Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 30

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

Project Titles and keywords

1. **Investigating cartilage disease and repair in sheep**
    - Cartilage, defect, repair, stem cell

2. **The behavioural and nutritional control disease**
    - Behaviour, Disease, Livestock, Nutrition

3. **Evaluation of avian myxoviruses & mammalian influenza virus infections**
    - Avian myxoviruses, mammalian Influenza disease

4. **Studies of porcine epidemic diarrhoea virus**
    - PED virus; Porcine diarrhoea

5. **Provision of Biological Materials**
    - Biological Materials

6. **Improving understanding of lameness in sheep**
    - Footrot, sheep, persistence, environment

7. **Producing antibodies for tests to diagnose animal diseases**
    - Antibodies, Test(s), Diagnosis, Animal Diseases

8. **Gene therapy for canine diabetes**
    - Dog, diabetes, gene, therapy

9. **Enteric pathogens of poultry**
    - Gut health; broilers

10. **Assessing new livestock viruses in UK breeds**
    - New Virus Livestock UK

11. **The faecal microbiome of obese and geriatric ponies**
    - Equine obesity, gastrointestinal microbiome, weight loss, equine nutrition

12. **Parental effects on offspring health and fitness**
    - Maternal, parasites, disease, immunity

13. **Is Schmallenberg Virus Still Circulating in the South of England**
    - Sheep, schmallenberg , transmission

14. **Genotype, health and fertility in dairy cows**

- Phenotype, SNP, immunology, disease, milk

15. Immunobiology of fertility in mares

- Reproductive biology, pregnancy, placenta, immunosuppression

16. Feed conversion efficiency in cattle and sheep

- Feed efficiency; cattle; sheep; methane; animal behaviour

17. Development of a novel vaccine vector in cattle

- Cattle, vaccine delivery system

18. Detection and control of fluke in ruminants

- Fluke, diagnosis, vaccination, treatment, challenge

19. Rabbit Vaccine Development

- Rabbits, myxomatosis, haemorrhagic disease, vaccine

20. Unravelling the Aetiology of Contagious Ovine Digital Dermatitis

- Sheep, lameness, contagious ovine digital dermatitis

21. PPRV pathogenesis and immune response to PPR vaccines

- Sheep, goats, vaccines, disease, protection

22. Translation of Bovine Tuberculosis Biomarkers to the Point of Care Setting

- Bovine tuberculosis, diagnostic test

23. Refinement of anaesthetic protocols for research animals

- Anaesthesia, Refinement

24. Smart tagging of fish to determine fish behaviour, biometrics, biomass and escapes using sonar

- Tagging, biomass, salmon, *Salmo salar*, sonar

25. Quantification of pain associated with pneumonia in calves

- Pneumonia pain welfare calf

26. Ruminant Welfare Studies

- Welfare, ruminant, stress, behaviour, pain

27. Novel acaricidal options for control of sheep scab disease

- Sheep scab mite disease acaricide

<table>
<tr><td>Project 1</td><td colspan="3">Investigating cartilage disease and repair in sheep</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Cartilage, defect, repair, stem cell</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Defects in the cartilage surface of joints can be very painful and often require surgical treatments. Because cartilage does not repair itself very well, cell grafts are also used to repair joints with defects. Currently cells have to be taken from the injured knee, grown in a laboratory, and then re-implanted into the knee to repair the defect.

We propose to establish, in sheep with experimentally created defects in the cartilage of the knees, whether a special type of cell, called a stem cell, could be used to repair joints in this way. Stem cells can replicate and produce cartilage very efficiently. The stem cells we wish to test can be manufactured on a large scale in a cost effective manner. This means that, if the stem cells work, many people could be treated with them quickly, without the lengthy process of having cells taken from their knee and grown in a laboratory.</td></tr>
<tr><td>What are the potential benefits likely to derive from</td><td colspan="3">In the first part of this project, we hope to show that human stem cells are able to live in the</td></tr>
</table>

this project (how science could be advanced or humans or animals could benefit from the project)?	knees of sheep without being rejected by the immune system. If they can, the work will have the potential benefit of showing that human stem cells are a viable treatment for cartilage defects, which would support their use in clinical trials for people with these injuries.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, up to 20 depending on whether the immune system rejects the cells or not. The work will take up to 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 end?	The level of severity is moderate because these sheep will undergo surgery. We expect the level of pain to be moderate and short lived, and the likelihood of any post-operative problems to be low Should any animal have an adverse effect, that cannot be treated, then they will be euthanatized by a schedule 1 method. The animals will be euthanatized at the end of the study by a schedule 1 method to allow harvest of their tissues.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The cells must be tested in the mechanical and biological environment similar to that in which they would be expected to be used clinically. This cannot be done with an in-vitro model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use a minimal number of animals to establish whether the cells will be rejected, and if this does occur, whether it will be overcome with drugs that suppress the immune system. We have used a power calculation based on existing data for a similar model to determine the numbers of animals we should use in 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.	Sheep are the ideal animal model for biomechanical studies aimed at human treatment, as they offer the optimal size, both in terms of the physical size of the animal and the cartilage thickness. Their welfare will be maximised through the provision of

Explain the general measures you will take to minimise welfare costs (harms) to the animals.	anaesthesia and postoperative analgesia.

Project 2	**The behavioural and nutritional control disease**	
Key Words (max. 5 words)	Behaviour, Disease, Livestock, Nutrition	
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)	The overall aims of the proposed work are: (1) to identify how nutrition and behaviour affect the outcome of disease in livestock; and (2) to investigate how disease affects animal behaviour and use this for diagnostic purposes. Both the understanding of how nutrition affects the outcome of disease and identifying the consequences of disease on animal behaviour would lead to better methods of control of infection in livestock species. Our past work has unequivocally shown that host nutrition has the potential to affect the outcome of infection. We now want to identify which nutrients are involved in this effect and how they interact with animal genetic background. Behavioural changes during infection would be used for early identification of health and welfare problems in livestock. Our recent work has identified several behavioural changes that could be potential candidates for this purpose. Through the use of most up to date technology, we should be able to capture subtle changes in behaviour as a consequence of disease and thus detect disease early.	
What are the potential benefits likely to derive from this	Early identification of sick livestock or animals whose welfare has been compromised will improve the	

project (how science could be advanced or humans or animals could benefit from the project)?	impact of corrective action, such as treatment effectiveness, and reduce the costs of disease. In addition, subclinical health challenges, which by definition are difficult to detect and may escape early diagnosis, compromise greatly livestock welfare and performance, and may lead to serious problems at a later stage, including treatment costs. Currently we know that health and welfare challenges affect livestock behaviour; what we do not know, however, is the magnitude of these behavioural changes, whether they are challenge-specific and what their diagnostic relevance is. This is one of the main aims of this project. Nutritional control of disease is an attractive complementary control to current methods of control; it has the potential to reduce sub-clinical disease which constitutes the major health and welfare challenge to livestock. It has thus the potential to either reduce the inputs and reliance from pharmaceuticals or the incidence of subclinical disease. The use of livestock that has been intensively selected for productive traits, enables us to extend our previous work that accounts for interactions between nutrition and immunity to pathogens, to include effects of genetic selection, and thereby the effect of selection for high performance. This work will enable us to predict the consequences of breeding animals for high production traits on the interaction between nutrition and (the consequences of) infection.
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use up to 80 cattle annum; up to 250 pigs per annum and up to 600 chickens per annum. The project will last for 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 end?	Animals will be challenged with pathogens (such as worms or bacteria) or live vaccines, whilst they are fitted with monitoring sensors. In addition their nutrition may be manipulated so that they may be fed different amounts of diets of different composition. As we are interested in subclinical disease any adverse effects are expected to be mild to moderate. These may include reductions in performance, diarrhoea and mild discomfort. In all cases animals will be monitored closely if the symptoms persist animals receive appropriate

	veterinary care. No adverse effects are expected to arise from the fitting of the sensors (all the proposed sensors have been used previously). Animals will be either kept alive at the designated establishment for reuse or discharged from the controls of the Act and returned to the farm
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The experiments investigate the effects of health and welfare challenges on animal performance, behaviour and health, and the effects of nutrition on the outcome of disease. Such objectives can only be achieved by experimentation on animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will use statistical analysis (such as power analysis) based on our previous data, that will identify the optimal number of replicates required to on one hand minimise the number of animals used but on the other hand maximise expected effects to be observed. We will make use of experimental design that will utilize a number of treatments simultaneously (e.g. different feeding treatments and different challenges) to maximise the efficiency of knowledge gain through the possibility of strategically combining treatment groups In addition the fact that the consequences of the challenges on performance and behaviours will be compared to baseline ones, i.e. before the administration of challenges takes into account individual variation and increases the statistical power of the 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.	The project addresses specific questions that are relevant to specific livestock species and their respective pathogens. For example, what are the consequences of enteric pathogens on the behaviour of cattle and can this be used for diagnostic purposes? We are interested in the induction of subclinical (as opposed to clinical) disease as they are usually undiagnosed and result in a reduction in animal welfare. The aim of the project is to identify changes in the behaviour and performance of animal that have such value.

Project 3	**Evaluation of avian myxoviruses & mammalian influenza virus infections**	
Key Words (max. 5 words)	Avian myxoviruses, mammalian Influenza disease	
Expected duration of the project (yrs)	Five	
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)	The aim of this programme of work is to evaluate avian myxovirus diseases and mammalian influenza infection processes in order to improve animal health and welfare. Key objectives are to determine and evaluate the factors contributing to (i) disease pathogenesis (virus and host), (ii) virus dissemination and transmission dynamics (inter and intra species), (iii) improve disease intervention strategies (traditional and novel immunisation/vaccination) and as a result reduce the potential impact on animal and public health. Plus the identification and development of new diagnostic tools and maintain fitness for purpose of those currently in use.	
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 results of this research have a number of potential benefits contributing to an improved understanding of disease processes and the causative organisms. A key area of interest is in disease intervention via mechanisms such as vaccination, antivirals and/or prevention of transmission. This is very much an evolving process with new and emerging viruses being regularly detected and resultant implications for understanding disease development, interspecies transmission including animal to animal, host-virus interactions and their responses. All areas have application for	

	improved disease control methods and strategies for animal health and welfare status.
What species and approximate numbers of animals do you expect to use over what period of time?	• Chicken 2150 • Turkey 1000 • Duck 1000 • Pheasant 950 • Partridge 950 • Pigeon 50 • Embryonated birds' eggs 3500 (2500 ck/ 500 ty/ 500 dk) • Pigs 1150 • Cattle 20 Over the five year period of the licence 2015-19.
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 majority of the animals will experience influenza like illness relative to their species. This will range from asymptomatic to mild, moderate and severe depending on the strain of virus used and how it reacts in the chosen animal species and protocol. A proportion of animals (<20%, may be higher for novel viruses) may die as a result of the infection process in severe protocols, however, any sick animals will be humanely killed prior to being found dead wherever possible, i.e. the last inspection for the day prior to overnight within the high containment animal facility. Interventions studies such as the use of vaccines or other immunising agents will on the whole reduce clinical disease signs. The procedures under taken on animals are in themselves relatively mild and sedation / anaesthesia and pain relief is provided where appropriate to limit distress e.g. administration of substances, withdrawal of blood, swab collection. All animals that are suffering from severe disease will be humanely killed, all other animals will be euthanized at the end of each experiment. Very few animals (<5%) will be re-used (only those that have only experienced moderate or mild severity as a result of their first use and have fully recovered) and the second application is mild.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	A complete biological system is frequently required to study the course of clinical disease and the whole body response to infection. The mechanisms of virus transmission from one animal to the next and disease interventions such as vaccination cannot be studied in non-animal alternatives. The development and use of *in vitro* and *ex vivo* methods where appropriate, for

	example: continuous cell lines and organ or tissue explant cultures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduction measures include the design of animal studies to maximise collection of biological materials/data from each study. Use of a statistically valid minimum number of animals per study will be determined via resource equation and/or power analysis.
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 species chosen are those for which the disease is most relevant in the field. Avian viruses are used in the most relevant bird species, ditto swine influenza in pigs. Pilot experiments will refine protocols – dose, route and timeline of infection required to establish infection and transmission. They also provide data that allows the severity of disease to be minimised, refinement of the humane endpoint(s) including timelines to minimise overnight deaths, for future studies on each particular virus isolate. We will also use some animals (chickens and pigs) that have been genetically altered in relation to their potential ability to have increased resistance to infection by the viruses under investigation e.g. naturally occurring anti-viral factors that have been increased in the host animal, no innate adverse effects of the GM have been described. All species have their own specific and disease refined clinical observation criteria and score sheets, no animal will be allowed to progress beyond the described humane end point using a 2-3 times daily monitoring system. We have on site veterinary teams and animal welfare officers who are engaged in each study. We use early clinical signs as endpoints when the scientific objective does not require progression of disease.

<table>
<tr><td>Project 4</td><td>Studies of porcine epidemic diarrhoea virus</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>PED virus; Porcine diarrhoea</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td></td><td>No</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
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<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The aim of the project is to produce defined material for use in optimising and validating surveillance and diagnostic tools for multiple strains of porcine epidemic diarrhoea virus, while evaluating their effect following infection of European pigs.</td></tr>
<tr><td>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)?</td><td>This project will enable the proper validation of surveillance and diagnostic tools for PED, suitable for use in Britain, and will help to ensure that experience and updated knowledge of the disease is established and locally available in the event of disease incursion.

Furthermore, archives of sera, infected faeces and fixed tissues will be established, with plans to provide such material to collaborators in Europe, the USA and elsewhere.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td>Up to 60 post-weaning pigs will be used over a period of five years.</td></tr>
<tr><td>In the context of what you propose to do to the animals,</td><td>The animals that become infected with PED virus may show signs of the disease such as vomiting</td></tr>
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what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	and diarrhoea, but these signs will not be allowed to progress beyond moderate severity before the animals are humanely killed. All pigs will be subjected to regular blood sampling and oral and rectal swabbing. After recovering, a proportion of the animals will be re-infected with a second variant of the virus. It is expected that they will show milder signs of disease than the first time. All of the pigs will be humanely killed at the end of the project.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	In order to realistically and meaningfully evaluate and optimise diagnostic tests for PED, material must be available that replicates the sample types to be tested from field submissions. Such material can only be obtained through the sampling of infected animals and their environment, and cannot initially be re-created through artificial means. The timing of sample collection must extend beyond the onset of any clinical signs in order to confirm the start and end times of virus or antibody detection. Based on the data obtained from this project, it is hoped that it will be possible to generate some material for future testing through laboratory techniques rather than continued procedures in pigs.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The study design allows all of the aims and objectives to be met through a single study rather than multiple studies with elements of repetition for each aim. The experiments to be carried out are peer reviewed to allow a thorough examination of the experimental design. In this way, the experiments will be scientifically designed to use the minimum number of pigs possible to produce the data and material required. For example, control animals are shared for each experimental group. Information from the first study will be used to optimise any further studies.
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	No animal species besides pigs are known to be naturally infected with PED virus, so pigs have been chosen by default for use in this project. While a mouse-adapted virus has been generated elsewhere, it is very different to that found in pigs. Older pigs will be used in this project, as they are known to show less severe signs of the disease than very young piglets. While diagnostic samples

(harms) to the animals.	are required from various time-points after infection to replicate the range of samples expected from field submissions, the infected pigs will be closely monitored to ensure they do not experience more than moderate signs of disease. Where required, qualified veterinary advice will be followed to minimise any harm to the animals, including the provision of treatment as required, such as re-hydration. Should any animal begin to experience more than moderate clinical signs, which is not expected, they will be humanely killed to prevent further suffering. Laboratory tests will be used to confirm whether animals are suitable to be re-infected in the second phase of the study.

<table>
<tr><td>Project 5</td><td>Provision of Biological Materials</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Provision Biological Materials</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>~~Yes~~</td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>This project is to provide a service for the supply of blood and other biological materials for use as controls in diagnostic testing, quality assurance, and research projects requiring biological materials. The samples supplied from the various farm animal species will be used in evaluating scientific tests for a variety of animal diseases.

The project is demand driven and all requests for samples will be ethically approved and only supplied once a written case outlining why the samples are required and why no alternative source is possible. Whenever possible blood collection at post mortem will be used. However there are some tests and projects which require fresh blood free from any post mortem changes.

The number of animals used will be kept to a minimum by combining the needs of research groups and other users. The samples will be used to further develop and improve animal diagnostic tests related to the required species for a wide variety of farm animal diseases. Blood collection will be from superficial vessels and the techniques used will be of mild severity with no</td></tr>
</table>

	expected adverse effects. This provides control material for a large number of different tests some of which are statutory and also a number of research projects involving farm animal species.
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 main benefits in the licence is allowing peer reviewed research to be undertaken with known control material resulting in a variety of scientific publications and outputs. The tests (including Quality Assurance functions) and products that require blood or sera to underpin vital statutory functions and animal health benefits.
What species and approximate numbers of animals do you expect to use over what period of time?	Cattle 50 Sheep 50 Pigs 50 Goats 10 Horses 5 The use of these animals will be spread over the 5 year life of the licence with lot of the animals being sampled intermittently over periods of months or 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?	Mild severity, the only effect should be insertion of needle to remove the blood sample. Animals will either be killed by a schedule 1 method at the end or kept alive at the Designated Establishment if they are assessed as not suffering or not likely to suffer as a consequence of the regulated procedures applied. The criteria for this assessment will have been determined by the Named Veterinary Surgeon in advance and records will be kept in a form agreed with the Home Office Inspector. Animals will only be re-used in this protocol if: a) The actual severity of any series of regulated procedures that have previously been applied to the animal, is no more than mild; b) A veterinary surgeon with knowledge of the lifetime experience of the animal or animals has advised that their general state of health and well-being is likely to have been fully restored following the application of the previous series of regulated procedures and that the animal is free of adverse effects arising from the previous regulated procedures.
Application of the 3Rs	
1. Replacement	Blood and faeces are the basis for the majority of laboratory tests involved in animal health and

State why you need to use animals and why you cannot use non-animal alternatives	veterinary diagnosis. This licence supplies material to tests that are already established for control purposes or in the process of being developed. Wherever possible sample collected post mortem will be used to supply the need for material, however as these laboratory tests are used to diagnose disease in the live animal, there is often the requirement to use fresh material, to avoid post-mortem changes and contamination issues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used is minimised by the re-using them. This allows a small number of animals to provide control blood across the establishment and other research establishments. The experimental design is based on matching demand for blood through the request form to numbers of animals maintained to supply the blood.
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 institution and its partner organisation deal with a wide range of farm animal diseases both in a research and diagnostic capacity. Requests for samples from different groups will be combined whenever possible. The department has a group of experienced and knowledgeable staff who are able to perform the necessary techniques quickly and efficiently. Having licence holders who are proficient at the techniques required is essential for good sampling practice, which minimises any animal stress and keeps animal handling to a minimum. Analgesia/anaesthesia will be used where appropriate. The severity limits of this protocol are mild.

<table>
<tr><td>Project 6</td><td colspan="2">Improving understanding of lameness in sheep</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="2">Footrot, sheep, persistence, environment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="2">4</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>
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<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">Footrot is a disease of sheep which causes severe lameness. This is a concern for animal welfare and also has economic consequences for the sheep farming industry. Footrot is an infectious bacterial disease transmitted between animals via the environment. Disease can reappear in flocks after long periods of absence, and it is currently unknown where the bacteria persist in the environment during this time. The bacteria have also been found in the mouths and faeces of sheep, but it is unknown whether they persist in these sites longer term, thereby providing a source of infection. Dichelobacter nodosus is the bacterium that causes footrot, but a second bacterium Fusobacterium necrophorum is thought to be involved. It is not yet clear what the role of F. necrophorum is in the disease process. The aims of our research are:

• To identify sites in sheep and the environment where D. nodosus and F. necrophorum can persist
• To determine how the persistence of these organisms influences occurrence of disease
• To better understand the role of F. necrophorum in the disease process
Contagious ovine digital dermatitis is a cause of lameness with unknown aetiology, whilst it is</td></tr>
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	attributed to bacterial infection the evidence for this is weak. One alternative hypothesis is that it is caused by an unknown virus. The objective is to investigate whether there are viruses present in the scabs that form during the development of CODD.
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)?	Footrot is currently estimated to cost the UK sheep industry approximately £80 million per year, and is reported to account for over 80% of lameness in the UK. It is therefore a major concern for animal welfare. Greater understanding of the epidemiology of footrot will help farmers to better manage this disease, which will benefit animal health, welfare and productivity. This project will also provide information as to whether eradication of this disease on a larger scale in the UK is possible, or whether control is a more realistic target. The CODD project could lead to a change in management / treatment for this condition.
What species and approximate numbers of animals do you expect to use over what period of time?	The project will use sheep on commercial farms. They will be sampled regularly (approximately weekly) for periods of up to 8 months. We will enrol a maximum of 210 sheep and their lambs from each farm for the footrot trial. We will enrol a maximum of 10 sheep from a maximum of 6 flocks for the CODD viral study.
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 propose to take faecal samples from the rectum of sheep. This is a mild procedure, and usually causes very minimal discomfort. This will require restraint of animals for a short period which may cause minor/transient stress however we will minimise this by using appropriate handling facilities, and fully trained staff. We will also keep sheep in groups to minimise any stress caused by isolation. We propose to take scab samples from sheep with CODD and to swab the mouth and interdigital skin. For both studies, the sheep will remain on their farm during the study, and at the end will be returned to the care of the farmer.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	We are investigating how disease occurs in a natural population of sheep, and where bacteria / viruses can be found in the natural environment of these sheep.

use non-animal alternatives	We therefore cannot avoid using sheep.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have performed statistical calculations to determine the minimum number of animals necessary for our results to be significant. We have based these calculations on evidence from a previous study. We will not use the same sheep in both studies.
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 chosen to study the species for which our proposal is relevant. We will minimise harm to the animals by: - Using good knowledge of sheep behaviour to handle the sheep for example using good handling equipment and careful restraint: http://www.eblex.org.uk/wp/wp-content/uploads/2013/06/Manual-13-Improving-sheep-handling-for-better-returns.pdf - Using an appropriate lubricant to collect faeces from the rectum - Using swabs and scissors to carefully collect a scab sample from CODD lesions.

Project 7	**Producing antibodies for tests to diagnose animal diseases**	
Key Words (max. 5 words)	Antibodies, Test(s), Diagnosis, Animal Diseases	
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
		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 objective of this programme is to produce antibodies in small animals which can be used in tests to identify pathogens which cause diseases in many different species of animals. This is done by vaccinating them with inactivated or dead pathogen.	
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 antibodies produced are used in tests worldwide and help authorities around the world to apply the most appropriate disease control and eradication programmes to limit animal stress and suffering, improving animal welfare.	
What species and approximate numbers of animals do you expect to use over what period of time?	Rabbit - 200 Guinea pig – 600 Length of project – 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 end?	The severity limit for this work is mild and no adverse effects are expected. Occasionally a local reaction at the injection site may be seen, which may result in a small swelling and possible increase in body temperature. At the end of the study the animals will be humanely killed and their blood collected for use in the tests.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no other methods available to produce these antibodies. However, much of our research is aimed at developing techniques to reduce the future need for animals in diagnosis.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This is an established procedure and the minimum number of animals will be used in each experiment to produce the amount of antibody required for use in the tests.
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.	Rabbits and guinea pigs very rarely develop any signs of the pathogens used to vaccinate them with. The tests require antibody from two separate species of animals to work. The combination of these two species has been shown to be the most optimal. During the study the animals will be closely monitored by well trained, experienced animal staff.

Project 8	**Gene therapy for canine diabetes**	
Key Words (max. 5 words)	Dog, diabetes, gene, therapy	
Expected duration of the project (yrs)	Three	
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)	We aim to develop a treatment for diabetes that will involve giving a single, one-off intravenous injection that will stimulate the liver of diabetic dogs to make insulin, thereby negating the need for the diabetic dog to have twice daily injections for the rest of their lives	
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 treatment could remove the need for diabetic dogs to be given daily insulin injections, which would improve their quality of life immensely. We also anticipate that their length of life will increase due to a reduction in complications associated with having diabetes. If successful in dogs, there may also be a possibility of using this data to translate into early work in human medicine too.	
What species and approximate numbers of animals do you expect to use over what period of time?	6-12 naturally occurring diabetic dogs over three 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	The possible adverse effects will be the blood glucose going too low and also possibly of the dog's liver becoming inflamed. The impact on a dog's wellbeing of its blood sugar going too low could be moderate in that the dog could develop a hypoglycaemic seizure. The degree of liver inflammation that might arise is	

end?	likely to cause only mild abdominal discomfort and so should only be of mild severity. At the end of the trial, the dogs will be discharged back home to their owners or euthanised
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have reached the point where we need to assess whether this potential treatment works in a real patient, so there is not a non-animal alternative to this work. We intend to investigate this treatment in naturally occurring diabetic dogs so that no purpose-bred experimental animals will be used
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will undertake this treatment in one dog at a time and reflect on the results before considering treatment in the next dog. We therefore intend that we will be able to establish clear results quickly and with clarity
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.	Diabetes is a common problem in domestic dogs, so the primary aim is to try to develop a successful, permanent treatment for this species. We have established that this treatment is successful in diabetic mice, so we now feel confident to move the work to naturally occurring diabetic dogs, recruited at one of the leading veterinary specialist centres in the country. We are also of the opinion that this is the optimal model to investigate at this stage. No . noxious or painful events will occur and the treatment simply involves giving a single intravenous injection, so we think that this treatment actually has a lower welfare cost that the current standard of care for diabetic dogs, which involved twice daily injections of insulin at home

Project 9	**Enteric pathogens of poultry**	
Key Words (max. 5 words)	Gut health; broilers	
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)	The main objectives are: - Investigate how the chickens interact with their pathogens under different environmental conditions. - Develop new ways to control disease in chickens of different genotypes - Find new ways to diagnose enteric disease in chickens early so that they do not suffer from it.	
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 potential benefits of the programme of work include: - Develop novel control methods for intestinal disease in chickens, such as feed supplements - Reduce the use of antibiotics in chicken production without penalties in chicken health and welfare- increase chicken production to help with food security in the coming years. - Improve the health of humans by reducing antibiotic use In animals - Improve the health of humans by controlling better chicken disease that could be transmitted to humans	

What species and approximate numbers of animals do you expect to use over what period of time?	We will be using broiler chickens throughout the programme of work. Maximum of 1500 chickens will be used 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 end?	The severity of procedure is moderate. Action points are in place to minimise severity as much as possible. In most cases, chickens will be reared under standard commercial practices and will benefit from increased monitoring of their health and performance. At the end of the experiments, animals will be euthanized for further sample analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	This program of work aims to study the interactions between the chicken and its environment, including the pathogens and the diet to achieve health and welfare. Some non-animal alternatives will be used prior to studies with the animals, for example tissue culture to investigate the pathogen itself. But ultimately how the chicken responds to the pathogen and the novel therapy will depend on host's physiology, including its immunology, endocrinology, digestive physiology and neurology. The involvement of such a large range of host bodily functions in their response to disease reduces the possibilities to use non-animal experimentation, and thus would justify the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Each experiment will be assessed for its suitability and necessity by SRUC's AWERB. Furthermore, statistical advice will be sought for each experiment to ensure the minimum number of animals are used required to provide meaningful data that will help in the control of disease in chickens.
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 want to enhance the health of chickens by providing protection against specific diseases and maintaining gut health, therefore poultry are proposed for use. Our team has extensive experience with this type of studies and have over the years refined experimental protocols to ensure minimum suffering of the animals. For some procedures the animals are anaesthetised and thus do not suffer once anaesthesia is established. Prior to that, we have refined induction protocols to reduce handling and as a consequence anxiety of the birds (e.g. induction by being placed in an induction chamber where they will be allowed to inhale the anaesthetic agent prior to intubation)F. Most of the other procedures are

	standard for commercial practices and animals benefit from increased monitoring of their health status.

Project 10	**Assessing new livestock viruses in UK breeds**	
Key Words (max. 5 words)	New Virus Livestock UK	
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)	The objective of this project is to assess new viruses which may infect and cause disease in UK livestock breeds. This will help the UK government establish transmission mechanisms and develop control and preventative measures, diagnostic tools and isolate the causative agent.	
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 work is very beneficial to UK livestock and may help prevent thousands of animals getting infected with obvious welfare benefits. It may also help prevent huge financial losses to the UK agricultural sector.	
What species and approximate numbers of animals do you expect to use over what period of time?	The work needs to assess new viruses in susceptible animals therefore it may need to use, cattle, sheep, goats, pigs or horses. Only small numbers of animals are required to complete these initial studies and the likelihood of this licence being used is very low	
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 unknown as this work is to assess new viruses and we cannot predict the clinical effects in susceptible animals. The animals will be monitored very closely during any studies and the home office kept fully informed.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is a need to see the effects of new viruses in susceptible animals to help make decisions on control measures. As new viruses no small animal models will have been established.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals will be used by using experienced statisticians to design studies.
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.	Susceptible livestock species need to be used to assess the effects of a new virus in UK breeds. To ensure that welfare costs to the animals are kept to a minimum the animals will be closely monitored to prevent unnecessary suffering.

Project 11	**The faecal microbiome of obese and geriatric ponies**	
Key Words (max. 5 words)	Equine obesity, gastrointestinal microbiome, weight loss, equine nutrition	
Expected duration of the project (yrs)	3 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)	Improvements in equine nutrition and husbandry have shifted demographic in the UK equine population has increased demand for improved nutritional management of senior (>20 years) and obese (body condition score >7/9) animals. Horses depend on the bacteria which populate their guts to convert forage-foods to substrates they can use as energy sources. This 'microbiome' is altered in obese and old horses in a way that leaves them susceptible to metabolic disease when they are challenged with 'energy-rich' foodstuffs. This study seeks to understand how the microbiome is altered in old and fat animals, how these differences affect the microbial responses to diet change and whether there is any diet intervention we can use to limit this change. We also know that the microbiome differs in fat and thin animals. We will gather data to describe how the microbiome changes as obese animals undergo controlled weight loss and whether this is different in animals that are sensitive or resistant to diet-induced weight loss	
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)?	i) Improved nutritional management of elderly and obese animals will follow from a greater understanding of how age and body fatness alter the gut microbes and digestive function when animals are forage fed and how this can be perturbed by 'energy-rich' feeds. ii) We hope to show that specific dietary interventions	

	could ‘protect’ the microbes in the face of ‘inevitable’ dietary challenges and possibly serve to reduce the incidence of some metabolic diseases iii) A greater understanding of Improved management advice/owner compliance for the weight loss management of obese animals should the gut microbes and/or c marker substances in blood samples prove to be easily measured markers to help identify weight loss resistant animals. iv) The results of this study will feed into the ‘one-medicine’ ethos as these questions are important to other species including man, where controlled study and dietary compliance are less readily assured.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use up to 36 ponies over the course of the study. In each year. Each pony will be studied for up to 30 weeks. Ponies will be individually stabled and will be allowed to exercise freely at pasture daily.
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?	Most of the time, the ponies will be managed under standard husbandry conditions. To collect data to describe the animals ability to handle carbohydrates, calculate their body fat contents and measure the concentrations of various blood factors, we will need to place catheters into their jugular veins. Very occasionally, even though this procedure is conducted by experienced vets, some animals may have catheter site reactions/infections. These will be treated immediately and are generally of a mild nature and highly responsive to treatment. None of the procedures are likely to cause lasting harm and it is our intention that all ponies will be re-homed into domestic settings on completion of the study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The work is being performed to improve the welfare of ponies. Currently there are no methods available that would allow us to measure these changes in laboratory models. Also, because the body systems and chemistry of horses are unique, even among herbivores, we cannot usefully substitute another animal species to gain this understanding.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have extensive experience of this type of work in horses. We have used information gathered from earlier studies to work out the smallest number of animals needed to make sure that we generate useful data.
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 study specifically addresses questions in nutritional physiology which are unique to the horse and of importance to equine welfare. Nutritionally-related metabolic disease is a particular

objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	concern among *Equidae*. The equine industry in Industrial Nations has seen a rapid increase in the numbers of 'high risk' obese and aged equines. Novel approaches to reduce disease risk are urgently required. The unique digestive physiology of *Equidae* prohibits species substitution. Animals will be obtained from domestic sources and well-habituated to the routine management protocols to be used in this study. All intended procedures are classified 'mild'.

Project 12	**Parental effects on offspring health and fitness**	
Key Words (max. 5 words)	Maternal, parasites, disease, immunity	
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
		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)	We are investigating the impact of an individuals early environment on their health and ability to deal with disease in later life. In particular we are examining how different conditions a mother experiences, impacts on the health of different family members. Recent evidence has shown that different breeding conditions can result in female birds altering levels of hormonal, nutritional and immunological components passed into the embryonic environment. We also know the transfer of essential components required during embryo development to the egg is key to successful offspring development. Yet we have very little information on the effects that these changes have on the long term success of offspring or the extent to which different mothers are affected. Potentially, information about how these parental effects operate could be used to predict changes in animal health and productivity under different environmental conditions or employed directly to influence health and productivity in both captive, commercial and wild bird populations. However, to be able to utilise this approach we need empirical information on how much allocation of different constituents to the egg and resources to the young vary in response to quantitative changes in external factors, how females vary in their ability to allocate	

	these resources, the actual effects that changes in these different constituents have on offspring success, or for how long reported effects may persist. This study aims to measure these effects to identify the extent to which animal health and productivity can be affected.
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)?	Potentially, information about how these parental effects operate could be used to predict changes in animal health and productivity under different environmental conditions or employed directly to influence health and productivity in both captive, commercial and wild bird populations.
What species and approximate numbers of animals do you expect to use over what period of time?	We work with gamebirds and seabirds and will monitor several hundered birds and their offspring throughout the 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?	Our work mainly involves taking blood samples and examining our birds to monitor their disease status and vaccinating or challenging birds to monitor their immune responses to diseases in the population. The level of severity for all our procedures is classified as mild. Our birds are released back into the wild or in the case of captive birds released from the act.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The effects we are interested in very much depend on the biological context so studying these effects in living individuals in a natural setting is key to understanding how they may impact on populations of animals in the wild. Specific responses we are interested in can only be measured directly *in vivo* as neither the environment or the physiological and immune responses that operate *in vivo* can yet be captured *in vitro*. We are interested in the long term impact of early life effects on individuals exposed to 'normal life' so long term monitoring of individuals under natural conditions after our procedures to measure resource allocation and disease status is an important component of the study. As yet, there is no empirical data on which to base theoretical models on how maternal allocation changes might affect animal success. We have considered how we can use theoretical models to simulate the mechanisms we are currently investigating. The work of our last licence has allowed us to reach this stage for adults but now

	require similar data to do the same for juveniles in the population. The overall aim of this work is to collect this required data to model the impact of these effects on a larger population scale.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Our experimental protocols are based on the minimum number of animals per treatment group we estimate will generate reliable results based on previous findings from our own work and that of colleagues. We use statistical techniques that allow us to maximise the information we can extract from any experiment. Several of our study populations have been monitored for over twenty five years so there is considerable background biological data that can be utilised in the analysis to explain background variation in other traits to increase the power of analyses allowing minimum numbers to be utilised.
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.	While some specific questions about the consequences of maternal allocation on offspring success can only be answered blood sampling or examining animals directly, we are trying address more general questions, such as how variation in maternal responses are generated, in less invasive ways. These include using avian model systems where maternal responses can be monitored non-invasively and using dead vaccines to imitate pathogen challenge without the associated cost of infection for the host. Seabirds are an ideal system for moving this work to the field as they are extremely tolerant to human disturbance during the time the procedures will be conducted. The main population we are working with has been monitored for over twenty years so there is considerable background biological data that can be utilised in the analysis to increase the power of analyses allowing greater explanatory power in our models and they are the group in which our findings can be directly applied to model the effects of our variables of interest on breeding success at the population level. The listed procedures have already been utilised successfully in these species without any detectable adverse effects.

Project 13	**Is Schmallenberg Virus Still Circulating in the South of England?**	
Key Words (max. 5 words)	Sheep, schmallenberg , transmission	
Expected duration of the project (yrs)	3 years (maximum)	
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)	We would like to find out if Schmallenberg virus is still circulating on sheep farms in the UK. We are going to look at a large number of sheep farms in the South of England as we know that the number of sheep infected was higher here than in the North of the country. These counties also have an increased risk of continued disease exposure due to the continued spread of infected midges from Europe. The data from this study will help epidemiologists to develop models of disease spread which can be used in this country and abroad to develop improved disease control strategies. It will also inform us of our risk from future Schmallenberg outbreaks.	
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 information from this study will tell the farmer if his flock has the disease and whether to expect problems at lambing time. Also, the data from these studies will be used by epidemiologists to develop models of disease spread which can be used in this country and abroad to develop disease control strategies.	
What species and approximate numbers of animals do you expect to use over what period of time?	We wish to sample 12 sheep from 131 farms across Southern England for antibodies to Schmallenberg virus. We will visit each farm once and take a single blood sample from 12 randomly selected sheep on the farm. A total 1572 sheep will be tested. We plan that the study will take place over 3 months	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	We have used the minimum number of sheep possible, the procedure of blood sampling is mild, quick and with minimal adverse effects it will be carried out by licensed and trained staff only, the	

level of severity? What will happen to the animals at the end?	sheep will remain on the farm with their flocks
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Cattle, sheep, goats and deer and camelids are the only animals affected by the disease. In order to study whether a sheep farm has Schmallenberg virus the only test available is a blood test from the sheep on the farm.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have used statistics to calculate the minimum number of sheep we can sample to answer our research question of whether a farm is infected or not.
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.	Cattle, sheep, goats and deer, as well as camelids, are the only animals affected by the disease. We have chosen to study sheep as they are common, they live outside most of the year and are most likely to be exposed to the virus. In order to study whether a sheep farm has Schmallenberg virus the only test available is a blood test from the sheep on the farm. The procedure is considered to cause only mild transient discomfort to the sheep. To minimise any discomfort all staff will be trained and experienced in handling and blood sampling sheep.

Project 14	**Genotype, health and fertility in dairy cows**	
Key Words (max. 5 words)	Phenotype, SNP, immunology, disease, milk	
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)	UK dairy farmers are currently under considerable pressure to produce more milk at a lower cost in order to stay in business. However, this is only acceptable if it does not compromise the welfare of the animals in their care. At present cows only survive about 3 lactations on average, each lasting about 10 months. Cows could survive for much longer if they remain both healthy and fertile, as a pregnancy is essential to stimulate the onset of milk production. They are particularly vulnerable to diseases in early lactation as their natural immunity is reduced at this time. The overall aim of the project is to increase our understanding of the relationships between the genetic make-up of the individual cow (her genotype) and the way in which she is managed on the farm (diet, type of housing etc) on her health and fertility. There are three different elements proposed. The first involves a large multinational study organised with EU funding to identify how differences in genetics affect susceptibility to disease. This will assist us to develop better breeding programmes. This project will also identify compounds in milk which aid early detection of disease so that cows can be treated appropriately before the condition becomes serious. The links between genetics and disease	

	resistance will be clarified by testing the ability of immune cells taken from cow's blood to respond to different bacteria. We will also study the effect of liver fluke on the cow's immune system. This is an increasingly common parasitic disease of cows. We think that when cows become infected from grazing fluke-infested pasture then they become more likely to develop other diseases too. Understanding this interaction better will aid farmers in deciding how it is best to treat cows against fluke infection.
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)?	Our work will help us to understand how the genetic make-up of individual cows affects their health and fertility. This will help breeding companies to select bulls for use in breeding programmes which produce healthier cows and to assist farmers to target the breeding of cows within a herd for characteristics which are most suitable for the particular type of farm management system employed. We will also develop the use of novel tests based on compounds present in milk which assist the early diagnosis of disease so that cows can be identified and treated promptly. Another aspect is to determine whether treatment of cows against liver fluke, a very common parasite, also improves other aspects of the cow's health. Overall we wish to work closely with vets and farmers to optimise breeding and management strategies that will benefit animal health, consequently improving cattle welfare.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will involve dairy cows using an epidemiology approach. This means that quite large numbers are required to show significant effects and we anticipate recruiting about 2,000 animals during the 5 years of the project.
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?	All of the work will use cows kept according to normal husbandry procedures on dairy farms. Occasional blood samples will be taken for analysis, the animals may be genotyped using a small piece of ear tissue and the reproductive tracts may be examined after calving. All of these are standard procedures and the severity throughout will be mild. The cows will remain on the farm after use.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot	The entire proposal relates to understanding the influence of genetics on health and disease in dairy cows. This can only be achieved using live animals. We will work with cows on farms which are already

use non-animal alternatives	being kept for milk production.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Epidemiologists will be consulted to determine the number of animals and the number of farms which we need to recruit on each individual experiment in order to maximise the likelihood of finding a significant effect with the minimum of animals. Our sampling strategy will be based on previous experience to target the key time points. Individual blood samples will be split so that each can be used for a variety of tests.
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 project is only relevant to cattle. Animals will be exposed to normal husbandry procedures as used on UK farms. Only mild protocols will be used. We also wish to validate potential non-invasive methods of monitoring health, using compounds in milk, which will benefit animals in future.

<table>
<tr><td>Project 15</td><td>Immunobiology of fertility in mares</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Reproductive biology, pregnancy, placenta, immunosuppression</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The overarching goal of our research program is to improve fertility and reproductive health in mares through a better understanding of reproductive events such as conception and early pregnancy. Ultimately we aim to reduce the occurrence of early pregnancy failure and thus improve reproductive efficiency. To address this issue, we focus on understanding the cellular and molecular mechanisms that regulate three key processes; i) early conceptus development and function, in particular development of the specialised cells of the placenta, ii) modulation of the immune response in early pregnancy, and iii) regulation of hormone production and behavioural changes by the uterus.</td></tr>
<tr><td>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)?</td><td>The research performed under this licence will use horses to explore aspects of early equine placental development at a molecular level, immunity during equine pregnancy, the endometrial environment in early pregnancy and methods of improving behavioural health due to dysfunction of the reproductive tract. Chorionic gonadotropin hormones are produced by both horses and humans during pregnancy, but not by most other species used experimentally. This means that results gained from studies in the horse may also be valuable beyond equine health to provide new knowledge relevant and applicable to human reproduction. Thus potential beneficiaries of this research extend across both veterinary and human</td></tr>
</table>

	spheres, with social impact from advances in both healthcare and animal welfare. One of the aims of this project is to understand the mechanisms that regulate the differentiation of cells that produce eCG and ultimately eCG production. Thus a shorter term impact arising from the work will be the enabling of the *in vitro* production of equine Chorionic Gonadotropin, as a commercial product to induce superovulation in farmed and domestic animals. Currently commercial sources of eCG are produced by purifying the hormone from the serum of farmed pregnant mares, a procedure that can be associated with stress at the time of blood collection and terminating the pregnancy. An in vitro method of production of the hormone would represent initially a form of reduction and ultimately replacement. In the longer term, a greater understanding of placental development, immunity in pregnancy and plant oil regulated endometrial function will contribute to management of early pregnancy losses. At present 10-20% of early pregnancies are lost. Currently, only approximately 20% of such losses are accounted for by the identification of pathologies (eg infection or developmental abnormalities). This research project may provide insights into disrupted mechanisms accounting for currently unidentified losses and either lead to a means of prevention, or at least provide explanations for why the body may terminate a pregnancy that is not viable. This would lead to an improved ability to monitor embryo implantation, and will enable equine veterinarians to provide improved health management, regardless of the outcome. The development of a new more ethical treatment for reproductive behavioural disorders of mares has real advantages for the mares being treated and also reduces the risk to human health over other currently available therapies.
What species and approximate numbers of animals do you expect to use over what period of time?	Horses over the age of 3 years will be used for these studies. We expect to use a total of 15-20 horses over a 5 year period.
In the context of what you	Adverse effects are very unlikely and not expected

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?	for the procedures proposed. The reproductive procedures proposed are used in routine clinical reproductive medicine in practice and adverse effects are likewise uncommon. There may be mild and transient inflammation experienced at suture sites and this will be treated by removing the sutures. At the end of the study, horses are usually rehomed to owners with extensive experience handling horses under the approval of the named veterinary surgeon.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There are currently no in vitro models to study the placenta and immune response in horse pregnancy. Attempts have been made to develop a cell line to study trophoblast cells of the placenta but these studies have failed and the tissues of interest are complex and can't be made in the laboratory. Studies of behavioural responses in mares during different stages of oestrus involved multiple body systems and as a result there are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Preliminary experiments previously performed have allowed us to establish the predicted variation in our experiments and using this we have been able to predict the number of animals we require to measure certain responses. A resident statistician at the Establishment has been consulted and will be continue to be consulted throughout the project to ensure the number of animals used it appropriate. Excess tissue generated from one experiment is archived and can then be made available for other experiments without the use of further 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.	Horses are used as it is horse fertility that we are studying. The placentation of the horse is very different to other species (for example one of the cell types in the placenta we study are unique to the horse) and as such no other species can be used for these studies. The more complex procedures are performed by trained veterinary surgeons that in addition to holding a personal licence, have extensive experience performing the procedures in veterinary practice. The animals are regularly and closely monitored by a team of veterinary surgeons and animal technicians and kept in herds in paddocks with regular human intervention which provides a more enriched environment for the

	animals. All animals are carefully and slowly introduced to new equipment and procedures with the support of a reward system such as feed.

Project 16	**Feed conversion efficiency in cattle and sheep**	
Key Words (max. 5 words)	Feed efficiency; cattle; sheep; methane; animal behaviour	
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 project will develop knowledge and tools that will be used to breed cattle and sheep that are more efficient at converting feed into beef and lamb. It will underpin a UK-wide programme of work to breed for more efficient (and so more cost-effective) production of beef and lamb. The second objective is to develop ways to monitor feed efficiency and its components on commercial farms in real time, so that farmers have new information for managing their herds and flocks.	
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)?	Feed is the largest single cost in beef and lamb production systems, so reduced feed consumption (for the same amount of weight gain) will lead to increased farm profitability and reduced food costs. A reduction of £100 in feed costs for a finishing beef animal is realistic and this would represent up to a £10 million annual saving if there were 5% uptake across the approximately 2 million prime cattle slaughtered in the UK each year. The project will also contribute to targets for reduction in UK emissions of greenhouse gases, both through direct effects on methane emissions as well as reduced feed use resulting from more efficient conversion of feed into products. A key objective of this project is the development of minimally intrusive alternative methods to estimate FCE or methane emissions; these will contribute to long-term reductions in animal use.	
What species and	We expect to use 800 cattle and 200 sheep over the	

approximate numbers of animals do you expect to use over what period of time?	5 year project. These will represent a range of breeds and studies will be conducted at different physiological stages from soon after weaning up to finishing.
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 are concerned to ensure that animals perform well, according to their genetic potential, and all procedures in this project are mild. Dietary treatments are only likely to cause mild, transient digestive upsets. Animals will be carefully monitored during studies involving transport stress, to ensure that the intended low levels of stress are not exceeded. Sampling procedures (blood, faeces), methane measurements in respiration chambers and administration of rumen boluses are minimally intrusive, whilst sampling of rumen fluid through naso-gastric tubes is non-surgical and causes only transient discomfort. Animals will return to our herds and flocks, subject to veterinary certification and them not having received treatments that exclude them from the human food chain.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not possible to evaluate between-animal variation in feed conversion efficiency, nor to test the use of new proxy/sensors tools for the monitoring and management of livestock without working with the relevant farm animal species under normal management conditions.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Protocols for animal studies are scrutinised by our Animal Experiment Committee and this includes evaluation by a statistician.
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.	Methods for recording FCE and methane emissions follow international guidelines (International Committee on Animal Recording; International Panel on Climate Change). All sampling procedures are mild and designed to be minimally intrusive because we need to avoid experimental procedures compromising the interpretation of results. The duration and conditions of the transport stress procedures have been designed to impose only mild stress on the animals. Regular automated monitoring of feed intakes alongside excellent stockmanship means that any problems would be identified quickly.

Project 17	**Development of a novel vaccine vector in cattle**	
Key Words (max. 5 words)	Cattle, vaccine delivery system	
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)	Bovine infectious disease has huge health, welfare and economic consequences globally and, in some cases, potential for disease transmission to humans. Major agents of significant impact span viral, bacterial and parasite groups and, in many cases, control is restricted by a lack of effective vaccines. Where drugs exist, control is affected by resistance. For pathogens for which there exist vaccines, these are often expensive to produce and subject to variability and a short shelf-live. A number of cattle vaccines cause problems in diagnostic screening programmes because of cross-reactivity. In other cases, vaccination has proved a problem where effective pathogen-derived ('native') antigens have been identified, yet synthetic versions fail to stimulate immunity. All of these issues indicated that improved strategies for vaccine delivery in cattle are required. Against this background, this project aims to further develop a novel vaccine delivery vehicle for cattle to stimulate immune responses against a wide spectrum of infectious agents. The organisms we are using as the basis of the vehicle do not cause clinical signs of disease and were isolated from natural bovine blood in culture. These organisms have been adapted to make proteins from other disease causing agents. Having successfully optimised growth and	

	modification of the vaccine vehicle in the laboratory, we established that vaccination with the vehicle in cattle can generate specific immune responses to the delivered protein. Now, we aim to test a refined version of the vehicle in validation experiments required to evaluate the practical application of the vehicle for commercialization.
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 project will help us evaluate a vaccine vehicle of wide ranging potential benefit to bovine welfare. The experiments planned are designed to address very specific questions, which will allow further assessment of the viability of the proposed system for developing a novel, and much needed, method of cattle vaccination. The work will provide the essential information required to evaluate and optimise this technology, which has considerable commercial potential and industrial interest.
What species and approximate numbers of animals do you expect to use over what period of time?	The vehicle under investigation shows complete specificity for cattle hosts. We need to determine that the vehicle establishes in cattle, that sustained antigen delivery occurs and if this elicits immune responses to delivered antigen. These experiments will therefore be carried out cattle, up 36 animals 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 end?	The vaccine vehicle is non-pathogenic in its unmodified and modified state and we anticipate no adverse effects from its inoculation and growth in cattle. Nonetheless, all animals will be closely monitored for adverse effects. The amplitude and effects of any resultant proliferation by the vehicle will be closely monitored until the end of the study. Animals will be sampled by jugular venepuncture and the effect of this will be mild discomfort at sampling. The level of severity is mild and the animals will be euthanized at the end of the experiment because of the genetically modified nature of the vehicle.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We must use animals because we are testing the effect of a vaccine vehicle to generate antigen specific immune responses in the definitive host. We undertake many in vitro studies that inform and help optirnise the in vivo work, but because of the nature of the specific scientific question (‘Will the vehicle work in cattle?’), we cannot do these studies in an alternative model.

2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers to be used are based on successful published studies by our group and on power calculations performed by a qualified statistician to ensure that the experiments are designed to use the minimum number of subjects consistent with statistical robustness and to facilitate rigorous analysis of data.
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 need to use cattle because we are testing the effect of a vaccine vehicle to generate antigen-specific immune responses in the definitive host. We use calves as we need to them to be unexposed to the pathogens against which we are attempting to generate immune responses. It also optimises comparison with our earlier studies. We will minimize suffering by basing our vaccine dose on our previous study in which we did not observe clinical signs after immunisation using the vaccine vehicle. The animals will be handled by technicians, stockmen and veterinary staff with extensive expertise in handling such species and the sampling procedures have been specifically designed to ensure maximal opportunity to detect the vehicle with minimal experimental intervention. Our protocol is categorised as mild.

Project 18	**Detection and control of fluke in ruminants**	
Key Words (max. 5 words)	Fluke, diagnosis, vaccination, treatment, challenge	
Expected duration of the project (yrs)		
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
		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 aim of the proposed project is to establish an experimental challenge model of liver fluke infection in its natural sheep host. The model will be used in 3 ways, i.e. (i) to propagate the life-cycle of a confirmed triclabendazole-resistant liver fluke isolate of UK origin and other isolates of interest; (ii) to evaluate existing and novel diagnostic tests for detecting fluke infection and determining treatment efficacy, and (iii) to determine the protective effect of candidate liver fluke vaccine antigens. We would also intend to establish a similar experimental challenge model for rumen fluke, which would be the first of its kind in the UK, primarily for diagnostic test development, but also to facilitate studies into the pathological and production effects of rumen fluke.	
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)?	Improved understanding and control of liver fluke and rumen fluke will help reduce the parasitic burden on our livestock and improve their health and welfare status. Liver fluke disease also has severe negative impacts on food production. Control of the disease will improve the biological efficiency of livestock production. It will help to maintain food security whilst reducing waste and mitigating the environmental impact of disease.	
What species and	Sheep and cattle, approximately 30 per year for ~5	

approximate numbers of animals do you expect to use over what period of time?	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?	There may be transient discomfort due to animal handling during challenge and sampling but all handling and sampling will be conducted by trained individuals to minimize this risk. The infection is unlikely to results in overt disease because we will use a low challenge dose. Animals will be monitored at least twice a day to detect any discomfort and/or clinical signs that may occur. Any animals judged to have more than moderate signs of infection will be treated appropriately or euthanized by appropriate approved method as advised by the Institute's named veterinarian.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Non-animal alternatives are not a realistic option because it is not currently possible to complete the life-cycle of any trematode (fluke) parasite outside of a permissive definitive host.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The present application will involve using the minimum number of animals possible because we are dealing with controlled infection of the natural host with well-characterised parasite isolates under experimental conditions, rather than with natural infection of large numbers of animals in the field.
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.	All applications are subject to scrutiny and approval by our local Animal Experiments Committee, including detailed statistical consultation to identify the minimum number of animals required to produce statistically robust results. Experimental animals will also be monitored closely for clinical signs of disease throughout any procedures and will have the best of veterinary care. By using a well-characterized challenge model, we can minimize the risk of adverse effects in the animals.

Project 19	**Rabbit Vaccine Development**	
Key Words (max. 5 words)	Rabbits, myxomatosis, haemorrhagic disease, vaccine	
Expected duration of the project (yrs)	5	
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)	The aim of the work carried out under this project licence is to develop and support vaccines against the major diseases of the rabbit namely myxomatosis and rabbit haemorrhagic disease.	
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)?	Vaccination is probably the single most effective measure taken to reduce disease and suffering in animals and man. By developing new and improved vaccines we aim to improve animal welfare and reduce the suffering caused by disease. Furthermore by maintaining a surveillance of the incidence of disease outbreaks and examining the likely causes we hope to be able to identify new viruses and develop the necessary vaccines more quickly.	
What species and approximate numbers of animals do you expect to use over what period of time?	All the work will be carried out in rabbits. We expect to use ~400 rabbits 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	We expect few if any adverse reactions in the majority of rabbits since the procedures that are being carried out are routine vaccinations and the taking of small volumes of blood (~1-2ml), which are minimally invasive. Efficacy studies however will require that some unvaccinated control animals are	

end?	exposed to the pathogenic agents of myxomatosis and haemorrhagic disease. These rabbits will be euthanased when it is clear that the disease causing nature of the challenge virus has been established. Consequently the level of severity for this protocol will be severe.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To demonstrate that a vaccine preparation is both safe and efficacious it has to be administered to the animal for which it is intended. This is especially true of live viral vaccines such as myxoma virus, as the viruses show a high degree of host specificity. This means that they can only work in the animal which the virus normally infects. Although we can show that the viral vaccine will grow in cell culture (this is after all how we will produce the vaccine), behaviour of the virus in vitro does not indicate whether the vaccine will be too attenuated (weak) or too pathogenic (aggressive).
2. Reduction Explain how you will assure the use of minimum numbers of animals	Registration of veterinary vaccines is carried out in accordance with European Pharmacopoeia monographs; these documents state the minimum number of animals that must be used. Our challenge models are very robust which allows us to use just the minimum number of animals to obtain statistical 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 objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	As stated above, all our animal work will be carried out in rabbits as they are the target species for the vaccine and the only host for myxoma virus. Demonstration of vaccine efficacy requires that both vaccinated and non-vaccinated control animals are exposed to the disease causing myxomatosis and haemorrhagic disease viruses. We will euthanase any sick animals as soon as it is clear that they have contracted either myxomatosis or haemorrhagic disease.

Project 20	**Unravelling the Aetiology of Contagious Ovine Digital Dermatitis**	
Key Words (max. 5 words)	Sheep, lameness, contagious ovine digital dermatitis	
Expected duration of the project (yrs)		
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)	This study aims to identify the microbiological agents, vaccine targets and the routes of transmission of a cause of severe sheep lameness known as contagious ovine digital dermatitis.	
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)?	Contagious ovine digital dermatitis (CODD) is a very severe cause of lameness in sheep in the UK which affects hundreds of thousands of animals every year. Currently the disease is poorly controlled by injecting affected sheep with antibiotics. It is a relatively new disease, and although several bacteria have been found in CODD foot lesions we do not know which bacteria are the most important causes and how the bacteria cause disease. We also don't know how the disease spreads between sheep. This study aims to answer these questions so that we 1) can inform future vaccine development 2) advise farmers how to stop the disease spreading and 3) reduce antibiotic use in food producing animals.	
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 100 sheep in a study which will last a maximum of 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	In this study we will mix sheep with and without CODD and monitor the disease spread between sheep. This is a natural occurring process which happened on farms normally. However, we will do this in a regulated and closely monitored environment. We will collect blood samples and	

end?	microbiological swabs, closely observe the animals and collect samples from their environment. If a sheep acquires CODD it will become lame. However, its welfare will be closely monitored by a vet and it will be treated with antibiotics. At the end of study all the sheep will be humanely put to sleep by a vet. This study is classed as moderate severity, a maximum of 40 animals will be used in this part of the study In order to obtain bacterial samples from sheep from different environments and from different parts of the foot we aim to biopsy a maximum of 60 sheep with CODD on their own farms. Under local anaesthetic, these animals will have a small piece of CODD infected material removed from their foot. To prevent any pain and infection they will be given long acting antibiotics and pain killers. After the biopsy sample has been taken they will return to their flock on their farms.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We have to use sheep as our model of disease they are the only animals affected by CODD and we wish to understand which bacteria are causing the disease we observe, how the animal's immune system responds and how the disease spreads. We can only do this by studying the disease in sheep.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have taken advice from a statistician regarding the number of sheep we have used and have kept them to the smallest number we can.
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 animals will have their welfare closely monitored at all times and we will stop the study when most of the sheep develop the disease. We will keep the sheep in the highest welfare standard accommodation and if any animal becomes unwell it will be removed from the study and be examined by a veterinary surgeon.

Project 21	**PPRV pathogenesis and immune response to PPR vaccines**	
Key Words (max. 5 words)	sheep, goats, vaccines, disease, protection	
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)	The prime aim of the project is to improve vaccines for use in controlling “Peste des petits ruminants” (PPR), which is a severe disease of sheep and goats that has a major economic impact on poor livestock keepers in the developing world. We do not know how the current vaccines work, and the major aim of this project is to measure different parts of the immune response to PPR vaccines and determine which is the part that is important in protecting animals from disease. The second part of the project is to characterise new vaccines against PPR, vaccines which will allow us to distinguish vaccinated animals from those that have been infected with PPR, something we can’t do at the moment. In addition, some experiments will track the movement of the virus through the body of infected animals at the start of infection, to find the target organs most affected by the virus.	
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)?	If we do not know the part of the immune system that provides protection against a disease, the only way of testing new vaccines, or even quality controlling vaccines, is to expose vaccinated animals to disease. It is better for the animals if we could simply measure a specific immune response, rather than risk making them ill. It is also better for disease control, and	

	therefore for both the livestock and their owners, if vaccinated and infected animals can be differentiated, as this allows vaccination to be carried on at the same time as disease monitoring. The work described here will contribute to the control, and eventual eradication, of PPR, to the benefit of the people for whom it is currently a significant threat to livelihoods.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use goats, and approximately 200 animals, over the next 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?	Most of the animals we test will be vaccinated, and will not develop disease. A few animals in each study (approximately 1/6) will be unvaccinated and used as controls to establish that the infection with PPR has been applied correctly. These animals will be allowed to develop only the initial stages of PPR, roughly equivalent to a very bad cold, after which they will be euthanized. Animals which remain healthy will be culled at the end of the study, as they cannot be released back to the herd for biosecurity reasons.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Unfortunately, any study of the immune response of an animal to a vaccine requires the use of the whole animal, as there is no ex vivo model of the whole, integrated immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have calculated the minimum number of animals that will give us a statistically relevant result, given the variation that is found naturally between outbred animals, such as livestock.
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 is a natural disease of small ruminants, so it is most appropriate to use the normal target species, which is what we are doing. The animals are housed in contained rooms with good ventilation and temperature control, a carefully balanced diet and environmental enrichment as far as possible. Livestock animals which are social are never kept in isolation. Animals which become sick are monitored frequently, and are euthanized before any severe disease can develop.

Project 22	**Translation of Bovine Tuberculosis Biomarkers to the Point of Care Setting**	
Key Words (max. 5 words)	Bovine tuberculosis; diagnostic test	
Expected duration of the project (yrs)	2 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)	This project is part of a larger programme of work. The objective of this strand of the project is to provide samples from non-Holstein-Friesian cattle to be included in an analysis of potential biomarkers (measurable indicators of a biological condition) of bovine tuberculosis (BTB) infection in cattle. The overall aim of the main programme of work is to develop a new test for BTB The objectives of the programme of work are: - To infect experimental Holstein-Friesians with *Mycobacterium bovis* (the causative agent) and collect blood samples to enable determination of biomarker levels - To collect blood samples from infected and non-infected cattle of other breeds to ensure the validity of the biomarkers in non-Holstein-Friesians. - To draw up a list of biomarkers to detect bovine tuberculosis - To develop molecules which can bind to and detect the chosen biomarkers (aptamers). - To use these aptamers within a handheld device which can give an immediate	

	diagnosis. The device will be connected to the internet for accurate data recording. The particular element of the project for which permission is being sought in this application relates to the collection of blood from non-infected, non-Holstein-Friesian control animals.
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 benefits of this project will be to provide samples which will help to validate the usefulness of the potential biomarkers by demonstrating whether levels in uninfected cattle are similar between different breeds. If levels were found to vary greatly between breeds, a test using these biomarkers would need to be validated separately for each breed. Bovine tuberculosis, caused by the bacterium *M. bovis*, is a cause of chronic ill-thrift and is a potentially fatal disease of cattle. Furthermore, it can be transmitted to, and may also cause severe disease in humans. As a result, the UK Government spends a large amount of money on testing cattle and culling any animals which test positive for *M. bovis*; the expenditure for the financial year 2013-14 is expected to be £99 million. A major drawback in the control of this disease is the nature of the tests used. The tuberculin skin test involves the intradermal injection of proteins derived from *M. bovis* and measurement of the reaction this causes in the skin by measuring skin thickness at 72 hours. The test is time consuming and somewhat subjective. A more serious problem is that the test is negative in 20% of infected animals and thus such animals are not removed from the herd and may continue to shed the organism into the environment. A second test which measures the response of immune cells taken from the animal's blood to proteins derived from *M. bovis* is costly, time consuming and may respond to other related organisms, thereby resulting in false positives. A new test which has improved detection capabilities and reduces costs and time constraints, and which is very easy to use and connect to national databases would be a major benefit in the control of bovine tuberculosis and would improve animal welfare.

What species and approximate numbers of animals do you expect to use over what period of time?	The animals used will be non-Holstein-Friesian cattle. Data from up to twelve animals over a time period of 2 years will be collected.
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?	It is predicted that the animals will experience stress due to handling and constraint during the sampling process. However, it is anticipated that this will be minor because the cattle are regularly handled by experienced personnel who are known to the animals. Should an animal become overly distressed, it will be withdrawn from the project. The process of blood sampling will cause pain, but this is of mild severity and will be minimised by correct sampling technique. The volume of blood taken (up to 60 ml) is very small in this species and will not be sufficient to cause anaemia. Other possible sequelae include bruising and infection at the sampling site. The risk of such outcomes will be minimised by correct aseptic sampling technique. The cattle will be examined by the Named Veterinary Surgeon following the procedure to check that there is no injury or pain at the sampling site. At the end of the study the animals will be released from the project and will return to the herd. The individuals used in this project will not be used in further studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The systemic responses to infection are extremely complex and cannot be modelled by non-animal alternatives at the current time. It will be necessary to use cattle rather than other species in this study because biomarkers vary between species and cross-species similarities cannot be predicted with certainty. In addition, the data collected in this study will be used in comparison with data collected from other experimentally infected bovines.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Collection of blood from up to 12 animals will yield data which will be used for statistical comparison with data from experimentally infected animals.
3. Refinement	During the course of this procedure blood will be collected from the jugular vein. This is the routine

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.	way in which veterinary surgeons collect blood from animals and causes no more than momentary and mild pain and distress. The volume taken will be up to 60 ml; this is a very small volume in this species and thus there will be no risk of causing anaemia. The cattle will be handled by experienced herdsmen and thus the distress due to handling will be minimal.

Project 23	**Refinement of anaesthetic protocols for research animals**	
Key Words (max. 5 words)	Anaesthesia, Refinement	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	N	Basic research
	Y	Translational and applied research
	N	Regulatory use and routine production
	N	Protection of the natural environment in the interests of the health or welfare of humans or animals
	N	Preservation of species
	N	Higher education or training
	N	Forensic enquiries
	N	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Anaesthesia of laboratory animals should represent a refinement of research methods, as it should prevent pain and distress caused by research procedures. However it is important that the best anaesthetic methods are used. This project aims to develop improved methods of anaesthesia for a range of different species, and suitable for a variety of different research projects.	
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)?	Improved methods of anaesthesia would benefit animals used in research, as it would reduce the incidence of side-effects such as prolonged recovery periods, reduced food and water consumption after recovery. Improved methods of anaesthesia would also reduce complications such as slow recovery from the anaesthetic, which can increase the risk of death during or after an anaesthetic. These complications can also affect the quality of scientific data obtained from the animals, so the potential benefits are better science, as well as better welfare for the animals used.	

What species and approximate numbers of animals do you expect to use over what period of time?	The numbers of animals used, as well as the species, will be determined by the need for development of particular anaesthetic methods, but will require no more than 270 rats, 270 mice, 44 rabbits and 44 guinea pigs over the 5 years of the project.
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?	Most animals will undergo an anaesthetic, and some will be allowed to recover. The anaesthesia may be repeated. Some anaesthetics can cause slight pain on injection, or can be unpleasant to inhale, but these effects should only cause mild distress and the animals would rapidly become anaesthetized. These procedures would be classified as mild. A very few animals may need surgery to implant monitoring devices to measure the effects of anaesthesia, or to allow infusion of materials or withdrawal of blood. This could cause pain or infection, but we expect to be able to prevent these adverse effects by administering pain relief, and antibiotics, and by carefully monitoring that these are being effective. These procedures would be classified as moderate. Some animals would receive anaesthetics and would not be allowed to recover. These procedures would be classified as non-recovery. Other animals would recover, so that the longer term effects of anaesthesia could be assessed, and these animals would usually be humanely killed once the study was completed. These procedures would be classified as either mild, or moderate. However, when possible, animals that have not undergone any surgery may be rehomed.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Since the project aims to develop improved methods of anaesthesia, this needs to be undertaken in living animals, although some aspects of the work (eg developing new apparatus) can be done without the use of animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	At each stage, the number of animals used would be minimised by: a) Conducting pilot studies using very few animals (typically I or 2). This is often sufficient to determine whether a new anaesthetic regimen represents an improvement over existing techniques. b) Using statistical calculations to determine the minimum numbers of animals needed to show whether a new method is an improvement on older methods of anaesthesia.

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 age and species of animals selected will be those that match the animals in which the new information obtained on the project will be applied. When possible, animals will not be permitted to recover from anaesthesia. When recovery is needed, animals would not normally have undergone any surgical procedure. Surgery (to implant telemetry devices) would only be carried out when no alternative approach could be used, and these animals would receive post-operative analgesia to alleviate pain. All of the animals would receive high standards of care during anaesthesia, for example provision of warmth, and monitoring of body temperature to ensure these measures are effective. Animals would also be carefully monitored to ensure no unintended complications occurred during anaesthesia, for example respiratory distress because of inadvertent obstruction of the airway.

Project 24	**Smart tagging of fish to determine fish behaviour, biometrics, biomass and escapes using sonar**	
Key Words (max. 5 words)	Tagging, biomass, salmon, Salmo salar, sonar	
Expected duration of the project (yrs)	1 year	
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)	Microchip tags are frequently used to identify animals and are also used to identify fish. The information obtained is generally limited to identification rather than providing more information on the individual. The project is developing a smart tag that can be inserted in the abdominal cavity of farmed fish to give more accurate biomass estimates,to assess the welfare and health status of the fish and to identify if fish escape from the pen. This information can make management of the fish on the farm more efficient, make feeding and daily ration more accurate, and permit identification of welfare issues and disease at an early stage and so enabling remedial action. Escape of fish from farms is a controversial area with potential consequences for genetic interaction with wild fish populations. Escapes have to be notified to Government and means taken to minimise the likelihood of fish escaping. The tag under consideration would be carried in each fish and any escape would be detected by the sonar system by a tagged fish falling outwith the sonar range of the system. Immediate action could be taken to seal or repair the location where the fish are escaping from, and action could be taken to recover escaped fish.	

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 benefits would be more accurately estimating fish biomass, more accurate feed management, and improved health and welfare of fish. Eliminating escaped fish would reduce any risk of impact on wild fish stocks.
What species and approximate numbers of animals do you expect to use over what period of time?	Atlantic salmon, *Salmo salar*. 100 fish would be tagged and monitored over 3 months.
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?	Non-lethal adverse effects such as the effects of tagging on fish growth would be measured during the experiment. The adverse effect would be the insertion of the tag inside the abdominal cavity of the fish which would require anaesthetising the fish and making an incision with a scalpel into the body wall and then insertion of the tag. The incision would be repaired with a suture and an adhesive sealant would be applied to the incision area. The fish would euthanised using a scheduled method at the end of the 12 week trial period.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The smart tag is intended for routine use with farmed fish.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The numbers of fish have been given as a minimum number of 100 to carry out the trial, rather than tag all 5000 fish in the pen. This number represents the typical number of fish sampled monthly from each pen to measure weights and this would fit the sample number required to give a representation in the distribution of fish weights from one pen on a fish farm.
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 smooth external design and suitable size of the tag were determined following initial laboratory based fish welfare trials in Germany.

Project 25	**Quantification of pain associated with pneumonia in calves**	
Key Words (max. 5 words)	Pneumonia pain welfare calf	
Expected duration of the project (yrs)	3 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)	Pneumonia is a very common complaint affecting calves, causing illness, poor growth and sometimes death. At present there is no information about how painful this disease is to the animal. Assessing pain in cattle can be problematic as the behavioural response to pain shown by prey animals such as cattle is less evident than that in predators. Behavioural changes are widely used to assess pain, for example the degree of head shaking and ear flicking following dehorning, however this method has limitations. Firstly it is a subjective measure, which requires training of the observer and secondly it may not be useful in diseases such as pneumonia where the illness itself will cause behavioural changes. Pressure algometry has been used to provide a more quantitative measure of pain which is less subjective. A handheld device can be used to apply pressure to the animal until it trys to avoid it – e.g. by kicking out, moving away, flinching etc. The pressure at this point is the mechanical nociceptive threshold (MNT). In cattle mechanical nociceptive testing has been used to assess pain in dairy cattle with mastitis, lameness and following dehorning but not pneumonia. At present there is no information on how reliable this method may be for measuring chest pain. The aim of the proposed study is to determine whether pneumonia is a painful condition in dairy calves and if this pain can be measured using a	

	pressure algometer. These aims will be achieved through two separate studies: Objective 1: Can pressure algometry be used in different calves, by different operators and in different sites on the chest to give repeatable results. Objective 2: Do calves with pneumonia have a lower pain threshold than calves without pneumonia? i.e. Is pneumonia a painful condition?
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 study could have a range of benefits, firstly if pneumonia is found to be painful, it may result in farmers and veterinary surgeons being more likely to treat these animals with pain killers. In addition it would provide the baseline data needed to carry out further research on the efficacy of drugs in the mitigation of this pain. Finally, if the chest pain threshold correlates well with the degree of pneumonia the calf is suffering from this may provide a useful monitoring tool for farmers and veterinary surgeons in aiding easy early detection and treatment decisions.
What species and approximate numbers of animals do you expect to use over what period of time?	148 dairy calves between 2-12 weeks of age. Each calf will only be used on one occasion lasting approximately 20 minutes.
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?	There are no predicted lasting adverse effects from this procedure. As the pressure algometer will be removed from the chest when the calf makes an attempt to avoid the pressure only very mild momentary discomfort will occur during the procedure. The calves will remain on the farm of origin throughout the procedure and will continue to be farmed as normal.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is necessary to use bovine youngstock as the study is specifically investigating whether they feel pain associated with pneumonia. Behavioural observation to identify signs of pain is not appropriate as calves with pneumonia will likely show signs of disease which are similar to markers of pain. Therefore it would not be possible to identify changes in behaviour due to disease and those due to pain.
2. Reduction Explain how you will assure the use of minimum numbers	We have calculated the number of animals required to give meaningful results for objective 1. This pilot study will give information regarding the repeatability

of animals	of MNT and the likely average difference in reading between calves, this will be used to determine the required sample size required to fulfil objective 2.
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.	No lasting adverse effects are associated with this procedure. There will only be transient minor discomfort, as soon as the animal shows an avoidance reaction the force will be immediately stopped. The force applied will be stopped at 25N even if the animal does not react to negate any possibility of trauma to soft tissues. Animals will be handled and restrained for the procedure by experienced veterinary surgeons or animal technicians. Personal licensee's carrying out the procedure will be experienced veterinary surgeons. Severity of the procedure is mild.

Project 26	**Ruminant Welfare Studies**	
Key Words (max. 5 words)	Welfare, ruminant, stress, behaviour, pain	
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 aim of this project is to provide a better understanding of the recognition, interpretation and mitigation of ruminant stress and pain, particularly focusing on the mother and offspring. This will be used to develop practical information to modify husbandry practices to improve welfare. There is growing evidence that maternal stress, nutritional status and health during pregnancy can influence fetal development with consequences for progeny postnatal biology. Offspring can also be affected by early postnatal experiences, including pain, which can in turn influence development. Together, these effects can be important for animal welfare outcomes, and for farm productivity. However, there are still many unknowns. The type and level of prenatal stress (PNS) as well as the time in pregnancy when this stress occurs are all crucial to the type and magnitude of effects that can be expected. The longer term impact of maternal care on the development of their offspring is also not well known outside the rodent literature, which may be very different in comparison to livestock species. Additionally, our ability to reliably recognise good and poor welfare on farm, particularly for sheep, goats and beef cattle, is not well developed and studies to validate indicators of welfare are still required.	

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 likely benefits that will accrue from this work are a better understanding of the impact of early life expereince on later animal welfare, and an improved ability to assess and measure animal well-being. This will lead to better information on the husbandry of ruminant livestock to improve their welfare. Alongside these practical benefits, the work will also provide more basic information on the control of animal behaviour and the development of neonates, which will be relevant to other species, including humans.
What species and approximate numbers of animals do you expect to use over what period of time?	The work will use mainly sheep, with some studies conducted in cattle and goats, as the main ruminant livestock species in the UK. The work will use no more than 200 animals of each species per year, with most procedures being similar to those experienced by agricultural animals on commercial farms.
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 majority of the work involves exposing animals to conditions that simulate what might occur in commercial farms. The impact of this on the animal is assessed by behavioural testing and the collection of biological samples (e.g. blood, saliva etc.) that will allow us to understand the impact this has on the animal, or on their offspring if the exposed animal is pregnant. The likely severity of these procedures is largely mild, although behavioural testing may involve brief separation of mother and offspring which both may find distressing. Some parts of the work involve deliberately exposing animals to conditions that they will find unpleasant, although these are designed to replicate normal animal husbandry, and for some animals this may reach moderate severity. For most procedures the effects are short-lived, and the animals will return to be treated as commercial farmed livestock at the end of the procedure. For a small number of animals, where treatments mean that they cannot return to a situation where they may end up in the human food chain, they will be humanely euthanized 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	The ultimate goal of these studies is to generate information about ruminant animals (sheep, cattle and goats) which has direct relevance for the management of these species under commercial farm production systems. As a consequence animal use is required. As these studies all have behavioural outcome measures, and behaviour is species specific, the option of using other species to achieve

	these endpoints is not viable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	These studies build on existing work, using similar procedures, thus we have a good understanding of the likely between-animal variation that exist. This information will be used, in conjunction with our statistical advisers (BioMathematics and Statistics Scotland, BioSS), to conduct power analyses to reduce animal numbers to the minimum compatible with our experimental designs. We will work with BioSS to develop rigorous experimental designs that maximise the value of the data from each animal to the overall objectives.
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.	Most of the studies will be conducted in sheep, where we already have a substantial body of knowledge on behavioural responses which will facilitate the design of behavioural tests that minimise animal stress. Most of our studies take the commercial management of livestock as the control condition and seek to develop methods that will improve (e.g. improved housing conditions, pain relief with management procedures etc.) rather than reduce the welfare of animals within these conditions. Thus our studies generally do not impose a greater cost to the animals than they would experience in normal commercial practice, and where possible the experimental condition is improved welfare. As an animal welfare research group our aim is to provide information to improve the welfare of farmed animals. We do, therefore, seek to minimise the welfare costs to any animals in our studies and have developed more refined protocols for sampling, testing and euthanizing animals (e.g. through the use of sedation prior to euthanasia).

Project 27	**Novel acaricidal options for control of sheep scab disease**	
Key Words (max. 5 words)	Sheep scab mite disease acaricide	
Expected duration of the project (yrs)	3 – 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)	The project aims to investigate the potential of novel control solutions for sheep scab mite, *Psoroptes ovis*, and sheep scab disease that are selective, targeted and should satisfy biological, toxicological and environmental requirements, whilst contributing towards a safer and sustainable control strategy. There are four main objectives: 1) To assess acaricidal activity (acaricides are pesticides / miticides that kill mites) and barriers/opportunities for exploitation of active ingredients that may be developed as early replacements for current products as novel sheep scab control agents 2) To likewise assess developmental potential and acaricidal acivitity of a range of newer compounds for development as control agents for sheep scab disease 3) To investigate attractancy / aggregation factors, and a range of natural compounds as potential synergists for enhancement of acaricidal and ovicidal actives identified in 1 & 2 for novel sheep scab control, and 4) To Exploit genomic transcription data and molecular techniques for mode of action studies on novel acaricides and to identify potential new	

	targets for sheep scab control.
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 are to identify alternative treatment options for sheep scab disease, and therefore to improve the health and welfare of sheep. Investigation of various physiological processes in the sheep scab mite through exploitation of molecular techniques, together with behavioural and mode of action studies and bioassay of a wider range of alternative, potentially 'safer' acaricides may lead to new control strategies for sheep scab disease and improved animal welfare. The knowledge gained from the work could also be used to advance control of other related mite and ectoparasitic pests that cause both animal and human welfare problems.
What species and approximate numbers of animals do you expect to use over what period of time?	Sheep, approximately 20 per annum (max. 25), and 100 - 125 in total 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 end?	Due to the frequency of examination and regular removal of scab mites from the sheep it is not expected that the population of scab mite will increase considerably to pose a serious welfare issue, however susceptibility of sheep to scab mites varies. The protocols are categorised as 'moderate severity'. The general welfare and Intervention Criteria will be monitored against defined severity limits, which in most cases (>90%) would not exceed one or more of the following: Head twitch and nibble when handled, Spontaneous biting or scratching, Fleece displaced (pulled/tagged) or scab infestation covering <25% of the total body surface area, but with feeding and demeanour normal. The animals will be killed at the end of the procedure by a schedule one method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Mites are required in large numbers and in good condition for off-host assays and smaller numbers for on-host assays to achieve the objectives as specified The sheep scab mite (*P. ovis*) is parasite that lives on the skin mostly of sheep and cattle but also other ungulates. It is entirely dependent upon its host for

	feeding, reproduction and survival. Although previous studies have shown that it is possible to maintain mites off-host for a limited period, with some developmental progression, a complete life cycle *in vitro* has yet to be achieved. It is therefore necessary to generate experimental mites using deliberately infested sheep.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In the majority of cases, mites harvested from individual sheep will provide sufficient material for numerous off-host efficacy studies. Multiple experiments can thus be conducted at the same time, in order to maximise data yield from each animal, and minimising the overall number of animals used. For the proposed on-host studies, these will be restricted to those few compounds likely to be progressed further, and the numbers of animals used minimised by deploying multiple arenas (test areas) on a single sheep.
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.	<u>Choice of species</u> The overall objective is to find potential alternatives for the control of sheep scab disease. The sheep scab mite is an obligate parasite of its predominant natural host is the domesticated sheep. The ear canker mite of the rabbit provides a close comparator, but there is no realistic alternative to using the artificially infested sheep as the most refined source for the supply of mites and for on-host testing of candidate novel acaricides. <u>Minimising suffering</u> Due to the frequency of examination and regular or terminal (post-mortem) removal of scab mites from the sheep it is not expected that the population of scab mites would increase sufficiently to pose a serious welfare threat, however the susceptibility of individual sheep to scab mite can vary considerably. . The measures that are used to assess welfare/well-being include degree of head twitch and nibble when handled, spontaneous biting or scratching, fleece displaced (pulled/tagged), scab infestation covering <25% of the total body surface area and demeanour and feeding. The NACWO is kept informed at all times. Symptoms exceeding these, such as superficial open wounds, more extensive lesions (>25%), or secondary infection are notified to the NVS for advice / palliative treatment. Sheep would

	normally be removed from the trials before this, but if symptoms persist they would be culled to prevent undue suffering.