Non-technical summary: Supply of ruminant and porcine blood for virus infection research and diagnostics

Project duration
5 years 0 months

Project purpose
(a) Basic research
(b) Translational or applied research with one of the following aims:
(ii) Assessment, detection, regulation or modification of physiological conditions in man, animals or plants
(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 Blood, natural host cells, viruses, immunity, livestock

Animal types	Life stages
Cattle	aged, pregnant, adult, juvenile, neonate
Sheep	neonate, juvenile, adult, pregnant, aged
Pigs	neonate, juvenile, adult, pregnant, aged
Goats	neonate, juvenile, adult, pregnant, aged

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 licence is to “provide blood of cattle, sheep, goats and pigs to scientists in support of research and diagnosis of viral pathogens of livestock (including those also affecting humans termed zoonotic pathogens)”.

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?
Critical research and diagnostic testing rely on the provision of blood from natural hosts of highly important exotic viruses of livestock such as Foot-and-mouth disease virus (FMDV), bluetongue virus (BTV) and African Swine fever virus (ASFV). The ability to use natural host blood and specifically blood-derived cells within our research as well as for diagnostic assays is essential to prevent the incursions of these viruses into the UK and to increase the scientific capability to control these viruses worldwide.

Blood from pigs and ruminants is required to isolate live cells derived from the natural host to be maintained as cell cultures in the laboratory.

Within the laboratory these cells will then be utilised to study the replication and immune response of highly important viral pathogens e.g. African Swine fever virus, Bluetongue virus, Lumpy Skin disease virus, Peste des Petits Ruminants Virus, Foot and Mouth Disease virus, Bovine Respiratory Syncytial virus as well as vaccine candidate antigens. Furthermore specific markers present on these immune cells and the immunogenetic background of individual animals and breeds will also be further characterised from this material to enhance our understanding of immune responses to viral infections.

By using these natural host cells in vitro the overall need of studying these viruses and their respective immune responses directly in animals is reduced, leading to an overall reduction in the use of animals.

What outputs do you think you will see at the end of this project?
Natural host cells obtained from blood of pigs and ruminant are a vital resource to carry out research and diagnoses for numerous important livestock and zoonotic pathogens. Additionally the immune response of blood immune cells derived from natural hosts both towards vaccine candidates against viral pathogens, as well as more fundamental understanding of how immune cells generate a response, will be analysed. Overall, the research conducted using these cells, as well as the use of these cells in diagnostic assays such as virus isolation, is vital to keep the UK free from important livestock and zoonotic viral pathogens.

Who or what will benefit from these outputs, and how?
The diseases studied cause major social and economic impacts in affected countries and if introduced to the UK will result in a ban on the movement of animals and loss of international trade in addition to causing animal suffering and welfare issues. Hence it is extremely important that appropriate diagnostic assays as well as new research aimed at preventing and controlling viral disease outbreaks are carried out.

How will you look to maximise the outputs of this work?
The materials collected as part of this PPL will be used to generate data which will, as appropriate, be published in open source peer reviewed papers specific to the objective of that study.

Species and numbers of animals expected to be used
Cattle: 200
Goats: 60
Pigs: 60
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.
We will use cattle, pigs, sheep and goats raised on commercial farms and/or licenced establishments for which we have additional availability on this licence, which allow normal farm husbandry of these animals. None of the animals are genetically modified however some cattle and pigs are specifically inbred to reduce the variations in immune response determining genes The animals will be bled for the supply of blood from livestock allowing the isolation of cells or blood factors which need to be utilised fresh or from specified individuals Some animals might be held long-term (years) at the named additional availability

site in typical farm environments and husbandry. During this time these animals will donate blood in a tightly regulated frequency and under strict monitoring of adverse effects and stress responses.

Typically, what will be done to an animal used in your project?
The overall severity is mild and adverse effects to blood sampling are extremely rare. The animals will be blood sampled by experienced animal technicians or veterinarians and will be placed under veterinary care should an unexpected event (such as inflammation of the vein) occur. Any animal showing adverse effects to blood sampling will not be sampled for an appropriate time defined by a veterinarian. Animals might also be vaccinated using appropriate routes, volumes and adjuvants for the respective species to allow the usage of their blood derived cells for specific immune assays. Antigens which may be used are not expected to result in any adverse effects other than potential swelling/ inflammation at the injection sites. Following the procedures, animals might be released from the licence and returned to the national herd. Long term donors might also be euthanised humanely.

What are the expected impacts and/or adverse effects for the animals during your project?
Overall we are expecting 10-15 cattle bleeds each month, and 3-5 sheep, pig, and goat bleeds each month based on previous useage. Where animals are only bleed once for a specified purpose each bleed will count as single animal. Hence over the course of this licence we have asked for 200 cattle, 60 sheep, 60 pigs, and 60 goats. The majority of these animals will be released back to the national herd.

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)?
The overall severity is mild and adverse effects to blood sampling are extremely rare. The animals will be blood sampled by experienced animal technicians or veterinarians and will be placed under veterinary care should an unexpected event (such as inflammation of the vein) occur. Any animal showing adverse effects to blood sampling will not be sampled for an appropriate time defined by a veterinarian. Animals might also be vaccinated using appropriate routes, volumes and adjuvants for the respective species to allow the usage of their blood derived cells for specific immune assays. Vaccine preparations used are not expected to result in any adverse effects other than potential swelling/ inflammation at the injection sites. Following the procedures animals might be released from the licence and returned to the national herd. Long term donors might also be euthanised humanely.

What will happen to animals at the end of this project?
Killed
Kept alive
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? Many of the viral pathogens in question do not replicate within established cell lines or blood cells from model hosts, hence cells established from the blood of natural hosts are a requirement. Moreover, host cellular responses cannot be modelled or analysed using immortalised cell lines. The

blood samples required under this licence will be used to immediately generate primary cell cultures of blood leukocytes by well-established protocols for use in fundamental and applied research into immune cell responses as well as use of these primary immune cells for use in diagnostic assays such as virus detection. These cells will have to be generated within 12 hours from taking the blood samples as cell death occurs progressively once the blood sample has been taken. The short window of opportunity to isolate viable cells makes the use of commercially available blood unrealistic as leukocytes would not be alive once the blood has been received.

We have arrangements in place with local abattoirs to obtain blood for some experimental studies were possible. However cellular viability in obtained blood varies and obtaining blood post mortem can affect the activation status of blood derived cells. Therefore, for validity and comparability reasons, blood samples need to be freshly harvested and cannot be taken in sufficient quantity or of adequate quality after death. Furthermore obtaining blood from abattoirs does not allow us to obtain blood from the same individual on several occasions which is important to reduce experimental variability and allow the investigation of seasonal and age effects.

Which non-animal alternatives did you consider for use in this project?
Creation of permanent cell lines to replace the need for primary cell cultures created from ongoing use of animals has been considered for both isolation and culture of viruses for both research and diagnostic purpsoes, but also for assessment of cellular immune responses.

Predecessors of this licence still covered the need for mice to maintain arthropod colonies. Over the years these Culicoides biting midge and mosquito colonies have been adapted to an artificial hemotek® membrane feeding system, allowing the reduction of mice needed. In the previous licence the provision of mice as emergency cover only was still included should valuable arthropod colonies suddenly cease to feed on these artificial membrane-blood systems. This requirement was removed 5 years ago, and is an example of how ongoing attempts for replacement are considered in this PPL.

Why were they not suitable?
The propagation of animal viruses often requires primary cells and/or cell lines from the target species for both use in research as well as a primary diagnostic assay. For example, African swine fever virus does not replicate in established cells lines or cells from species other than pigs. Many field strains of African swine fever virus will only replicate in primary porcine macrophages while failing to even infect porcine cell lines, meaning primary cells are required to produce virus for use in research platforms as well as to be used themselves for diagnosing ASFV by isolation.

Continuous attempts are in progress to establish cell lines to reduce the need for primary cells. The development and validation of a pig macrophage cell line for ASFV research and diagnosis is currently being carried out within the ASFV group with the long-term goal of replacing or reducing the requirement for primary cell cultures.

Furthermore primary host cells are essential to establish cellular immune responses to respective viral infections which are vital to inform appropriate vaccine design or to identify immune-pathogenic mechanisms. The usage of primary cells obtained from the same individual over time further allows addressing specific research questions such as variation of cellular immune responses to different viruses, influence of genetic backgrounds and

difference in immune responses attributable to age and seasonal effects. These materials are not available from other sources such as commercial suppliers.

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?

Reviewing the numbers of animals used during the course of the previous PPL, the number of animals used of each type varied from year to year based upon the individual projects however for cattle it was around 20 per year. The use of goats, sheep and pigs was substantially lower, and therefore estimates of animal use have been reduced substantially compared to the previous version of this PPL to reflect this. The numbers estimated for this PPL allow, on average, for each of 1 pig, sheep and goat to be bled per month for the duration of this PPL, and 3-4 cattle to be bled per month.

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

Specifically, when individual animals are bled regularly, they will have no more than 10% of the total blood volume on any one occasion removed and no more than 15% of the total blood volume taken in a 28 day period (estimated as 60 ml per Kg body weight). This is monitored by an internal process and rolling documentation of animal weights and volumes being taken.

For certain scientific studies it may be necessary to obtain blood on several occasions from the same animal to monitor responses over time and minimise variations within experimental repeats.

Specific animals might also be required as blood donors due to their specific genetic background. Therefore an individual animal might be bled multiple times for a single scientific purpose. In addition this animal may also be bled for a different scientific purpose as a re-use.

Ruminants: Samples will not be taken more than once a week from the same animal
Pigs: Samples will not be taken more than twice a month from the same animals with a minimum interval of 1 week.

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

Each request for blood is made visible to the PPL holder in advance to it being taken, and the PPL holder also ensures that each person requesting the blood is aware of the uses and objective permitted under the PPL as well as the requirement to minimize volumes taken.

The scientific acceptable justification for the provision of blood from healthy animals under this licence is the need for fresh blood which cannot be fulfilled by commercial blood supplies (for example isolation of primary blood derived cells or obtaining blood from specified individual animals). Cells, isolated from blood, which are excess to requirements may be frozen to enable them to be used later without the need for a further blood sample

request. The volume of blood requested will, however, be the minimum required to achieve the objective of the request.

The capability to use genetically defined cattle and inbred pigs may enable fewer animals to be used to achieve the same experimental goals.

Most importantly the use of these primary cells obtained from the blood of livestock will reduce animals needed to be directly infected with the respective viral pathogens which both constitutes a reduction as well as a refinement since direct viral infections of the natural hosts with respective viruses would lead to clinical disease and higher severity categories of the respective procedure.

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.

It is of significant benefit to science that immune responses to viral infections of blood derived cells can be carried out using cells from the respective natural hosts. By using natural host target cells scientific results obtained are of most practical relevance and transferability to natural infection scenarios and subsequent vaccine development within the same host.

The use of these primary cells obtained from the blood of livestock will reduce animals needed to be directly infected with viral pathogens. This not only leads to less animals being used but also constitutes a refinement as direct viral infections of the natural hosts with respective viruses would lead to clinical disease and higher severity compared to the mild procedure of obtaining bloods samples from superficial veins.

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

The animals required to have blood collected under this licence are used as they are the natural hosts to the pathogens being studied / vaccines being developed and / or immune mechanisms being investigated.

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

As this PPL will be acquiring either blood samples or a simple vaccination only, which are very mild procedures, extended monitoring of animals after application of these procedures will not be required. Each animal will be monitored by the responsible person after blood samples and / or vaccination is undertaken until a time that the animals health has returned to normal. Where possible, animals will be trained to facilitate blood sampling, with positive reinforcement offered to encourage this behaviour.

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

The principals of "Diehl, K. H., et al. (2001). "A good practice guide to the administration of substances and removal of blood, including routes and volumes." J Appl Toxicol 21(1): 15-

23" will be applied to blood sampling and volumes taken, and monitored by an internal system at the establishment for each individual animal.

How will you stay informed about advances in the 3Rs, and implement these advances effectively, during the project?
By referencing online email groups, reviewing information circulated by the institute NIO, communicating with other establishments doing similar procedures, and referencing sites such as the NC3Rs, I will stay informed about advances in the 3Rs in this area.