Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 28

Projects with a primary purpose of: Translational and Applied Research - Animal Diseases and Disorders

Project Titles and keywords

1. **Bacterial carriage in the bovine nasopharynx**
    - Bovine, respiratory, disease, bacteria, virus

2. **African swine fever virus: Development of tools for control**
    - African swine fever, pigs, vaccine, diagnosis, pathogenesis

3. **Defining bovine protective immune responses**
    - Foot-and-mouth disease, vaccines, transmission, persistence

4. **IBDV Pathogenesis and Control**
    - IBDV, pathogenesis, virulence, vaccine, immunoprophylaxis

5. **Infectious disease in pigs**
    - Pigs, infectious disease, vaccines, prevention, pathogenicity

6. **Sexual transmission of Maedi Visna from Rams to Ewes**
    - Lentivirus, Sheep, Sexual transmission

7. **Genetics of one-carbon metabolism in sheep in relation to productivity, fertility and health.**
    - Sheep, genetics, trace elements, health, epigenetics

8. **Mycotoxicosis in ruminants**
    - Dairy, mycotocin, silage

9. **The use of therapeutic mRNA in companion animals**
    - Dogs, renal, anaemia, mRNA

10. **Development of Bacterial Vaccines for Poultry**
    - Vaccines, poultry

11. **Novel Therapeutics for Veterinary Oncology**

12. **Supply of materials to develop and maintain tests for BSE in sheep animal**
    - BSE, sheep, supply, positive, material

13. **Novel interventions for poultry red mite**
    - Poultry, vaccine, parasite

14. **Ecology and Epidemiology of Infectious Disease in Wildlife**
    - Wildlife, Epidemiology, Demography, Management

15. **Understanding Batrachochytrium dendrobatidis and ranavirus infection dynamics to develop mitigation strategies**
    - Amphibian, disease, conservation, mitigation

16. **Supply of materials to develop and maintain tests for animal diseases**
    - Transmissible, Spongiform, Encephalopathy, Characterisation, Endoparasite

17. **Parasitic infections of fish**
    - Parasite, Disease, Teleosts, Aquatic Health, Invasive Species

Project 1	**Bacterial carriage in the bovine nasopharynx**	
Key Words (max. 5 words)	Bovine, respiratory, disease, bacteria, virus	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Respiratory disease is a an important health problem in both beef and dairy cattle leading to financial losses in the UK estimated at over £60 million per annum as a result of animal deaths, costs of veterinary treatment, adverse impacts on carcass quality and life-long decrease in productivity of affected animals. Affected animals may become chronically ill, causing stunted growth, long-term respiratory distress, increased risk of fertility disorders, reduced milk production and compromised animal welfare. There are also costs relating to improving building design to reduce disease risk and increased farm labour. Bovine respiratory disease also increases the need for antibiotic treatment of livestock and therefore potentially contributes to the growing problem of antimicrobial resistance. It also prolongs time to maturity and productivity and hence emissions of greenhouse gas and other environmental pollutants. Like the common cold in humans, there is no single cause and the disease is associated with a number of known types of bacteria and viruses. The bacteria involved mostly belong to the family *Pasteurellaceae*, which are often found living without ill effects on the mucous membranes of the nasal cavities, but can on	

	occasion multiply deep within the lungs causing the severe, sometimes fatal condition bronchopneumonia. The circumstances resulting in this disease are poorly understood, but it is believed to occur in animals with immune defences weakened by environmental stress or viral infections. A similar situation pertains in humans, in whom usually harmless bacteria living without ill effects in the nasal cavity can spread and cause more severe infections including pneumonia. It has recently been shown in children using a live intranasal 'flu vaccination as a 'model', that viral infection is associated with an increase in density of carriage of these nasal bacteria, which may in turn increase the likelihood of transmission of these bacteria among individuals. We intend to investigate the analogous situation in cattle, that upper respiratory viral infection may lead to an increase the density of *Pasteurellaceae*.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Improving our current understanding of bovine respiratory disease (BRD) and hence our ability to control both so-called 'shipping fever' in beef cattle in feedlot systems and 'enzootic pneumonia' in dairy replacement heifer calves has the potential to mitigate against the significant economic, welfare and environmental impacts of these diseases. Understanding of the interactions between viruses and bacteria in the origin and spread of BRD is currently very poor. The short term benefit of the proposed work should be improved understanding of these interactions. In the medium to longer term this could lead to improved approaches to vaccination.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will be conducted exclusively using cattle, and may involve use of up to 90 cattle per year for up to 5 years. Individual cattle will be used for a maximum of approximately 1 year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	This work will only involved procedures that are expected to be of mild severity. Nasal swabs will be collected from cattle to obtain samples of the bacteria resident in their nasal cavities. We shall generally use short (15cm) swabs to minimise discomfort, but occasionally we may use longer (30cm swabs) to confirm that the shorter less invasive swabs provide the required information. Animals may experience mild transient discomfort while the samples are being collected, which should take only a few seconds. Blood samples will be collected from cattle for testing

	for evidence of viral infection. Animals may occasionally experience very mild transient discomfort while the samples are being collected. Intranasal vaccines or an equivalent quantity of diluent (physiological saline) may be administered to animals. This procedure is widely used in commercially farmed cattle and not expected to have any adverse effects. After use cattle will be returned to their herd of origin.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We are investigating the effects of respiratory viral infection on bacteria naturally colonising the nasal cavities of cattle. While viruses can be grown in cell culture systems, and bacteria can be grown in artificial culture media, the conditions required for culturing bacteria and viruses differ, and even if a single system were available for both this would not be a substitute for experimental animals which posses a functional immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We shall use medical statistical methodology as applied to randomised control trials to determine the minimum number of animals required to obtain meaningful results. Our intention is to use the so called the stepped wedge study design because it should allow a smaller number of animals to be used to obtain the same statistical power as more traditional parallel study designs using larger numbers of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	This project will be conducted using cattle as results obtained in other species might not reliably be applicable to cattle. Cattle to be used will be under approximately 1 year of age, which is the age group in which the disease we are investigating occurs naturally. Cattle will be managed in a purpose built state-of-the-art facility to maximise their welfare, they will be managed by experienced stockmen and all the procedures used will be of the mildest severity.

Project 2	**African swine fever virus: Development of tools for control**	
Key Words (max. 5 words)	African swine fever, pigs, vaccine, diagnosis, pathogenesis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	African swine fever virus (ASFV) causes a severe disease in pigs which results in death of most infected animals. The disease is present in Africa, Sardinia and recently has spread through the Trans Caucasus, Russian Federation and parts of E. Europe. This increases the risk of further spread within the EU and globally. The objectives of this project are to further develop methods for control. There is no vaccine for ASFV and a main objective is to further the development of vaccines by constructing and testing novel mutant ASFV by deleting genes that help the virus to evade the host's defences. This can result in the host eliminating the virus without disease signs and induction of an immune response that protects the pigs against challenge with a virulent virus. In addition we will identify the ASFV proteins that can induce a protective response and incorporate the genes for these into safe non-replicating virus vectors to produce candidate vaccine not based on replicating ASFV. These studies will lead to improved knowledge of virus host interactions by understanding the role of virus genes in evading host defences, the immune mechanisms that lead to protection against challenge	

	and the virus proteins that are involved in inducing protection. A second objective is to study the dynamics of virus transmission between pigs in order to develop a mathematical model to predict virus spread within a farm. This will provide information that will help in planning responses in the event of an outbreak. Although most ASFV viruses causes an acute disease with death of most pigs, some can cause reduced signs and some pigs survive infection (30-50%). Some isolates cause little sign of disease. Another objective will be to study the disease caused in pigs by isolates of the virus which pose a particular threat to the UK and EU. Samples collected from these experiments will be used to test the standard diagnostic procedures used to identify the virus and antibodies induced by ASFV. A fourth objective will be to investigate if pigs genetically modified by gene editing may be more resistant to ASFV. This could lead to development of alternative control strategies based on breeding of resistant pigs.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project will lead to improved understanding of the mechanisms by which ASFV evades the hosts defences, and the host immune responses that are important in inducing protection against challenge. Key virus proteins important for inducing protection will also be identified. The project will advance development of vaccines for ASFV. This would be of immense value in control of ASF disease. This would contribute to welfare of pigs and wild boar and would limit economic losses for pig farmers and the pork industry. It would also help secure global supplies of pork and pigs.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will last for 5 years and involve use of pigs. The approximate numbers of animals used will be 540 over 5 years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the	Most animals (up to 300) will be used to investigate immunity to ASFV (protocol 1). A proportion of these will be immunised with non-infectious immunogens at APHA (protocol 2) and then transferred to Pirbright. This will involve inoculation of pigs with different vaccine formulations Following inoculation the great

end?	majority of animals will experience mild or sub-threshold suffering. We will confirm that an immune response has been induced before challenge with strains of ASFV that can cause severe disease or death. In parallel a small group (usually 3) of control animals that have not previously been inoculated will be infected as a control for the challenge. A high proportion of the animals will be protected from challenge and experience mild signs. Those not protected will be terminated at a moderate severity level. The control pigs will experience clinical signs of ASFV and will be terminated at the moderate severity limit. We will investigate the type of disease that ASFV strains representing a risk of introduction to the EU and UK cause (protocol 3). To distinguish between strains that cause moderate disease and highly virulent ASFV that kill almost all pigs, we will terminate pigs suffering severe signs. We will also investigate genetically modified pigs that are predicted to be resistant to ASFV. We will expect to terminate these pigs when they develop moderate suffering. However since the pigs will be different from the usual pigs we study possibly some may develop a more rapid disease progression and possibly the moderate severity limit could be exceeded unexpectedly. We will investigate the disease caused by new genetically modified ASFV (protocol 4) that are predicted to cause mild signs of disease. The severity limit will be moderate. Pigs from this protocol may continue to be used in protocol 1 to determine if they are protected against challenge. We will also study transmission from infected to uninfected pigs and terminate pigs at moderate severity (protocol 5).
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The only species susceptible to ASFV are domestic and wild pigs. Therefore to investigate immunity and pathogenesis these are the only species that can be use. Although we will carry out experiments in cell culture to characterise the viruses and other materials we use it is not possible to predict if protection is induced or the pathogenesis of viruses from cell culture results. The experiments carried out in cell culture will include tests using immune cells from pigs inoculated with ASFV strains that can induce protection. We can use these cells from a limited

	number of pigs to predict which the ASFV proteins that are important for protection. We can also predict if pigs will be protected against challenge with a different ASFV isolate. We have developed assays in cell culture which can indicate the virulence of an ASFV isolate. These include the measurement of virus replication levels and the activation of host antiviral defences.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will reduce the numbers of animals used by ensuring that experiments are carried out with numbers statistically calculated to give significant results. We will also carry out experiments in cell culture prior to in animals to prioritise and only test what is necessary in animal experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Pigs are the only species susceptible to ASFV and therefore must be used for experiments to understand pathogenesis, immunity and transmission. We will carry out preliminary experiments in cell culture prior to animal experiments. Furthermore, one group of immunised pigs can be used in many concurrent projects by different research groups. Inbred pigs will be used if possible since the variation between responses in individual pigs is greatly reduced meaning that fewer pigs are required to produce statistically significant results. A clinical and pathogenesis scoring system is used to enable comparison between experiments. Pigs housed on longer term studies will be trained with positive reinforcement to reward desired behaviour e.g. cooperating with procedures which could include swabbing without restraint. This is a refinement in animal handling methods to improve animal welfare and the value of animals in research.

Project 3	**Defining bovine protective immune responses**	
Key Words (max. 5 words)	Foot-and-mouth disease, vaccines, transmission, persistence	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	X	Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This programme will provide an understanding of the cells involved in the immune response in cattle that will underpin infectious disease programmes. It is a necessary step in devising effective vaccination strategies that can be applied to cattle and that result in appropriate protective immune responses. The focus of the activities performed under the authority of this licence is the control of exotic virus diseases of cattle. Exotic virus diseases of animals constitute a major threat to the livestock industry of the UK. Domesticated animals in the UK are not vaccinated against the exotic viruses as this would preclude export trade with many countries. Consequently we have completely susceptible animal populations and the entry of any exotic virus of animals could be devastating unless quickly diagnosed and eradicated. The disastrous impact of the 1967-68, the 2001 and 2007 foot-and-mouth disease epidemics serves to illustrate the point in that to eventually eradicate the disease the slaughter of over 400,000 and 4 million animals respectively was necessary. In disease outbreaks the key task of eradication and prevention of transmission may require the use of	

	vaccination. Particularly when outbreaks occur in areas of high densities of domesticated livestock the, logistics of slaughter and the concerns of the general public over welfare may require alternative strategies. However, current vaccines are not completely effective, for example they may stop animals becoming seriously ill, but they are not long lasting and do not always stop them infecting other animals. Extensive investigation of immune cells are carried out in cell culture systems before animal experiments are performed. The use of laboratory based studies has resulted in a significant reduction in the number of experiments and the number of animals used in each experiment.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Under previous licences we have made significant advances in vaccine design and testing procedures to limit pain and suffering. We believe further development will result in advanced products for the control of animal diseases.
What species and approximate numbers of animals do you expect to use over what period of time?	Computer modelling to design vaccines is used increasingly to reduce the number of vaccines to test. A maximum of 1200 mice and 425 cattle are to be used over a 5 year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	We expect to use cattle and mice for vaccination studies and to collect cells from the immune system. In the majority of experiments animals receive vaccine and are blood sampled. Superficial surgery is performed under general or local anaesthetic in approximately 10 cattle per year. Animals may receive challenge with live virus, animals will be monitored frequently to ensure moderate severity is not exceeded.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our current knowledge of the immune system in any vertebrate species does not allow protection afforded by different vaccination strategies to be predicted. The long term goal of this programme of work is to identify measurable correlates of protection and so remove the need to perform challenge studies with virulent pathogens. It is difficult at this time to see how *in vitro* systems will completely remove the need to perform *in vivo* studies.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of animals used in experiments will be the minimum possible to achieve statistically robust data. Group sizes will be determined using a Power calculation. The availability of inbred animals means that there is less variation in responses than when using animals from an outbred population and therefore smaller group sizes can be used. While each experiment will require the inclusion of appropriate controls, wherever possible these will be kept to a minimum that does not jeopardise the reliability or integrity of the experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have developed a detailed understanding of correlates of protection for foot-and-mouth disease infection of cattle that can be measured using blood samples. These correlates of protection will be used to assess the new antigen formulations, instead of live virus challenge, where possible. We are confident we can assess the protective immune responses in cattle without carrying out challenge with live foot-and-mouth disease virus on all occasions. The vaccinated animals will be housed in conventional facilities, rather than high containment, with all the benefits of improved animal comfort and environmental enrichment.

<table>
<tr><td>Project 4</td><td colspan="2">IBDV Pathogenesis and Control</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">IBDV, pathogenesis, virulence, vaccine, immunoprophylaxis</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)</td><td>x</td><td>Basic research</td></tr>
<tr><td>x</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
<tr><td></td><td>Preservation of species</td></tr>
<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td></td><td colspan="2">Infectious bursal disease virus (IBDV) infects chickens, and can cause severe disease. Birds that recover from infection are often immunosuppressed, more susceptible to secondary infections, and less responsive to vaccines. Classical strains were identified in the 1960s, but very virulent (vv) strains emerged in the 1980s with a higher fatality rate (up to 100% in some flocks). These viruses continue to circulate worldwide despite current vaccination programmes, and IBDV remains economically important to the poultry industry, and a welfare concern.

There are two objectives to the project:
1. To develop new strategies to control IBDV infection
2. To advance our knowledge of IBDV disease.

In order to meet the first objective, we plan to develop new vaccines that do not cause disease but generate immune responses that protect birds against subsequent infection. In order to achieve this aim, we will:
1. Introduce mutations into IBDV viruses to make them replicate less well, and not cause disease.
2. Take existing vaccines that currently protect against Newcastle Disease Virus and IBDV, and apply them to new strains of IBDV.
3. Develop virus-like particles that are empty ‘shells’ of IBDV that are not capable of replicating or causing disease, but can still induce immune responses.
4. Inoculate birds with the gene encoding an antibody against IBDV to protect them from infection.</td></tr>
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	In order to meet the second objective, we plan to characterise chicken and virus factors that influence disease. Some inbred lines of chickens are more resistant to IBDV disease than others, but the reason remains unknown. We plan to identify possible chicken genes that are responsible for IBDV resistance. In addition, why vv strains have a higher mortality rate than classical strains is poorly understood. We will address this by characterising the disease caused by mutant viruses designed in the laboratory.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The expected benefits of the project are: • New strategies for controlling IBDV. • An increased understanding of how IBDV causes disease. These benefits are worthwhile to the following: • **Poultry health and welfare**: By controlling IBDV, fewer birds will suffer from disease caused by the virus, or from secondary infection. • **Farmers**: The profit from flocks will increase if IBDV is better controlled. • **Supermarkets, the consumer, and UK exports**: If IBDV is better controlled, the amount of high quality poultry meat will increase leading to increased sales by supermarkets, increased supply to consumers and increased overseas exports and associated revenue. • **Vaccine/pharmaceutical companies**: The vaccines developed could be commercialised. • **The scientific community**: A greater understanding of the mechanism of IBDV disease may lead to novel avenues of research. For example, if chicken genes are identified that enhance IBDV resistance, they could potentially be bred into the flocks to produce birds that are resistant to IBDV.
What species and approximate numbers of animals do you expect to use over what period of time?	We plan to use 600 chickens per year for 5 years, making a total of 3,000 birds, and 360 embryonated eggs per year for 5 years, making a total of 1,800 embryonated eggs.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	Birds infected with IBDV may show a variety of clinical signs and symptoms: ruffled feathers, depression, dehydration, diarrhoea, soiled vent feathers, and prostration. Different strains of IBDV vary in their severity, and the adverse effects that will be observed during a

level of severity? What will happen to the animals at the end?	study will depend on the strain used. From previous experience, we anticipate that all birds inoculated with IBDV will show mild symptoms from approximately 24 hours post-infection, and birds inoculated with very virulent (vv) strains of IBDV will show moderate disease from approximately 48 hours post-infection that progresses to severe disease. During the course of this project, it is necessary to infect some chickens with vvIBDV strains. However, in most experiments, it will be possible to humanely cull birds before they experience severe disease without affecting the conclusions. All remaining birds will be culled by a humane procedure at the end of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where possible, alternatives to animals will be used, for example cells grown in the laboratory. However, when IBDV is grown in cells, it changes (mutates) and no longer behaves in the same way as the natural infection. Consequently, it is necessary to grow stocks of some IBDV strains in chickens. It is also necessary to use embryonated eggs to quantify how much virus is present in these stocks. We plan to study IBDV disease, how IBDV suppresses the immune system, and how the virus is transmitted between birds. We will also determine how immunogenic and efficacious vaccines are. Unfortunately, it is necessary to infect birds to achieve these aims.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of birds and embryonated eggs used in this project will be kept to a minimum, while ensuring meaningful data is obtained. In order to achieve this goal, we have consulted publications for guidance on the minimum number of animals per experimental group, and statisticians at The Pirbright Institute to ensure studies are suitably designed and data is appropriately analysed. Inbred lines of chickens will be used for some experiments that will reduce biological variation and keep the number of animals needed to a minimum.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	Turkeys and chickens are the only species known to be naturally infected with IBDV. As this project is concerned with controlling the disease in chicken flocks, the most appropriate species in which to conduct the planned studies is the chicken.

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	All infected birds will checked at least daily and a clinical score kept. Birds will be culled by a humane procedure when humane end-points are reached, and we anticipate no birds will die because of infection. To ensure sick birds are identified as soon as possible, the frequency of checks will increase to 8 hourly intervals when birds experience moderate disease, and 3 hourly intervals when the birds experience severe disease. In most experiments, it will be possible to humanely cull birds before they experience severe disease without affecting the conclusions. The immune responses induced by vaccines will be measured in the blood of inoculated birds. Birds that failed to mount an antibody response will not be subsequently infected with IBDV as they are unlikely to be protected. This will minimise the number of animals that could potentially experience severe disease.

<table>
<tr><td>Project 5</td><td colspan="2">Infectious disease in pigs</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Pigs, infectious disease, vaccines, prevention, pathogenicity</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">5 years</td></tr>
<tr><td rowspan="8">Purpose of the project as in ASPA section 5C(3)

(Mark all boxes that apply)</td><td></td><td>Basic research</td></tr>
<tr><td>X</td><td>Translational and applied research</td></tr>
<tr><td></td><td>Regulatory use and routine production</td></tr>
<tr><td></td><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td></tr>
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<tr><td></td><td>Higher education or training</td></tr>
<tr><td></td><td>Forensic enquiries</td></tr>
<tr><td></td><td>Maintenance of colonies of genetically altered animals</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="2">(1) To investigate the disease causing mechanisms of micro-organisms associated with general, enteric or respiratory symptoms in pigs of different breeds. We may identify new mechanisms of action of microorganisms or find new ways how micro-organisms are spread from pig to pig that are important.

(2) To investigate the influence of micro-organisms on each other when they enter a pig at the same time but also when they enter a pig at different time points. This may involve testing several viruses and bacteria or a combination of viruses and bacteria in the same model. This is done to determine differences of importance of order of infection on the clinical symptoms a pig may develop (i.e. exposure to virus A first and 7 days later exposure to virus B causes more severe clinical symptoms compared to exposure to virus B first and 7 days later exposure to virus A).

(3) To investigate ways to reduce or prevent clinical signs and disease and vaccine development. Knowledge on proper control of infectious disease is critical in order to prevent unnecessary harm and pain for pigs.

(4) To develop diagnostic tools for studying infectious diseases in pigs. Correct and timely diagnosis of</td></tr>
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	infectious diseases allows treatment and opportunities to prevent disease spread within a farm and across farms and geographic regions. (5) To conduct surveys to study how infectious diseases change over time and to monitor new disease causing agents. Knowledge of the main structure of a micro-organism in combination with regular sampling over time and in certain geographic regions will allow us to identify occurrence of new variants and variants not included in current vaccine preparations.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This research directly benefits pigs and in the long run will result in healthier pigs thereby reducing usage of antibiotics and contributing to pig welfare, a healthier environment and better meat quality. Specifically, the benefits of this project are a better understanding of infectious pig diseases which ultimately will lead to improved intervention strategies such as vaccination or changes in pig husbandry. Concurrent infection of pigs with two or more micro-organisms (viruses, bacteria) is a common scenario and often increases clinical signs. Knowing which micro-organism interact will help to add in decisions on reduction or even eliminate certain pathogens. Timely demonstration of diseases is important for pig herds in order to implement correct strategies. Closely monitoring field pathogens is important as even minimal changes in the pathogen genome sometimes are associated with increased severity or lack of vaccine efficacy. Earlier detection of such changes will help determine consequences and prevention at a time when the effect on the pig population is still marginal. Finally, improving diagnostic tools and establishing new diagnostic tools for early pathogen detection are needed to monitor disease trends and pathogen spread. Having the correct tools available can be critical to prevent rapid pathogen transmission when emerging pathogens enter the UK pig population or new pathogen subtypes appear.
What species and approximate numbers of animals do you expect to use over what period of time?	Pigs Approximately 5000 over 5 years which include experimental pigs and pigs sampled for epidemiological investigations on farm.
In the context of what you propose to do to the animals, what are the expected adverse effects and the	Mechanism studies and vaccine trials: Expected adverse effects: • Lumps or redness at the vaccination site

likely/expected level of severity? What will happen to the animals at the end?	• Haemorrhage during blood collection • Clinical signs after challenge: Fever, diarrhoea, weight • loss, respiratory signs, lethargy. • Likely/expected level of severity: Mild-to-Moderate • At termination the pigs will be euthanized. • Field studies; controls sample derivation for test development: • Expected adverse effects: • Haemorrhage during blood collection • Lumps or redness at the vaccination site • Likely/expected level of severity: Mild • At termination the pigs will be rehomed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Studying the development of a disease, determining if vaccines protect a pig and generation of positive controls for diagnostic tests can only be done in live animals. Cell culture systems are not suitable to study interactions between the animal's immune system and the disease causing agent. Similarly, antibody responses ("titre development") can only be studied in pigs and not in cell culture systems.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will seek advice from a statistician to determine the minimum number of animals needed to accurately determine if there is a true difference between treatments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Under this project license we will study pigs. The majority of the bacteria and viruses in pigs under investigation only infect pigs and a lower species such as mice or rats cannot be used. Refinements in place: Pigs will be kept in social groups whenever possible. If this is not possible, pigs will be able to see each other through glass parturitions. All pig rooms/pens will be equipped with environmental enrichment. Other refinements that are in place include usage of real-time PCR cycle threshold numbers or virus genome calculation to inform endpoints. A statistician will be consulted when planning individual studies to determine the minimal number of pigs needed to have enough statistical power to determine significance if there are true differences between groups.

Project 6	**Sexual transmission of Maedi Visna from Rams to Ewes**	
Key Words (max. 5 words)	Lentivirus, Sheep, Sexual transmission	
Expected duration of the project (yrs)	4	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Maedi Visna is a serious disease of sheep and goats that is endemic in the UK. The virus is mostly transmitted by respiratory tract secretions in animals housed in close contact with each other. There is strong evidence that the virus can be transmitted sexually. This study will monitor ewes artificially inseminated with semen from Maedi Visna infected rams to examine this question scientifically robustly	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Current control programmes for the virus rely on culling of infected individuals. In flocks of high genetic value (or rare breeds) this can result in considerable loss of valuable genetic diversity. Semen collection for later artificial insemination is a logical way to conserve this genetic resource but without data on the potential risk of transmission of virus via this route the safety of this option cannot be assessed.	
What species and approximate numbers of animals do you expect to use over what period of time?	Species: sheep Number of animals: 72 Time period: 5 years	
In the context of what you propose to do to the animals, what are the expected adverse	The procedures applied to the animals are of minimal stress (blood sampling, collection and deposition of semen, hormonal treatment of ewes). The onset of	

effects and the likely/expected level of severity? What will happen to the animals at the end?	the clinical signs of the disease is typically not seen for years after viral infection (it is present in many UK sheep flocks). The ewes inseminated in this study will only be kept for 2 months and are very unlikely to suffer adverse effects from the virus in this time period. The rams will be monitored closely. There is minimal risk of viral infection, much less clinical disease to the teaser ewes used in semen collection. Any animals showing signs of viral disease will be euthanised. The rams and the inseminated and control ewes will be euthanised at the end of the study; other animals will be returned to normal husbandry conditions in the universities sheep flock. The virus is not transmissible to people or animals other than sheep or goats.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This virus only affects sheep and goats therefore it is necessary to study it in sheep and goats. Cell culture experiments cannot replicate all the physical and chemical barriers to sexually transmitted viruses present in a sheep's reproductive tract.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The rams involved in this work were naturally infected (the virus was detected on routine screening). The number of ewes used for insemination has been calculated as the minimum number need to demonstrate viral transmission at a comparable rate to the one previous published experiment (via a more invasive insemination route, ie ultrasound guided laparascopic intra-uterine insemination) examining sexual transmission of Small ruminant lentiviruses.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Laboratory experiments and clinical studies have demonstrated as far as possible that MV could be sexually transmitted via other insemination methods. There is no way to further examine this question without actually performing semen transfer into the ewes reproductive tract under controlled conditions to allow rigorous monitoring for virus.

Project 7	**Genetics of one-carbon metabolism in sheep in relation to productivity fertility and health**	
Key Words (max. 5 words)	Sheep, genetics, trace elements, health, epigenetics	
Expected duration of the project (yrs)	3	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	Yes	Basic research
	Yes	Translational and applied research
	No	Regulatory use and routine production
	No	Protection of the natural environment in the interests of the health or welfare of humans or animals
	No	Preservation of species
	No	Higher education or training
	No	Forensic enquiries
	No	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project will undertake two 'proof of concept' studies (one with weaned lambs and the other with breeding ewes) to demonstrate that selection on the basis of identified markers in genes regulating a key set of metabolic processes in these animals leads to real improvements in embryo development, lamb growth, and ewe/lamb health.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The outcome of this project will lead to the development of strategies for the judicious targeting of trace-element supplements within and between flocks, and/or for the selection of genetic tolerance within breeding programs. This in turn will lead to improvements in animal health and reduce suffering associated chronic trace-element deficiencies. It will also benefit the sheep industry financially, both in terms of reduced costs and from improved productivity. The project will also develop genetic and statistical tools that can be applied to investigate similar interactions between genes and diet within human populations.	
What species and approximate numbers of animals do you expect to use	Sheep (200)	

over what period of time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	These animals will be maintained and used in moderate dietary studies to assess metabolic status and dietary intake. Based on previous experience, no adverse effects are anticipated. Detailed endpoints such as assessments of early embryo development will be conducted post mortem following euthanasia using a Schedule 1 method. Procedures will be terminated if animals show adverse effects associated with loss of appetite and weight loss. They may be given appropriate dietary supplements at that point or humanely euthanized by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This project is specifically interested in dietary nutrient x genetic interactions as they affect metabolism, embryonic development and health in the species of clinical interest (i.e. the sheep). Nevertheless, findings from this study will be directly translatable to clinical studies in humans. Studies leading up to the current proposal were conducted without the need to undertake regulated procedures in animals. The current proposal is proof of concept. Having identified possible genetic markers, it will be necessary to establish the extent to which selection on the basis of these markers confers real advantages with regard to tolerance to deficiencies in dietary trace elements. To achieve this objective we need to work with live animals, and have identified weaned lambs and breeding ewes as the two most appropriate classes of sheep to work with.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have conducted and published studies of a similar nature in sheep before, and have undertaken statistical power calculations to determine the minimum number of animals required for study in each of the two planned experiments.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Work to be undertaken in this project builds on previously published studies conducted at our centre with sheep. For this proposal sheep represents the species of primary clinical interest. That is, information gained from this study will be used to better target dietary interventions to genetically susceptible animals and/or be used to select animals that are genetically tolerant to deficiencies in key trace elements; thereby improving the nutritional

(harms) to the animals.	wellbeing of subsequent generations. As an outbred species, information gleaned from sheep will also be of interest to those concerned with the nutritional wellbeing of humans. Procedures to be undertaken in live animals are the minimum necessary to test our hypothesis that we can identify animals that are either genetically susceptible or tolerant to dietary deficiencies in key trace elements. That is, our live animal assessments are largely limited to analysis of regular (weekly or fortnightly) blood samples in sheep and indirect measures of appetite. At the earliest indication of dysfunction we will consider our experimental endpoints to have been met and the studies will be terminated. At this stage animals will be killed using a Schedule 1 method. Subsequent analyses of tissues (e.g. liver and/or embryonic) will be conducted post mortem. We will need to precisely record dietary intake as part of these studies. To facilitate this, animals may be kept for the minimum period necessary in adjacent individual pens, in sight and contact of one another. Pen size will conform to Home Office Code of Practice.

Project 8	**Mycotoxicosis in ruminants**	
Key Words (max. 5 words)	Dairy, mycotoxin, silage	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Mycotoxins are found in ~80% of maize silage in the UK. The symptoms of mycotoxicosis in cows are similar to that of many other diseases due to their high variability. This makes determining the cause of the illness very difficult and mycotoxicosis is often the last treated cause. If ingested in high enough quantities, the mycotoxins will be passed into the milk and as they are not effected by pasteurization, pass in to the human food chain. There is currently no rapid diagnostic tool for mycotoxicosis in cattle. This work aims to identify mycotoxin metabolites that could be potential biomarkers of mycotoxin poisoning in the urine, blood and/or saliva of dairy cows and subsequently developed for use in a diagnostic test.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Symptoms of mycotoxicoses are very varied and often similar to that of other diseases, thus they are often undiagnosed and/or incorrect treatment given. The problem does not end in animal disease or production losses. Mycotoxins in the feed of dairy animals can lead to their metabolites appearing in	

	dairy products, which pose a risk in human health particularly for infants. A rapid early detection method of mycotoxicosis for ruminants is therefore required. It is not possible at this stage to say what form the test may take as it will depend on what metabolite and from which sample matrix it is to be analysed.
What species and approximate numbers of animals do you expect to use over what period of time?	Dairy bull steers ≤ 1 year old 150 over the term of the PPL
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Expected adverse effects: a decrease in feed intake, body weight and/or condition score is likely to occur to moderate severity. At the end of each Stage or if an animal exhibits symptoms in excess of those expected at moderate severity, the animals will be withdrawn from the trial and killed using Schedule 1 method. Samples will be taken from the animals post mortem.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Whilst *in vitro* work will provide some metabonomic data on the effect of mycotoxins and their metabolites on the cow, the interactions within the gut of the cow are highly complex involving the host immune response, the diversity and stability of the gut microflora, age, breed, health and nutritional status of the animal. These cannot as yet be replicated satisfactorily *in vitro*.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The sample sizes are based on previous work that was successful. It is not possible to calculate a standard power calculation due to the complexity of the metabonomic analysis however this does not prevent the calculation of profile difference significance.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the	The rumen is a unique environment where food is partially digested before being regurgitated, again chewed and then sent back into the rumen for further digestion. No other animal is similar enough in this way to provide high quality data. The metabonomic analysis is highly sensitive and data even from other

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	ruminant species would not necessarily be relevant to the aim of this study. Animals will be acclimatized to handling before the study and minimal restraints used during sampling other than that required for the safety of the handlers. Animals will be housed within sight of each other. Animals will be checked twice daily for signs of reduced feed intake, diarrhoea, respiratory distress and lameness. Condition score and body weight will be checked weekly.

Project 9	**The use of therapeutic MRNA in companion animals**	
Key Words (max. 5 words)	Dogs, renal, anaemia, mRNA	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	Yes	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Kidney diseases are a common cause of suffering in pet dogs. The cause of these diseases is frequently unknown and there are few therapies which significant improve patient outcomes. One of the most common complications of renal diseases in dogs is anaemia. This can be highly debilitating and significantly reduce the quality of life of dogs with chronic renal failure. There are no effective licensed treatments for anaemia associated with chronic renal failure in dogs. The administration of mRNA has been demonstrated to be an effective way to increase protein production in experimental models of disease. The key objective of this study is to establish the most appropriate dose to administer canine erythropoietin, the key hormone which increases red blood cell numbers in dogs, mRNA in order to successfully treat dogs with anaemia associated with spontaneous renal diseases.	
What are the potential benefits likely to derive from	The study will also allow the wider biomedical community to understand more about the therapeutic	

this project (how science could be advanced or humans or animals could benefit from the project)?	potential of mRNA therapies which will inform similar studies in human patients. We hope that the study will define the most appropriate dosing regime of canine erythropoietin mRNA to manage anaemia associated with chronic renal failure. This will then allow other dogs with renal disorders to have their anaemic condition successfully treated.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to treat 50 dogs over the course of five years.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The adverse effects are likely to be mild and transient. There might be mild, transient irritation around the injection site. The number of red cells may exceed normal levels and the blood pressure may increase. Both these complications should be transient and can be managed medically if they cause clinical signs. As with any injections of any substance, there is a very small risk of anaphylaxis. We are very well prepared to deal with that as the clinical studies will take place in a well equipped veterinary hospital which has on site veterinary care 24 hours/day. Appropriate treatment for renal disease will be continued for the animals on the study and owners can withdraw their animals at any time. Aside of out-patient visits to the clinic, the animals will remain with their owners during the course of and at the end of the study
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Our aim is to establish better treatment regimes for the management of anaemia in client owned dogs with spontaneous chronic renal disease. We believe this approach is the epitome of 3Rs best practice since we are studying disease processes which have already spontaneously developed rather than inducing them in otherwise healthy animals
2. Reduction Explain how you will assure the use of minimum numbers	As the animals have developed renal diseases spontaneously, we are avoiding the need to induce illnesses in otherwise healthy animals. We will use the minimum number of animals required to establish the

of animals	best treatment regime for canine erythropoietin mRNA
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We aim to develop better treatments for dogs with spontaneous renal diseases. Consequently, the therapeutic trial has to be undertaken on dogs with spontaneous renal diseases. The study will take place in a well equipped veterinary hospital which has 24 hour veterinary care and outstanding facilities and staff so is very well placed to deal with any adverse events.

Project 10	**DEVELOPMENT OF BACTERIAL VACCINES FOR POULTRY**	
Key Words (max. 5 words)	VACCINES POULTRY	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The licencing of bacterial vaccines for the prevention of disease in poultry and the reduction in the prevalence of zoonotic organisms in the human food chain.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A reduction in the numbers of cases of human food poisoning caused by zoonotic organisms in the food chain. Increased health and welfare of poultry due to a reduction in disease. A reduction in the need for the use of antibiotics as a preventive measure in poultry production.	
What species and approximate numbers of animals do you expect to use over what period of time?	Chickens - 9000 Rabbits - 100 Mice - 400 Guinea pigs - 100	

	Pigs - 50 Cattle - 50 Rats - 50 Pigeons - 40
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The studies which will involve the majority of the animals will not cause any adverse effects and the level of severity will be mild. However, especially in cases where the efficacy of vaccination can only be shown by a reduction in disease or where safety or reversion to virulence is being investigated, the symptoms may include dullness, reduced feeding, diarrhoea, morbidity and mortality. The level of severity in these cases will be severe. At the end of the studies the majority of the animals will be humanely euthanased. It may be possible in exceptional circumstances to rehome birds which have been a source of control materials.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In the study of infectious diseases the effect on the animal as a whole is important as is the transmission between animals. It is not possible to reproduce the immune responses to vaccination *in vivo*, and animals must be used. The regulatory requirements for the licensing of vaccines require that studies of efficacy and safety are carried out in target species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Where there are not pre-existing specific models the minimum numbers of animals will be used to ensure the statistical significance of the resulting study data as determined by pilot studies. Where minimum numbers of animals are specified by regulatory requirements these will only be exceeded to meet the necessary statistical criteria or where a repeat study may be avoided by the use of larger groups. The development of *in vitro* alternatives to some tests

	used for vaccine release will reduce the long term use of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The use of chickens is appropriate for most of the studies as they are the target species. The alternative species will be appropriate for the parameters under investigation, either as the best indicator of the effect or most likely to be exposed environmentally. The models used will be the most refined in that the harm to the animal will be negligible or that the application of humane end-points will ensure that any disease progression will be the kept to a minimum.

Project 11	**Novel Therapeutics for Veterinary Oncology**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Our objective is to develop novel and safer therapeutic options for cancer in animals, in particular dogs, The ultimate aim is to improve the safety of cancer treatment options and efficacy and thus long term survival. In addition our goal is that these treatments will have minimal side effects Specifically, for this license we wish to determine the effective dose of novel cancer agents that can be given safely to patients to effect a biological response in their cancers.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	Safe and effective treatments for cancer in companion animals in dogs are lacking. This project is taking new, targeted therapies for cancer in dogs towards clinical application by determining safe and effective biological doses. The potential benefits could be realized in 3-5 years for the general dog population if these drugs are shown to be effective and reach full registration. Potentially human medicine could also benefit from this study. If the drugs being developed prove effective in	

	dogs, then it is likely that this could be translated into human clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use pet dogs that have naturally occurring cancers. In the trial we have designed, we anticipate recruiting around 50-60 dogs over a 2-year period.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity is moderate and animals will be at home with their owners during and after the trials. They will visit the veterinary hospital for treatments and monitoring only. For the agents to be used in the trials we have significant preclinical evidence that they have a good toxicity profile and the risk of adverse reactions is therefore low. In the event of any adverse drug reactions, such as anorexia, vomiting or diarrhoea, animals will be treated by our specialist veterinary team. Where clinical signs cannot be controlled, or there is progressive disease, then euthanasia will be performed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It would be impossible to answer these research questions in tissue culture or computer simulations. This is because we need to establish the safety and efficacy of cancer drugs in the context of a real tumour (in association with an intact tumour microenvironment) and in the context of a living animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• Through appropriate statistical methodology and experimental design: i.e. we are using standard phase zero clinical trial design. This design allows for 89% power1 at a particular dose level, to detect a 60% response rate at the patient level. Utilizing expertise in veterinary anaesthesia, imaging and oncology to ensure high welfare standards.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will	• I in 3 dogs get cancer, the aim of treatment being to maintain the dog's quality of life and to ensure that they live a normal life. • Current treatments are less than ideal, having a high potential for significant side effects. • We have developed what we consider to be safer and

take to minimise welfare costs (harms) to the animals.	more targeted treatments and have tested them as far as possible in preclinical models. However, for these treatments to become clinically acceptable, we now have to determine the effective and safe doses we can use in dogs that are affected by cancer. • We will be using dogs who have failed conventional treatments or for which no treatments are available. However, these dogs will still be well and have a normal quality of life. • The study may not yield clinical benefit for some of the dogs, but this will be monitored closely and these dogs may be removed from the study early. Because of this we have designed significant safeguards (in terms of humane endpoints and clinical monitoring) to ensure that we minimise any welfare costs. This is supported by utilizing expertise in veterinary anaesthesia, imaging and oncology to ensure high welfare standards.

Project 12	Supply of materials to develop and maintain tests for BSE in sheep animal
Key Words	BSE, Sheep, Supply, Positive, Material
Expected duration of the project	1 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Transmisible Spongiform Encephalopathies (TSEs) are a group of fatal neurodegenerative disease affecting humans and animals, and include BSE in cattle and scrapie in sheep. Bovine Spongiform Encephalopathy (BSE) has been identified as a food-borne risk to man and animals. There is a potential risk that BSE could infect sheep and this needs to be distinguished from scrapie due to its ability of BSE to infect humans (scrapie does not infect humans). As field case material is not available the aim of this project licence is to produce BSE positive tissue sheep brain material to be used in EU-wide Proficiency Testing exercises.

The project licences is for one sheep from a group of animals that was challenged with BSE under a previous project licence.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Exercises provide assurance that laboratories testing for TSEs are able to correctly identify BSE in sheep , therefore protecting both human and animal health. This is the only opportunity for many laboratories to demonstrate their ability to detect unusual strains of TSE. Knowledge of test performance also enables test development and maintenance.

What types and approximate numbers of animals do you expect to use and over what period of time?

1 Sheep

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Most sheep challenged with BSE will progress to clinical disease and the most likely adverse effects will result from their clinical illness. The sheep will be clinically monitored to detect early signs of BSE infection in the brain , once this is confirmed the sheep will be euthanased.

Application of the 3Rs

Replacement

There is no in-vitro alternative for the production of BSE infected brain material.

Reduction

This is a single sheep from a previous project licence so it is the minimal number.

Refinement

For the generation of TSE infected materials the clinical score sheet we will be used ensure the scientific endpoint is captured (tissues are infected) but minimise the period with the animal is showing moderate clinical signs Clinical score sheets (Annex 1) are used to determine endpoints CCTV may be used during TSE experimental studies in order to detect clinical signs earlier whilst the animals are not observed by the animal care staff or the neurologist.

Although only single sheep remains from the previous project licence it is housed with a companion.

Project 13	Novel interventions for poultry red mite
Key Words	poultry, vaccine, parasite
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Overall aim: This project will progress novel interventions for the control of poultry red mites

Key Objectives:

- Which red mite antigens are most appropriate for vaccine-induced protection?
- What is the efficacy of prototype vaccines in in vitro/in vivo models?
- What are the most appropriate immune correlates of protection?
- Which methods of delivery, including adjuvant selection, are most appropriate for vaccine-induced protection?
- What is the field efficacy of prototype vaccines?
- Are vaccination strategies compatible with other, novel and existing, interventions in integrated management strategies?

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

From this work we will develop a clearer understanding about whether or not vaccination is possible to control mites which feed on the blood of their hosts. In addition we will develop a better understanding of what types of immune responses we need to develop in birds to allow these vaccines to work. In the longer term this may result in an effective vaccine to control a parasite which is a serious welfare issue in the egg industry.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use adult hens (approximately 1100 in total over 5 years). In initial studies we will use small numbers (approximately 25 per year) but for studies to examine the effects of vaccines in integrated management strategies in the field we will use larger numbers to replicate field conditions in hen houses.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

For small-scale initial studies the hens will be kept in coups in barns on a farm. They will be immunised with vaccines using standard routes of delivery (e.g. injection) and blood will be taken from their wing veins to monitor the immune response. In addition, small areas of their skin (after feather plucking) will be exposed to low numbers of parasites for short periods (up to 6 hours) . For larger scale experiments hens will be housed in commercial-style egg production systems for immunisation, blood sampling and exposure to mite infestations which reflect those that they may encounter in normal field conditions. At the end of the small-scale experiments hens will be euthansed by anaesthesia . At the end of the larger scale experiments the hens may be kept alive at the establishment (with the agreement of a vet) with a view to re-homing them if appropriate.

Application of the 3Rs

Replacement

Poultry red mite needs to regularly feed on blood to complete its lifecycle, and has many similarities with ticks. The population of the poultry red mite is thus maintained through a complex interaction with the host, and in the local environment and animal densities of a commercial egg laying unit, these populations can become critically high. There is thus no practical alternative to the use of animals (hens) to test the efficacy of a vaccine to control this parasite. Searches for potential alternatives to the hen model using the current (20th October 2016) resources of the Fund for the Replacement of Animals in Medical Experiments (FRAME http://www.frame.org.uk)

and the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs http://www.nc3rs.org.uk) have not yielded any useful alternative models.

Reduction

All animal studies are planned in consultation with statisticians prior to submission to the local ethics committee in order to provide adequate group sizes for the most appropriate statistically robust analyses while minimising the number of experimental animals.

Hens may be vaccinated with a cocktail of antigens in order to reduce the number of experimental groups. Pilot studies with reduced numbers of individual birds, accompanied by some laboratory-based analyses will provide a method to minimise the use of hens.

Refinement

The poultry red mite is a specific problem for the hen industry, and particularly for egg laying birds. The hen is thus the only realistic choice for these studies. The parasites to be investigated in this project can only survive by feeding on hen blood. We have developed and refined a mite challenge model such that we test antibodies against mites in vitro. While this requires a source of hen blood for the model, it reduces the numbers of hens required as a single blood sample can be used for testing antibodies (derived from eggs) against a range of antigens. The field efficacy of a vaccine can only be determined by immunisation and challenge experiments on hens with live parasites. We will be advised by vets who specialise in poultry diseases throughout the project.

Project 14	Ecology and Epidemiology of Infectious Disease in Wildlife.
Key Words	Wildlife, Epidemiology, Demography, Management
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;
Yes	(ii) assessment, detection, regulation or modification of physiological conditions in man, animals or plants;
Yes	(iii) improvement of the welfare of animals or of the production conditions for animals reared for agricultural purposes.
Yes	(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 aims mentioned in paragraph (b);
Yes	(d) protection of the natural environment in the interests of the health or welfare of man or animals;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

This project aims to provide data to inform our understanding of the dynamics of an infectious pathogen in wildlife and approaches for controlling transmission to domestic animals. Wildlife are a potential source of infectious disease in livestock but the dynamics of infection may vary in response to local differences in host density, behaviour and habitat structure, and such variation is likely to impact on the outcome of management interventions. Hence, the development of sustainable and effective methods of disease control in wildlife is challenging. Long-term and intensive studies of wildlife host populations are required to provide information on vital

demographic parameters (e.g. survival, fecundity, immigration), to identify drivers of pathogen persistence, spread and disease progression, including physiological and behavioural correlates, and to develop and assess methods for managing infection in wildlife and risks of transmission to livestock.

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Pathogens of wildlife can cause serious disease in livestock with significant financial and social impacts on the UK farming industry. The control of disease in livestock is made more challenging by the involvement of reservoirs of infection in wildlife. Data from our studies will be used to further develop understanding of the dynamics of infectious pathogens in wildlife host populations, to provide insights into disease dynamics and risks to livestock, to produce and parameterise mathematical simulation models, to assess approaches for the control of pathogens in wildlife populations, and ultimately to inform policy on sustainable options for the management of disease in both wildlife and livestock.

What types and approximate numbers of animals do you expect to use and over what period of time?

Wild animal hosts over 2 kg in weight are the subject of these studies. During the five year term of the licence we anticipate capture, examination and release of no more than 800 different individuals.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

The expected level of severity of procedures is mild. No lasting adverse effects are expected as a result of capture, examination or monitoring of wild animals. Nevertheless, we have detailed procedures in place to address any welfare issues arising from unexpected outcomes. At the end of the study animals will be set free into the wild.

Application of the 3Rs

Replacement

Complete replacement of the use of live animals is impossible as the purpose of the research described here is to investigate natural ecological and epidemiological processes in a wild animal population. However, the data available from our studies can be used in simulation models to investigate the likely effects of different management scenarios and the dynamics of disease under other conditions, which may reduce or obviate the need for use of live animals.

Reduction

Population-level studies require that a sufficiently large and representative sample of the target population is monitored and long-term studies require that the same level of effort is deployed so that data is comparable across years. Also, we are studying disease transition events using imperfect diagnostic tests and can only intermittently monitor animals by trapping and sampling, so it is necessary to maximise sample sizes. However, in the interests of animal welfare trapping is only carried out four times per year at each capture location at intervals of no less than one month. Statistical advice will be sought on likely sample sizes required to test specific predictions with adequate statistical rigour whilst ensuring that the minimum number of animals are involved.

Refinement

The research team are highly experienced in trapping and handling wild animals and since the inception of this programme of work in 1976 we have continued to develop and improve field and sampling methodology in the interests of animal welfare. The team includes a veterinarian and full time NACWO. All staff involved in trapping and sampling have undergone extensive and documented training, and are involved in annual refreshers. Trapping procedures, trap design and sampling protocols have all been refined over time and are continuously reviewed to ensure we apply the highest standards of animal welfare. Trapping is carried out under licence from Natural England.

Project 15	Understanding Batrachochytrium dendrobatidis and ranavirus infection dynamics to develop mitigation strategies
Key Words	Amphibian, Disease, Conservation, Mitigation
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose	
Yes	(a) basic research;
	(b) translational or applied research with one of the following aims:
Yes	(i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

The overall purpose of this project is to identify under which conditions amphibian pathogens pose conservation threats and use this information to design conservation strategies and management plans for disease in captivity, the wild and the amphibian trade. To do this we will continue to investigate what pathogens cause threatening disease and how their genetic structure may affect disease. We will also investigate how pathogens compete with each other in hosts, as evidence suggests that pathogen competition leads to elevated disease. Our third objective is to understand how amphibian community composition affects transmission and disease dynamics, so that we may identify communities that are more resistant to, or tolerant of, infections. Last we will continue to identify environmental factors that can exacerbate effects of disease, or more hopefully provide a refuge from more threatening disease dynamics. We will do this for several European amphibian species, including UK species and communities

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

We have proven ability to translate experimental work into mitigation strategies in captivity and, more recently, in the wild. Our interventions in the wild have been focussed on simple and relatively closed amphibian host systems, so we hope to extend to more complex amphibian communities and, therefore, more general situations. This is an extremely urgent problem, as amphibians are confronting an

increasing array of pathogens and pathogen genotypes and as a result increasing rates of population decline.

What types and approximate numbers of animals do you expect to use and over what period of time?

We will use ten amphibian species, all of which are threatened by at least one of the pathogen groups we will work with. We have requested to use a maximum of 15,000 animals over the 5 years. We expect to use fewer than the requested amount, as during the course of our work certain species will prove to be more focal to our research questions and conservation imperatives.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

These infectious agents cause lethal disease and in the case of one (ranaviruses), significant signs of ill health before death. Because of this, and the fact some animals do die before we can intervene with humane euthanasia, our procedures are classed as severe. With few exceptions, animals involved in our procedures will be euthanized. Some may be treated with antifungals and afterwards retained in our facility.

Application of the 3Rs

Replacement

To understand infection and transmission dynamics animals must be used. Because transmission and infection dynamics are known to be species and life history stage-specific, the results from experiments using one species and/or stage cannot be extrapolated to another. Each species and stage must be examined individually. To understand how environmental correlates may affect the development of infections and mortality, animal hosts must be used.

We will be submitting a proposal to the NC3Rs this year, the subject of which is replacement, investigating if we can examine pathogen growth rates in cell culture and if this can be used as an estimate of virulence.

Reduction

We have experience with measuring host responses in 8 of the European amphibian species listed in this license application and have estimated sample sizes based on experiments to date. Based on experience and statistical analyses of experimental data aets, we conclude that we need to replicate treatments in experiments 30±5 times to detect biologically meaningful differences in death associated with infectious disease. Some of the variables we are interested in vary subtly; variables such as growth rate, mass gain and developmental timing. In these cases we need to increase replication by a factor of 10.

Refinement

Our list of species represents the predominant native species in the UK and the most predominant nonnative species, as well as the nonnative species most implicated by the data generated through the 2008 UK chytrid survey as being a potential vector host and European evidence for mortality due to ranaviruses. We also have included European species that are common in communities affected by both pathogen groups. Our methods of exposing and infecting animals have been trialled for multiple European species. We also have extensive experience with stocking densities of larvae, juveniles and adults of all species listed, as well as feeding requirements and habitat enrichment (e.g., retreat tubes for aquatic newts and caudate larvae, cover objects for adult and juvenile anurans). Further, humane endpoints have been identified for all species, life history stages and both pathogens, as well as general signs of health and well-being, and are listed below.

We are actively seeking ways to improve our husbandry and welfare methods. We have already established UV-B light regimes and expanded dietary regimes for post-metamorphic amphibians to improve nutrition, tested various cover objects and have implemented group housing for animals outside of licensed procedures. We have already identified pre-experimental stocking densities that improve survival and growth and implemented water aeration strategies for developing tadpoles. We have submitted a full studentship application to the NC3Rs to develop validated indicators and validate the existing ones for assessing ill health and poor welfare due to husbandry practice and infectious disease. Specifically, the aims of this project are to (i) establish dietary and housing guidelines that will improve NMA welfare in licensed procedures, (ii) determine early predictors of disease-related and other forms of experimental mortality and (iii) validate existing and new indicators (growth rate, developmental timing) with respect to survival for use in welfare monitoring and implementation of appropriate humane end points that reduce procedure severity.

Project 16	Supply of materials to develop and maintain tests for animal diseases
Key Words	Transmissible, Spongiform, Encephalopathy, Characterisation, Endoparasite
Expected duration of the project	5 year(s) 0 months

Purpose of the project (as in ASPA section 5C(3))

Purpose

(b) translational or applied research with one of the following aims:

Yes (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their effects, in man, animals or plants;

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):

Part 1) Transmissible Spongioform Encephalopathies (TSEs) are a group of fatal neurodegenerative disease affecting humans and animals, and include Bovine Spongioform Encephalopathy (BSE) in cattle and scrapie in sheep. BSE has been identified as a food-borne risk to man and animals. The incidence of both diseases has been declining due to feed bans and selective breeding, respectively; however, atypical forms of both have been recently identified in cattle and sheep with unknown risk to humans. As field case material is not available the aim of part 1 is to produce all naturally occurring forms of atypical TSE positive tissue material to be used in EU-wide Proficiency Testing (PT) exercises to ensure the disease can be identified should it occur in the field

Part 2) Control samples collected from animals which have already contracted a disease in the field. Control samples have two important functions, they show if the test has worked correctly and they provide a diagnostic comparison to the test sample. The aim of part 2 is to provide the opportunity to collect positive field case samples from naturally infected animals rather than experimentally infect animals.

Part 3) Endoparasites are gut parasites that cause disease and poor health in animals. Farmers regularly give their livestock drugs to kill these parasites, but some strains become resistant, meaning that research and development into this area is required to maintain effective treatments. Part 3 is demand led and aims to grow and provide a number of uniques strains of endoparasites to other institutes and research centres for use as efficacy tests and reference controls.

Part 4) New and emerging diseases are a constant threat to UK livestock. If required, susceptible animals would be infected in a controlled environment to study the disease course and presentation, with the aim of increasing understanding and preventing the disease from spreading. For all work in part 4 this licence will be amended and submitted to the Home Office for their approval. This is a requirement as the infectious agent is not defined in part 4; therefore the appropriate analysis cannot be done

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?

Part 1) Proficiency Testing exercises provide assurance that laboratories testing for TSEs are able to correctly identify all known strains of TSE, therefore protecting both human and animal health. This is the only opportunity for many laboratories to demonstrate their ability to detect unusual strains of TSE. Knowledge of test performance also enables test development and maintenance. Part 2) Control material can be very difficult to gather and plays a vital role in test assurance processes. Having the opportunity to take material from animals which are brought to our attention because they are already naturally infected provides this benefit, it also may enable us to better understand the disease if it is not commonly found in the UK. Part 3) The helminth collection is unique and without these strains of parasitic nematodes much important research and development could not take place around the world. Outcomes of this work enhance control strategies with the associated improvement in animal welfare. Part 4) Understanding a new and emerging disease presentation is necessary for its diagnosis and control, therefore protection of the UK's farmed animals.

What types and approximate numbers of animals do you expect to use and over what period of time?

Part 1) • 11 sheep • 4 cows Part 2) This work is reactive to the presence of field cases and the need for control material. Estimated 20 animals selected in the field on the basis of the previous diagnostic test results or clinical signs exhibited and on-farm examination by Veterinary Officers. The numbers of wild birds (200) is maximal and will be demand led by the number of disease outbreaks occuring in wild birds and the volume of sera required. Part 3) 10 lambs (2 per year over the life of the licence) Part 4) This is demand led and specific protocols will be added to this licence as and when necessary detailing animal numbers required.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?

Part 1) The animals will be injected intracerebrally under general anaesthesia with appropriate analgesia. There should be no clinical effects from this technique obvious on recovery with animals will progressing to clinical disease and the most likely adverse effects will result from their clinical illness. All animals will be clinically

monitored to detect signs of disease as early as possible and to minimise suffering. The animals will be euthanatized at the end of the study by a schedule 1 method to allow harvest of their tissues. Expected severity moderate . Part 2) This work is reactive to the presence of field cases and the need for control material. The animals will have already suffered from clinical disease and in a lot of cases will then appear healthy. In some cases the main adverse effect will be that the animals may be kept alive longer than they would have if no samples or tests had been required. If healthy, the animals will be released back to stock, if not they will be killed by a schedule 1 method. Wild birds are used to provide species specific negative control material to allow accurate testing for notifiable disease. Expected severity from this procedure is mild Part 3) Clinical signs or adverse effects are unlikely to result from the doses of nematodes given which are calculated to reduce the risk of clinical signs so expected severity will be mild. Occasionally, blood sucking nematodes such as Haemonchus may cause anaemia. The animal will be treated with appropriate anthelmintic at the end of the trial to remove the parasite infection, and then returned to stock after a health check by the NVS. Part 4) See aims and objectives.

Application of the 3Rs

Replacement

Parts 1 & 2) The purpose of this licence is for the generation and collection of infected animal tissue, no other techniques to produce this material are possible at the current time.

Part 3) Endoparasites have complex lifecycles and cannot survive and reproduce outside of their specific host species.

Part 4) This is about characterising the disease which can only be done in the animals

Reduction

Part 1) Known volumes of tissue are required to fulfil external quality assurance schemes each year, stocks are reviewed annually and animals only challenged when stocks are low.

Parts 2 & 3) This work is either opportunistic (part 2) or demand led (part 3).

Part 4). The organisation will have information about the spread of the disease in the field, this information will be used with biostatistical advice to minimise numbers of animals used.

Refinement

Animals will be chosen depending on the species susceptible to disease. For field cases this will be by natural infection.

If transport to our laboratory is required it will be carried out by our own trained staff and animals will only travel if assessed as fit to do so.

The humane end points will be based on current knowledge of diseases.

Project 17	Parasitic Infections of Fish	
Key Words (max. 5 words)	Parasite, Disease, Teleosts, Aquatic Health, Invasive Species	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
	x	Regulatory use and routine production
	x	Protection of the natural environment in the interests of the health or welfare of humans or animals
	x	Preservation of species
	x	Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The five main objectives are to: 1) Assess how fish genetic and/or epigenetic background influences disease resistance. 2) Assess how fish stress and diet impact parasite infections. 3) Determine how parasite transmission is influenced by environmental factors. 4) Assess how different fish assemblages impact parasite transmission. 5) Identify fish parasite strain variation in relation to virulence.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This licence covers a series of experiments on the epidemiology of infectious disease in freshwater fish. Parasites pose a significant economic cost to aquaculture and as a potent evolutionary force they often threaten wild fish populations. Understanding the basic biology of fish parasites is essential for optimizing farming practices, more accurate diagnosis and design of control programmes to	

	improve the health of fish stocks.
What species and approximate numbers of animals do you expect to use over what period of time?	Much of the work will be conducted using common, tropical and temperate freshwater fish that have relatively short generation times (6 weeks in the case of the guppy) and can easily be maintained under laboratory conditions. For our more applied research, we will use farmed fish such as Tilapia or salmonids. We use ca. 2000 individual fish each year.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	During the course of this project, all experiments will follow a similar design in which fish (naïve to infection or with a known number of parasites) will be briefly anesthetised and given a specific dose of parasites. The fish will then be monitored at regular intervals during the course of the infection to assess parasite population growth (the number of ectoparasitic worms can be countered on the surface of the fish) and possible effects on the host assessed (such as alterations in feeding and reproductive behaviour). Gyrodactylids cause high mortalities in wild and farmed fish (>90%) and a related species (namely *Gyrodactylus salaris*) has been defined as one of the most invasive fish parasitic worms having caused mass epidemics in Norwegian salmon. However, most of the research conducted under this licence will be categorised as mild in severity because many fish shed their experimental load of parasites through induction of an immune response, and susceptible fish (which would die under natural conditions) are usually treated to remove potentially lethal doses of parasites. Therefore, the experimentally infected fish used in the current study are actually healthier than most wild or farmed fish. Some fish will die of natural infections, most will recover and either enter breeding stocks or (if suitable) be returned to the wild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Gyrodactylids are obligate parasites that cannot survive for any length of time away from the host (i.e. *in vitro* culture is not an option, although we have developed protocols for culturing other parasites). Because gyrodactylids are ectoparasitic worms the entire course of infection can be monitored on a

	single fish (thus reducing the number of animals used, while still gaining quantitative data on population growth). Where possible, we seek to derive multiple endpoints from each cohort of animals, combining behavioural and infection studies with morphological and genetic studies. An inherent problem in studying parasites is the huge intra-specific variability in host responses, necessitating large sample sizes, but appropriate experimental design ensures minimum numbers of animals are used to obtain statistically significant results.
2. Reduction Explain how you will assure the use of minimum numbers of animals	From our past experience and power analyses, we can minimize the numbers of fish used to achieve statistically significant results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We use fish species, which are the natural hosts of the parasites being studied. All fish are maintained in an enriched environment and our behavioural studies provide a further check on optimal maintenance conditions.