Animals (Scientific Procedures) Act 1986

Non-technical summaries for project licences granted during 2016

Volume 17

Projects with a primary purpose of: Translational Applied Research - Human Infectious Disorders

Project Titles and keywords

1. **Diagnosis of Toxoplasmosis**
    - Toxoplasma, Toxoplasmosis, Diagnosis

2. **Host defence peptides: modulators of inflammation**
    - Inflammation, Infection, Antimicrobial

3. **Development of Malaria Vaccination and Control**
    - Vaccines; mosquito; vector control; immunisation

4. **Systemic host-pathogen interactions and disease**
    - Infection; malaria; bacteria; sepsis; severe

5. **Evaluation of novel treatments for disease**
    - Emerging viral infections, neutralising antibodie**s**

6. **The Production of Normal blood and Tissue**
    - Production, normal blood, tissue

7. **Pathogenesis and Prevention of Bacterial Sepsis**
    - Sepsis, bacteria

8. **Immunopathology of experimental malaria infection**
    - Malaria, immunity, brain, immunopathology

9. **Isolation and propagation of virus in eggs**
    - Influenza, viruses, hen’s eggs

10. **In-Vivo Models of Infection and Inflammation**
    - Infection, inflammation, immunology

11. **The Production of Antiserum, Protozoan Antigens and Tissues**

Project 1	**Diagnosis of Toxoplasmosis**	
Key Words (max. 5 words)	Toxoplasma, Toxoplasmosis, Diagnosis	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	Provide Toxoplasma cells and Toxoplasma antigen for human diagnosis and for molecular epidemiological surveillance of infection in the UK population with toxoplasmosis.Human diagnosis includes approximately 14000 patients per annum and direct support for the UK National External Quality Assurance Scheme (UK NEQAS) for the serogiagnosis of Toxoplasma infection which provides quality assurance for diagnostic laboratories throughout the UK and also elsewhere in Europe and more widely. Obtain new isolates of Toxoplasma from human patients to add to knowledge on the relationship between strain type of the parasite and disease in humans.	
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)?	Early and accurate diagnosis of toxoplasmosis can reduce the impact of severe or life-threatening disease in vulnerable clinical groups including unborn children, organ transplant recipients, cancer patients and patients with immunodeficiency.	
What species and approximate numbers of animals do you expect to use	Mice: 1500 per year for 5 years (7500 total) Chicken eggs: 200 (average) per year for 5 years	

over what period of time?	(1000 total)
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Mild discomfort associated with inoculation of mice (typically manifesting as short-term increase in vocalisation limited to within the duration of the procedure) may occur in less than 1 in 100 cases and some mild symptoms of infection (decreased movement, tremor or the very early stages of abnormal posture) may occur in less than 1 in 1000 cases, resulting in an overall moderate level of severity. Animals will be killed 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	Toxoplasma cells are parasites that grow inside animal hosts. Toxoplasma grows less well in tissue culture and is not suitable to provide the necessary accurate diagnosis of patients that underpins potentially life-saving treatment. This is because the Toxoplasma cells produced in this way are more morphologically heterogenous and typically smaller than cells produced by in vivo culture so that microscopic evaluation for cell killing (a key step in the Dye Test) is technically difficult and unreliable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Human diagnostic testing has been streamlined to require the minimum number of Toxoplasma cells. This ensures the minimum number of animals is used for growing Toxoplasma. Success to date has been a 12% decrease in the number of mice required to offer and maintain a comprehensive national reference service, and this remains under constant review with a view to further reduction as advances in technologies permit.
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.	Mice are chosen for culture of Toxoplasma since these are the competent host species for this parasite with the lowest degree of neurological sensitivity where whole tachyzoites can be collected in a form suitable for human diagnostic testing. Animals will be housed in an enriched environment for the minimum time and will be monitored regularly when infected so that any early signs of infection can be identified, and action taken to minimise suffering, at the earliest opportunity.

Project 2	**Host defence peptides: modulators of inflammation**	
Key Words (max. 5 words)	Inflammation, Infection, Antimicrobial	
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)	Infectious diseases, and our ability to treat them, are an area of urgent concern internationally, particularly given the rise of antibiotic-resistant bacteria and the potential for rapid global spread of viruses for which no vaccines are immediately available. Targeting successful natural host defences represents an attractive strategy for developing novel preventative and/or therapeutic approaches. This requires knowledge of naturally-occurring modifiers of inflammation/immunity in health and disease. The overall goal of this project is to understand the highly successful mechanisms by which “Host Defence Peptides” (HDP; antimicrobial agents produced naturally by all animals) function to prevent infection in the healthy state, both through their direct effects on disease-causing infectious organisms and by modifying the natural responses of the host to infection. Studies will focus on naturally-produced HDP in lung, gut and skin infections, then assess new treatment approaches based on increasing the levels of naturally-produced HDP, or therapeutic use of novel HDP-based drugs. The main objectives are:	

	• Determining how HDP enhance protection of the lung against infection. • Determining how HDP enhance protection of the gut against inflammation and damage. • Determining what cells are most important for the production and effects of HDP. • Determining whether aging, and/or the specific composition of normal healthy gut bacterial populations can regulate HDP to influence susceptibility to infection. • Determining how HDP enhance protection of the skin against bacterially-mediated damage. • Determining the effectiveness against infection of increasing the levels of naturally-produced HDP. • Determining the effectiveness against infection of treatment with novel HDP-based drugs.
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 future benefits of this project are expected to be the development of new treatment approaches for infectious diseases, which can avoid problems of microbial resistance by promoting successful natural host defences (therefore not targeting killing of microbes directly with the new treatment), and complement and/or replace current treatments (such as antibiotics).
What species and approximate numbers of animals do you expect to use over what period of time?	Approximately 5200 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?	For the vast majority of mice, the expected level of severity is mild (or subthreshold), with 3000 mice used purely for breeding purposes (maintaining and generating genetically-modified animals with no overt harmful characteristics, but altered susceptibility when challenged in disease models). A proportion of mice will experience consequences of moderate severity in disease models of a) lung (e.g. acute bacterial infection causing decreased spontaneous activity, decreased body temperature, and laboured breathing, or weight loss during longer, milder infections), b) gut (e.g. weight loss, diarrhoea and abdominal discomfort), c) skin (tenderness to a small area of skin) or d) associated with aging (e.g. spontaneous generation of tumours). A small percentage (~1-2% of all mice used on the project) may experience more severe consequences (e.g. a few hours of minimal activity and laboured breathing,

	greater weight loss, or bleeding with diarrhoea). However, all models will be of the minimum severity possible, while ensuring the use of the animals has maximal value in addressing the scientific questions. Mice will be closely monitored and culled in the event of unexpectedly severe outcomes. All animals will be humanely killed 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	This research has a very substantial emphasis on preliminary and complementary studies, evaluating HDP on cell lines, primary cells (e.g. blood cells), and tissue samples (e.g. whole human skin samples). However, the aims of this project can only be completely addressed by incorporating studies using animals, because the protective effects of HDP are reliant on specific cellular interactions/responses that are restricted in time and place within a whole live animal in a manner than cannot currently be reproduced in other ways.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The number of animals used will be minimised by effective use of 1) experiments with cultured cells to generate and test hypotheses before using animals, 2) experimental approaches that provide repeated measurements to minimise culling animals at multiple timepoints (e.g. weight loss, skin water loss, lung function, *in vivo* imaging), 3) experimental approaches that maximise the number of outcome measurements assessed per animal, and 4) strong experimental design (utilising pilot/dose-finding studies, local and published data to enable power calculations) via collaboration with an expert in biostatistical and experimental design to use fully crossed factorial designs, using sibling matching and incorporating block effects (for cage factors and experimental repeat variability), enabling statistical analysis by general linearised model, assessing all major variables and increasing power to detect significant effects with fewer mice.
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	Mice are extensively characterised with regards to immune responses, lung and gut physiology, and infectious lung diseases and colitis models, with validated models available for this project that replicate key aspects of human immune processes and responses to infection. Importantly mice also produce HDP that are very similar to the human HDP of interest, with highly conserved functions, and the genetically-modified animals required for these

(harms) to the animals.	studies are available and characterised. The disease models chosen are well characterised and provide very clear outcome measurements that are the least severe which discriminate between experimental groups (and in which lethality is predicted not to occur), minimising animal suffering. Specifically, lung infection models will utilise a) bacteria, for which antibiotic resistance is an increasing problem, in acute clearance models in which effects of HDP can be clearly observed in short time-course studies, and b) viruses, for which treatments are urgently required, in models that do not cause severe symptoms and in which the effects of HDP can be clearly observed by serial assessment of small weight losses. Gut disease models will be restricted to short protocols that have been demonstrated to minimise adverse effects, and skin models will only induced altered levels of skin water loss, assessed by a non-invasive assay and expected to be non-symptomatic. In addition to choice of refined models, and specific recent refinements to these models (e.g. use of a heated cage rack for mice with acute lung infections), welfare costs will be minimised through the use of careful monitoring, clearly constructed clinical scoring systems, analgesia where appropriate and introduction of any further refinements identified during the course of this licence, following discussion with the named vet and home office inspector.

Project 3	**Development of Malaria Vaccination and Control**	
Key Words (max. 5 words)	Vaccines; mosquito; vector control; immunisation;	
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)	To understand key features of mosquito biology that are essential for the success of this insect in being such a good vehicle for the transmission of the malaria parasite. To understand the role of key proteins on the surface of the parasite that cause many of the deadly symptoms associated with the parasite.	
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 could lead to novel mosquito control methods that rely on either interfering with the ability of the mosquito to reproduce or the ability of the parasite to successfully mosquito and human cells.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use approximately 2450 mice over a 5 year period and approximately 25 rats	
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 will use the animals as a source of blood that is required by the mosquito to reproduce. This is a mild to moderate procedure. In order to maintain the parasite we will occasionally need to propagate it in a mice or a rat. We intend to kill the animal using humane methods as soon as there are sufficient levels of the parasite and before the animal shows obvious symptoms of parasite infection.	

Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Maintenance of mosquito colonies requires regular provision of a blood meal for egg production, and this is best done on live animals due to mosquito behavioural biology. It is also necessary to propagate malarial parasites in mice. Mice have to be used for some procedures such as the microinjection and establishment of new transgenic lines (which require high feeding rates and a large number of progenies) and also when infections with malaria parasites are part of the experimental plan. We have halved the number of animals we expect to use over this project cycle as we have put in place procedures to replace animal feeding with membrane feeding using donated blood where possible. Although it is also necessary to propagate malarial parasites in mice, these can subsequently be used in cultures for short periods of time to perform specific experimental procedures.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We aim to be able in time to use membrane feeding more widely so that mice are only used in cases where membrane feeding proves prohibitively inefficient. We will be aiming to reduce the number of mice used by using the whole mouse blood to maximise the number of mosquitoes that can be fed per mouse. Successful preservation by freezing of parasites is possible and will be used to avoid animal wastage as the parasite strains do not then need to be maintained by continuous breeding.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	We have made several refinements to the protocol to ensure minimal pain and distress during the procedure (such as the use of eye drops). Animals will be monitored regularly for signs of any adverse effects and aseptic techniques will be used to minimise potential for infection. Animals showing signs of adverse effects will be killed using humane methods to minimise suffering and where appropriate, treated under veterinary advice.

Project 4	**Systemic host-pathogen interactions and disease**	
Key Words (max. 5 words)	Infection; malaria; bacteria; sepsis; severe	
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)	Infectious diseases like malaria and bacterial blood poisoning (sepsis) kill millions of people each year. Despite this, we don't have a good understanding of why some people get severely ill and die with a certain infection, whilst others make a full recovery. We are using data from humans with severe infections, in conjunction with mathematical models, to identify biological mechanisms which determine the outcome of infection. In order to validate these mechanisms, this project aims to manipulate these mechanisms in mice with the same types of infection and examine their effects on outcome. This is the first step to harnessing them as new treatments for malaria and sepsis.	
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)?	At the end of this project we hope to have identified and validated the role of a variety of biological mechanisms which determine the occurrence of severe infection, and which determine specific features of severe infection. We believe that identifying these mechanisms is essential to develop new therapies for malaria and sepsis in humans, which can be used alongside current antimalarials and antibiotics. Our discoveries may also provide novel solutions for the increasing threat of drug resistant infections.	

What species and approximate numbers of animals do you expect to use over what period of time?	The experiments will be conducted in mice. Over a 5 year period we expect to use up to 3250 animals
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 order to study life-threatening infections, we need to infect animals with malaria parasites or bacteria which are capable of causing severe illness. With careful monitoring we expect that the vast majority of animals will have a moderate outcome, experiencing relatively few adverse effects such as moderate reduction in activity, change in appetite and moderate loss of weight. At the end of experiments animals are humanely killed in order to collect specimens. Approximately 25% of animals will undergo procedures where they are at risk of developing more severe manifestations of disease; we expect less than 2% of these animals to die. Animals in these severe category protocols may develop abnormal movements, lethargy, more severe weight loss, and fast breathing. Our experiments incorporate a monitoring schedule which allows us to promptly identify and humanely kill animals when they manifest the first signs which indicate they will progress to severe disease, rather than allowing them suffer severe adverse effects or die from the infection. Our previous work has shown that we can still obtain the data we need by stopping these experiments before there is severe suffering. However, there is some variability in responses between animals, which means there is a chance that a very small proportion of animals will suffer these more severe adverse effects, despite our best efforts to prevent them.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We use a variety of non-animal alternatives wherever possible, but severe infection is one disease which affects almost every organ of the body, and the outcome of infection is determined by the concerted response of these organs. It is impossible to mimic this in any way other than inside a live body. Our original identification of biological mechanisms will rely heavily on data from humans with infection, mathematical modelling, and work done with cultured cells and tissues in the lab. However, when the mechanism appears to involve the effects of multiple organ systems, there is no viable alternative but to validate it in live animals.

2. Reduction Explain how you will assure the use of minimum numbers of animals	We have strict rules to define the situation when a biological mechanism of interest should or should not be tested in animals. It must involve a mechanism which cannot be tested in cell culture, and it must involve a mechanism which is known to be active during the same type of infection in mice. We will not conduct uninformative experiments, where the mechanism of interest in humans is not relevant in the animal model. We work closely with a statistician to plan the design of our experiments, calculating the numbers of animals which will be required for each experiment to give confidence that the results will demonstrate a statistically significant difference between groups where a difference really exists. By designing experiments with multiple groups of interest, we can reduce total numbers of mice, without compromising the statistical validity of the experiments. We follow best practice guidelines for experimental design, to ensure that results are robust with the minimum number of animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Mice are the least sentient and best studied animal models of human malaria and sepsis, and there is extensive literature available for about the biological mechanisms involved in their responses. This means that for any given mechanism identified in humans, there is more likely to be data or literature on the equivalent mechanism in mice than there is for any other species. We have chosen infection models which have the simplest and most reproducible methods, and highly predictable courses of infection, allowing us to work with the smallest possible numbers of animals to achieve our objectives, and to use humane endpoints for our experiments which are scientifically robust. To minimise welfare costs to animals, we use the fewest procedures possible, and terminate experiments at the earliest possible stage compatible with a valid scientific outcome. We provide environmental enrichment to ensure that animals have the best possible welfare when they are healthy.

Project 5	**Evaluation of novel treatments for disease**	
Key Words (max. 5 words)	Emerging viral infections, neutralising antibodies	
Expected duration of the project (yrs)		
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)	x	Basic research
	x	Translational and applied research
		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)	Emerging viral infections are a global threat to public health. As evident from the recent outbreak of Ebola virus in Western Africa, the absence of treatment strategies and the genetic diversity of many emerging viruses underpin the requirement for better first line treatment strategies. Others and we have recently shown that broadly neutralising antibodies are among the most potent methods to prevent infection or even treat an already established infection. Having developed broadly neutralising antibodies against HIV and hepatitis C virus we could demonstrate that these antibodies not only efficiently block infection but also can control infection once infection is established. We now aim at translating our knowledge to other emerging viral infections including Phlebo-, Arena-, Hanta-, Filo-, Flavi-, Henipa- and Bornaviruses. Generating broadly neutralising antibodies against these viruses will provide the first viable treatment strategy against the diseases caused which result in severe illnesses and death.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the	Currently, most emerging viral diseases have no clinical treatment and supportive therapy is the only option for many people. Identifying broadly neutralising antibodies against these infections would provide an important first line treatment to prevent the initial spread of infection. Additionally, knowledge of	

project)?	how broadly neutralising antibodies are generated would greatly benefit vaccine development.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice, 9000 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 majority of this PPL is centred on immunisation of mice with viral antigen. Expected adverse effects of this are mostly associated with inflammation. One protocol in this PPL is infection of mice with Flaviviridae as a prototype family of emerging viral infections. The model used results in 10% to 20% weight loss of mice and development of symptoms of infection. This protocol is classified as moderate and all animals enrolled in these experiments will be closely monitored (at least once daily and more frequently once symptoms develop) and appropriate endpoints applied to minimise suffering. All animals will be humanely killed at the conclusion of the experiment and their tissues used for further analysis.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The study of immune responses, especially the production of antibodies, cannot be recapitulated without the use of small-animal models because of the complexity of such responses and the involvement of different cells/tissues in the whole animal. We intend to immunise animals with substances eliciting immune responses to viral particles (immunogens), after which we will use in vitro models of virus infection to develop our understanding of immune response and also for efficacy testing of novel treatments.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Recommended sample sizes were calculated using statistical analysis to ensure that excess animals are not used. We will compare multiple immunogens to a control population, thus reducing the total number of animals required in this study. To further reduce number of animals used for immunisation studies we will re-evaluate statistical power and group size requirements after each immunisation to adjust our group sizes, should immunisations reach higher immunological response rates.
3. Refinement	Only mice will be used in the studies proposed. They represent the lowest vertebrates with a considerable

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.	degree of physiological and genetic homology with humans. The aim will be to ensure experimental outcome can be satisfied in the minimum possible time using the minimum number of animals, thereby reducing the suffering of the animals. Where possible, oral gavage or dietary supplementation may be used instead of intraperitoneal injections. This is considered as a method of refinement as in some cases oral gavage is less invasive compared to intraperitoneal injection and does prevent problems with possible irritation by compounds given by the i.p route. However, inhibitor availability would dictate if oral gavage could be used. Adverse effects will be prevented by close monitoring of the animals during administration of the anaesthetic and throughout the study/research. To reduce severity, animals will be monitored through the experiments. Animals may be comforted with fluid and warmth. The risk of infection will be minimised by the use of aseptic techniques whilst dosing and a (local or general) anaesthetic will be used when blood sampling if more than transient pain is anticipated. Veterinary advice will be sought in the event of any cause for concern; any animal failing to respond to non-invasive treatment or whose condition deteriorates will be humanely killed.

Project 6	**The Production of Normal blood and Tissue**	
Key Words (max. 5 words)	Production, Normal blood, Tissue	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	X	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to provide red blood cells for diagnostic, surveillance and reference work.	
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 benefit from this project is great as far as diagnosing new and emerging strains of influenza (flu); the work also contributes to UK vaccine development. Influenza viruses are responsible for a spectrum of respiratory virus disease in humans. Each winter it is estimated that several thousand excess deaths are associated with seasonal influenza virus circulation in the UK. During influenza pandemic excess deaths are much higher as was seen in pandemics of 1957 and 1968. The animal red blood cells that we use are essential for analysing influenza, which in turn helps to produce a picture of which influenza strains are circulating in the community. More thani 5 million doses of influenza vaccine are given in the UK annually, and it is an important part of the global vaccine program to perform detailed analysis of virus	

	strains in a timely fashion.
What species and approximate numbers of animals do you expect to use over what period of time?	We use turkeys and guinea pigs in the main as their red blood cells are highly compatible with certain strains of influenza (flu). We also have authorisation to withdraw blood from mice, rats, hamsters, rabbits and ferrets.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The level of severity is mild and once the animals have reached the authorised amount time on licence some may be transferred i.e. reused on a different licence, although this is contingent on the overall health of the animals and this will be confirmed by the Named Veterinary Surgeon who knows the animals' history of health and wellbeing. Some animals are culled by a schedule one method which is accredited by the Home Office.
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 HI and HAl assay at present. There is no replication or synthetic blood replacements on the market.
2. Reduction Explain how you will assure the use of minimum numbers of animals	A minimal amount of animals are used for these tests, as we only require a small volume of blood to carry out the tests.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	Animals are kept in harmonious groups although the stocking density is low and this affords the animals more access to space, food and water. Environmental enrichment is also provided and this alleviates boredom and gives the animals an occupation which allows them to display natural behaviour. The animals have a long period of rest between bleeds e.g. the birds have 7 weeks rest between bleeds. Avian red blood cells are vital for the diagnosis of certain strains of influenza and the same can be said for the guinea pig red blood cells. Animal welfare is optimum and a high priority, all of the animals are healthy and given a variety of foods and enjoy human interaction. As a

	refinement general anaesthesia is used briefly to obtain samples of blood from the animals as this eliminates contingent suffering. The animals are brought around to recovery with no lasting effects and this method avoids unwanted and unnecessary stress being placed on the animals.

Project 7	Pathogenesis and Prevention of Bacterial Sepsis
Key Words	Sepsis, Bacteria
Expected duration of the project	5 year(s) 0 months

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

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

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

The project aims to determine whether specific features in bacteria and patients can determine whether an infection is prevented, or not. This hopefully could assist in development of public health interventions to reduce such infections, as well as treatment and vaccine development in the longer term.

Certain bacterial infections are increasing in prevalence, for reasons that are unknown. Subtle changes in bacteria can lead to an increase in the community ‘reservoir’ in healthy people thereby providing a larger supply of bacteria that can cause more lethal infections. Understanding the factors that promote such infections could help us to detect and prevent such infections affecting the community in the future

Unfortunately there is no vaccine for some of the most common bacterial infections. Unlike viruses, we understand very little about the normal immune response to bacteria. This project seeks to determine the early immune response events following exposure to bacteria to see if the bacteria can avoid the immune response in lymph glands. Understanding these events might allow us to develop better vaccines against such bacteria.

Though rare, if not prevented, deep-seated infections due to pathogenic extracellular bacteria have a 20% mortality. Treatment with life-saving antibiotics sometimes is insufficient because the bacteria make chemicals that destroy our immune system and body tissues. Part of this project will determine if we can develop better novel

treatments aimed at the bacterial chemicals (virulence factors) and evaluate new mixtures of old antibiotics.

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 should help to inform and potentially identify sensible public health strategies that could reduce the burden of serious bacterial infection, focussing on the commoner bacterial pathogens. In the longer term, new treatments or vaccine strategies developed during this project may be developed for clinical use. The findings should be of wider benefit to other academic groups and those working in the commercial field.

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

We plan to use approximately 1000 mice per year over the course of the project (5500 over 5y). About 25% will not undergo any infection, but will be part of a colony of mice that have humanised immune responses, which is an important trait when studying infection in mice.

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

Bacteria will be given as drops into the nostrils; this results in only minor discomfort though is done under brief anaesthetic. In some cases bacteria are given by injection into the soft tissues. This too is of mild severity as the injection incurs only minor discomfort. If the experiment lasts longer than 6h, the injection site can cause moderate discomfort, and studies are curtailed if this is the case. All injections will follow good practice (LASA) guidance on administration of substances. The research aims to measure the progression of infection in different circumstances, so at the end of each study, all mice are humanely put down following LASA guidance, and tissues are saved for culture of bacteria and measurement of immune responses.

Application of the 3Rs

Replacement

Bacterial infection results from the outcome of a variety of interactions between the pathogen and host that are currently difficult to model in the lab or in non-vertebrates. Bacterial pathogens behave quite differently during infection in warm-blooded animals Nonetheless, we have made significant progress in using alternative methods to answer some of the research questions we have, including using databases of patient outcomes to link disease severity with bacterial type, meaning we can use patient information to answer some questions. We also have developed a human whole blood model of infection as well as using the wax worm

Galleria to examine resistance to killing by certain types of white blood cells called phagocytes.

Reduction

Longitudinal monitoring methods are non-invasive and will allow us to measure bacterial infection daily in the same individuals, thereby reducing numbers required for different time points considerably. By reducing variation between groups at the start, (for example and weight sex) we can also reduce the numbers needed for each study. Where possible we will design experiments that provide the maximum amount of information 'in one go' rather than a long series of different experiments; this can allow us to use just a single control group for comparison with a number of treatments, thereby reducing numbers used overall. We will design studies so that our work can be reported in accordance with the NC3Rs' ARRIVE guidelines.

Refinement

Mice are to be used as they provide a faithful representation of the infections being studied and they also enable use of humanised models. Pathologists have shown that infections in patients are similar to the modelled infections, and it is also possible to use antibiotics in the same way as they are used in hospitals. Veterinary advice will always be on hand to give advice on use of analgesia (painkillers) and a brief anaesthetic can be given to avoid any discomfort.

Project 8	Immunopathology of experimental malaria infection
Key Words	malaria, immunity, brain, immunopathology
Expected duration of the project	5 year(s) 0 months

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

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

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

The overall objective of this project is to identify the key pathways responsible for the development of immunopathology (i.e. tissue damage caused by the immune system) during malaria infection. It is becoming clear that the most severe complications of malaria infection, including cerebral malaria, respiratory distress and severe anaemia, are due, in part, to over activation of the host's immune response to the parasite. However, in the case of cerebral malaria, we still do not understand the interplay and interactions of the malaria parasite with cells of the immune system and with the endothelial cells that form the cerebral vasculature.

Consequently, we still do not know the mechanism of vascular damage that leads to leakage, brain swelling and fatal outcome during the condition. Moreover, we do not know how anti-malarial drug therapy promotes recovery from the cerebral malaria syndrome in some individuals but not others, or the responses within the brain that resolve tissue damage and neuronal dysfunction following successful treatment of the condition. Crucially, in general, we do not understand how pro-inflammatory and anti-inflammatory immune responses develop and function during malaria infection, and why immunological homeostasis (i.e the balance between protective and pathogenic immune responses) sometimes fails to be achieved, leading to immunopathology. In particular, we have very limited knowledge of the behaviour of effector and regulatory T cells within tissues during malaria infection and how their interactions with other cells within the tissue determine their protective vs pathogenic activity.

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

This project may significantly advance our understanding of how protective and pathogenic immune responses form during malaria infection and will help to identify the specific events that lead to development of immunopathology, including cerebral malaria. These results should enable the development of targeted therapeutic and adjunct treatments for severe malarial disease, as well as identifying mechanisms through which to augment protective immune responses.

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

The general project plan will involve infecting resistant (for example BALB/C) and susceptible (for example C57BL/6) strains of inbred and transgenic (for example IL-27R KO, IL-10-GFP reporter) mice with different species of Plasmodium parasites that cause specific types of immunopathology. We expect to use approximately 8600 mice during the course of this 5-year project, with 500 used or parasite maintenance / establishment; 5000 in different experimental designs; 3000 in GAA colony breeding and 100 for obtaining GAA animal tissue.

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

Depending upon the species and strain of malaria parasite and the strain of mice utilised, malaria infection may lead to mild, moderate or potentially severe suffering. P. yoelii XL, P. berghei NK65 and P. berghei ANKA parasites have the potential to cause severe suffering due to high parasitaemia and associated anaemia or cerebral pathology (the latter in case of P. berghei ANKA) in susceptible mouse strains. However, of the experiments involving infections that have the potential to cause severe suffering in animals, not all infections will be allowed to progress to the stage where severe suffering occurs. For example, animals may be euthanized at time points of infection preceding development of severe suffering to examine immune cell activation, regulation or the early events in pathogenesis of severe malaria infection. We expect that 5% of animals that enter into experiments on protocol 2 of the licence may experience severe suffering. To help us to definitively address the importance of specific immunological pathways, we will employ different immunomodulatory techniques within the protocols, including localised and systemic administration of blocking and activating compounds, at precise stages of the experiment in relation to malaria infection. This will allow us to delineate the sequence of events that are necessary for the development or prevention of severe malarial disease and those important for parasite control. Most immunomodulatory techniques should not directly promote animal suffering. Irradiation with cell reconstitution will be performed under established protocols with appropriate monitoring to detect acute radiation sickness or reconstitution failure or more chronic radiation-induced suffering, such as dental complications. Anticipated effects due to

anti-parasite chemotherapy will be monitored and animals exhibiting non-transient and non-recoverable levels of severe suffering will be quickly identified and euthanized. We will perform behavioural tests (such as novel object recognition or Y maze), to assess cognitive capacity of animals following malaria infection, specifically cerebral malaria. The behavioural tests are non-invasive and will not cause animal distress or suffering. The final fate of animals on the licence will be either the transfer of animal to other project licences with the authority to receive animals that have undergone the procedures specified in the individual protocol or animals will be killed by exsanguination under terminal anaesthesia, a schedule one method, or following intravital (in animal) imaging under terminal anaesthesia.

Application of the 3Rs

Replacement

We can only address the majority of our questions when a complete immune system is present in its normal anatomical and physiological configuration (for example within the spleen, the major site of immune priming and parasite killing during malaria infection), or when parasites and immune cells can interact with the complex architecture of the intact brain (leading to cerebral malaria); the use of animals is, to a significant extent, unavoidable.

Reduction

We calculate the required group size using data from pilot experiments, previous experience, and published work to ensure that we have sufficient power to detect a biologically relevant effect using as few animals as possible. *In vitro* assays, such as co cultures of parasites with endothelial cells or T cells with antigen presenting cells, will be utilised to replace animal experimentation where possible; however, animals will frequently be required to obtain materials for use in these *in vitro* experiments.

Refinement

Mice are the most appropriate species for this work as murine malaria infections are the most well-characterised of the various animal models and so much is known about their immune systems and all the reagents that we require are available. In terms of cerebral malaria, there is accumulating evidence that the nature of blood brain barrier disruption and the relative importance of the perturbation in driving cerebral pathology, are very similar in mice and in humans, validating the animal model for the study of the human condition. Animal suffering will be minimised by closely monitoring all animals in relation to a well-defined grading system and providing analgesia, when required. Using our well-defined grading system, of the 5% of animals that may experience severe suffering during the course of our experiments, all of these animals will not experience prolonged suffering for more than a few hours.

Project 9	Isolation and propagation of virus in eggs
Key Words	Influenza, viruses, hens' eggs
Expected duration of the project	5 year(s) 0 months

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

Purpose

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

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

The objective is to isolate and propagate influenza viruses in eggs for development into influenza vaccines.

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 egg-isolated virus will be shared with other laboratories to be developed into a virus that has better growth characteristics to make them suitable for consideration as candidate vaccine viruses. If appropriate, candidate vaccine viruses can be selected as vaccine viruses for use in vaccines.

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

Avian eggs, approximately 15,000 embryos will be used over a five-year period.

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

The level of severity is deemed to be moderate. Infection of eggs with some viruses can result in death of the embryo although spontaneous death can also occur during normal embryonic development. Where virus pathogenicity is suspected, but this is not frequent in our work, batches of eggs will be examined for viability at regular intervals and if lack of movement or closure of blood vessels is detected in individual

eggs then the egg will be killed by a Schedule 1 method but the other eggs will continue to be incubated and monitored. However, should the viability of eggs sampled be lower than 50% all eggs will be killed by a Schedule 1 method.

Application of the 3Rs

Replacement

The vast majority of influenza vaccines currently in use are propagated exclusively in hens' eggs. For some human influenza viruses, much improved isolation has been observed by using eggs aged 14 days or older for inoculation.

Reduction

Following an isolate being successfully propagated in 14-day old embryos it will be subsequently passaged in 10-day old embryos, an age not included in the act.

Refinement

The chicken is the basis for the vast majority of influenza vaccines. It is the usual species chosen for the propagation of virus in eggs and has been used for over 70 years.

Project 10	In-Vivo Models of Infection and Inflammation
Key Words	Infection, inflammation, immunology
Expected duration of the project	5 year(s) 0 months

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

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

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

We wish to develop and deploy standardised and refined animal models of infectious disease, focusing mainly on the skin, the airways and the digestive tract.

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)?

Microbial and viral infections are major health problems, even in the developed world. There is growing concern about the emerging multi-drug resistance that many bacterial infections exhibit, making the development of new strategies for their effective treatment an urgent priority. Infectious agents interact with and evade the host immune system in a variety of ways, not all of which are well understood. In particular, micro-organisms signal among themselves in the coordinated production of three-dimensional colonies, or “bio-films”. These often behave differently from the individual bacteria in their response to antibiotic treatment; bio-films are often more resistant. This project sets out to provide standardised mouse model systems in which hypotheses about microbial resistance to treatment can be tested rigorously, with the aim of identifying completely new targets for therapy. In addition it will allow the testing of novel chemical entities (NCEs) emerging from drug discovery programmes for “proof of concept” activity against microbial infections. Agents which show such activity can then be further optimised as potential treatments for human and animal disease.

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

Wild type (WT) and genetically altered (GA) mice will be used. Small pilot studies (group sizes of about three mice) will be used to establish each model with regard to dose and time point, starting low and increasing the dose until the scientific aim is met. Using published data, our own historical experience and statistical power tests, hypothesis testing is expected to use group sizes of around 6–8 animals. A total of approximately 4000 mice will be required over the course of the five-year project.

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

The experimental infections of the airways, digestive tract or skin are expected, in most cases, to result in outward signs of adverse welfare (e.g., fever, lassitude) but these will be controlled within the “moderate” severity limit. Scoring systems will be devised in order to make this control as unambiguous and robust as possible. The use of standardised models by specialist members of a central service is expected to result in very few unanticipated welfare problems. The agents to be tested for their effects on the course of infection will not be expected, in themselves, to cause harm. All animals will be killed humanely at the end of the procedure. In almost all cases, tissues or cells will be harvested after death for detailed laboratory analysis.

Application of the 3Rs

Replacement

Animals have to be used because there are no reliable alternatives currently available to achieve the objectives of this licence. Experimental agents will have been tested for useful properties (lack of toxicity, effectiveness against the target micro-organism, etc.) in simpler systems, but the site and three-dimensional nature of bacterial colonization in animals can have major effects on the efficacy of therapeutic agents like antibiotics. The case for moving each project to animal studies will be made in the internal request documentation, and submitted to the AWERB for confirmation that adequate justification does indeed exist.

Reduction

Provision of these models as a service allows us to reduce the numbers that would be used overall. Not only will there be better control over experimental design and analysis than there might be if these studies were being carried out by scientists whose primary expertise is not in this field, but the experiments will be carried out and monitored by a small and stable team of people with the requisite expertise and training.

The minimum number of mice required to generate robust and biologically significant data will be used. Group sizes will be based on statistical power calculations. Where no pilot data exists, pragmatic numbers will be decided formally based on group experience and statistician advice.

Refinement

The chosen mouse models of infection have a predictable and reproducible outcome and will be refined to ensure maximal scientific output with minimal adverse welfare. The choice of dose and route of infection will be made to mimic the natural progression of infection, but with the lowest degree of clinical symptoms possible. Many microorganisms induce fever and general symptoms of disease transiently that subsides as the microbe is eradicated by the immune system. The possibility of a faster disease progression in genetically altered mice will be taken into account when designing the experiments and monitoring and endpoints will be determined. Where genetically altered microorganisms are being studied, these are expected to be less virulent than the wild type standard strains.

Advances in imaging technology enable models to be refined and mouse numbers reduced by the use of bioluminescent tagged bacterial strains. Infection can be monitored by non-invasive imaging in real time in individual mice, avoiding the need to kill animals simply to measure the stage of infection.

In addition to each project requiring formal ethical approval, each experiment will be planned in detail as to the monitoring and specific end points to be used. These will be agreed in conjunction with the named vet (NVS) and will use the minimum severity possible in order to address the scientific hypothesis to be tested.

Project 11	**The Production of Antiserum, Protozoan Antigens and Tissues**	
Key Words (max. 5 words)		
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3) (Mark all boxes that apply)		Basic research
	x	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this project is to produce reagents (substances used for biological or chemical analysis) for the diagnosis, control or prevention of communicable (and other) disease either directly by established in the laboratory procedures or indirectly by using animals in experimental procedures.	
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The potential benefits of this project is the production of antisera which is used in the diagnosis, control or prevention of communicable (and other) diseases such as E.coli, Salmonella, Influenza and other bacterial ,viral or protozoan disease either directly by established in- vitro procedures or indirectly by research using in-lab procedures. In order to carry out this work antisera is generated against specific microbes, components, proteins and/or have neutralising activity as required for a range of organisms to assist in diagnosis, typing and assessment of human immune responses. Such antisera may be prepared to defined levels of protein	

	activity in human immune responses, and many are not commercially available. Additionally it is important that we have access to reagents whose supply is independent of the commercial market.
What species and approximate numbers of animals do you expect to use over what period of time?	Very few animals are used for antisera production. The numbers found in the protocols are there as a contingency and act as a surge capacity. We use ferrets for antisera production during the influenza season. Rabbits are used for enteric antisera production and this is currently in decline due to technology being capable of doing some of this work. Which is in line with the principles of the 3R's
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 seventies of the protocols in the main are mild/non recovery, one is classified as moderate. There are few adverse effects for mild and non-recovery or moderate protocols; these effects are ameliorated as soon as and if they arise. This could be an adverse reaction to the inoculum, for example. All of the animals are euthanised at the end of term; this is mainly due to the nature of the final procedure being non recovery. Non recovery is where the animal is anaesthetised and it is precludes recovery or resuscitation. Where a classification of moderate has been given this is because the animal has to endure pain suffering or lasting harm greater than that of mild in order to reach the objective.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	All requests under the licence will be tested for the possibility of the in-vitro (non- animal lab based work) being performed without the use of animal products by specific questions and justification in the request form. There is no substitute for ferret antisera-it is not commercially available for the assays that we conduct here.
2. Reduction Explain how you will assure the use of minimum numbers of animals	To minimise the number of animals used, a request is made for permission to reuse specified animals from the other Project Licence - 'Production of Normal Blood and Tissues' where they will have been used for the production of normal blood, after a sufficient period of rest which will not be less than the minimum

	interval between bleeds applicable to the last volume of blood sampled and subject to determination of fitness for reuse by BSD staff. Animals will only be 'determined fit for reuse' if they are healthy i.e., they show no adverse clinical signs, and are exhibiting signs accepted as normal for the species (e.g. normal body weight, food and water intake, behaviour patterns.) A veterinary Surgeon will assess the animal's health before they are transferred for reuse. Overall the use of animals to produce antiserum (a blood serum containing antibodies against specific antigens, injected to treat or protect against specific diseases) has and will continue to decline as molecular and other methods are developed. For example, molecular methods are currently being evaluated alongside antiserum methods for the typing of Salmonella spp. The commitment to this reduction is defined in the above statement. There will be a commitment to use the least amount of animals per inoculum normally and the most appropriate species i.e., the largest to maximise the volume produced. On prolonged storage, bacteria may reduce the expression of somatic antigens which affects the quality of vaccine preparations used for generating new batches of typing sera. Injecting rabbits with bacteria which are expressing incomplete somatic antigens (a somatic antigen is an antigen located in the cell wall of a bacterium) may result in low titred/low levels of antibody in the sera. Therefore to reduce the overall number of rabbits used, mice are first injected to maximise the expression of somatic antigens by type- strains. This process also establishes the authenticity of the titre before injections into rabbits; this also enables the production of high-titred/high levels of antibody in sera (the blood serum of an animal used to provide immunity to a pathogen or toxin by inoculation or as a diagnostic agent) and makes best use of rabbits employed for serum production.
3. Refinement Explain the choice of species	Some animal use will remain necessary to achieve this aim for the foreseeable future. To ensure such

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.	use is ethical and that animal welfare is protected during such use the we are committed to the following principles in line with the Animals Scientific Procedures Act 1986 (ASPA): Any suffering caused to animals and the number of animals used should be the lowest necessary to achieve the identified objective. To this end then we will; Encourage the development of alternative methods through co-operation with or participation in national and international initiates aimed at refining, reducing or replacing the use of animals.