Non-technical summary: Testing and assessing FMD vaccines

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
(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)

Key words
cattle, pigs, FMD, foot-and-mouth, virus

Animal types	Life stages
Cattle	adult
Pigs	juvenile, adult
Guinea pigs	adult
Rabbits	adult

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?
The aim of this project is to generate tools and data to assist in the global control Foot-and-Mouth Disease Virus (FMDV). This largely includes the assessment the immunogenicity and efficacy of FMDV vaccines at all stages of development and production. It also will include generation of reagents to assist in diagnosis of disease.

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?
Through vaccine testing and diagnostic reagent generation, we allow the UK to be better equipped to deal with any future incursions of FMD. Not only are these advantages equally important internationally (quality vaccines and diagnostic tool provision), but assessing cross-reactivity of vaccines will allow us to advise countries which vaccines are more efficacious for the strain they may be dealing with and whether there is any cross-protection to other strains that may be circulating in neighbouring regions.

What outputs do you think you will see at the end of this project?
This project will contribute to national, European and global control of FMD through provision of diagnostic reagents, as well as vaccine performance qualification. Vaccination is a

fundamental part of FMD control measures and there is a need to confirm the efficacy of existing vaccines for field use, as well as to continually develop and improve upon current vaccines. There is a regular need to identify new vaccine candidates to ensure that there are suitable vaccines for emerging FMDV strains. Once identified, these require additional testing to produce safe, potent, efficacious and stable vaccines.

Further vaccination with new adjuvants and constructs or manipulated capsids may increase the duration of immunity to FMD and the development of DIVA (differentiating infected from vaccinated animals) or marker vaccines for FMD that will protect target species and act as efficient discriminatory tools to support serological surveillance and confirmation of disease free status.

This work, both by assessing vaccine efficacy and performance as well as diagnostic tool provision, will have a global impact on animal health as it will help with international control and eradication of this economically devastating disease of cloven hoofed domestic and wild animals.

Who or what will benefit from these outputs, and how?
A reliable supply of safe, potent and effective vaccines is essential for the maintenance of animal health and welfare and the successful operation of animal health programmes. Immunization of animals with high quality vaccines is the primary means of control for many animal diseases including FMD. Supply of high quality, verified diagnostic reagents is of equal value in assisting in the control of FMDV outbreaks.

The information gathered from these studies will ensure that the establishment and World Reference Laboratory are in a strong position to offer national and international advice on FMD. We will also have the ability to supply materials to research and diagnostic groups within the establishment to enhance our research activities into the infectious processes and immune responses associated with FMDV in the target species and our diagnostic capabilities.

How will you look to maximise the outputs of this work?
The approach to the data generated from non regulatory studies carried out under this PPL, whether successful or unsuccessful, will be published in peer reviewed journals to enable the immune responses to FMDV infection and or vaccination to be disseminated to the community.

Regulatory data remains confidential however this will be utilised to enable market authorisation for vaccines to be obtained, which has an obvious direct maximisation of output as this will lead to increased vaccine uptake being available across the globe.

The reagents created to assist in diagnosis of FMDV will be made available globally.

Species and numbers of animals expected to be used
Cattle: 620
Pigs: 362
Guinea pigs: 50
Rabbits: 3

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.
The animal species and ages being used to assess vaccines are either in line with the international OIE and EU standards for assessing Foot-and-mouth disease vaccines for cattle, and in other peer reviewed literature for pigs. The animal species and ages (adults) being used to generate diagnostic reagents (rabbits and guineapigs) are those which have been verified and validated to generate optimal diagnostic tools over many decades.

Typically, what will be done to an animal used in your project?
For vaccine assessment, animals will have blood samples taken at periodic time points throughout the course of the study, typically before vaccination and after vaccination. Animals may then have a vaccination. If the immunity of vaccinated animals is also being tested, animals will be injected with foot-and-mouth disease virus and may have blood and swab samples collected after challenge. A typical batch test of vaccine will take 21 days, and a potency test (assessing protective ability of the vaccine) will typically take 29 days.

For reagent production, animals will have blood samples taken at periodic time points throughout the course of the study typically at day 0, at the time of boost, and at the end of the study. Animals will be vaccinated, and then typically boosted around day 24-28. Animals will then have a large blood sample collected at the end of the study before they are humanely culled, typically 42 days after the initial vaccination (21 days post boost).

What are the expected impacts and/or adverse effects for the animals during your project?
Animals which are only vaccinated with vaccines are expected to only experience mild severity. Animals which are challenged with live virus may develop clinical signs of foot and mouth disease and are expected to experience moderate severity.

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)? All animals which are only injected with vaccine are expected to only experience mild severity. All animals which are challenged with live virus are expected to experience moderate severity. For vaccine assessment studies in cattle and pigs it is expected that around 70% of animals on this licence will experience moderate, and 30% mild severity. For the reagent generation in rabbits and guinea pigs, all animals are expected to experience mild severity.

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?
The testing of vaccines requires the host species to be used under regulatory and licencing authorities. However, prior to animal testing, vaccine strains are screened using cell culture techniques to help match them to field viruses against which protection is sought and to check that they have growth and stability characteristics suitable for vaccine manufacture and storage.

Research is ongoing to develop improved methods to evaluate vaccine performance in the field, reducing reliance on the use of experimental animals; some of the work from this project will generate data that can be used in models already developed to assist with their validation.

Antibodies collected from vaccinated rabbits and guinea pigs are currently validated as the most effective reagents used in diagnostic platforms for foot-and-mouth disease virus detection. Antibodies from 2 species are required to allow one to be used to immobilize the antigen, and another to act as a detection antibody. In parallel, immune cells from both vaccinated guinea pigs and rabbits will be collected post mortem and stored to enable the ability to generate antibodies in vitro which may then be compared to the polyclonal antisera. This second output is not the primary aim but will be explored as a potential future replacement.

Which non-animal alternatives did you consider for use in this project?
It is not possible to assess vaccine efficacy and immunogenicity for regulatory purposes without using animals as the set international procedures stipulate the test. Titration of cattle adapted virus is now undertaken in vitro, animals are not used. The use of antibodies generated from pre-existing animal-free antibody libraries, which are monoclonal, is being explored. However, results demonstrating equivalent or better performance of monoclonal compared to polyclonal antibodies has not yet been demonstrated. Cross comparison of the major diagnostic assays has been undertaken, and continues to be assessed for this purpose.

Why were they not suitable?
The regulatory tests could not be achieved without the use of animals as the law stipulates the test required.

The performance of currently available monoclonal antibodies has not been proven to be equivalent to or better than polyclonal serum against all strains of FMDV.

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 is based on for cattle:
2 Potency tests conducted per year, (17 animals per test) for 5 years = 170
8 batch tests per year (5 animals per test) for 5 years = 200
5 x Immunogenicity studies per year (10 animals per test) for 4 years = 200
3 generalisation studies per year (2 animals per test) = 30
2 production studies per year (2 animals per test) = 20 Total = 620

for pigs:
4 potency tests (17 animals per test) during the course of this project = 68
3 x challenge model studies (18 animals per test) = 54
4 immunogenicity tests per year (15 animals per test) for 4 years = 240
Total = 362

For rabbits and guinea pigs:
150mls of hyperimmune rabbit antisera is required for each serotype
280mls of hyperimmune guinea pig sera is required for each serotype
FMDV serotype O-specific antisera is required from both species, and serotype A from guinea pigs only
Guinea pigs used are on average 400-600g, and rabbits 3-5kg, and based on previous experience 20- 30% of circulating volume (assumed to be 60ml/kg body weight) can be routinely collected at the end of the study.
Therefore (assuming 20% of circulating volume can be collected consistently with the midpoint mass for each range for each species) the numbers required are:
Guinea pigs = 25 per serotype and as 2 serotype specific antisera is required = 50 guinea pigs total Rabbits = 3 per serotype and as 1 serotype-specific antisera is required = 3 rabbits total

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?
These are the minimum number of animals used according to the OIE manual and EU Pharmacopeia for cattle. For pigs, statistical advice will be sought from the institutes' statistician using approaches such as power calculations.

The immunization regimen for producing antibodies in the rabbits and guineapigs has been optimized to maximize the concentration of antibodies in the serum. This includes the optimum interval between immunizations as well as the optimum preparation of vaccine. The volume of sera (and therefore the number of animals as described above) was determined based on generating enough antisera to last 5 years using historic demand data (and likely antibody concentration). The level of antibodies has been deemed to be maximum achievable using a vaccination regimen, with further boosts unable to increase concentration further.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?
Whilst the numbers of animals used in these studies is fixed by regulatory requirements for cattle, we will continually review the published literature with the Institute statistician to ensure optimal number of pigs and cattle are used when not being used under fixed regulatory guidelines. Also, 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.
For safety and potency testing of these veterinary vaccines the target species for the products have to be used. To minimise suffering, we will ensure that the vaccines have been formulated in such a way as to maximise its chances of being efficacious before it is put into cattle or pigs, or that preliminary studies have been carried out to demonstrate immunogenicity before challenge. We can also use medicines under direction of the on call veterinary surgeon to reduce clinical signs.

When quantifying the virus in animals, all animals will be heavily sedated to reduce stress, and also allow more precise administration of virus to be given into the insensible target area of skin, thus reducing pain. Pain relief may be given before and during infection to reduce some of the clinical signs.

The use of stringent scientific and humane endpoints, in addition to provision of highly trained staff will prevent unnecessary suffering.

Rabbits and guineapigs used in this licence will only experience the least pain, suffering, distress or lasting harm as this will only be related to the brief pain caused by a needle used to immunise and take blood samples. Published guidance will be referenced when undertaking the administration of substances "Refining procedures for the administration of substances. Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement 2001".

Why can’t you use animals that are less sentient?
International regulatory requirements stipulate cattle must be used to assess foot-and-mouth disease vaccines. The same approach is recognized by Global FMD experts in so far as using pigs. This ensures that there is maximum confidence that the vaccine will protect cattle and pigs when they are vaccinated with it on farms around the world from FMD.

Rabbit and guinea pig antiserum is required as they are the species referenced in the OIE terrestrial manual in the FMDV diagnostic assay section, and so no other species can be used to produce this.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Animals will be housed together with bedding and other items of enrichment. Highly trained animal technicians will monitor these animals throughout the day, ensuring they are comfortable and to maximise their welfare status. We have 24/7 CCTV surveillance which can be used to monitor the animals behaviour over time. Antibodies will be measured in vaccinated individuals, and if deemed too low for protection these animals will not be challenged with virulent virus. Anti-inflammatories may also be applied prophylactically before virus challenge where it is known they will not interfere with the outcome. Analysis of a biomarker in the blood for heart damage (Troponin) will also be assessed (where possible) to assist in monitoring the prevalence of FMDV induced heart damage.

Rabbits and guinea-pigs will be housed in floor pens, as a group, with bedding and enrichment items to allow them to express natural behaviour and also to provide shelter for use by the animals. They will be fed species specific diet, and lighting and temperature adjusted to fulfil their specific needs.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
Adherence to the ARRIVE guidelines for reporting these studies, as well as to the FELASA guidelines for both large animal and rabbit / guineapig health monitoring to help ensure the most robust health assurance for animals used in this study.

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

Through continued CPD and frequent review of the CAAT (Center for Alternatives to Animal Testing) I will keep informed about advances in the 3Rs. Included in CPD will be annual attendance at national lab animal science conferences as well as naturally reviewing the current literature surrounding infectious disease research, as well as attending relevant FMD conferences where updates and best practices are discussed.