Non-technical summary: Immunity, pathogenesis and transmission of Culicoides-borne viruses of ruminants

Project duration
5 years 0 months

Project purpose
(a) Basic research
(b) Translational or applied research with one of the following aims:
(iii) Improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes
(i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants

Key words
viruses, insects, transmission, vaccination, control

Animal types	Life stages
Cattle	juvenile, adult
Sheep	adult, 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?
The overall aims of this project are to contribute to developing control and prevention measures against those viruses spread between animals by the biting flies, Culicoides biting midges. Protection of the individual ruminant host against midge-borne viruses is either achieved through immunisation of individual animals or by protecting the ruminant population through disruption of virus transmission by biting insects.

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?
Some viruses do not spread directly between their human or animal hosts but instead infect bloodfeeding insects or ticks, which then pass the virus on during feeding. In Europe, the most important animal viruses transmitted by insects are those spread by Culicoides biting midges, which infect ruminants such as cattle, sheep, goats and respective wildlife. Of midge-borne viruses, Bluetongue virus (BTV) poses the biggest risk to ruminants and in outbreaks has already caused substantial animal suffering and economic losses of millions of Euros in many European countries. The costs associated with the 2006-2010 BTV-8 outbreak have been estimated at 180 million Euros in Germany alone. Other midge-borne viruses such as epizootic haemorrhagic disease virus (EHDV), Schmallenberg virus (SBV) or bovine ephemeral fever virus (BEFV) have also either recently occurred in Europe or are considered potential risks as they are transmitted by the same insects.

Viruses transmitted by insects are very difficult to control as Culicoides biting midges are very small and infected insects can fly or be blown vast distances.

Within this license we want to address key knowledge gaps to develop better control measures so that either less ruminants become infected by midge-borne viruses or less ruminants develop painful disease. Our objectives are to gain a fundamental understanding of the immune response of animals to BTV vaccination and the factors that determine virulence and onwards transmission of midge-borne viruses.

What outputs do you think you will see at the end of this project?
Control measures against midge-borne viruses are carried out through two principles: Protecting the individual mammalian hosts from infection through vaccination (of specific importance for BTV) and disrupting or preventing virus transmission by the insect vectors thereby protecting the population of mammalian hosts. The objectives of this licence address both of these principles.

Studies undertaken in this licence will allow us to identify why a proportion of cattle do not appear to develop detectable BTV antibodies after vaccination even when tested across multiple assays. We hope to establish indicators of appropriate vaccination status, thereby reducing risks associated with importation/trade of these animals from regions where BTV circulates and animals are vaccinated. Additionally, the study will enable us to compare cattle immune responses following BTV vaccination to those previously identified in ruminants during BTV infection, thereby generating further insight into whether immune responses to BTV infection and vaccination differ and identify potential future opportunities to improve BTV vaccines.

In addition, our studies will aim to identify virulence mechanisms of midge-borne viruses such as BTV and factors driving transmission to and from blood-feeding insects.

It is currently impossible to determine virulence, pathogenicity and transmissibility traits of midge-borne viruses (specifically across different BTV strains) in vitro as they involve multiple interactions within the host. It is important to investigate phenotypic differences of BTV and EHDV strains, including those that represent an emerging threat to the UK livestock industry. Assessing phenotypic characterisations of viral strains will also greatly help to identify the genetic basis of virus virulence and transmissibility.

Determining the efficiency of virus transmission to and from insect vectors and ruminant hosts is key across the midge-borne viruses (BTV, BEFV, EHDV and SBV) will enable the implementation of control measures that aim to reduce viral spread. Determining the time frame of infectiousness of ruminant hosts to blood-feeding insects and its relationship to detectable systemic viremia in the host is critical to be able to predict viral spread by mathematical modelling which might influence vaccine deployment, removal and protection of individual ruminants and the implementation of control zones. Data from animal studies contextualise data from artificial feeding of insects where the impact of the immune response on infectivity of virus within the host cannot be replicated.

We will publish the data sets acquired through the outlined experiments in open-access, peer reviewed journals. Further dissemination of the knowledge gained will be achieved through presentations of the work at scientific conferences, interest groups and specific disease policy meetings.

Who or what will benefit from these outputs, and how?
Results obtained in studies under this licence will be used by policy makers, the livestock agriculture sector, the wider veterinary and medical arbovirology research community and might influence product development in the long term. Data from immune response to vaccination and pathogenicity and transmissibility of exotic midge-borne viruses will be of direct benefit to policy makers (Defra) in risk assessments of incursion and mitigation prior to and during an outbreak in the UK. This will be communicated directly with the Department of science and policy leads through study reviews, established contacts and disease expert groups. Many of the activities performed under the authority of this licence will be highly relevant not only for arboviral diseases of livestock but also to those of humans e.g. Zika virus. Human arboviruses often need to be studied in less relevant rodent models while research into ruminant arboviruses has the benefit of being investigated in its natural host.

How will you look to maximise the outputs of this work?
Outputs will be maximised through publication in open access journals. Our network of collaborators and interested parties, through the vector-borne disease research community and National Reference Laboratory activities will be used to promote our work to a national and international audience.

Species and numbers of animals expected to be used
Cattle: 240
Sheep: 60

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.
Cattle and sheep represent the host species of the midge-borne viruses to be studied. As such they represent the natural animal model for investigations of these viruses, providing data that is directly relevant to the field. In vaccination studies animals of sizes representative of UK herd composition will be used. In transmission experiments, cattle will be used as young animals of less than 120 kg on receipt for ease of handling in our animal facilities. Adult female sheep typically demonstrate greater clinical disease than younger animals and ewes at the end of reproductive productivity will therefore be used in most studies.

Typically, what will be done to an animal used in your project?
All animals will have blood samples taken on farm to screen for virus-specific antibodies and suitability for our studies.

Vaccination and immunology: Animals may be vaccinated with a commercial BTV vaccine to assess immune responses. Responses will be compared to those reported in previous, virus infection studies. Blood samples will be taken throughout the duration of the project at time points up to a year from vaccination. Vaccinated animals will be returned to their herd following the experiment.

Virus infection: Animals may be infected with a midge-borne virus, primarily through the bites of infected Culicoides midge vectors or alternatively through subcutaneous needle inoculation. Blood samples will be taken throughout the experiment. Further exposure to the feeding of naïve Culicoides biting midges on animals may take place at intervals through the

infection cycle. Animals in infection/transmission trials will be euthanized at scientific or humane endpoints, if reached.

What are the expected impacts and/or adverse effects for the animals during your project? Vaccination and immunity:
The vaccination used under this licence will not result in any adverse reactions, except possibly a local reaction at the injection site such as erythema and/or swelling. Systemic allergic responses to vaccination are rare but a mild and transient rise in body temperature might occur following vaccinations.

Virus infection: Of the midge-borne viruses used under this licence (BTV, EHDV, SBV and BEFV), pronounced clinical disease is only expected for BTV infections of sheep. Disease will be determined by the inherent virulence of the most virulent virus strains used.

Typical signs of BT in sheep develop normally between 5-14 days post infection and are characterised by: rises in body temperature resulting in temperatures classed as fever, reddening of mucosal membranes occurs, including the conjunctiva of the eye. Ulcers and small petechial bleedings may occur on the nose, gum and dental pads. Subcutaneous oedema may develop in the face and lips. There may also be inflammation and reddening of the coronary bands possibly causing some lameness. Nasal discharge and excess salivation may occur.

Sheep infected with EHDV are mostly asymptomatic, however should clinical signs develop these would mirror a BTV infection.

Cattle very rarely show clinical disease to infections with BTV or EHDV. Occasionally they may show a rise in body temperature. Highly virulent strains of BTV or EHDV may rarely lead to similar clinical signs as described for sheep.

Cattle may develop mild disease to BEFV infections namely fever, depression, reluctance to move and salivation.

SBV infections of adult ruminants are either clinically unapparent or mild with raised body temperature and diarrhoea.

Local responses to arthropod blood-feeding vary greatly between individual animals and range from small petechia, typical bite nodules to a substantial local oedemic inflammatory response.

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)?
Vaccination and immunity: Mild severity, 100% of animals. Clinical impact from vaccination and repeated blood sampling is expected to be transient and mild.

From previous studies with BTV infection we expect: 50% of infected sheep to experience moderate severity, with 50% experiencing mild severity. In cattle, we expect 100% to experience mild severity. Animals will be euthanised prior to the development of pronounced disease, but are likely to exhibit clinical signs of BTV or EHDV infection. All animals within each trial may be infected, with the exception of contact transmission controls, if required.

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

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?
It is extremely challenging to examine the complex interactions between mammalian hosts, insect vectors and transmitted viruses experimentally and not possible to achieve *in vitro.*

One of the main aims of this work is to gain a fundamental understanding of the role of the immune response in resistance to and pathogenesis of midge-borne viruses in order to improve and develop vaccines and prophylactic and therapeutic strategies. The immune system is highly complex and interrelated and an understanding of the immune response in infectious disease requires the use of living animals. Additionally, our current knowledge of the immune response of ruminants to these viruses does not allow protection afforded by different vaccination strategies to be predicted. In alignment with other research groups, the long-term goal is to identify measurable correlates of protection and so reduce the need to perform challenge studies with virulent pathogens in the future.

Furthermore, it is currently not possible to assess the virulence of emerging orbivirus strains in vitro or within a mouse model system. Several studies investigating the virulence of genetically modified viruses have demonstrated that phenotypic behaviour of specific viruses in cell culture is not correlated with replication characteristics in mice, which in turn does not reflect the true pathogenicity observed in ruminants. Additionally, a complete biological system is required in order to study transmission, especially for vector-borne pathogens where successful transmission requires the interaction between vertebrate hosts, blood-feeding arthropod vectors and the pathogens. Therefore in vivo studies have to be performed in order to be able to elucidate transmission mechanisms, risk periods and efficiencies.

Which non-animal alternatives did you consider for use in this project?
We are investigating if ruminant host derived organ explants and/or primary cell cultures can be used to reflect the phenotype of these viruses in the future. These techniques could be used to study pathogenesis and specific cellular responses to arboviruses and arthropod saliva, potentially aiding in the development of genetic markers for virulence or transmissibility.

Why were they not suitable?
Use of primary cell cultures and organ explants for determination of virus phenotype is at a very early stage of development and has not yet been able to provide the data required for these studies.

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?
For the vaccination studies, the number of cattle was chosen: (i) to be similar to a UK dairy herd, so the range of responses post vaccination would likely be similar to that observed in the field; and (ii) to make sure there would be sufficient animals present throughout the duration of the study, so not too many would be lost through turn-over as part of routine herd management. The aim of the study is to construct typical profiles of how a range of immune parameters change with time post vaccination which may subsequently be used to infer the vaccination status of an animal and when it was likely to have been vaccinated.

For transmission studies where we aim to characterise pathogenicity and transmission parameters of a virus strain, fewer animals are required. We rely on our previous, similar studies, where a treatment group of 6-7 individuals was sufficient to capture the range of individual response to infection. Our infection protocol has provided very high efficiency of infection and therefore we are confident of all animals exposed in studies exhibiting infection and viraemia, reducing the need for additional animals to ensure infection.

What steps did you take during the experimental design phase to reduce the number of animals being used in this project?
Specialist statistical advice is a critical element of our animal experimental design. In the past decade we have built up a significant data catalogue which we use to estimate variation between animals in response variables and inform appropriate effect sizes for use in power calculations. The numbers of animals used in experiments will be the minimum possible to achieve statistically robust data (typically we will aim to detect differences with 80% power and 95% confidence), but previous comparable studies have been between 7-9 animals per treatment group depending on most variable outcome used for power analysis. For example, groups of 8 sheep were used to successfully characterise differences in pathogenicity of a re-emergent strain of BTV -8 in sheep. While each experiment will require the inclusion of appropriate controls, these will be kept to the minimum that does not jeopardise the reliability or integrity of the experiment.

What measures, apart from good experimental design, will you use to optimise the number of animals you plan to use in your project?
Initial proof-of-principle studies will be carried out on a smaller number of animals in those studies were this is appropriate. These proof of principle studies will then be used to inform power calculations used to determine animal numbers for follow–up studies, while unsuccessful proof-of principle studies might result in the aim not being investigated any further.

The phenotypic behaviour will firstly always be investigated in insect-derived cell cultures and subsequently in adult vector insects. Only once specific replication characteristics within the insect vector have been confirmed (low or high replication efficiency) will the transmission sequence between ruminant host and insect vector studied *in vivo.*

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 perceive it as a specific strength that the studies into the immune response and pathogenesis of midge-borne viruses proposed under this licence will be carried out in ruminants, the natural host species. Most experimental studies of vector-borne viruses utilise either needle-inoculation of the virus into the host and/or the use of non-natural model species such as rodents. However, we are able to transmit the midge-borne viruses directly between the natural ruminant host and relevant blood feeding insects, an experimental design that is exceptionally representative of natural infection.

Why can't you use animals that are less sentient?
While a mouse model of clinical bluetongue has been developed using mice deficient in a major antiviral mechanism, striking differences between the murine and the ruminant immune system and the deletion of an important anti-viral mechanism renders such mice unsuitable for our studies. Additionally, arthropod feeding on small rodents is likely to result in different cell migration dynamics partly due to the small size of the animals. For example, in certain studies of mosquito-borne arboviruses it was demonstrated that feeding of infected arthropods on mice would immediately result in detectable viraemia simply due to the small blood volume. Time course feeding of arthropods through the cycle of infection means that animals under terminal anaesthesia cannot be used.

How will you refine the procedures you're using to minimise the welfare costs (harms) for the animals?
Through the experience gained under previous Project licences, specific scientific endpoints have been designed for protocols which will significantly reduce the impact on the animal. Animals in virus characterization studies will be euthanized upon confirmation of viraemia and initiation of clinical signs of disease rather than development of pronounced disease. Throughout previous projects the bloodfeeding of insects on cattle and sheep has been continuously modified. Blood-feeding success rates of exposed insects on sheep is highest when allowed to feed on the inner thigh. However only 10 minutes exposure results in high feeding rates and only very rarely are longer exposure times (max. 30 minutes) on sheep required. On cattle, insects need longer to successfully blood-feed presumably due to the thicker skin. However, here insects feed best on the rump, neck or side which allows cattle to be minimally restrained in a feeding head-lock. Therefore, cattle exposed to blood-feeding insects for an average of 20 min (max 30 min) do not display any aversion or stress behaviour.

What published best practice guidance will you follow to ensure experiments are conducted in the most refined way?
The Laboratory Animal Science Association (LASA) guidance will be consulted prior to each experiment to ensure best practice is known and adhered to.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
Guidance on 3Rs implementation will be reviewed prior to each animal experiment. Science investigators will explore the literature published by other groups working with BTV and communicate with animal unit staff to ensure each is updated with relevant developments within the type of studies undertaken.