Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2015

Volume 8

Projects with a primary purpose of: Translational and applied research – Human infectious disorders

Project Titles and keywords

1. **Animal models for bacterial pathogenesis and therapies**
    - Treatment infection development imaging antimicrobials

2. **Tuberculosis pathogeneses & treatment in zebrafish**
    - Zebrafish, tuberculosis, *Mycobacterium marinum*, therapeutics

3. **Pathogenesis of prion diseases in sheep**
    - Prion, BSE, vCJD, transfusion

4. **Immunopathogenesis and drug resistance in African trypanosomes**
    - Trypanosomes, pathogenesis, immunity, drug resistance

5. **Training in murine polio intra-spinal inoculations**
    - Training polio intraspinal inoculation

6. **Evaluation of avian and mammalian influenza virus infections in animals as models for human disease**
    - Avian and mammalian influenza, model for human disease

7. **Malaria: Development of Vaccines, Drugs and TBIs**
    - Malaria, mosquito, vaccines, drugs, transmission

8. **Biology and control of protozoal infections**
    - Malaria, *Leishmania*, Trypanosomes, drug, vaccine

<table>
<tr><td>Project 1</td><td>Animal models for bacterial pathogenesis and therapies</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Treatment infection development imaging antimicrobials</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5yrs</td></tr>
<tr><td>Purpose of the project (as in Article 5)</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>
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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>As we run out of ways to treat bacterial infections new approaches need to be developed before we are unable to cure diseases which we used to be able to.

The aim of this project is to identify new antimicrobial targets for the treatment and control of bacterial infections.

Also to develop new ways of detecting bacteria with imaging technology, so infections in hard to reach places such as hip replacements can be identified and treated.

We will use imaging techniques for target validation, lead optimisation and safety assessment in the discovery and development of new antimicrobials for the treatment of infection in man</td></tr>
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	and 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 the identification of new antimicrobial targets and treatments for the bacterial infections. This will provide us with new specific targets to generate novel antimicrobial compounds and so enable us to treatment infection.
What species and approximate numbers of animals do you expect to use over what period of time?	All the animals in this project will be mice, a total of approximately 5,000 mice are expected to be used over the 5-year period of this licence will be in force however there will be continued efforts to minimise these numbers
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?	In all cases the animals used within these studies will be closely observed all animals will be routinely anesthetised for imaging and bacteria administration. Substances will be administered to treat the symptoms of disease states, so testing the effectiveness of the new antimicrobial. The imaging employed is non-invasive and combined with our experience in both imaging and research of new medicines any potential adverse effects will be managed and minimised. Using this imaging technology we can reduce the amount of animals we need to use as we can see the disease change within the same animal over time. The duration of infections will be the minimum required to establish a clinical disease and assess the antimicrobial drug action.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Although we can use tests in a tube and tests in an invertebrate mini-host model they cannot replace the complexity of the infection response in a mouse or a human.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Using whole animal imaging technology we can track the infection process within the same animal at multiple time points. This substantially reduces the numbers of animals required compared to existing models and also allow us to observe changes in bacterial numbers and the effects of the

	antimicrobials non invasively.
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 infection models permit the assessment of multiple parameters and also permits the observation of real time changes in the infection process within the same animal. We have clearly defined end points, with experimental protocols that follow regulated standard operating procedures and are performed by trained staff. We are continually refining the sensitivity of the imaging technology enabling a reduction of the bacterial load required for the validation of the infection process and screening of antimicrobials. Small-scale pilot studies will be employed to investigate novel genetic mutations, new imaging markers and new antimicrobials to minimize the animal numbers during early investigations when unexpected outcomes are most likely. All imaging and infection events are carried out under anaesthesia, so reducing stress to the animal and mice are routinely monitored and scored regularly for signs of distress.

<table>
<tr><td>Project 2</td><td>Tuberculosis pathogeneses & treatment in zebrafish</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Zebrafish, tuberculosis, Mycobacterium marinum, therapeutics</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)</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>
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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>We are trying to understand the basic disease mechanisms of tuberculosis, a complex infectious disease where both host and bacterial determinants interact in a choreographed fashion. Tuberculosis remains a serious health problem globally, with ~ 2 millions deaths and 10 million active cases a year. The existing vaccine BCG is not very effective and the antibiotics though effective take 6-9 months for reliable cure. This long treatment time is a significant barrier to the eradication of TB. Therefore there is a major unmet need for fundamental data about the way TB invades and establishes itself in the host organism and to find new effective treatments.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td>By using our zebrafish model our work will provide fundamental data about how the TB bacterium</td></tr>
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project (how science could be advanced or humans or animals could benefit from the project)?	evades and exploits host immune mechanisms to persist and cause disease. This work could lead to new approaches to TB treatment and vaccination.
What species and approximate numbers of animals do you expect to use over what period of time?	6,460,000 larva and 109,000 adults 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 animals will become infected and in the case of the adults eventually suffer from the consequences of a TB-like disease manifesting lethargy, surface hemorrhages, and weight loss. Animals will eventually become moribund and die from the infection, within weeks to months depending on the inoculum. However it is anticipated that no more than 7.5% of animals will die and in all other cases intervention will occur such that animals showing clinical signs are killed before they become moribund and are likely to die. All animals will be euthanized at the end of predetermined infection protocols.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Tuberculosis is a complex disease involving interactions of the bacteria with many different types of cells and tissues in the host. This mileu is virtually impossible to replicate outside the host.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We have taken measures to reduce the variability of infection developing techniques to deliver similar numbers of bacteria into each animal. In the case of the larval fish, which we use to the extent possible, we have developed a technique to sequentially monitor infection within the same animal over time, thus reducing the number of animals needed for each experiment. For all experiments, we use the minimum number of animals required based on prior experience. For new experiments, we run small pilot studies to assess the impact of a condition and how much it varies among the animals. This then allows us to

	use statistical calculations to determine the minimum number of animals that we can use.
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 zebrafish is a natural host to tuberculosis caused by *Mycobacterium marinum* that is a close relative of *Mycobacterium tuberculosis*, the agent of human tuberculosis. The mechanisms of disease appear to be conserved between zebrafish and humans. The zebrafish has proven to be an ideal model for the study of tuberculosis and has enabled us to address questions that have been elusive in the more traditional models of tuberculosis - mice, guinea pigs, rabbit and more recently nonhuman primates. We have further refined the system by using larval rather than adult fish to the extent possible and limiting the studies to a few days where possible. We are always seeking to refine our techniques so as to use fewer animals for shorter time periods.

Project 3	**Pathogenesis of prion diseases in sheep**	
Key Words (max. 5 words)	Prion, BSE, vCJD, transfusion	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	√	Basic research
	√	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	This project aims to better understand the potential risks posed by transmission of infectious brain diseases, called prion diseases, through blood transfusions. Prion diseases include variant Creutzfeld-Jakob disease (vCJD) in humans and bovine spongiform encephalopathy (BSE) in cows. Individuals infected with prions can remain healthy for years before developing signs/symptoms of brain disease. They may even remain disease-free during their natural lifespan. Recent studies of tonsil and appendix samples that have been removed from otherwise healthy patients estimate that as many as one in 2000 people in the UK may be carrying vCJD without outwards signs of disease. There are currently no tests that can easily detect individuals with these “silent” infections, known as subclinical infections. In the human population they	

	pose a potential risk to others through blood and organ donation. There have been several vCJD cases attributed to known exposure to infected blood products. We have previously used sheep infected experimentally with BSE as a model to study the risk of transmitting prion infection by transfusion of blood components that are commonly used in humans, such as red cells and plasma. These experiments showed that BSE could be transmitted by transfusion of different blood components. However, a significant number of sheep that were transfused with components from infected donors did not develop disease, even after follow-up over several years. This project will continue to monitor the surviving sheep for clinical signs of disease. We will perform in-depth sensitive analyses of their tissues after death to determine how many of the animals had been infected by the transfusion. In some circumstances, it may be necessary to confirm the levels of prions in infected tissues by inoculating tissue extracts into mice. The aim is to determine the frequency of subclinical infections in a population that has been exposed to prion disease through blood transfusions.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project offers a unique opportunity to study the long term outcome(s) of known exposure to prion infection, in a population of sheep that has been closely monitored for signs of disease, and from which multiple tissue and blood samples have been stored and are available for further analysis. The results will allow us to determine the frequency with which infections occur without causing symptoms of disease and the factors that control these infections. They will also help us to develop new tests to identify infected individuals before they show signs of disease. The results will help us to design better strategies for the control and prevention of vCJD in humans, and also prion diseases of farmed and wild animals including sheep, cattle and deer.

What species and approximate numbers of animals do you expect to use over what period of time?	Sheep – 43 over 2-3 years Mice – 300 over 2-5 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?	For sheep, the expected adverse effects are: • Discomfort associated with blood sampling (mild severity) • Neurological signs associated with prion disease (e.g. behaviour changes, nervousness, itching, loss of coordination - moderate severity) • Age related health issues – as most of the sheep are > 7 years old, they may develop health problems due to their age rather than their infection status e.g. cancer, arthritis etc. (mild severity). For mice, the expected adverse effects are: • Infections or toxic effects following inoculation (mild) • Neurological signs associated with prion disease (e.g. lethargy or hyperactivity, gait changes, itching – moderate severity)
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	There is no alternative to the use of animals in studying the complex and long term interactions between prions and host tissues that will determine the outcome(s) of infection. Since such studies are impossible in humans, sheep represent a good experimental model. They are natural hosts of prions and show a similar pattern of infection and disease in body tissues as patients with vCJD. In addition, the large size of sheep allows collection and transfusion of volumes of blood comparable to those used in human clinical practice. For many years, inoculation of mice with tissue extracts has been used to detect prion infection, with few alternative methods available for growing prions in the laboratory. More promising techniques for prion detection that do not require mouse inoculations are now emerging, but they are still being adapted for use in sheep and other farm animals, and require validation against more established methods. We aim to adopt and develop these techniques, where possible, to reduce/or

	replace use of sheep and mice in our work.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project does not propose the use of any additional sheep, as the animals that will be used received blood transfusions under previous Project Licences. They are currently being monitored for clinical signs and providing blood samples for archiving. Mouse lines (transgenic or non-transgenic) will only be selected for detection of prion infectivity in tissues and/or body fluids if there is sufficient evidence to believe that they provide the most sensitive method available, and there are no alternatives e.g. cell lines. Experiments will be designed to use the minimum number of mice consistent with producing statistically meaningful results.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	BSE-infected sheep were chosen because they are a natural host of prion diseases, and they show a similar tissue distribution of infection as humans with vCJD. The ability to collect large volumes of infected blood from a single individual is a particular advantage. Transgenic mice that express high levels of prion protein (PrP^{C}) from another species (e.g. sheep) are more susceptible to infection with prions from that host. They are therefore often used to provide sensitive and accurate measurement of the levels of infection in tissues and body fluids from prion-infected animals/humans. Every effort is made to reduce or eliminate pain or distress associated with experimental procedures by routine use of analgesia and local or general anaesthesia, where appropriate. Animals are carefully observed for adverse effects during and following procedures. They are humanely killed if any persistent or severe effects are identified. Clinical signs associated with prion disease are carefully monitored and animals are humanely killed at the earliest time points consistent with accurate diagnosis. These end points are regularly reviewed and will be revised as new data becomes available.

<table>
<tr><td>Project 4</td><td>Immunopathogenesis and drug resistance in African trypanosomes</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Trypanosomes, pathogenesis, immunity, drug resistance</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>
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<tr><td>Preservation of species</td><td>Yes</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>Trypanosomes are parasites that cause disease in people and animals. They are transmitted by the bite of an infected tsetse fly. Some individuals can resolve the disease themselves whilst others become seriously ill. These outcomes are influenced by both host and parasite factors but little is known about what these are. The first aim of the project is to identify host and parasite factors that interact to determine disease severity in trypanosome infections. How the parasite spreads through the body after the initial infection in the skin will be investigated. The second aim of the project is to understand how and how quickly strains of the parasite that infect farmed animals become resistant to new drug treatments, and whether this development of resistance can mean the parasites</td></tr>
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	are also resistant to existing drugs in use or other classes of drug in development ('cross-resistance') (and vice versa), in order to inform the development of improved therapies.
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)?	Key benefits are likely to include an increased understanding of how the parasite causes disease, which has the potential to inform both therapeutic and diagnostic approaches. Additionally, informing on resistance/cross-resistance is essential to optimise identification of candidate therapies, and to maximise the lifetime of existing therapies. Ultimately, we hope to gather information that will lead to better treatments for people and farmed animals living in areas where the disease is present.
What species and approximate numbers of animals do you expect to use over what period of time?	We anticipate a maximum use of 1500 mice 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?	Most procedures, such as manipulating immune cell populations in the animals, do not cause discomfort. Trypanosome infection is classified as a moderately severe procedure. Adverse effects include anaemia, lethargy and reduced appetite. Any animals displaying signs of disease will be killed by an authorised method. All animals will be killed by an authorised method 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	For the drug resistance studies, the relevant species and strains of trypanosome unfortunately do not grow in cell culture, necessitating the use of animal studies. For the disease severity studies, the species can be grown in cell culture but this significantly alters the parasite such that it no longer reflects parasites that circulate in nature. Additionally, when studying the immune response and the stages of disease under particular consideration (e.g. vector bite & spread of the

	parasite through the body), unfortunately there is no system that can mimic this situation.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Good principles of experimental design and previous experience/data will be used to ensure minimum numbers of animals are used. This will include approval by internal Institutional ethics review committee for each experiment.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Most trypanosome strains grow in mice, and a significant amount of tools (e.g. transgenic mice expressing fluorescent labels, mice that are deficient in particular genes – and therefore biological processes) are only available in the mouse model. This means that detailed and specific information (e.g. what cell types are interacting and how with trypanosomes) are only possible in the mouse model. Good technique (e.g. aseptic) for each procedure as well as extensive monitoring and defined endpoints will minimise harm.

Project 5	**TRAINING IN MURINE POLIO INTRASPINAL INOCULATIONS**	
Key Words (max. 5 words)	Training polio intraspinal inoculation	
Expected duration of the project (yrs)		
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)	Oral polio vaccines (OPV) have been the principal tool used in the polio eradication programme of the World Health Organisation (WHO) so far. They imitate natural infection of the gut, immunising recipients with a high degree of safety and efficacy. They are manufactured mainly in Europe where they are subject to EU regulations, which require testing of a proportion of batches in animals by Official Medicines Control Laboratories (OMCLs) designated by the licensing authorities. Transgenic mice carrying the human receptor for poliovirus are increasingly used as a substitute for the non-human primates that were previously the only adequate models for testing vaccine safety. The overall objective of this project is to enable a small team of staff to develop and maintain manual skill in the inoculation of substances into discrete areas of the mouse spinal cord: this needs to be achieved with sufficient accuracy and reliability to satisfy the criteria for the polio vaccine test as detailed in WHO and European Pharmacopoeia regulatory documents, which specify the procedure, numbers of animals and the particular type of mice to be used. The test has been designed to minimise animal usage and suffering while producing statistically reliable results. The test is also used to qualify new attenuated polio strains to be used for new vaccine production as part of the global polio	

	eradication programme.
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 most immediate benefit of the proposed training programme will be that we will maintain a pool of appropriately qualified and competent practitioners able to perform this test in-house and perform our essential role to the continued success of the polio eradication programme, which depends on the supply of vaccines of high quality and safety. Therefore, the benefits of having these trained staff, and having the ability for them to maintain their competence, will have ramifications at the national, European and global level.
What species and approximate numbers of animals do you expect to use over what period of time?	Mouse, including genetically altered. Up to 4000 mice may be used in 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 training programme involves the inoculation of an inert dye into the spinal column of anaesthetised mice. No adverse effects are expected as the animals will not be permitted to recover from the general anaesthesia. Animals will be killed by a Schedule 1 method as part of the procedure.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Some aspects of initial training such as identifying the anatomical landmarks and making the skin incision can be carried out by looking at pictures and videos or using cadavers. However, the aim is to train the prospective operator(s) to develop the required manual skills to make the inoculation into the correct region of the spinal cord, i.e. into the grey matter. Correct and precisely placed inoculation has been shown to correlate well with 'twitch' or 'tremor' reactions in the hind limbs of test animals. Our experience and that from other organizations that have implemented this test, strongly indicates that it is not possible to observe the correct reactions on non-sentient (freshly-killed) animals; it is essential that the animals are alive (correctly anaesthetised).
2. Reduction Explain how you will assure the use of minimum numbers of animals	The training process is repeated until a satisfactory percentage positive score is reached. The ability to perform the training process on-site, coupled with our full control of the local training process means that potential trainees can be selected based on genuine experience of proficiency in Regulated Procedures, and can then be monitored closely and constantly by

	experienced operators to determine genuine rate of progress. Training can be planned carefully to maximise success in a reasonable time frame. Unnecessary repetition of aspects of training can be avoided.
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 non-recovery severity procedure so that opportunities for further refinement are limited to good induction and maintenance of general anaesthesia. It is not necessary for animals to recover from the anaesthesia and they are killed immediately following the inoculation. The vertebral column and spinal cord are then dissected out to allow for precise assessment of the accuracy of inoculation by viewing the distribution pattern of the dye.

Project 6	**Evaluation of avian and mammalian influenza virus infections in animals as models for human disease**	
Key Words (max. 5 words)	Avian and mammalian influenza, model for human 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 and mammalian influenza infection processes and disease transmission between livestock species and animal models of human disease (mice, ferrets, pigs) in relation to zoonotic and reverse zoonotic infection in order to improve animal health and welfare and reduce their impact on public health. 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. The identification and development of new diagnostic tools and maintaining fitness for purpose of those currently in use will also be progressed.	

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 particularly at the occupational exposure interface between animals and humans. This is very much an evolving process with new and emerging viruses being regularly detected and resultant implications for understanding disease development, interspecies transmission, host-virus interactions and their responses. All areas have application for improved disease control methods and strategies for animal welfare and animal and human health.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice 600 Ferret 500 Pig 300 Chicken 300 Turkey 150 Duck 150 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 and moderate depending on the strain of virus used and how it reacts in the chosen animal species and protocol. Animals will not be allowed to progress beyond a moderate severity, close monitoring and humane end-points will be employed to prevent this wherever possible. However, any sick animals will be humanely killed prior to being found dead wherever possible, i.e. at 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 and 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 A minor aspect of this work is to assess whether genetically altered mice which will be have been generated under another project licence are more resistant to influenza virus infection. No other adverse phenotypic change is expected in these mice.
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. The animal models of human disease have been chosen on the best face validity information, only relevant cross-species animals and models of human disease have been chosen. 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. 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.

Project 7	**Malaria: Development of Vaccines, Drugs and TBIs.**	
Key Words (max. 5 words)	Malaria, mosquito, vaccines, drugs, transmission	
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)	This project aims to facilitate the discovery and development of novel anti-malarial interventions by the development of vaccines, drugs or transgenic mosquitoes that target the transmission of malaria to mosquitoes. Studies to examine the basic biology of this clinically important protozoan will also be performed.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The project aims to contribute to the control and elimination of malaria. With 3.3 billion people at risk of infection, 250 million people infected with malaria every year, resulting in over 584,000 annual deaths - the development of novel anti-malaria blocking strategies is a major issue, specifically when considering the crucial status of current anti-malarial research. Our groups have crucial ongoing links with NGOs, clinicians and field sites, and our research is used at the global level to inform the use of current and future anti-malarial interventions. Additionally, malaria is a valuable model for analysis	

	of host interaction with parasites, there is inevitable overlap between basic biological studies and our primary aims (vaccine and drug development). Discoveries in malaria have had implications for immune responses to other pathogens and to immunity, and have contributed to the study of insect vectors of disease in general.
What species and approximate numbers of animals do you expect to use over what period of time?	Over 5 years: Mice = 20100; Rats = 750; Rabbits = 50.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Expected adverse effects generally include distress caused by malarial infection, specifically; pallor, piloerection, reduced mobility, lethargy/weakness, respiratory distress. Animals will be culled immediately if adverse effects are experienced. Likely severity levels are Mild/Moderate. Animals will be checked daily following infection with malaria. Increased frequency of monitoring may be undertaken for certain procedures and remedial action taken as advised by the NVS. Animals will be humanely killed at the end of the studies.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Human malaria is caused by several species of Plasmodium most of which are specific for humans and some can also infect other primates. However, due to ethical and safety restrictions, the transmission stages of human malaria are experimentally intractable, and animal models are the only models for such research. The rodent malaria parasite P. berghei is a safe, versatile, biologically relevant and reliable model to study malaria transmission in laboratory mice and rats. In vitro culturing of this parasite is not feasible except from very few stages of its lifecycle, therefore replacement of the mouse model is currently not possible or experimentally relevant. In addition, maintenance of mosquito colonies requires regular provision of a bloodmeal for egg

	production, which is best done through feeding on live animals due to the mosquito physiology and behavioural biology. Therefore, although much of the mosquito maintenance is carried out through artificial blood feeding on human blood products, mosquito feeding on live of animals remains an essential component of this research. Furthermore, feeding on human blood products cannot precede feeding on live animals for P. berghei infection and testing of antimalarial drugs and vaccines due to immunological interference or feeding on humans for testing the efficacy of transmission blocking interventions due to ethical and safety restrictions. For all the above reasons, feeding of mosquitoes on live animals is required for some of the mosquito maintenance procedures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals proposed to be used under this project licence will be reduced by using well-established, robust systems for propagation and generation of material. The robustness of these refined systems greatly reduces the number animals used, as -100% of animals become successfully infected upon inoculation. Our long experience of working with these systems enables us to reduce the number of animals used by careful planning, using appropriate controls and replicas to avoid unnecessary repetition. Mosquito colonies are maintained on rats or mice. By using the appropriate rodent species according to colony size we can minimise the number of animals used. Regulated re-usage of the same rats for colony feeding at no more than six feeding occasions enables us to further reduce the number of animals we use. Animals are used in rotation so that no animal is used more than once every four weeks Experience indicates that no adverse effect, long or short term, results from repeated exposure to mosquito bite.
3. Refinement Explain the choice of species and why the animal model(s)	In almost all our work, infecting parasite inocula are large (to ensure rapid establishment of infection), animals are monitored daily and are used within a few

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.	days of infection. Under these conditions infections are well tolerated, causing little discomfort to hosts, with animals typically displaying a normal behaviour. The procedures outlined above inhibit establishment of chronic infection. Different species of mosquitoes display different preferences for blood sources. Rats and mice are well tolerated by our colonies as a blood source. This maximises progeny output following feeding, which intrinsically reduces animals usage. The fact that mice and rats are well accepted by our colonies also reduces feeding time, allowing feeding under conditions of gentle anaesthesia. Light anaesthesia, in combination with rehydration following feeds ensures that animals rapidly recover and experience minimal discomfort. During antibody production, resulting immune responses are monitored by taking small (-3Oul) blood samples by the least invasive method. Different immunization programs are established to raise robust immune responses, ensuring maximum chance of success using the smallest number of animals under minimal duress.The appropriate animal species will be chosen to best serve the procedure whist using the least number of animals. Rabbits and rats will be favored over mice for larger scale antibody production, as dictated by naturally available serum quantities at the end-point. Vaccines which do not produce ulcerative effects will be preferentially used. Any animal showing symptoms exceeding w mild severity limit will be killed using a humane method. For the study of potential anti-malarials, animals will be given doses based on data derived from in vitro studies, and initial doses used are not anticipated to cause health problems since compounds have been tested for cytotoxicity and genotoxicity. Known anti-malarials, which are investigated for their effects on transmission or liver stages, will be used at doses equivalent to those already routinely used for humans and are unlikely to result in toxicity effects.

Project 8	**Biology and control of protozoal infections**	
Key Words (max. 5 words)	Malaria, *Leishmania*, Trypanosomes, drug, vaccine	
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)	The overall objective of the programme is to improve our knowledge of the biology of malaria, *Leishmania* and trypanosome parasites and to exploit this knowledge in the development of new tools for the control of these diseases. Currently, drugs are the mainstay for control of these diseases, however, there is an over-reliance on a limited number of effective compounds and drug resistance is a serious threat to their use. This programme seeks to screen for new compounds or therapeutics and will evaluate their mode of action or in combination with other drugs. To date there are no licenced vaccines for human use against these diseases. Focusing on *Leishmania*, we seek to evaluate new and existing experimental vaccines to determine the immune mode of action and correlates of protection. As part of this we will develop methodologies to use infected sand fly vectors to deliver infection – to select for antigens or immunomodulators that can protect against natural	

	challenge. Understanding the pathogenesis of infection that most closely resembles the course of infection in the wild will be essential for identifying targets for control. To achieve this, we will investigate the interactions between the parasite, the vector, and the mammalian host. Specifically, we aim to dissect the immune responses that control infection and pay close attention to the role of parasite- and vector-derived virulence factors that permit sand fly transmission and promote 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)?	• Identify new drugs or new modes of anti-protozoal action. • Identify effective drug combinations. • Provide new data on the activity of drugs and mechanisms of resistance. • Evaluate the immune mechanisms that govern parasite infection, disease progression control and immunity to reinfection. • Evaluate experimental vaccines against *Leishmania*. • Dissect the interactions between parasite and insect vector that enable transmission and insect-born infection, that may identify novel transmission-blocking opportunities. • Develop new methods to evaluate vaccines and prophylactic drugs, using non-invasive, real-time bioluminescent/fluorescent imaging. • Develop new methods to initiate infections from the bite of infected arthropod vectors, i.e. mimicking natural infection. • Study the pathogenesis and immunology of natural infection initiated by insect bite.
What species and approximate numbers of animals do you expect to use over what period of time?	Approximate yearly animal usage (for 5 years): Mice: 2500 Hamsters: 64
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	Protocols requiring the provision of cells or tissues or to maintain colonies of arthropod vectors are unclassified in their severity level because the animals will be humanely killed.

end?	The majority of the project is expected to be of mild or moderate severity. Most procedures involve injection of agents or compounds that result in transient irritation. The majority of infections are moderate severity in which parameters of infection, such as weight, size of cutaneous lesions or parasite load in the circulating blood will determine endpoints for experiments. At the end of the experiment animals will be humanely killed in order to determine (i) the level of infection, e.g. to assess the protection offered by an experimental vaccine, (ii) the transmissibility of parasites to arthropod vectors, (iii) the pathology associated with infection and/or (iv) the immune response of the host to infection. In those parasite species in which the onset of disease is known to be rapid and vigorous, and may involve the central nervous system (CNS) the severity limit is considered severe and will be closely monitored to prevent undue suffering.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	At present animals are an essential part of pathogenicity, drug and immune-related research. Many *in vitro* systems lack the complexity of pharmacokinetic and immunological parameters that are inherent to whole animal models and vital information will be missed. The group is involved in evaluating *in vitro* 3D models of both skin and liver, which we hope, in time, will replace some *in vivo* experiments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	• Random allocation of animals to treatment groups to reduce bias. • Statistical tests for significance will be performed. • Number of animals used in experiments will be the minimum needed to provide sufficient cells/tissues/parasites for *in vitro* assays, for determination of immune function and to achieve adequate statistical power. • Dressings may be employed to evaluate topical treatment in cutaneous leishmaniasis to prevent rapid removal of the formulation by licking and reduce the need for repeat

	experiments. • Non-invasive bioluminescent/fluorescent imaging will reduce the number of animals required for evaluating the success of long and short-term interventions. • Sample sizes will be continuously reviewed in the light of analysis of the data as it becomes available. • Sharing control groups or using control groups for analysing multiple outcomes, where appropriate, will reduce animal numbers further.
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.	Rodents are natural hosts *Leishmania* and the mouse is the host species of choice for *Leishmania* and trypanosomes. Since there are no suitable models for human malaria species, rodent malaria models are considered essential for anti-malarial drug efficacy evaluation and immunological studies. It is currently impossible to maintain all the different stages of the complex malaria life cycle *in vitro* therefore animal passage is unavoidable at present. Mouse models have been used and validated extensively in the past and continues to provide much of our knowledge to date on protozoal infections.