doses for each disease can be costly and repeat vaccination programmes also stressful to animals. We aim to improve the effectiveness and multivalency of poultry vaccines, so one or two vaccines administered at the hatchery can provide long-lasting protection against AIVs along with other important avian viral diseases.

Additionally, we plan to explore the use of cost-effective antiviral and probiotic strategies to minimize the impact of avian viral diseases on poultry. This project will also contribute to pandemic preparedness by evaluating the safety of candidate vaccine viruses in chickens for large-scale production in low-containment facilities, posing no adverse risk to animals, humans, or the environment.

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

This research aims to reduce the impact of major avian viral diseases on poultry production. The consequences and repercussions of these diseases on trade, food security, public health, and the livelihood of millions of farming and associated communities around the world are evident from the continued global prevalence and spread of avian viruses such as high pathogenicity AIV. The research outputs will include (i) improved knowledge of the factors that facilitate fitness, pathogenesis, and persistence of AIVs in different avian hosts and the risk of zoonotic infection by AIVs; (ii) improved disease control tools (vaccines and antivirals) with greater ability to reduce the production losses, zoonotic and pandemic threats; and (iii) new data and publications leading to further improvement in disease control systems and animal welfare, (iv) professional development of the next generation of scientists and (v) socio-economic wellbeing.

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

Our research aims to understand how viral and host factors increase the transmission and disease severity of avian influenza viruses (AIVs). In the short-term, these findings will improve our fundamental knowledge of AIVs, which will aid in the development of disease control tools such as vaccines, antivirals, and probiotics. These tools will target multiple

avian viral diseases affecting poultry and will provide strong and long-lasting immunity against AIV together with other major avian viral diseases.

In the mid-to-long term, we will develop more potent and efficacious disease control tools, reducing economic and welfare issues associated with administering multiple doses of viral vaccines to a single bird. We aim to enhance vaccine potency and multivalency through novel approaches such as designing highly effective multivalent vaccines that can be delivered via mass delivery methods including vaccination to embryo in eggs before hatching, or via spray or drinking water. Our research also aims to develop novel antiviral therapeutics such as recombinant antiviral compounds that can also be administrated using the mass delivery methods, providing immediate protection against the target avian viruses.

Overall, this project will contribute to the reduction of poultry production losses, promoting global food security and improving animal welfare. This research will benefit various stakeholders in the poultry value chain, including commercial and backyard poultry farmers, animal and public health bodies, and the veterinary product development economy. By providing direct benefits to farming communities and substantial indirect economic, public health, environmental, and social benefits, the effective control of AIVs in poultry will also reduce their transmission to humans.

How will you look to maximise the outputs of this work?
The research will advance our knowledge of the pathogenesis of AIVs, and of vaccines, antivirals, and probiotics for improving controls against major avian viral diseases affecting poultry production. Specifically, the project will generate a large volume of new data on molecular markers linked to AIV evolution, virulence, vaccine failure, and zoonotic infections.

The research will also inform new approaches to the development of improved multivalent vaccines for different avian species (such as chickens, ducks, and turkeys) that can be exploited for other livestock and human diseases. The outputs of the project will be disseminated primarily via scientific publications and conference presentations. We are working on several collaborative projects with the poultry industry, and national, and international partners working to improve disease control systems for viral diseases of poultry and livestock. We will openly discuss and aim to publish “negative” data, which in regard to this project, involves the identification of viral and host genome factors and mechanisms which do not contribute to the pathogenesis of the disease, or that are not immunogenic determinants of AIV, and novel multivalent vaccines, antivirals or probiotics that do not provide protection.

The methods and reagents developed through this research will also be made available to scientists, veterinarians, and public health officials who are concerned with reducing the impact of infectious diseases affecting animal production and welfare, human health, and food security.

Species and numbers of animals expected to be used
Domestic fowl (Gallus gallus domesticus): 2750 Post-hatch and 500 embryos.
Other birds: No answer provided

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.

This research programme involves the use of chickens, ducks, and turkeys, including embryos and post-hatched birds (neonate, juvenile, and adult). These birds at different stages of their development experience disease outbreaks from the target avian viruses in the field and for which we want to improve disease control systems. There are no alternative less sentient model species available that provide the required assessment of AIV pathogenesis and identification of immune correlates of protection against target avian viruses.

Typically, what will be done to an animal used in your project?
The experimental procedures will involve:
1. Embryonated eggs will be used for assessment of the infectivity, replication, and virulence of AIV; for evaluation of the immunogenicity of subunit or recombinant vectored vaccines expressing antigens of AIV, and other important avian viral pathogens, or for evaluation of the efficacy of antivirals. The age of the chicken embryos will be from 0-21 days. The age of turkey embryos will be from 0-28 days and duck embryos will be variable (0-28 days) depending on the specific breed of duck. Typically, a virus, vaccine, or antiviral will be inoculated into each egg via pipette tip or with a syringe into a small hole in the side of the egg made with either an 'egg gun' or a drill. Following inoculation, eggs will be incubated at 29 – 42oC in a humidified rocking incubator or a warm room. Embryos will be monitored by candling (daily) during the appropriate incubation period required for the specific virus strain used in the experiment (usually 72 hours post-inoculation). The embryos will be killed humanely one day before hatching for studies on the pathogenicity of AIV or will be allowed to hatch for studies on the immunogenicity and protective efficacy of vaccines, or for studies on antiviral efficacy.
2. Inoculation/vaccination of birds (chickens, turkeys, or ducks) with substances (virus, vaccine, antivirals, or immune modulators/enhancers or probiotics) via the appropriate route of administration. Birds will be closely monitored for any adverse effects (clinical signs) on their health on a regular basis (monitoring intervals will be adjusted according to the phenotypic characteristics of the virus strain or vaccine). Samples (swabs, blood) will be taken at intervals to analyse virus replication and/or immune responses. Swabs will be taken from oral and cloacal cavities, appropriate to species and challenge virus and vaccine. The birds may be culled humanely at set intervals (post-vaccination and/or postinfection) to monitor host immune responses or to investigate the changes, presence, and distribution of the virus in infected animals. The birds may be kept up to 32 weeks post-vaccination, post-antiviral treatment, and/or virus challenge to assess the impact of the treatments. Since the challenge viruses will be pathogenic, emphasis will be placed on animal welfare and provisions have been made in the specific protocols.

What are the expected impacts and/or adverse effects for the animals during your project?
Embryos in the final third of gestation may show signs of haemolysis of blood vessels, reduced or lack of movement. Embryos showing these signs will be killed immediately.

Birds (chickens, turkeys, or ducks) and mice will experience mild and transient pain associated with vaccination, blood sampling, or swabbing.

Following infection with virulent viruses, birds may develop clinical signs of disease depending on the virus strain. Clinical signs of disease may include weight loss, sneezing, coughing, ocular and nasal discharge or sitting alone, and reluctance to evade capture. Generally, the birds take about 72 hours to recover from these typical clinical disease signs. However, infection with some AIVs may result in death in a proportion of birds without any

clinical signs appearing beforehand. The birds will be monitored for clinical disease signs at regular intervals so that the animal does not cross the defined severity limit for the virus strain used for infection.

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)? Mild more than 70%
Moderate: 15-20%
Severe or sudden death without clinal signs: 5-10%

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

A retrospective assessment of these predicted harms will be due by 18 October 2028

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?
There are no alternative less sentient model animal species available that provide the required in vivo animal models for investigation of virus-host interactions in the pathogenesis of and immune responses to avian viruses. Chickens, ducks, and turkeys were selected because they are natural host species affected by different avian viruses (AIV, NDV, IBV, IBDV, FAdV, ARV, CAV, AMPV, ILTV) in the field. Therefore, we have chosen these avian species to model how AIV strains induce disease and persist in poultry. Similarly, vaccination studies ultimately require the establishment of immune correlates of protection for the target host species to be protected from disease. As there are differences in the immune systems between different avian species, it is therefore important to use target host species for the groups of viruses being studied here, where biological and antigenic variation is often related to, and dependent on the host of origin.

Which non-animal alternatives did you consider for use in this project?
Where appropriate, cell culture and other relevant in vitro techniques such as ex vivo tracheal organ cultures (TOC) or embryonated eggs (up to 14-day-old embryos) will always be used as the initial methods for assessing virus infectivity and replication efficiency. Studies have indicated that the majority of avian-origin viruses prefer embryonated egg culture as a growth medium. Therefore, embryonated eggs may be used as an alternative to tissue culture. Culturing viruses in cells can allow mutations to develop in the surface glycoproteins altering the antigenicity and receptor binding preference of the virus. By using eggs as a growth media or investigating pathogenicity or host responses to infection, this vastly imitates a more natural infection with reduced selective pressure that can be exerted by tissue culture cell lines. The use of eggs is the efficient way to produce most avian viruses with no genetic changes altering virus behaviour and pathogenicity.

In vitro techniques including single-cell sequencing, and phage display techniques will be investigated for the generation of recombinant antibodies. Antibody sequences will be

derived from immune cells (B cells from blood or tissues) collected from naturally infected animals or animals used in other virus infection or vaccination studies.

Why were they not suitable?
There are no alternative less sentient model animal species available that provide the required virus phenotype (infectivity, tissue dissemination, and transmissibility protective efficacy) data for avian species against selected viruses.

A retrospective assessment of replacement will be due by 18 October 2028

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?
A statistician is consulted prior to each study to ensure that an appropriate number of animals is used to generate meaningful results. The number of animals 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 avian viral diseases.

Group size could vary depending on experimental design aspects such as non-infected controls, virus strain or genotype, host species, challenge dose, immune status, and route of inoculation. However, studies usually involve groups of 6 to 10 birds.

The group size advised by the World Organisation for Animal Health (WOAH) will be adopted for testing the pathotype of avian influenza viruses known as the Intravenous Pathogenicity Index Test (IVPI).

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?
We always consult relevant publications describing the sample size in pathogenicity and vaccinationchallenge studies. We have taken into account the results from the many years of in vivo studies using different avian viruses that have been carried out previously. In addition, each animal study is reviewed by a statistician.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?
Pilot experiments will be undertaken in studies using viruses with unknown characteristics that differ in their infectivity and replication profiles in embryonated eggs in order to estimate an appropriate virus dose for productive infection in inoculated birds. The data from pilot experiments will be used for sample size estimation for follow-on virus infection, transmission, and vaccination experiments. We will continually review the published literature with a statistician to ensure the optimal number of birds for each experiment. Multiple studies will also be integrated in such a way as to utilise a minimum control group of

experimental animals. Samples such as post-mortem tissues can be shared between different studies and with other researchers.

In the studies that investigate the IVPI test of AIVCVV, the standard WOAH (World Organisation for Animal Health) advised procedure will be employed. For these tests, a minimum of 10 birds per group has been estimated to provide statistically valid data.

A retrospective assessment of reduction will be due by 18 October 2028

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.
We are using the natural host (chicken, turkeys and ducks) for which we wish to contribute to better disease control against selected avian viruses. The experiments investigate the pathogenesis, transmission, vaccine and antiviral efficacy evaluations in chickens, turkeys or ducks. The precise number of groups will be dependent on the precise information required in each experiment.

Before in vivo studies, the infectivity, replication and virulence of candidate viruses will be examined in embryonated eggs of chicken, turkey or ducks. This will determine whether the virus is lethal to the embryo of the target avian species and provides some indication of the virus behaviour in vivo when we compare it to the strain that we have previous information about. Lethality will be monitored by candling of infected eggs, to look for the death of the embryo or indicators that the embryo should be euthanised (no movement of the embryo or blood vessel disruption). The mean death time from published work will always be considered when infecting eggs from known genetic and pathological backgrounds and the infected eggs will be euthanised at least three hours before this time. This means that virus-induced embryo death will be avoided. If the mean death time is not known, a pilot experiment using a minimum number of eggs will be undertaken to determine the approximate mean death time.

The harm to animals caused by procedures such as injection, swabbing and bleeding are mild and transient. The greatest harm to poultry will be the development of clinical signs of disease following infection with virulent viruses. In designing an experiment, close consideration will be given to the likely severity, ensuring moderate severity is not exceeded for a known virus (by administering a defined dose). In the case of viruses with unknown severity, a design will be applied which will ensure that clinical signs of disease will occur predominantly during normal working hours, thereby facilitating increased inspections (minimum of four times/day) and accurate assessment using a clinical score sheet. Overall, these measures should result in the severity limit not exceeding moderate. However, a number of experiments will require the use of the most virulent virus strains in order to produce scientifically valid results. However, the number of animals that may experience

severe disease signs will be kept to a minimum. In some circumstances, animals infected with virulent viruses show little or no apparent clinical disease signs, and up to 20% per group may die unexpectedly. All unexpected deaths (regardless of virus challenge or not) will be investigated by post-mortem examination.

The birds used in this research will be housed either in open raised floor pens with solid floors which were specifically designed in consultation with our Animal Technicians and NACWOs or housed in negatively pressured poultry isolators which are designed to protect personnel and the environment from cross-contamination. Whilst isolators present inherent challenges, we are committed to providing high standards of animal welfare. The enrichment provided to all animals is a priority and this is no different for poultry in isolators. Birds are social animals and so they are housed in groups to allow for normal social interaction. Where possible, they are also afforded more space than required within the Home Office Code of Practice. Foraging, scratching, and pecking are all important behaviours to chickens and turkeys, so we provide our birds with substrate on the floor to allow foraging and dustbathing and toys to enable them to express their species-specific behaviour regardless of whether they are housed in open pens or in sealed isolators. Additionally, for ducks, plastic containers filled with water and a sandpit will be provided to enable them to express their natural water paddling/bathing behaviour.

Why can’t you use animals that are less sentient?
There are no alternative less sentient model species available that provide the required immunopathological parameters or immune correlates of protection of avian viruses for target avian species. Where appropriate, cell culture, embryonated eggs (at immature life stage), and other relevant in vitro techniques such as ex-vivo tracheal organ cultures will be used as the initial methods for assessing infectivity and replication efficiency of selected avian viruses.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Our procedures for determining pathogenicity, transmission, vaccine, and antiviral efficacy in avian species have been used for many years by us and others and have been published (e.g., where appropriate experimental designs include both positive and negative controls. Increased monitoring regimes when birds start to show clinical signs of disease). Further to refine the procedures, advice is taken from the Named Veterinary Surgeon (NVS). Pre-study meetings involving the researchers, Named Animal Care and Welfare Officers (NACWOs) and animal technicians 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 design humane endpoints for future experiments. Pain and distress scoring sheets specifically designed for each virus will be used. Highly trained animal technicians will monitor the animals as per experimental procedures requirements defined in the study protocols, ensuring they are comfortable and maximise their welfare status. All experiments will be followed by a wash-up meeting to discuss all aspects of the study and to ensure lessons are learned.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
We will adhere to the ARRIVE (Animal Research: Reporting of In vivo Experiments) guidelines. We will also adopt the PREPARE (Planning Research and Experimental Procedures on Animals) principles. In particular, the allocation of birds to each study group

will be random, and where possible observer bias will be managed by blinding of treatment groups.

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 and available resources including guidelines, training materials, and practical information. We will maintain an open dialogue with the animal technicians and NACWOs at the establishment in relation to the enrichment that can be provided.

A retrospective assessment of refinement will be due by 18 October 2028

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?