Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 7

Projects with a primary purpose of: Translational and Applied research

- Animal Diseases and Disorders
- Animal Welfare

Project Titles and Keywords

1. **Drugs and diagnostics for animal trypanosomiasis**
    - **Animal trypanosomiasis; trypanosome; parasite; livestock disease; genetics**

2. **Short term behavioural effects of pergolide (Prascend) in normal horses**
    - **Equine Cushing's Syndrome, PPID, Equidae, pergolide**

3. **Fish diseases: risk factors and control measures**
    - **Fish, disease, control, treatment, pathogenesis**

4. **Ruminant vaccine development**
    - **Ruminants, vaccines, parasites, bacteria**

5. **Investigation of natural diseases in dogs and cats**
    - **Dog, spontaneous diseases, human model**

6. **Research into TSEs using genetically altered mice**
    - **Transgenic mice, prion, BSE, scrapie, characterisation**

7. **Assessment of nutritional status in dairy cattle**
    - **Dairy, Cow, Nutrition, Energy, Rumen**

8. **Reagent Production in Support of Diagnostic Tests**
    - **Reagent Production Diagnostic Tests**

9. **Equine Vaccine Development**
    - **Equine Vaccine Development**

10. **Novel vaccines for Johne's Disease**
    - **Vaccine, Johne's Disease, Mycobacteria**

11. **Locomotor activity and myopathy in meat poultry**
    - **Myopathy, activity, exercise, turkeys, broilers**

12. **Reducing the incidence and spread of digital dermatitis in cattle and sheep**
    - **Bovine and ovine digital dermatitis, Treponema, infectious disease**

13. **Development of sustainable aquaculture protocols**
    - **Sustainable aquaculture, pelagic-spawning, angelfish**

14. **Development of a novel humane constricting device for the castration and tail docking of lambs and castration of young bovines**
    - **Lamb, calf, castration, tail-docking**

PROJECT 1	**Drugs and diagnostics for animal trypanosomiasis**	
Key Words (max. 5 words)	Animal trypanosomiasis; trypanosome; parasite; livestock disease; genetics	
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[1]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Trypanosomiasis is a major livestock disease that severely constrains agricultural production in the tsetse-infested region of sub-Saharan Africa by destroying both draught and production animals. Available drugs for treatment are few and they have been in use for decades ; drug resistance is increasing and widespread. The overall purpose of this programme of work is to develop experimental systems and tools to study the organisms (trypanosomes) that cause the disease. Specific objectives are to 1. Develop an experimental system to investigate whether trypanosomes can swap genetic material, e.g. genes for drug resistance. 2. Develop methods for *in vitro* culture of bloodstream	

	form (BSF) trypanosomes to enable testing of candidate drug targets and inhibitors.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This research will increase knowledge about the causative agents of animal trypanosomiasis - livestock trypanosomes. In the longterm, this will contribute to the better use of existing drugs, the development of new drugs and lead to improvements in control of the disease.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse. Approximately 20 mice per year or 100 mice over the course of the 5 year 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?	No adverse effects are expected. Overall severity is mild. Animals will be killed to harvest parasites from the blood. As soon as the parasitaemia reaches a high enough level, animals are fully anaesthetized and the parasites harvested from the blood without the animal recovering consciousness.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is essential to use bloodstream form (BSF) trypanosomes for development of new drugs or diagnostic methods as this is the life cycle stage found in the mammalian host and is therefore the clinically relevant stage. Currently there are no reliable *in vitro* culture methods for BSF trypanosomes of the important species of livestock trypanosomes. Hence the BSF need to be obtained from experimental animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Animals are used for the growth of parasites, so statistical considerations do not apply. The minimum number of animals will be used to grow the different parasite strains required in sufficient amount. Where possible, in vitro culture will be used to obtain parasite material.
3. Refinement	It is necessary to use a mammal to grow the

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.	parasites as other animals are not susceptible to infection. Mice are the smallest suitable species as only small numbers of parasites are needed, but from a range of different trypanosome strains. Mice are assumed to be at the low end of the neuro-physiological range for mammals. Animal suffering will be minimised by maintaining infected animals for the minimum period of time required to obtain parasite material. This varies from a few days to 4 weeks, depending on the growth characteristics of each trypanosome strain. Mice are kept in groups for company, because there is negligible risk of the parasites being transmitted from mouse to mouse. A humane end-point is reached when the animals have moderate parasitaemia but no obvious signs of ill health.

<table>
<tr><td>PROJECT 2</td><td>Short term behavioural effects of pergolide (Prascend) in normal horses</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Equine Cushing’s Syndrome, PPID

Equidae, pergolide</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[2]</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[3]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Up to one in 5 competition horses 15 years and older are likely to have PPID. This disorder is associated with aging with signs such as muscle wastage, poor performance and laminitis. Pergolide has been shown to restore function in affected horses allowing them to continue active lives including competition. The ability of these horses to remain in competition will increase their productive life and reduce the welfare compromise associated with retirement shown in epidemiological studies. However, the drug is currently banned for use in competition horses by the FEI (Federation Equestrian International) due to perceived</td></tr>
</table>

	advantages to performance in normal horses including potentially calming the normal horse. Due to the ban, currently owners must choose whether to treat their horse and retire it from competition or not treat and risk the development of severe complications of PPID including muscle wastage and laminitis. Therefore our study aims to: 1. To investigate the behavioural response to pergolide in normal horses. 2. To investigate the effect of pergolide on heart rate at rest, during stimulation during the novel horse test and during submaximal exercise on the lunge.
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)** If our hypothesis is correct, demonstrating that Pergolide does not have calming or other behavioural effects will support its ability to be used in competition horses and ponies. **ii)** Up to one in 5 competition horses 15 years and older are likely to have PPID and the ability of these horses to remain in competition will increase their productive life and reduce the welfare compromise associated with retirement shown in epidemiological studies.
What species and approximate numbers of animals do you expect to use over what period of time?	Ideally, we will need to use 4 horses for two study periods of two weeks duration separated by two weeks of rest where no drug is given. Including the two weeks to acclimate the horses to their surroundings the whole study is planned to be complete in 8 weeks.
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?	Other than blood sampling to screen for illness and underlying disease (PPID), all of the procedures in the study are non –invasive and for well handled horses would represent no more than routine husbandry. For this reason we will select horses that have had a history of being well handled so that horses will be accustomed to handling, body condition scoring, application of a surcingle/girth and exercise on a lunge. It is our intention that the animals will be discharged

	from the act at the end of the study and re-homed into private domestic settings.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Horses and ponies have a unique metabolism and other animals or isolated tissues cannot therefore be used to derive the information we seek. This work on horses will provide clear benefits directed towards improving pony welfare.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Prospective power calculations were performed and incorporating the crossover design which strengthens the power and reduced the requirement for more 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.	Our questions can only be answered by normal horses. The work is being performed within the Veterinary Hospital and the people conducting the study are experienced veterinary surgeons. The horses will be monitored continuously and any issues will be immediately addressed. The staff charged with the day to day care of the animals are experienced equine technicians. All animals will be habituated to the essential handling methods at outset and the horses will be allowed to exercise at liberty daily with a companion wherever possible

<table>
<tr><td>PROJECT 3</td><td>Fish diseases: risk factors and control measures</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Fish, disease, control, treatment, pathogenesis</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)[4]</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>Yes</td><td></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[5]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objectives of this project are to evaluate the presence of known or unknown pathogens affecting fish health in Scottish waters, to measure the impact on the health and welfare status of Scottish fish farms and to develop and/or implement control measures to help minimising it.</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 outcomes of the project expect to contribute to the establishment of a sustainable fish farming industry in Scotland with a high health and welfare standard. In addition, the benefits will increase the scientific knowledge on interactions between host and pathogens and improve or establish a precise description of symptoms induced by known or emerging diseases in fish, respectively.</td></tr>
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What species and approximate numbers of animals do you expect to use over what period of time?	A maximum of 13,000 animals will be used per year. Most of them will be salmonid as this group includes a large majority of aquaculture species in Scotland. However, other species will be likely used depending on i) the development of farming industry in other fish species (for example gadoids - cod) and ii) wild species interacting with farmed species.
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 procedures used in this project will be classified as severe as they involve the induction and study of the development of diseases and associated clinical symptoms. All animals will be monitored and removed humanely before death occurs and it is expected that all animals will be killed humanely by schedule 1 method at the end of every procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	To date, there is no animal-free alternative to monitor the development of symptoms related to infectious diseases in fish. The applicant's institute is involved in several projects aiming at investigating alternative to animal use such as the development of suitable fish cell lines, the establishment of non-lethal correlates of clinical signs of disease or levels of efficacy of control measures, the development of ex-vivo organ systems. However, it is unknown whether these tools will be available during the life of the project and to what extent they can replace animal experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	In some cases pilot experiments involving small number of animals will be considered before large experiments are carried out. Statistical expertise will be consulted at an early stage before each procedure to maximise the success i.e. conclusive outcome that does not require repetition. The applicant's institute has been pioneering the development of a non-lethal infectiology model for fish within a NC3Rs project allowing 80% reduction in the number of animals used. It is likely that this alternative will be used in some experiments during

	the project.
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.	Relevant species are those used by the aquaculture industry or those in the wild that may affect the industry. In most cases, animals will be from commercial origin but in some case they will be collected from the wild. Husbandry parameters will be aligned to the RSPCA guidelines when maintaining livestock in captivity in the bio-secure facility. Anaesthesia will be used for a large majority of procedures and, when possible, in-tank anaesthesia will be used to minimise stress to animal related to handling (netting).

<table>
<tr><td>PROJECT 4</td><td>Ruminant vaccine development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Ruminants, vaccines, parasites, bacteria</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)[6]</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>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Vaccines are one of the most effective methods of controlling infectious disease. This project aims to develop vaccines to combat diseases in livestock species for which vaccines are either unavailable or lack efficacy. Diseases targeted in this project cause continuing and significant welfare and economic problems and, occasionally, are also a threat to human health. This work seeks to identify mechanisms by which animals become immune to such diseases, mimic such immune responses through the development of the appropriate immune-stimulating compounds (i.e. adjuvants), test new vaccine formulations and provide detailed information of transmission of disease between individuals to allow the impact of vaccination to be predicted.</td></tr>
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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 in response to national and international needs, contributes to biological, veterinary and medical knowledge and is in the public interest. The development of vaccines will reduce the disease burden of our livestock species, thus improving the health and wellbeing of farmer livestock, and in the case of disease which affects humans, improve human health. It will reduce the reliance on chemical treatments to control disease, resulting in reduced contamination of the environment.
What species and approximate numbers of animals do you expect to use over what period of time?	The animals used in this work (cattle and sheep) are the natural hosts for the diseases being studied. Laboratory animals (mice, rabbits) will be used in limited situations to provide reagents for in vitro testing, thus limiting the overall number of animals required. The numbers used are restricted to those expected to produce statistically significant answers to questions posed, using a number of statistical methods based on previous work and experience in conjunction with experts in the field. Over the 5 year project, up to 400 sheep, 400 cattle, 20 mice and 20 rabbits will be used, although wherever possible the use of non-animal systems will be employed to address research objectives.
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 experimental models used in this work have been developed over a number of years with great care and attention in order to minimise suffering by the animal. Work with cattle and sheep is not expected to be of greater than moderate severity, and in mice and rabbits to be of mild severity. It is not anticipated that the infection protocols used in the studies will result in clinical disease, with the animals remaining apparently healthy. Experienced observers, with access to veterinary advice and care at all times, monitor clinical signs of all experimental animals at regular intervals in order to quickly identify any animal requiring veterinary treatment. Any animal failing to respond to treatment will be killed humanely. By necessity, the majority of experimental animals will be killed at the end of procedures. Animals involved in on-farm studies will

	be returned to the farm stock.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Wherever possible, the use of non-animal models will be employed throughout this project. However, the development of vaccines requires firstly, an understanding of the mechanisms by which an animal becomes immune to disease and secondly, the characterisation of the immune response generated following vaccination. To address these questions, we need to study the immune response as a whole (i.e. *in vivo*), as many different components of the immune system interact to generate the final immune response. Furthermore, the use of animals is an absolute requirement for the assessment of the efficacy or effectiveness and safety of any new vaccine.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The careful refinement of experimental models ensures that only the minimal number of animals required to obtain statistically significant and biologically relevant outcomes will be used. Independent advice on the experimental design is provided by trained statisticians in advance of any experimental work being conducted. In addition, proposed experiments are reviewed by an ethical review committee to ensure that the minimal number of animals is used. Wherever possible, experiments will be conducted *in vitro* to minimise animal usage.
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.	Over the last 10 years or more, we have developed relevant, reliable and reproducible disease models in conventional cattle and sheep, which have been refined to be the least severe necessary for valid results to be obtained. Considerable care and attention has gone into refining the techniques employed to monitor the immune responses during animal studies in order to reduce the degree and duration of any suffering to a minimum. Trained teams of observers monitor animals at regular intervals, accurately evaluating the responses of individual animals and seeking veterinary intervention where necessary.

<table>
<tr><td>PROJECT 5</td><td>Investigation of natural diseases in dogs and cats</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dog, spontaneous diseases, human model</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)8</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[9]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>There are currently estimated to be 10 million dogs and 10 million cats in the UK which suffer from a wide range of illnesses. Despite recent advances in our understanding of the biology of many diseases in companion animals, we still cannot adequate diagnosis and treat many diseases resulting in significant suffering in client owned animals. Consequently, there is an obvious unmet need to improve our knowledge of the biology of the many diseases which afflict companion animals so that we can improve welfare in this very large population of animals.

There is also a clear benefit in understanding more about the biology of spontaneous diseases in companion animals since many diseases in cats and dogs are highly similar to diseases in humans; a</td></tr>
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	greater knowledge of the biology of diseases in dogs and cats is likely to be highly informative in our quest for a deeper understanding of disease biology in humans. The evidence of similarities between human and companion animals is now compelling from many different diseases including diabetes, liver disease. Given that there is growing evidence that mouse models to not replicate many features of human disease, there is a clear need to develop and validate superior animal models of human disorders. Finally, there is a need to reduce, replace and refine animal models of human disease. An obvious strategy to do this is to undertake additional studies on animals which develop spontaneous disorders rather than relying on chemically or surgically induced large animal models which may not mimic many of the features of the relevant human disease. Taken together, these benefits and needs can be addressed by performing clinical research on client owned companion animals which have developed spontaneous diseases. The general project plan will be to perform clinical investigations on client owned dogs and cats with spontaneous diseases and compare the results with those obtained in healthy dogs with the permission of the owners after we have explained the purpose of the study and the procedures their animals will undergo. No pressure will be placed on the owners to take part in the study and they may withdraw their animals at any time.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	A deeper understanding of disease pathogenesis in dogs and cats is likely to lead to development of superior treatments for client owned cats and dogs with spontaneous illnesses. In addition, a better understanding of diseases in dogs and cats may offer a better understanding of the important diseases in humans.
What species and approximate numbers of animals do you expect to use over what period of	Initially, we expect around 500 dogs will be used for our studies into the gastrointestinal disorders.

time?	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The procedures undertaken in this study will include blood sampling, diagnostic imaging (ultrasound/CT/MRI), endoscopy and tissue biopsy. The expected adverse effects will be limited to, and not be noticeably more, than the standard diagnostic tests and normal treatment for the conditions and will be managed by an experienced team of onsite veterinary specialists
Application of the 3Rs	
1. Replacement **State why you need to use animals and why you cannot use non-animal alternatives**	Our aim is to better understand spontaneous diseases in dogs and cats. We believe this approach is the epitome of best practice on compliance with the 3Rs since we are studying disease processes which have already developed rather than inducing them in otherwise healthy animals
2. Reduction **Explain how you will assure the use of minimum numbers of animals**	As the animals will develop the disease spontaneously, we are avoiding the need to induce illnesses in otherwise healthy animals. Control, healthy animals will be used as controls, where appropriate, to facilitate the meaningful interpretations of data gathered from the spontaneously ill dogs.
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.**	Our aim is to better understand the biology of spontaneous diseases in dogs and cats hence we may identify further diseases of relevance to these species that require improved scientific knowledge in order to produce effective treatments for them and thus the need to include dogs and cats in this licence.

<table>
<tr><td>PROJECT 6</td><td>RESEARCH INTO TSEs USING GENETICALLY ALTERED MICE</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Transgenic mice, prion, BSE, scrapie, characterisation</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)[10]</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[11]</td><td>Yes</td><td></td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The purpose of this project licence is to breed and maintain transgenic mouse lines, and wild-type mice where required, for use in the field of veterinary and biological science in the investigation of transmissible spongiform encephalopathy (TSE) or prion diseases affecting ruminants and other species.

TSEs are a group of fatal neurodegenerative disease affecting humans and animals, and include BSE in cattle and scrapie in sheep. BSE has been identified as a food-borne risk to man and animals. The incidence of both diseases has been declining due to feed bans and selective breeding, respectively; however, atypical forms of both have been recently identified in cattle and sheep with unknown risk to humans.

Transgenic models expressing different prion proteins</td></tr>
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	(PrPs), that are susceptible to TSEs, have provided rapid, sensitive bioassay models for human and animal prions compared with wild-type mice and natural hosts, such as cattle and sheep. Such models represent the most effective and reliable way to demonstrate TSE infectivity and determine strain characteristics and prion infectious titres. As there are currently no suitable alternatives to the use of animal models, transgenic mice are an important resource for TSE bioassay and strain characterisation. **Objective 1. Completion of ongoing mouse bioassays for TSE strain characterisation.** In this study, we propose to complete the bioassay characterisation of TSE field cases initiated in the previous Project Licence, including born-after-the-reinforced-ban (BARB) BSE, H-type BSE and L-type BSE cases from cattle, BSE-like sheep cases and goat scrapie, inoculated into a panel of transgenic and wild-type mice. Further BARB BSE cases, and ongoing inoculations of transgenic ovinised PrP ARR mice, mean that mice are still incubating disease and will be analysed. These studies will also identify optimised models for TSE bioassay, contribute to improved strain characterisation of TSE cases and could identify risks to human and animal health and the emergence of new TSEs, and provide policy-making bodies with relevant scientific information and evidence. **Objective 2. Ongoing surveillance of TSE field cases and maintenance of breeding colonies.** This project licence will support the ongoing surveillance and monitoring of TSE cases of special interest arising in ruminant species by bioassay and strain characterisation of cases, including BARB BSE occurring in cattle after the feed bans, atypical TSE cases, and newly emerging prion strains that may represent risks to public and animal health. It remains critical to be able to distinguish BSE from other animal source TSEs that are non-zoonotic, and these studies will provide policy-making bodies with important information regarding risks to human and animal health.

	Maintenance of transgenic mouse lines will be maintained so that requirements for TSE bioassay and strain characterisation can be met. **Objective 3. Development of alternative approaches to detect TSE infectivity.** In line with the 3Rs policies of reduction, refinement and replacement of the use of animal models, it is proposed in this project licence to continue development of *ex vivo* and *in vitro* alternatives to mouse bioassay in the detection and strain characterisation of TSEs. The development and application of prion organotypic slice culture assay (POSCA), protein misfolding cyclic amplification (PMCA) and TSE-susceptible cell assays, could contribute ultimately to faster, more economic and ethical methods to reduce or replace the use of mouse models in TSE bioassays.
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)?	Support for ongoing surveillance and monitoring of TSE field cases of special interest, including improved strain characterisation of BARB BSE and scrapie cases, using transgenic models. Identification of optimised transgenic models for TSEs. Determining TSE susceptibility of a transgenic ovine ARR allele. Development of alternative methods to mouse bioassay for TSE infectivity. Provision of relevant TSE strain information to policy-making bodies for risk evaluation.
What species and approximate numbers of animals do you expect to use over what period of time?	Breeding colonies: 3500 mice. TSE challenges: 1500 mice.
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	No adverse effects are expected from the breeding colonies. Surplus mice will be euthanized by a Schedule 1 method. To reduce adverse effects, mice will be TSE-inoculated under general anaesthesia with analgesic administered.

will happen to the animals at the end?	Mice with TSE clinical signs will be euthanized at a monitored clinical endpoint. Mice exhibiting any adverse effects will be euthanized immediately on the basis of welfare concerns and advice from the Named Veterinary Surgeon. For breeding colonies, a few mice may develop sore eyes or barbering of hair on the back, which will be treated appropriately. If the conditions do not improve the mice will be euthanized. For TSE-challenged mice, a small number may develop intracranial haemorrhage or exhibit the effects of inoculum toxicity at the time of inoculation and will be continuously monitored and euthanized if necessary on welfare grounds. TSE-challenged mice are expected to develop clinical signs of TSE disease, which could include marked affected gait, rough coat, vacant stare incontinence, weight loss, hunched posture. TSE-challenged mice will be euthanized at a defined clinical endpoint. In the case of <2% of TSE-challenged mice, random sudden death could occur during the TSE incubation period without the onset of TSE signs. This is not a desirable outcome, however, it cannot be predicted and it is not possible to reduce the occurrence by use of continuous monitoring.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	It is not feasible to replace the current use of transgenic models in TSE bioassays as animal bioassay models represent the most reliable method of detecting TSE infectivity and determining prion infectious titres. However, the development and application of alternative approaches to mouse bioassay is integrated into this application.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Transgenic lines will be bred as homozygotes, with one exception. TSE inoculation group sizes have been reduced from 20 wild-type mice to 8 transgenic mice (or exceptionally 12 mice) for post-mortem analyses. This represents the minimum number of mice that will be required for analyses, which will also allow for a small number of intercurrent losses.
3. Refinement	

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The use of transgenic models will facilitate TSE bioassays over shorter time periods compared to wild-type mice or the natural hosts. Optimised transgenic models for TSEs will be identified. Potential suffering during TSE inoculations will be minimised by use of general anaesthesia and analgesic administration. Mice exhibiting TSE signs, or adverse signs, will be euthanized when showing definitive clinical signs of TSE disease or for welfare reasons.

<table>
<tr><td>PROJECT 7</td><td>Assessment of nutritional status in dairy cattle</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Dairy, Cow, Nutrition, Energy, Rumen</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[12]</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[13]</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>Correct nutrition in dairy cattle is an essential requirement for milk production, in order to meet the high nutritional demands of lactation. For example an average dairy cow giving 30 litres of milk will be working at over three times her basic maintenance energy requirements, and a cow giving 50 litres (not uncommon in modern Holstein dairy herds) will be working at nearly five times her maintenance energy requirements. This places significant metabolic stress on the animal, which can be harmful for cow health, productivity and future fertility.

In order to try and meet the high demands of lactation and reduce the metabolic stress on the cows, they are often fed high energy diets supplemented with concentrate feedstuffs such as wheat, maize or molasses. However high levels of</td></tr>
</table>

	fermentable carbohydrates can result in a lowering of the pH in the rumen of the cow, which can also have harmful effects on cow health. This project seeks to assess nutritional status in dairy cattle with the aim of quantifying the effects of different nutritional strategies for the reduction in negative energy balance (NEB) and sub-acute rumen acidosis (SARA) in dairy cows.
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 welfare of the high yielding dairy cow is coming under close scrutiny, and there is increasing evidence that the high metabolic demands of lactation predispose cows to increased levels of disease such as fatty liver, mastitis and reduced fertility. This work will seek to more objectively assess nutritional status in dairy cows compared to traditional methods such as body condition score and weight changes, with the aim of monitoring and reducing the incidence of disease and improving cow health. In addition it will aim to develop existing models of dairy cow nutrition to improve the accuracy and precision of feeding dairy cows. It will also enable us to trial various nutritional supplements that are promoted to potentially reduce the effects of NEB and SARA in dairy cows.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use adult dairy cows kept on a commercial dairy farm under conditions that are commonly encountered on UK dairy farms. Over the 5 year course of the project, we expect to use 200 cows in the experimental work.
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 require to blood sample the cows on a weekly basis, to be able to analyse metabolites in the blood for the assessment of energy balance and metabolic status. The cows will also receive a bolus that sits in the rumen to measure pH, and we may require to take occasional samples of the rumen fluid to validate the measurements obtained from the rumen bolus. Any adverse effects will be mild, limited to local irritation from the needle puncture required to obtain the samples. At the end of the experiment, the animals will be returned to the commercial dairy herd to continue with their

	productive life.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Feeding the dairy cow is a very complex science, as she effectively contains a “fermentation vat” in her rumen that digests plant cell wall material to obtain nutrients. Non-animal models cannot replicate complex cow behaviours that affect the rumen environment such as feeding, feed intake and rumination activity, nor the holistic view of cow health, productivity and reproductive function that are affected by nutrition of the dairy cow, nor the complex pathways that determine energy balance in a high producing dairy cow. Dairy cattle are therefore the only animals that can be used in this work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of cows required will be based on extensive experience of feed trial work carried out previously, and other peer-reviewed research that has been published. Previous work has utilised similar number of cows to assess the efficacy of dietary supplements on milk production, rumination behaviour and rumen pH measurements. Through the use of good statistical methods we will minimise the number of animals required in our 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.	This project will seek to obtain information that will be applied to dairy cattle, and therefore this is the species and type of animal of choice. The methods used will seek to obtain data on nutritional status in dairy cows, using well established methodology for the assessment of nutritional status such as body condition scoring and measurement of blood metabolites to assess energy balance. The procedures involved (blood sampling and rumenocentesis) are classified as mild, and are designed to obtain samples with minimal suffering in order to assess energy balance and rumen environment.

<table>
<tr><td>PROJECT 8</td><td colspan="3">Reagent Production in Support of Diagnostic Tests</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Reagent Production Diagnostic Tests</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[14]</td><td colspan="3"><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[15]</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 colspan="3">The objective of this project is to produce reagents for either direct use in diagnostic tests or improvement of them.</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 colspan="3">The tests directly supported by or developed as a result of this project, in turn support disease diagnosis and health monitoring in both animals and humans. This enhances both disease management and control and welfare in both animals and humans.</td></tr>
<tr><td>What species and approximate numbers of animals do you expect to use over what period of time?</td><td colspan="3">Embryonated Hens eggs:1000 over 5 years.

Chickens : 750 over 5 years

Turkey : 40 over 5 years</td></tr>
</table>

[14] Delete Yes or No as appropriate.
[15] At least one additional purpose must be selected with this option.

	Mice : 40 over 5 years Rats : 20 over 5 years. Rabbits : 1000 over 5 years Sheep : 25 over 5 years Porcines : 6 over 5 years Bovines : 20 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 level of severity for all procedures covered by this licence is moderate or mild. It is expected that adverse effects will include depression, listlessness, inappetance and related symptoms, with limited inflammatory reaction at the site of inoculation when adjuvant is used. The inflammation maybe more marked on the infrequent occasions when Friends Complete Adjuvant is used. Other than for those animals that have solely been used as blood donors (protocol 8) all animals will be euthanized at the end of the procedure. Animals used as blood donors and that are in good clinical condition maybe released to stock at the end of the procedure
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Most reagents are produced for use in established diagnostic tests for which there is no recognised or suitable alternative testing method. Where reagents are produced for use in the development of new tests animals are used where it is considered that non-animal alternatives, such as recombinant antibodies which are targeted single antigenic epitopes, lack the ability to mimic the polyclonal antibody responses that occur in the field which would result in the test lacking the broad activity or sensitivity required.
2. Reduction Explain how you will assure the use of minimum numbers	Reagent stocks are monitored using an electronic stock management system and close contact is maintained with our customers in order to anticipate future demand and to ensure that reagents are

of animals	supplied in appropriately sized volumes to minimise waste. This, in addition to the centralisation of biological reagent production within RPU rather than it being carried out within in research groups at the APHA, has reduced the number of animals used and the amount of unnecessary “contingency” stock held. Methods to increase the recovery of product from individual animals have been investigated. One that was found to increase the yield in Turkeys was trialled in chickens but was not successful in these. The number of animals used per group are the minimum possible although single animals are not used in order to avoid the stress of single housing but the use of killed antigens means that individual animals within a study can be used to raise antibodies to different antigens rather than the necessity, when using live antigens, of all animals receiving the same antigen with the potential result being overproduction of antiserum. These actions has resulted in a significant reduction in the number of animals used in recent years.
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.	Animals are only used where routine in-vitro methods are not available and where possible purpose bred laboratory animals or birds rather than other animals are used. During the life of the previous project a significant improvement has been made in the procedure used to raise polyclonal antiserum to Swine influenza in chickens. The use of a killed inoculum with the addition Montanide (a non-ulcerative adjuvant) rather than a live inoculum has not only resulted in higher titered antisera but also in the antisera in all birds having an acceptable titre. Previously the serum from a significant number of birds had to be discarded as the titre was too low to be of use. The resulting reduction in the number of birds used will be ongoing. Additionally the use of a killed antigen prevents the birds experiencing signs of clinical disease.

<table>
<tr><td>PROJECT 9</td><td>Equine Vaccine Development</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Equine Vaccine Development</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)[16]</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>Yes</td><td></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[17]</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>This project has two aims:

1. To develop new vaccines for horses
2. To update and improve existing horse vaccines</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 key benefit of this project will be the development of up-to-date horse vaccines that are safe and work well.

As with diseases in other animals including man, vaccination to prevent disease is preferable to relying on treatment once the animal becomes sick. Without vaccination, contracting the disease would be debilitating or, at worst, fatal. In the long term, a horse may be left unable to breed, exercise or compete. For this reason, most UK equine</td></tr>
</table>

	governing bodies require that a horse has an up to date vaccination record card in order to race, enter competitions, affiliated shows or studs. This minimises the risk and spread of infectious disease in situations where horse to horse contact is high. This project will make sure that existing and newly developed equine vaccines protect the animal from currently circulating strains of disease without negatively affecting its overall wellbeing.
What species and approximate numbers of animals do you expect to use over what period of time?	Species – Equidae (Horses and Ponies) Numbers and timeframe – Approximately 26 over a 5 year period
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The regulated procedures required by this license are expected to be mild in severity and limited to intramuscular vaccination, blood sampling, nasal swabs and monitoring of temperature. The procedures are similar to those that a horse would experience during an annual vaccination and/or veterinary check-up. Since the procedures involved are those used in routine veterinary inspection, only minor and short lived discomfort is expected. For example: some tenderness and/or minor swelling at the site of vaccination which may last a few days. As far as is possible, horses will be rehomed after 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 aim of this license is to develop vaccines for horses and ponies. As such, the only clear way of showing that a vaccine works and is safe is its administration into these target animals. Vaccines work by mimicking a microbe and teaching the immune system to recognise and destroy it. This protects the animal from disease if it encounters the genuine microbe in the future. The immune system is a highly complex physiological network of cells, tissues and organs that is made up

	of a specific and a non-specific arm. This complex system of many different cell types and organs cannot be constructed or replicated in the laboratory. The way in which a horses (or any animals) immune system reacts to a vaccine or an invading microbe can only be studied by using the whole animal.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The minimum number of animals required in safety and efficacy studies are governed by specific European Pharmacopoeia and European Medicines Agency guidelines. The number of animals used in each study will be guided by the legal minimum number required.
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 studies in the target animal are the only sure way of showing that a vaccine is safe and works well, it is not possible to use an alternative animal model (e.g. mice, rats). The use of horses and ponies is therefore the most refined choice to fulfil the objective of this license. The severity of the regulated procedures required is expected to be mild with a very low degree of pain, distress or suffering anticipated. Since adverse reactions to vaccination are highly undesirable (from both the animals and owners point of view), studies will be conducted using vaccine formulations already shown to be acceptable in either the target animal or other species. Horses will not be subjected to any virulent disease organisms.

PROJECT 10	**Novel vaccines for Johne's Disease**	
Key Words (max. 5 words)	Vaccine, Johne's Disease, Mycobacteria	
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[18]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To discover If a new design of vaccine can be effective at stopping Infections with *Mycobacterium avium* subspecies *paratuberculosis* (MAP).	
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)?	MAP is a bacterium that causes a serious disease called Johne's in domestic animals. It is present in pasteurised milk and has been implicated as a factor In Crohn's Disease of humans. There is currently no effective vaccine available and the disease is spreading. Success of this project will aid in development of a new vaccine that could have benefits In animal health, contamination of food and the environment and have Implications for human health.	
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 200 mice over 5 years	

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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?	Previous experiments using similar methods showed that the adverse effects on animals was none. Vaccination and challenge with a bacterium requires handling and Injections. All measures and precautions will be taken to minimise animal suffering and distress. Where necessary anaesthesia will be used prior to initiation of a procedure and animal behaviour will be monitored for signs of pain and discomfort. The expectation therefore is that the likely expectation of the level of severity will be moderate during the experiment. if pain is suspected, appropriate strategies for the management of pain will be applied. If in doubt the Named veterinary surgeon or NACWO will be consulted. All animals will be humanely killed immediately after the experiment.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The use of animals is justified as this is the only manner In which vaccine immunogenicity and protection can be tested. The animal model is already a proven way of testing vaccines that will be ultimately used in larger animals.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments in this project are designed to take into account previous experience with similar work. The statistical aspects of design have been given careful consideration with power calculations In order to reduce the number of animals to a minimum whilst being able to provide meaningful results imperative, for the development process of these types of vaccines. By staging the project Into two separate procedures potentially ineffective vaccine candidates can be screened out and animal usages minirnised. Wherever possible the overall number of control groups will be minimised by simultaneously testing multiple vaccine candidates Into a single experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general	Mice are a valid animal model as the mouse strain we are using is susceptible to the causal agent (MAP). They also are able to raise immunological defences that are indicative of relevant mechanisms In the animals (cows and sheep) for which the vaccine is being developed. MAP infection following oral administration in these mice is not assured so MAP has to be given by lntra-peritoneal administration. The number of anaesthetic

measures you will take to minimise welfare costs (harms) to the animals.	episodes will be limited by performing blood samples at the same time as Innoculations / immunisations.

PROJECT 11	**Locomotor activity and myopathy in meat poultry**	
Key Words (max. 5 words)	Myopathy, activity, exercise, turkeys, broilers	
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[19]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	To determine if the locomotor activity of growing meat birds is related to the development of muscle damage (pathology) and whether this differs in different lines of commercial poultry. Also to determine if the incidence of leg or foot problems and lameness in commercial birds is related to locomotor activity and muscle pathology.	
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 studies will provide insight in to the relationship between bird activity and the development of spontaneous or stress induced myopathy and establish if there is any correlation between the degree of myopathy and locomotor capacity. It may provide insight as to the efficacy of increasing bird activity or exercise as a means of reducing the incidence or extent of growth associated myopathy. In turn this may provide the basis for strategies for the reduction or alleviation of the myopathic condition in commercial turkey production. This would have benefits in relation to both bird welfare and meat quality.	

What species and approximate numbers of animals do you expect to use over what period of time?	Turkeys (500) and broiler chickens (300) over 3 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?	Single blood samples will be taken from each bird by simple venepuncture in commercial sheds. Following adequate haemostasis and puncture cleaning the birds will be returned to the flock. All the birds will be slaughtered at a commercial plant at the end of the trial.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The nature of the required work necessitates that the study be undertaken in live animals under commercial conditions and the goals cannot be achieved by using any of the alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The design of experiments will maximize the information obtained from the minimum resource. The sample size has been determined using power analysis. Generally, the significance level will be 5%, and the power 80%. The numbers of animals required have been determined from estimates of the coefficient of variation based on previous experience and the scientific literature.
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 have been selected as the pathology which is of concern is found in commercially produced meat poultry. Each bird in the study will only have a single blood sample taken. Expert handling and sampling will minimise the adverse effects of the procedure.

<table>
<tr><td>PROJECT 12</td><td>Reducing the incidence and spread of digital dermatitis in cattle and sheep</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Bovine and ovine digital dermatitis, Treponema, infectious disease</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5 years</td></tr>
<tr><td>Purpose of the project (as in Section 5C(3)20</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[21]</td><td></td><td>No</td></tr>
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</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>Summary:

1) To compare cattle and sheep bacterial populations, both on normal skin and in digital dermatitis lesional skin to understand how they interact to produce lesions which cause pain and lameness.

2) To investigate whether the key infectious agents (bacteria called treponemes) can survive in oxygen and thus have more chance of spreading infections between animals.

3) Identify bacterial proteins suitable for vaccine production using modern genomic approaches.

4) To vaccinate cows with a digital dermatitis treponeme vaccine and assess immunity both before and after vaccination.

5) To carry out studies of beef cattle to determine their exposure to digital dermatitis treponemes.

6) To identify how digital dermatitis treponemes may</td></tr>
</table>

	evade the host immune system. 7) To identify and define the genetic component of host susceptibility/resistance to infections leading to 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)?	Digital dermatitis is a very common, very serious problem in cattle and sheep and the project will analyse the causative bacteria (treponemes) with a view to improving both prevention and treatment of infection. By generating an effective vaccine to stop infections with the treponemes we will prevent the disease and thus also stop transmission between animals. This approach will also mean that the need for treatments will be reduced and possibly eradicated. Such an outcome will have very beneficial side effects of reducing the need for antibiotics and toxic footbath products too. Hence the many beneficiaries include the cows, farmers (reduced treatment costs), agroeconomics, the dairy and sheep industries as a whole and food security for the nation (and worldwide) will be significantly enhanced.
What species and approximate numbers of animals do you expect to use over what period of time	Cattle – 1,400 Sheep – 500
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 procedures carried out as part of this study are classified as mild. The techniques involved are blood sampling, biopsy collection and vaccination which are routine procedures in veterinary practice. There are few adverse effects from the procedures involved. Good handling of the cattle or sheep and the experienced veterinary surgeons undertaking the procedure will minimise any discomfort to the animal. For blood collection and vaccination there will inevitably be the mild discomfort associated with venepuncture and the possibility of haematoma. Side effects from vaccine are potential granulomas But are greatly outweighed by the good that a vaccine may do to reduce the prevalence of this severe infectious disease.

	After all procedures all animals will be discharged from the controls of the Act and returned to stock on the farm, for disposal according to normal agricultural practice.
Application of the 3Rs	
1. Replacement **State why you need to use animals and why you cannot use non-animal alternatives**	Ruminants are the animals which suffer this severe infectious disease called digital dermatitis. Therefore they are the most appropriate animals to study the disease in especially since it is now endemic across the UK and therefore we will be sampling/vaccinating animals with naturally occurring DD so that these animals may benefit from future preventative measures such as farm management practices and vaccines that might begin to reduce this important infectious disease. No disease model exists for this disease and there is little need for it given the high prevalence of the disease throughout ruminants across the UK.
2. Reduction **Explain how you will assure the use of minimum numbers of animals**	We assure the use of minimal animal numbers by use of calculations based on statistical significance and use of power calculations as valid. We have also consulted with a biomedical statistician at Manchester University to minimise animal numbers in the project.
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.**	Ruminants are the animals which are continually suffering with this severe disease. Therefore given the high prevalence of this disease ruminants are the most refined choice for this work. The specific animal involved is of the upmost importance for example we are interested in the different immune responses in dairy cattle and beef cattle as these two livestocks have different prevalences of the disease. Understanding the differences in exposure may help us better understand the underlying pathogenesis and transmission routes of this disease. The general measures taken to minimise harm to the animals include Good handling of the cattle or sheep and the use of experienced veterinary surgeons undertaking the relevant procedures will minimise any discomfort to the animal. The procedures involved eg blood sampling, vaccination and biopsy are the kind of procedures normally conducted in veterinary practice.

PROJECT 13	**Development of sustainable aquaculture protocols**	
Key Words (max. 5 words)	Sustainable aquaculture, pelagic-spawning, angelfish	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
		Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals[22]
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The overall aim of the programme is to develop techniques for the commercial culture of pelagic-spawning (where spawning takes place in open water) reef fish that are common place within the global marine aquarium trade. Success will ultimately increase the diversity and availability of farmed species within the trade. This will reduce the number of fish collected from the wild. Protecting natural fish stocks from overexploitation, and the environment in which they inhabit from destructive collection practices. In addition, farmed fish are better adapted to life in captivity compared to wild collected fish. Key objectives will include: i) An investigation of the reproductive behaviour and spawning requirements of dwarf angelfish in captivity, including their environmental (temperature and light levels) and dietary requirements (food types and feeding frequency). ii) Research into rearing techniques for eggs and the early life stages of pelagic-spawning reef fish, examining a variety of aquarium designs and husbandry techniques in order to enhance development. iii) Assessment of novel diets for the early life stages of pelagic-spawning reef fish, The	

	provision of suitable food items for early life stages of reef fish has been identified as arguably the most important factor contributing to the success or failure of farming activities and is considered a 'bottleneck' in rearing efforts. IV) Identification of suitable egg and early life stage rearing conditions and housing requirements.
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)?	Farmed reef fish offer significant improvements for animal welfare over their wild counterparts. Wild collected animals are transported over long distances and for long periods of time. Fish often experience stressful conditions during transport, often leading to death. Fish require quarantining prior to sale in order to eliminate parasites and other diseases. Farmed fish however can be produced and distributed within the destination market and subsequently need only be transported over comparatively short distances and time periods, thereby reducing their carbon footprint. It is recognised that farmed fish are considered to be healthier, being preadapted to life in captivity having been weaned onto commercial diets from the early stages of their development. The scale of the ornamental fish trade is too vast to be accounted for by the farming of reef fish within the foreseeable future. However, establishment within destination markets could form part of a sustainable management strategy. It could off-set some of the environmental impacts of the traditional supply network, such as long-distance overseas air travel, multi-stage distribution network, extensive infrastructural demands, wild collection, handling and shipping mortality.
What species and approximate numbers of animals do you expect to use over what period of time?	Dwarf angelfish are one of the most popular and heavily traded of all groups of marine ornamental fish accounting for *c.* 8% of the total trade. Our primary aim is to study the flame, lemonpeel, coral beauty and bicolor angelfishes, all of which are popular within the global marine aquarium trade. Most research to date on the farming of pelagic-spawning reef fish has focused upon the flame angelfish, hence there are solid foundations from which commercial culture techniques can be built. In addition we will study the early life stages of other commercially desirable pelagic-spawning reef fish species (Regal and Yellow tangs). We plan to use approximately 75 adults, *500* juvenile and 5,000 larvae per dwarf angelfish species. For both species of tangs we plan to use 1,000 larvae and 300 juveniles per species.

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 procedures that are included are classified as mild in severity and in general all the methodologies are modifications to those that have been tried and tested. These will be performed by well-trained and highly experienced staff. Some observational work will be carried out on the development of embryos within the eggs and hatchlings younger than 3 days of development (prior to first-feeding hence too young to be under the Act). At the end of the project mature adults will be maintained as a source of eggs and larvae for future projects while juveniles will be reared to adulthood for use in future studies. Fish no longer required will be euthanised at the end of the project using a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The basis for this study is the development of culture techniques for live animals and therefore there are no non-animal alternatives.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Reduced numbers of animals is achieved by using a small group size over an appropriate number of replicates and where possible a factorial design will be used.
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 angelfishes are listed among the top ten families of fishes that make up the international trade of marine aquarium species. Within this family, the dwarf angelfish are one of the most popular and heavily traded of all marine angelfishes accounting for c. 8% of the total trade in marine aquarium fishes. Our primary aim is to study the flame, lemonpeel, coral beauty and bicolour angelfishes. Most research to date has focused upon the flame angelfish which has been described as a model species in the field of reef fish culture, hence there are solid foundations from which commercial culture techniques can be built. Fish showing signs of illness will be given the proper treatment or killed by a schedule I method.

<table>
<tr><td>PROJECT 14</td><td colspan="3">Development of a novel humane constricting device for the castration and tail docking of lambs and castration of young bovines</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Lamb calf castration taildocking</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)[23]</td><td colspan="3"><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>Yes</td><td></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[24]</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">To make the necessary procedures of castration and tail docking of lambs and the castration of calves more humane by reducing pain.</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 colspan="3">Reduction of the pain suffered by livestock during these normal, and in most husbandry systems, necessary ‘mutilations’. These farm procedures prevent uncontrolled breeding, male aggression and Blow Fly Strike.</td></tr>
<tr><td>What species and approximate numbers of</td><td colspan="3">Approximately 80 to 120 lambs per year (which can be returned to stock) and an estimated 40 to 80 calves per year (which also can be re-used with Home Office approval). The initial project is</td></tr>
</table>

animals do you expect to use over what period of time?	expected to take 3 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?	At worst the animals will undergo a husbandry procedure (castration, tail docking) which is common and legal practice in the UK. This is painful for up to 3 hours. The severity is moderate. At the end of the experiment the animals can be returned to stock.
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 assess efficacy of castration, docking and analgesia other than in the living animal of the target species.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Engineering test-rigs are used during the development process. Experiments will be designed to show the efficacy of both the procedure and the analgesia simultaneously. Group sizes will be advised by CSIRO / BIOSS statisticians.
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.	Lambs and calves are the farmed species which these procedures are practiced on in the real world. Efficacy must be demonstrated in the target species. Where novel analgesia delivery is seen to fail a veterinarian (each of the co-operating research groups include experienced large animal veterinarians) will administer analgesia by conventional methods.