Non-technical summary

Control of equine herpesviruses in the horse.

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

Vaccines, Herpesvirus, Equines, Abortion, Neurological Disease

Animal types                Life stages
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Ponies                      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.
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 research is to test the safety and protection afforded by new vaccines against herpesvirus infections in the horse.

A retrospective assessment of these aims will be due by 7 October 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 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?

Infectious diseases are very common in racehorses in training, breeding and in pleasure horses, the more serious of which can be devastating to the animal and the industry. Some of the well characterised infectious diseases are caused by members of the herpesvirus family and the most problematic of these are EHV-1 and EHV-4. EHV-1 and 4 are abundant viruses that affect horse populations on all continents. While the two viruses share a high degree of genetic similarity, they differ significantly in the disease they can cause. EHV-1 can result in respiratory disease, abortions, death of new-born animals and damage to blood vessels in the brain and spinal cord which can cause neurological disease. Neurological disease outbreaks have occurred with increasing frequency in North America and Western Europe and are a concern for the UK equine population. Outbreaks on stud farms in unvaccinated pregnant mares can lead to extremely high rates of abortion. Commercially available vaccines have been shown reduce the extent of virus shedding of EHV-1 and 4 from infected horses. However, they offer poor protection against abortion and none are licenced to protect against neurological disease. This was highlighted by the abortion outbreak recorded in Hertfordshire in 2016 and the later cases of neurological disease that occurred in Valencia in 2021, both in fully vaccinated animals. EHV-4 can cause severe respiratory illness in horses, these horses are placed in quarantine, cannot travel, and are unable to be ridden or compete causing financial impact sometimes globally.

The Fourth International Havemeyer Workshop on Equid Herpesviruses, held in 2018 in North Carolina, invited the most pre- eminent scientists from around the world working on equine herpesviruses. They highlighted the continued occurrence and impact of equine herpesviruses on equine health, welfare and the equine industry. The workshop concluded the most important direction for research continues to be the development of vaccines that can control the most serious types of
disease. Previously published work by other groups has indicated that a vaccine based on a partially disabled EHV-1 virus can offer protection against both EHV-1 and EHV-4 infection, but not the other way round. Therefore, the focus of this project licence is primarily EHV-1.

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

This project will develop and test the safety and effectiveness of new vaccines against EHV induced disease. This will also contribute to an improved understanding of EHV disease processes. Results will be published in peer-reviewed publications and findings will be presented at local, national, and international scientific meetings. In the longer term, there will be intellectual property that may support a planned patent application, support future funding applications/clinical trials or may aid in the licencing of any successful vaccine. The results will directly improve the health and welfare of horses worldwide. Knowledge gained in this project may be directly applicable to the development of vaccines for the prevention of other mammalian herpesviruses.

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

In the short and medium term we will determine whether we can design and make novel vaccine candidates in the laboratory. Data gained from these experiments will contribute to our understanding of EHV-1 infections in horse cells and ultimately in the horse. Long-term we hope to develop a more effective vaccine against EHV-1 which has the potential to benefit all equines, preventing respiratory disease, abortion and neurological disease. These improved vaccines will be of particular interest to the equine breeding industry in the UK which still suffers significant welfare problems and financial losses due to EHV-1 induced abortions.

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

This project has contributors from a number of international academic and commercial laboratories. Where applicable, results will be patented, published in peer reviewed journals and presented at international conferences. We will attempt to disseminate all findings, including unsuccessful approaches and non-significant data via open access publications or platforms such as F1000Research which offers rapid publication of data without editorial bias.

Species and numbers of animals expected to be used

- Ponies: 48

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.
Unfortunately, the use of ponies for our research is essential. EHV-1 and EHV-4 cause disease that is unique to this animal. We need to measure how the complex pony immune system responds to the vaccines and virus infection in order to maximise the level of protection achieved.

For pharmaceutical companies to licence and market vaccines commercially, they need to be tested according to regulations determined by the competent authority. These regulations state that the vaccines must be tested in the target species, in this case ponies.

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

Animals are kept at grass when uninfected. Blood samples will be taken from the jugular vein for the isolation of blood cells for the cultivation of vaccines and the development of tests. These are routine procedures conducted by experienced staff. Ponies will be maintained on site and may be rehomed at the end of the procedure.

Some animals will be vaccinated with experimental vaccines. Typically, this will involve the injection of the vaccine into the neck muscles in the same way as commercially available vaccines. When undergoing the first vaccination procedure these animals will be held in containment buildings. These animals will then have swabs inserted into the nose to obtain biological material (nasal swabs) and blood samples taken regularly to monitor the immune response induced by the vaccines. At the end of the study animals will be rehomed or humanely killed depending on the vaccine given. Once a dossier of data has been compiled for each vaccine, animal housing and rehoming will be reassessed to determine whether the animals could be vaccinated at grass and rehomed after the procedure. This procedure typically lasts 6 weeks per vaccination.

A pilot study will be conducted where animals housed within an open barn with bedding will be infected with EHV-1. This procedure typically lasts three weeks. Infected animals will be bled and swabbed regularly to determine viral titres and the immune response to virus infection. At the end of the experiment some ponies will be humanely killed. Others may be kept alive for continued bleeding to assess the on-going immune response to infection.

Some animals will be vaccinated and then infected with EHV-1 and housed within an open barn with bedding. This procedure consisting of a vaccination phase and an infectious phase typically lasts 8 weeks in total. At the end of the experiment all ponies will be humanely killed.

EHV-1 infected animals are typically not rehomed as they are thought to carry the virus for the rest of their lives. This virus may reactivate later in life, spread to other animals and cause disease. Animals that are fully recovered at the end of procedures may be kept alive at the establishment (with agreement of a vet) with a view to their reuse on procedures if appropriate and licenced. Otherwise, animals will be killed humanely using an approved method.

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

When blood samples are taken from equines this will result in temporary discomfort and no lasting harm.
Animals that are vaccinated may experience mild local reactions such as swelling or soreness. We expect any reactions observed will be no worse than those seen following the administration of current commercially licenced EHV-1 vaccines that are used in the general horse population.

Animals infected with EHV-1 typically have a self-limiting and mild illness. Unvaccinated ponies typically develop a fever from day 2 after infection which lasts for 1-3 days and they may develop nasal discharge. Naïve ponies can sometimes develop secondary bacterial infections after virus infection and any affected animals will be treated with antibiotics. There is a possibility that ponies infected with EHV-1 may develop neurological disease as a result of damage to blood vessels in the brain or spinal cord, similar to that seen in some outbreak situations in the field. In most animals this resolves on its own without treatment. Any affected animals will be observed closely for the duration of the clinical signs and appropriate supportive therapy administered. If they fail to respond to treatment or the severity limit is likely to be exceeded they will be humanely killed in order to minimise any suffering. At the end of the project, animals could undergo continuous use if we need to do more work to achieve our objectives. Animals could also be re-used on another project licence.

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)?

**Ponies**
- Mild: 90%
- Moderate: 10%

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

- Killed
- Rehomed
- Used in other projects
- Kept alive

A retrospective assessment of these predicted harms will be due by 7 October 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?
During the preliminary stages of the vaccine development programme we will use cell lines and horse cells isolated from blood to avoid the need to infect animals. These studies will enable us to select only those vaccines that are most worthy of further investigation in ponies. Post-mortem tissues from animals humanely killed for reasons not connected to this project will also be used in order to avoid the need to infect animals in the preliminary stages. Unfortunately, the use of ponies for our research is essential. EHV-1 causes disease that is unique to equines. We need to measure how the ponies’ immune system responds to the virus and vaccines in order to maximise the level of protection achieved by the vaccines.

For pharmaceutical companies to licence and market vaccines commercially they need to be tested according to regulations determined by the competent authority. These regulations state that the vaccines must be tested in a target species, in this cases ponies.

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

We will use commercially available cell lines for vaccine production and some of the laboratory based vaccine characterisation. Unfortunately, there are no non-animal alternatives that can be used instead of equines for the testing of the vaccines.

Why were they not suitable?

There is no non-animal model that can reproduce the complex interactions between the virus and the horse immune system.

A retrospective assessment of replacement will be due by 7 October 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?

This first part of the vaccine development programme requires horse blood from which we isolate primary cells. We have used 2 animals for this purpose on a number of different project licences over many years. This is because not all animals produce cells that respond in the typical way. We have previously seen up to 10% of cells not behaving as predicted, so we use the minimum biological
replicate to reduce the chances of having a non-responding blood donor. Blood will be tested at the beginning of the protocol and replaced with alternative donors if necessary. Using two animals also allows us to keep them in a social group at grass.

The second part of this programme is the establishment of the EHV-1 challenge model in Welsh mountain ponies. Historically, we have used 5 animals in these pilot studies for this purpose in order to get an accurate representation of infection in the animals, before we proceed with safety and efficacy studies later in the programme. Previously conducted studies have shown between 75-100% of control animals develop disease so we would expect at least 4 animals to become infected. These data obtained in this pilot study will be used to re-evaluate the number of animals to use in any vaccination study in the future.

The third part of this programme is the testing of candidate vaccines for safety and immunogenicity in Welsh mountain ponies. Typically, we use 3 animals per experimental vaccine group. We envisage we will test at least 3 candidate vaccines in the first instance alongside a commercially available vaccine for comparison purposes. These data will be assessed and used to select one or more experimental vaccines to take forward to the next stage where we will test vaccine effectiveness.

The fourth stage of the programme is the testing of vaccine effectiveness after infection with EHV-1. We typically use 7 animals per vaccine group, and we envisage we will test at least 2 candidate vaccines. These numbers slightly exceed the minimum requirements suggested in the European Pharmacopoeia monograph guidelines which are required for maintaining the official quality standards for medicines and their ingredients in Europe, and are more likely to obtain statistically significant data.

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

During the experimental design phase we consulted the PREPARE guidelines (https://norecopa.no/PREPARE) and the guidance on the NC3Rs website which gives advice on how to minimise the number of animals used per experiment. The design of all studies have been checked by a statistician to ensure that the smallest numbers of ponies are used in order to achieve statistically significant results. We have also used additional software such as GPower1 to confirm the experimental design numbers (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).


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

We use pilot studies to ensure we have the experimental conditions correct before we use larger numbers of animals later in the project. Pilot studies will allow us to assess the experimental design and identify potential problems, as well as implement improvements early on in the licence.
Animals are randomly assigned to control or treatment groups, while trying to maintain existing social groups within the ponies. The study director and staff involved in animal husbandry and the clinical monitoring of the animals are blinded. An experienced study co-ordinator maintains the study file with the key for the randomisation which can be accessed if required during the protocol.

We will also ensure that we freeze virus and blood samples in a -70C archive freezer in order to reduce the number of animals used in the future. This will also allow us to share samples with other researchers further reducing the number of animals used.

A retrospective assessment of reduction will be due by 7 October 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.

Basic experiments have been developed that help us characterise vaccines in the laboratory before we use them to vaccinate horses. In this way we can reduce the number of animals used for testing. However, we cannot model the complex interaction of the virus with the horse immune system so we must use the natural host to determine whether newly designed vaccines are safe and work. Extensive experience of animal handling and regulated procedures suggests that serious adverse effects of repeated blood sampling, vaccinating and EHV-1 infection are rare. Horses that undergo vaccination only are kept at grass in groups in grass paddocks with freedom to roam with shelter if they require. The research being addressed by the programme will contribute to the welfare of horses in the long term by providing new and improved vaccines. In order for pharmaceutical companies to obtain marketing authority for commercial vaccines they must be tested in the target species, in this case equines.

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

The only way to accurately determine how well an EHV-1 vaccines will work in the horse is to use the natural host. In order for pharmaceutical companies to obtain marketing authority for commercial vaccines they must be tested in the target species, in this case ponies.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
All ponies will be health-checked by an equine veterinarian prior to purchase. Ponies will be transported by a professional experienced transporter in a livestock lorry with plenty of floor space, the floor will be covered with straw bedding. These procedures ensure that the ponies arrive in good health with the minimum of transport-induced stress.

The ponies will start their acclimatisation process as soon as they have had a couple of days to settle in at the quarantine site. Ponies are easier to handle if they become confident of being around humans. Routines are quickly put into place and the ponies are run as a herd for all handling procedures. Typically any handling procedures are conducted first thing in the morning using the same experienced personnel. Initially the ponies are held in a safe holding pen, then driven through a race system into a crush / veterinary stocks. Once they are comfortable moving freely through this system they are individually held in the crush, with a backboard slid behind them to prevent them from reversing out. Initially each pony will be offered concentrate cubes from a scoop, but most at this early stage of training will not take any. This process (which can take a couple of weeks) then progresses to ponies being quietly approached in the crush and a halter placed. Following a handling session, the ponies are then released as a group and fed concentrates to provide a source of positive association. Visual assessments of each pony while enclosed in the holding area become part of the daily routine of husbandry management. When the ponies are confident, new procedures will be introduced such as weighing, having rectal temperatures taken, worming and bleeding.

Ponies form associations with other ponies and staff should be aware of these affiliations so that they are allowed to continue during the studies. Accidental splitting up of these attachments can be stressful, therefore animals are managed to avoid this occurring. Difficult or very nervous animals can be separated into pairs or smaller groups to enable a more intensive period of training to get accustomed to the facilities, handlers and procedures.

The procedures of bleeding, vaccination and challenge will be undertaken by experienced staff on site thereby reducing the amount of stress suffered by the animals. By the time the study commences, ponies are used to being handled and entering the handling system and crush. Animals are housed in social groups and acclimatised to their environment. We use environmental enrichment to help ensure the animals are less stressed. To mitigate the effects of containment indoors, environmental enrichment includes the radio, which provides background noise during the day and is thought to calm the animals. Lighting is adjusted to reflect seasonal and daylight-saving changes outside. Root vegetables and fruits are provided such as carrots, swede, white cabbage, turnips and apples with some strung from the ceilings. Apples are provided in buckets of water, along with hay blocks, hay nets, foodballs and licks to encourage play. Scratch mats are also provided for the ponies in easily accessible areas of the containment room or enclosure. These processes are constantly reviewed by the scientists and veterinarians involved to maximise the quality of life and minimise suffering.

Animals will be regularly monitored after EHV-1 infection by experienced handlers who know the animals well in order to minimise any suffering or stress as a result. This will ensure that the humane endpoints are adhered to. Checks will include measuring body temperature, appetite, demeanour, breathing, eye and nasal discharge and assessing the size of the lymph nodes in the neck. Non-abrasive swabs with flexible handles are used. If animals develop serious respiratory disease they will be treated using antibiotics and/or anti-inflammatory drugs usually given orally as a paste.

We also include a neurological assessment or each animal to determine if the central nervous system has been affected. Any animals that develop neurological disease will be observed by the named
veterinary surgeon or other competent person for the duration of the clinical signs and appropriate supportive drugs administered.

When the ponies become eligible for rehoming, they will be well handled but are usually relatively young, so extensive work is undertaken to find knowledgeable, experienced homes for them. Potential new owners are encouraged to visit the ponies prior to departure to ensure they are fully aware and comfortable with each individual and where they are in their training. Comprehensive details are provided for the new owner and advice given in any areas required.

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

We will follow the guidelines published by the NC3Rs which includes the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines. These guidelines make recommendations which help to improve the reporting of research involving animals, thereby maximising the quality and reliability of published research, and enabling others to better scrutinise, evaluate and reproduce it in the future. The Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) guidelines complement the ARRIVE guidelines. They cover the planning of experiments, dialogue between scientists and the animal facility, and quality control of the various components in the study. Advice on use of the guidelines is available on the Norecopa website (https://norecopa.no/PREPARE). The Laboratory Animal Science Association (LASA) also publishes guidelines that make recommendations on good practice (https://www.lasa.co.uk/current_publications/).

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

We will be working closely with the Named Veterinary Surgeon (NVS), the Named Animal Care and Welfare Officer (NACWO) and a number of experienced horse handlers which will help keep us up-to-date about the principles of the 3Rs when working with the equines.

We also have access to on-line resources such as the National Centre for the Replacement, Refinement and Reduction of Animals in Research website (https://www.nc3rs.org.uk/) which often reports on the development of new 3Rs approaches. The website of the Royal Society for the Prevention of Cruelty to Animals (RSPCA) has an Animals in Science section (https://science.rspca.org.uk/sciencegroup/researchanimals) which has advice on implementing the 3Rs. The Norwegian National Consensus Platform for the advancement of the 3 Rs (Replacement, Reduction, Refinement) in connection with animal experiments (https://norecopa.no/about-norecopa) hosts a large amount of guidance for planning animal research and testing. The laboratory animal science association (https://www.lasa.co.uk/) hosts the large animal research network meetings (LARN) which are an important source of 3Rs information and discussion.

**A retrospective assessment of refinement will be due by 7 October 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?