Animals (Scientific Procedures) Act 1986

Non-technical summaries for projects granted during 2014

Volume 10

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

Project Titles and Keywords

1. **Pathogenesis and Control of Mycobacterial Infections**
    - Tuberculosis, Vaccine, Drugs, aerosol

2. **Filovirus infection models for therapy evaluation**
    - Rodents, filovirus, Ebola, Marburg

3. **Genetics of African Sleeping sickness**
    - African Trypanosomiasis, mice, Trypanosoma congolense, Trypanosoma brucei rhodesiense

4. **Herpes simplex virus pathogenesis and control**
    - Virus, vaccine, antiviral, latency, herpes

5. **Modelling bacterial infection of the Cystic Fibrosis airway**
    - Infection, regulation, signal transduction, virulence

6. **Developing new drugs to treat sleeping sickness**
    - Sleeping sickness drug development

7. **Chemotherapy of Protozoal Infections**
    - Leishmaniasis, African Trypanosomiasis, Chagas Disease, treatment

8. **Drug discovery for neglected diseases**
    - Efficacy, Drug Discovery, Trypanosomiasis, Leishmania, Chagas' disease

9. **Assessment of novel treatments of infection**
    - Bacterial, viral, infection, respiratory

10. **Neonatal bacterial meningitis — infection and treatment**
    - in vivo imaging; Escherichia coil KI; blood-brain barrier; Group B streptococcus; neonatal bacterial meningitis

PROJECT 1	**Pathogenesis and Control of Mycobacterial Infections**	
Key Words (max. 5 words)	Tuberculosis, Vaccine, Drugs, aerosol	
Expected duration of the project (yrs)	Five	
Purpose of the project as in ASPA section 5C(3)	✓	Basic research
	✓	Translational and applied research
		Regulatory use and routine production
		Protection of the natural environment in the interests of the health or welfare of humans or animals
		Preservation of species
		Higher education or training
		Forensic enquiries
		Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	The objective of this license is to develop and evaluate new drugs and vaccines for the treatment or prevention of tuberculosis disease. In order to achieve this, new vaccine/drug candidates will be evaluated in a sequential series of pre-clinical evaluations in specialized animal models of the disease. Tuberculosis is one of the leading causes of death of humans from a single infectious agent worldwide responsible for around two million deaths each year. The largest challenge to the successful control of TB is the detection and successful treatment of individuals with latent *M. tuberculosis* infection who are at a high risk of relapsing with active, contagious disease. Many of the fundamental aspects of the host-pathogen relationship between *M. tuberculosis* and humans are poorly understood and there is an urgent need to define clear correlates of protective immunity and biomarkers of disease.	

What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The development of a safe, effective and affordable new TB vaccine or drug regimen would have a huge global impact upon human health. The studies proposed in this licence will have a direct impact upon the progression of novel candidates to early stage clinical evaluation. The need for new interventions (improved diagnostics, therapeutics, and vaccines) has been recognised as a priority by international agencies including the WHO and this programme of work will have a direct impact upon meeting targets laid out in the WHO global plan to stop TB.
What species and approximate numbers of animals do you expect to use over what period of time?	A total of approximately 1500 guinea pigs and 1200 mice will be used in this five year project, in order to study the pathogenesis of tuberculosis, and develop and evaluate new vaccines and drugs to prevent or treat global tuberculosis disease.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	All studies will have clear strict indicators of disease progression ensuring the lowest level of distress possible. Following aerosol challenge, *M. tuberculosis* infection progresses slowly and animals remain clinically well for long periods. Early time-points therefore, allow assessment of progression of infection in the absence of adverse clinical events. Signs of severe infection include significant weight loss, loss of appetite and laboured breathing. These adverse effects are minimised by using early readouts and careful monitoring of weight and eating habit that measure the progression of disease before the onset of severe adverse events. The expected severity level is moderate. Animals will be euthanised at the end of each study.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Where alternatives to the use of animals exist or are developed such approaches will be used. Use of animals in pre-clinical evaluation of vaccines and therapeutics is needed to determine important safety and efficacy performances prior to introduction of these products to clinical or field trials. In addition, vaccines are targeting specific states of tuberculosis disease that cannot currently be replicated *in vitro*

	due to complex infection processes with many unknown mechanisms of evading the immune system.
2. Reduction Explain how you will assure the use of minimum numbers of animals	This strategy will ensure that only the most promising candidates reach clinical/field trials thus reducing the numbers of animals used in these studies. Size of experiments will be such that will allow inferences to be made about pre-specified efficacy differences between groups.
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.	Vaccine and drug targets will be generated from a series of non-animal studies using defined culture conditions that mimic environments that *M. tuberculosis* may experience in humans during TB infection. The candidate vaccines will then be evaluated in a step wise progression of *in vivo* experiments increasing in model complexity, primarily assaying for immunogenicity in the mouse. Those vaccines showing significant immune responses in the mouse will proceed to guinea pig or mouse efficacy studies. The most efficacious candidates in short-term guinea pig studies, showing reduced bacterial burden in tissues compared to controls, will proceed to long term survival studies in the guinea pig. Similarly, the drug candidates will be evaluated in a series of *in vivo* experiments. Mice and guinea pigs are widely recognised as being suitable species for the early stages of screening of TB vaccines in order to demonstrate safety, immunogenicity and protection against virulent challenge. Mice may be used to enable detailed immunological analyses, which are not currently feasible in guinea pigs. Guinea pigs are the favoured model of TB disease because pathology and immunological responses are more similar to human disease compared to those seen in mice.

<table>
<tr><td>PROJECT 2</td><td colspan="3">Filovirus infection models for therapy evaluation</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Rodents, filovirus, Ebola, Marburg</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td>~~No~~</td></tr>
<tr><td>Regulatory use and routine production</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Preservation of species</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Higher education or training</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td>~~Yes~~</td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td>~~Yes~~</td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">To develop clinically relevant in vivo rodent models of filovirus disease that will enable pathogenesis studies and evaluation of potential intervention strategies.</td></tr>
<tr><td>What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?</td><td colspan="3">This project will enable in vivo models to be developed and utilised to enable the testing of vaccines and therapies against Ebola virus. Due to Ebola virus continuing to be responsible for causing sporadic outbreaks of disease, including the current largest one ever recorded, the virus continues to pose a public health threat. Presently, there are no approved vaccines or therapeutics against Ebola virus available for use in the human population. This research will help fill in a void in the provision and planning to protect human health from Ebola virus disease.

It is anticipated that application of this model will</td></tr>
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	ultimately lead to improved, effective treatments for Ebola virus. In addition, as filoviruses are considered biothreat agents, the studies will enable stockpiling of suitable treatments in the event of either a naturally occurring outbreak or a deliberate release.
What species and approximate numbers of animals do you expect to use over what period of time?	Over the 5 year length of the licence, up to 1500 guinea pigs may be used.
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?	Potential adverse effects to the viral challenge, the cannula or to the dosing of animals with vaccine or therapeutic agents have been identified. Animals will be anesthetised during procedures and will be closely monitored until a full recovery is made. In this licence, it has been recognised that due to the acutely pathogenic nature of the infectious agent some animals may die before they can be humanely killed so the relevant protocols have been categorised as severe. However, critical periods will have been identified and monitoring frequency will be increased to every six hours as a minimum during these higher risk periods. Humane clinical endpoints will be defined in each study, where animals will be culled to prevent unnecessary suffering. At the end of all studies, animals will be culled by a Schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	As much evaluation as possible of the efficacy of new treatments will be done using *in vitro* systems, such as cell-based assays of viral inhibition. However, to establish evidence of efficacy of new treatments it is essential to have the full range of host-pathogen-treatment interactions. This can only be achieved in an animal model. Additionally, due to the lethality of filovirus infection in humans, there is no present alternative to using animals in order to obtain licensure for these therapies

2. Reduction Explain how you will assure the use of minimum numbers of animals	Studies will be undertaken in a step-wise manner so that the number of animals used will be minimised if the treatment shows no likelihood of efficacy; for example, if a new vaccine does not elicit an appropriate immune response, then it would not progress to a challenge efficacy study. Assessment of novel molecules and/or formulations of antivirals will also be approached in a step-wise manner, in that only those treatments showing the greatest promise will be tested in larger cohorts of animals. Robust scientific quality control of the test materials and methods will ensure that studies are carried out successfully first time. This will remove the need to repeat studies and therefore reduce the number of animals used. The conduct of studies to high quality standards will also facilitate application of data generated to support future regulatory submissions and clinical application.
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.	Wherever possible, prior safety data will be provided for therapeutic substances, including any previous work conducted in other species such as mice. Data obtained in pharmacokinetic, dose tolerance or immunogenicity studies will be used to inform on the most appropriate dosing regime for future efficacy studies. In order to provide animals with maximum social interaction and environmental enrichment we will aim to keep the housing period within the high-containment facility to a minimum and throughout all the studies listed in this license we will maintain group housing of infected mice or pair-housing of infected guinea pigs. The initial adaptation studies will involve relatively small numbers of animals and as well as increasing the virulence of the virus for guinea-pigs, we will use the data obtained in these studies to inform our management of all subsequent studies with regards to health monitoring and improving our definition of end-points. For guinea pig studies where regular blood sampling or access to the intravenous route is

	required, then animals with catheters inserted into an appropriate vein will be used. Blood sampling via the catheter is less stressful for the animal where repeated samples are required and prevents the use of sequential culls as a means of collecting sufficient blood volumes. With the exception of animals which have surgically implanted catheters, animals will be anaesthetised for all procedures. A gaseous agent, such as isoflorane will be used as this allows rapid recovery and removes the use of needles within the high containment environment (to ensure staff safety).

<table>
<tr><td>PROJECT 3</td><td colspan="3">Genetics of African Sleeping sickness</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">African Trypanosomiasis, mice, Trypanosoma congolense, Trypanosoma brucei rhodesiense</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5 years</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td></td><td>No</td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Different breeds of cattle and different strains of mice respond differently to infection with Trypanosoma parasites that cause sleeping sickness. Some animals survive much longer after infection than others.

In previous work we have discovered 5 genes in mice and cattle that may contribute to those differences in survival, and we are searching for more genes that might be involved. We now want to test whether the differences between the genes in the different animals really do control the differences in response after infection. To do this we will obtain mice that have been genetically modified to carry the alternate version of each gene of interest.

We do not expect that any of the genetic modifications that we will use will be harmful to the</td></tr>
</table>

	mice because the variants will be forms that occur naturally in healthy animals. We will compare how long the genetically modified mice survive after infection compared with mice that have not been modified. If we see a difference in response to infection then that will be strong evidence that our hypothesis was correct. If we confirm that a difference in a gene really does cause a difference in survival then we will use a range of techniques to understand just how that gene causes that difference. To do this we will infect mice and then kill them once the infection has developed. We will collect tissues and test these to see what genes are responding to the genetic modification. Just as different animal strains differ in response to infection with the same parasite, different parasite strains can cause disease with greater or less severity. We have a collection of parasites obtained from patients who were suffering different forms of trypanosomiasis. We have previously found that these parasites also cause different forms of disease in mice, which correlates well with what was observed in humans. We are trying to identify the parasite genes that cause these differences in pathology. In order to do this we need to observe how the parasites grow in the mice and also to collect parasites from mice to sequence the parasite genomes to identify the differences between the strains.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	If we can confirm that versions of genes that appear to protect cattle against trypanosomiasis also protect mice against trypanosomiasis we will be justified in developing cattle to carry the protective version of the gene. We will also be able to analyse how the protection is given and an insight into that mechanism may enable us to predict that certain drugs would benefit cattle and possibly humans with trypanosomiasis. The identification of versions of genes that protect

	mice against infection will also help us understand how that protection is provided. Although it is unlikely that drugs will be suitable against the specific genes that we identify we may be able to show that particular metabolic or immune responses are protective and then predict drugs that might enhance those responses.
What species and approximate numbers of animals do you expect to use over what period of time?	We will use a maximum of 1,000 mice for this project over five years. We will also use a maximum of 100 rats to obtain large numbers of parasites for analysis of parasite genomes.
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?	Testing whether particular versions of genes are involved in controlling the response to infection involves finding out how long mice survive after infection. This is a severe procedure for the mice but we will minimise their suffering by monitoring them closely and as soon as they start to exhibit signs of terminal illness they will be killed by a humane method. In order to understand the mechanisms of gene action we will infect mice and then kill them at a predetermined time to obtain tissue samples. Most mice will only exhibit moderate symptoms of disease before we kill them and any that do exhibit more severe signs will be killed immediately.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	*Trypanosoma* live in the blood stream and consequently they interact with most organs in the body. There is no method of reproducing the highly dynamic conditions in the blood stream in the test tube. So in order to discover the animal's response to infection we need to infect mice.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Before undertaking any experiment we will make a statistical prediction about the number of mice that will be required to be confident of observing the size of difference in effect that we believe to be caused by the gene we are studying. This will enable us to use the minimum number of mice necessary and avoid using too few to obtain the

	results that we want.
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 will use mice for most experiments. In some cases this is because we are following up on previous experiments in mice. In other cases we are using mice to predict the effects of gene differences in cattle. We do not expect mice to respond to these gene differences in exactly the same way as cattle but there is likely to be sufficient similarity to demonstrate whether an effect has occurred. Experiments in mice can be undertaken far faster than experiments in cattle so we hope to be able to start breeding more resistant cattle sooner by undertaking experiments on mice. We will also use some rats, which because of their much larger body size enable us to obtain enough parasites for genome sequencing using fewer animals.

PROJECT 4	**Herpes simplex virus pathogenesis and control**	
Key Words (max. 5 words)	Virus, vaccine, antiviral, latency, herpes	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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
	X	Maintenance of colonies of genetically altered animals
Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)	There is currently no licenced vaccine to protect against primary herpes simplex virus infection and existing antivirals cannot eradicate latent virus from the nervous system. This project seeks to develop novel attenuated virus vaccines and gain an insight into what virus genes are expressed during neuronal latency and how host gene expression is altered in the latently infected cell.	
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 from the work proposed are a detailed knowledge of the molecular basis of herpes simplex virus latency which can be used in a practical way in the development of novel vaccines and antivirals for the prevention and treatment of neurotropic herpesvirus infections.	
What species and approximate numbers of animals do you expect to use over what period of time?	18,750 mice and 700 rats 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?	Adverse affects may arise following virus infection. Signs of disease are evident in no more than 10% of animals and occur during the acute stage of infection (between 3-10 days post infection)' such cases animals are expected to show and any animals that show two of these signs and/or weight loss of 20% will be killed. All affected animals are monitored daily to ensure clinical signs of disease do not exceed a moderate severity level. At the end of procedures animals will be killed by a schedule 1 method.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Understanding virus disease mechanisms involves a complex interplay between virus replication in distinct tissue types and the immune response to infection. Such complex anatomical and immunological systems cannot be reproduced *in vitro.*
2. Reduction Explain how you will assure the use of minimum numbers of animals	In vitro studies are used wherever possible in our studies on virus replication and latency either utilising established continuous cell lines or primary cell types. We constantly update our approach depending on new data, planning new pilot experiments before performing larger, definitive experiments required for statistical significance and therefore only when we are reasonably confident about the outcome. The number of mice used in each experimental group will depend on the magnitude of the effect expected and statistical advice will be sought to ensure that the minimal number of animals are used to, generate statistically significant and hence informative data.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The pathogenesis of herpes simplex virus infection in the mouse closely mimics human disease and virus latency is efficiently established in the peripheral nervous system. The mouse therefore provides an informative model to explore the molecular basis of herpes simplex virus latency and to evaluate vaccination and chemotherapeutic strategies. The use of non invasive imaging techniques will significantly reduce the numbers of animals used in studies of pathogenesis and harm to animals will be minimised by using the minimum doses of virus agents necessary to facilitate host colonization and through regular monitoring of disease signs should they occur.

<table>
<tr><td>PROJECT 5</td><td colspan="3">Modelling bacterial infection of the Cystic Fibrosis airway</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Infection, regulation, signal transduction, virulence</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td><s>No</s></td></tr>
<tr><td>Regulatory use and routine production</td><td><s>Yes</s></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><s>Yes</s></td><td>No</td></tr>
<tr><td>Preservation of species</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Higher education or training</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td><s>Yes</s></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td><s>Yes</s></td><td>No</td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Cystic Fibrosis (CF) is an inherited disease that causes thick, sticky mucus to form in the lungs and other organs. Progressive loss of lung function resulting from the inflammatory response to bacterial colonization is the leading cause of mortality in cystic fibrosis (CF) patients. It is now appreciated that infecting bacteria can act cooperatively to facilitate disease progression. Nevertheless, very little is known about the molecular mechanisms that underpin interactions between bacteria during such infections. Here we are examining bacterial interactions during infection and their contribution to antibiotic-resistance and poor disease prognosis.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td colspan="3">If specific signalling processes and proteins that regulate these interactions during infection can be</td></tr>
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project (how science could be advanced or humans or animals could benefit from the project)?	identified as important to antibiotic resistance and infection, they may become target for future therapeutic intervention.
What species and approximate numbers of animals do you expect to use over what period of time?	A total of approximately 300 animals (mice) a year (1500 / 5 year license) will be required over the course of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	A scoring system will be adopted to characterise the course of the bacterial infection, for both scientific and welfare reasons. The body condition, weight, rectal temperature and lung function of the mice will be evaluated. The usual level of severity is mild. In the majority of cases, the animals are killed humanely and the scientific analyses are carried out on tissues or cells harvested after death. These interventions are also expected, in the most part, to be mild, with a severity limit of moderate.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	**We try to reduce the number of animals by using** various invertebrate models and in vitro cell culture systems. However, these have now reached the limit of their usefulness due to their ill defined immune systems. Using animal models will allow us to analyse various phenotypic and immunological characteristics of infection. This would not be achievable with the basic models described above, as they do not have comparable immune systems, thus making the data from the animal experiments very valuable.
2. Reduction Explain how you will assure the use of minimum numbers of animals	The project detail was reviewed by a Medical Statistician who has defined the minimum number of animals needed in the experiments in order to gain meaningful data.
3. Refinement	I use the mouse as a model system to test because

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.	it is a small mammal that can be easily bred and tested in laboratory conditions. Non-mammalian, lower organisms do not provide a suitable model for the biochemical or signalling processes being analysed as they do not share a parallel immune system. The genome of mice has been well studied is they are genetically attractable. Throughout all experiments the state of the animals will be monitored closely to avoid unnecessary pain and distress. All animals will be regularly monitored for infection by typical pathogens and signs of discomfort, using humane endpoints to be agreed with the NVS.

PROJECT 6	**Developing new drugs to treat sleeping sickness**	
Key Words (max. 5 words)	Sleeping sickness drug development	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)		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)	Trypanosomes are protozoan parasites (single cell organisms) and are responsible for a tremendous burden of disease worldwide. They cause a range of diseases including Sleeping Sickness (Human African Trypanosomiasis) resulting in significant morbidity and mortality amongst the world's poorest populations. This places a serious health and economic burden on low-income countries where resources are already limited. Unfortunately, treatments against these diseases are inadequate. Effective vaccines are not available which means that chemotherapy is essential to reduce parasite burden. The existing drugs are all problematic: they are toxic, ineffective, require cumbersome dosing regimens, and are associated with drug resistance. The development of new improved drugs is therefore crucial if these diseases are to be controlled and eliminated. In this project we will use mouse models of parasitic disease to test novel anti-parasitic compounds. The main objective of this project is to develop at least one new compound to progress through the drug discovery and testing pipeline. This will help address the unmet need for safe, ideally orally administered and inexpensive drugs to treat Stage 1 and 2 Sleeping	

	Sickness.
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)?	Development and testing of new compounds in the best available animal models would enable efficacious tria[Thèiizyme being targeted in these experiments is also present in other trypanosomes responsible for Chagas disease (endemic in Latin America) and in trypanosome infections of commercially important livestock. Data generated from this project may prove readily transferrable to the other trypanosomatid diseases and may also provide new therapies for these neglected diseases. Such therapies could potentially contribute to an increase in both health and economic welfare for at-risk populations.
What species and approximate numbers of animals do you expect to use over what period of time?	Mice will be used for the majority of the experiments; an estimated 2400 mice will be used over the 30 month duration of the project. Rats will be used when required; an estimated 100 rats will be used over the 30 month duration of the project.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	The objectives of the project fall into two main areas: 1. Development and preliminary testing of compounds in mice to confirm that the compounds are safe to administer to a larger number of animals. 2. Treating mice infected with trypanosomes with the test compounds to test for a cure. Adverse effects associated with compound screening may include making the animal ill following administration of the compound. In extreme cases this may lead to the need to humanely kill the animal due to unacceptable side effects. All compounds are thoroughly tested in cell based assays and little compound toxicity is expected. The animals will be closely monitored and any showing signs of clinical distress following administration of compound will be euthanised. Adverse effects associated with infecting mice with trypanosomes include the animals carrying a high parasite burden and showing signs of being unwell as a result. The parasite strains used in the experiments cause a progressive and highly predictable disease. Infected animals will be checked regularly by experienced staff with end points clearly defined to minimise suffering and disease progression beyond the specified time.
Application of the 3Rs	
1. Replacement State why you need to use	In our *Ttypanosoma* drug studies we occasionally observe significant anti-trypanosomal effects *in vitro* that don't necessarily translate to *in vivo* data. This can be

animals and why you cannot use non-animal alternatives	attributed to drug availability in the host. Doing *in vivo* drug assessment is the only way of evaluating whether a compound would reach and kill parasites in the mammalian host. There is currently much interest to better define the pharmacokinetic and pharmacodynamic properties of existing and new drugs so that we may more accurately predict which drugs are likely to be efficient *in vivo.*
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments-are-designed-to-obtain-the maximum—information from the minimum resource while still allowing for statistical analysis. We will make use of existing data from our and other published work to ensure the minimum use of animals for design of new experiments. The experiments proposed in this application are already in use in other laboratories also developing drugs for trypanosomiasis and follow the guidelines for such studies set out in the Drugs for Neglected Diseases Initiative Manual. All the compounds to be tested in the project are novel and are targeted specifically at inhibition of *the Ttypanosoma* phosphofructokinase enzyme identified as essential for survival of the trypanosomes.
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.	Rodent models are well established and described for *Tiypanosoma.* Mice can be used for the majority of the studies, rats are used when a larger volume of blood is required for analysis and/or more time points are to be analysed from a single animal. In these circumstances this means that fewer rats could be used where a large number of mice would be needed to provide the required data All personal licence holders working under this licence are trained to a high standard to minimise animal suffering. Newly trained personnel are assisted by more senior members to ensure that this standard is maintained. We will work closely with staff at the animal holding facility to ensure that animals are regularly assessed for any signs of discomfort or distress. Humane end points are set and will be strictly enforced.

<table>
<tr><td>PROJECT 7</td><td>CHEMOTHERAPY OF PROTOZOAL INFECTIONS</td></tr>
<tr><td>Key Words (max. 5 words)</td><td>Leishmaniasis, African Trypanosomiasis, Chagas Disease, treatment</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td>5</td></tr>
<tr><td>Purpose of the project (as in section 5C(3)</td><td>
<table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals</td><td></td><td>No</td></tr>
</table>
</td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td>The objective of this project is to contribute to the identification and development of better medicines, vaccines and biological markers for 3 Neglected Tropical Diseases (namely Leishmaniasis, African Trypanosomiasis, Chagas Disease) which cause death and suffering in humans worldwide. They are threatening a total of more than 400 million people worldwide and collectively cause approximately 150 000 deaths per year.

Current medicines have considerable side effects, some medicines cannot be given to pregnant women and some medicines cause potentially deadly effects in treated patients. Most of the medicines have to be injected with a needle and cannot be taken by mouth. There are also growing problems with drug resistance and some of the</td></tr>
</table>

	current medicines cannot be used any more in certain areas of the world due to a lack of efficacy. So far there is no vaccine available for any of these diseases.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	The expected benefits of this project include: • Characterisation of how anti-leishmanial and anti-trypanosomal drugs work and how they are best used, e.g. optimal doses given • Characterisation of how a disease process can affect drug action or how drugs can affect disease processes • Contribution to the identification, evaluation and development of new medicines • Refinement of animal models and their use in studies to develop medicines
What species and approximate numbers of animals do you expect to use over what period of time?	We will use mainly mice and potentially a small number of hamsters. Over 5 years we estimate to use a total of 11 900 mice and 520 hamsters. This number of animals is shared by research groups working across the faculty.
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?	Mice infected with viscerotropic *Leishmania* parasites show no clinical signs of infection and those infected with dermotropic *Leishmania* parasites, albeit developing a lesion on the rump show no signs of discomfort or distress. Hamsters infected with viscerotropic *Leishmania* parasites will progress to clinical disease, but endpoints are set to not exceed a moderate level. Mice infected with *Trypanosoma* parasites will show clinical signs of disease such as piloerection and hunched posture. Endpoints are defined to not exceed a moderate level of severity. A small percentage of animals may experience a higher severity limit, e.g. in case when new strains of *Trypanosoma* are introduced, for which the progression of infection cannot be known. At the termination of each experiment all animals will be humanely killed by a Home Office approved method.
Application of the 3Rs	
1. Replacement	The programme of work aims to identify new treatment and vaccination strategies, to assess

State why you need to use animals and why you cannot use non-animal alternatives	pathology and host-parasite interactions in disease models and to identify tools to monitor treatment responses. These studies require the use of animals to answer specific questions, which cannot be investigated in isolated cell culture. Examples of such studies include the distribution and effect of a drug in a multicellular organism or the response to a treatment taken by mouth measured in blood or other tissues.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Experiments are planned in order to maximise data output with the least number of animals used and to minimise bias by allocating animals to treatment groups in random order. Where possible data is evaluated by a researcher unaware of the nature of treatment given to each group. In each experiment and at all stages of the work, control groups of mice (e.g. untreated or PBS treated animals in studies evaluating drug activity) will be included and where possible animal numbers will be limited by sharing control groups or using control groups for analysing multiple outcomes. As an example urine or blood samples may be collected from animals undergoing experimental procedures such as evaluation of drug activity to look for prognostic markers. The number of animals will be significantly reduced by our development and usage of non invasive imaging technologies.
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 mouse is the host species of choice for *Leishmania* and *Trypanosomes* and will be used wherever possible. Mouse models have been used and validated extensively and provided much of our knowledge to date on these infections. The parasite strains we use for infections are well characterised with respect to the disease they cause and all new parasite strains we use are assessed for their infection kinetics and virulence in small groups of animals and prior to this in cell culture. The use of non invasive imaging for *Trypanosoma* infections will reduce the total number of animals used and in some cases determine an endpoint for experiments to limit disease progression.

PROJECT 8	**Drug discovery for neglected diseases**	
Key Words (max. 5 words)	Efficacy, Drug Discovery, Trypanosomiasis, Leishmania, Chagas' disease	
Expected duration of the project (yrs)	5	
Purpose of the project as in ASPA section 5C(3)		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 seeks to discover better and safer treatments for parasitic diseases that afflict millions of poor people and their domestic animals living in tropical and sub-tropical regions of the world. Current drugs are unsatisfactory for a variety of reasons, including high cost; unacceptable (often serious) side effects to adults, pregnant women and children; the need to be given by injection rather than by mouth; and increasing treatment failures, often due to the emergence of drug-resistant strains of parasite. The diseases we study include malaria, which causes about 1 million deaths a year, mainly in young children in Africa, and three "neglected diseases" (African trypanosomiasis, leishmaniasis and Chagas' disease) which collectively cause about 150,000 human deaths a year. Trypanosomiasis is also a serious animal health problem in Africa. Better treatments for these diseases would lead to better health and greater	

	productivity thereby helping to alleviate poverty in developing nations.
What are the potential benefits likely to derive from this project (how science could be advanced or humans or animals could benefit from the project)?	This project will aid the discovery of potential new medicines. The data generated in this project will be essential to the development of new medicines as they provide a means to select substances for full preclinical development and thence into clinical trials.
What species and approximate numbers of animals do you expect to use over what period of time?	Model systems for the study of these serious human and animal diseases have been established in mice, rats and hamsters. The total number of animals to be used over the course of the project has been estimated based on a prediction of the likely number of new substances for investigation. It is anticipated that up to 5000 adult mice, 1000 adult rats and 500 adult hamsters will be used over the 5 year duration of this project
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Our interventions are of two types. We shall test the efficacy of novel compounds to treat the disease state in the rodent model systems. Of course, we shall only do this when there are robust data from laboratory studies to suggest that the compounds are active and not generally toxic. Secondly, in order to have parasite preparations that are representative of those that are infectious in animals, for use in these laboratory studies, we shall have to harvest parasites from infected animals. These are standard procedures, where the severity can be precisely controlled and in which unexpected events should be very rare. Our rodent models of infection closely mimic the course of the human diseases and the outcome of treatment with the currently available medicines. The early symptoms of these infections are flu like (fever, loss of appetite, general malaise), but, like human patients, can be ultimately fatal if not adequately treated. By frequent monitoring of the health of infected animals and by measuring parasite numbers in small blood samples, we can usually withdraw animals from the studies and kill them humanely before signs of serious illness or death from the disease occur. However, our initial

	studies on *T. cruzi* (the causative agent of Chagas' disease) have demonstrated that these observations and tests are not always predictive of the stage of disease, mainly because these parasites hide in the tissues of the body, rather than circulating in the blood. We are applying advances in whole animal non-invasive imaging technology to measure the total parasite load and thereby reduce the severity of the protocol for developing new medicines for Chagas' disease.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	Compounds that show promising activity in laboratory tests may still prove to be poor agents in vivo and therefore there is still no alternative to using animals to select promising compounds for preclinical and clinical trials in humans and the "target" animal species (e.g. cattle). It is indeed a legal requirement to demonstrate safety and efficacy in animal models before human clinical trials can begin. Parasites are sometimes difficult to obtain in sufficient amounts from in vitro culture and have to be obtained from animals. The nutritional environment in tissue culture is different to that of the animal host and it is important to confirm that parasite adaptation to in vitro conditions has not affected virulence in animals or susceptibility to experimental compounds.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Careful experimental design is used to determine that the correct number of animals (neither too many, nor too few) is used to obtain biologically significant results. Generally, five animals are used for each dose of an experimental compound, with up to six doses being evaluated.
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	The rodent models of infection closely mimic the course of the human diseases and the outcome of treatment with the currently available medicines. The early symptoms of these infections are flu like (fever, loss of appetite, general malaise). As noted above, observation and measurement of the numbers of parasites in small blood samples can usually be predictive of the onward course of the disease and therefore can allow the early termination of the experiment before the disease state worsens. The

(harms) to the animals.	model for Chagas' disease is currently an exception, but as explained above, we are working on systems that may improve the predictability in that system too and allow the early withdrawal of animals from the studies while still providing robust data.

PROJECT 9	**Assessment of novel treatments of infection**	
Key Words (max. 5 words)	Bacterial, viral, infection, respiratory.	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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 project is to identify novel treatments and therapies against viral and bacterial infectious diseases	
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)?	Identification of new vaccines and anti-microbials for use against the ever increasing incidence of resistant and mutating strains that cause high rates of ill-health and mortality amongst the population. We are able to do this in an efficient and effective manner due to our extensive experience with animal models.	
What species and approximate numbers of animals do you expect to use over what period of time?	We expect to use a maximum of 24000 animals in total over the next 5 years. Most of these will be mice (18000) and rats (3000), but hamsters (1000), ferrets (1000) and fertile chicken eggs (2000).	
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected	Most animals are expected to show signs of clinical disease as a result of infection which may include changes to body condition (including bodyweight loss), abnormal posture (e.g. hunched), coat condition	

level of severity? What will happen to the animals at the end?	(eg. piloerection), breathing, activity, and chromodacryorrhea in rats, Most animals are not expected to develop symptoms above moderate clinical signs. Where severe disease is an expected outcome as a result of, eg septicaemia following bacterial infection, humane endpoints are described to ensure least suffering occurs.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	The immune systems response to the presence of pathogens, involves multiple systems, multiple organs and multiple cell types. The complexity of the immune response cannot be reproduced in vitro. In vitro experiments on cell lines and ex viva experiments on cell cultures will be performed. However, the limitations of these methods do not allow them to replace the use of experimental animals: there is no alternative to the use of a living animal that would allow the objectives to be met.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Power analysis will be performed to establish the total sample size required to generate meaningful data. Typically, power value will be set at 80% in order to reduce the number of animals used in the studies. Animal suffering will be limited by ensuring the most appropriate, robust and well defined models are used in order to achieve the objective. The animal of least neurophysiological sensitivity will be chosen. Number of animals used will be the minimum required to ensure statistically meaningful data is acquired. Endpoints will be identified to minimise suffering. Where possible mild over moderate severity models will be used.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs	Infectious doses and humane end-points have been defined which ensures useful information can be obtained without causing un necessary suffering. Lead compounds will be administered prior to or from the start of signs of disease (prophylactic and therapeutic regimen, respectively). Animals will be monitored frequently and scored for clinical signs of disease. Blood, cells and/or tissue samples will be collected at the end of the experiment for ex vivo

(harms) to the animals.	analyses. Where possible bioluminescent bacteria will be used to reduce number of animals required.

PROJECT 10	**Neonatal bacterial meningitis — infection and treatment**	
Key Words (max. 5 words)	in vivo imaging; Escherichia coil KI; blood-brain barrier; Group B streptococcus; neonatal bacterial meningitis	
Expected duration of the project (yrs)	5 years	
Purpose of the project as in ASPA section 5C(3)	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)	Neonatal bacterial meningitis (NBM) is a disease affecting newborn babies in both industrialised and developing countries. With a mortality rate of 15-50% globally, even for survivors the consequences can be seriously debilitating. NBM is a complex disease which involves several steps of pathogenesis. As bacteria are normally taken up by the infant orally during birth, they must then colonise the gut and transition into the bloodstream where they replicate and may cause sepsis and/or migrate into the brain. We propose to use murine models of NBM to define the bacteria-host interactions that enable bacteria to breach the host's barriers. To do this we will set up in vivo imaging models where the infectious organism is genetically modified to express proteins that	

	will glow when the organism is imaged. Following infection, these glowing bacteria can then be tracked using whole animal non-invasive imaging. As the disease progresses the location and concentration of infection can be determined at multiple timepoints in the same animal. Importantly, how different treatments may alter disease progression can then be visualised in different experimental groups. We wish specifically to look at how the components of the host cell membrane modulate bacterial invasion of host cells, which in turn affects the way in which bacterial infection will progress. To alter these components we will use genetically modified or immunosuppressed mice, or we will test agents that will modify membrane constitution, or through a fatty diet. This might affect gut and brain barrier cells as well as macrophages in the blood, and we will look for effects at the different stages.
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)?	Based on preliminary experiments in cell culture, altering cholesterol levels in the host cells has the potential to prevent bacterial invasion into the brain. This would restrict bacteria to the blood and would make them more accessible for antibiotic treatment and should lead to a reduction in the severity of brain damage. Identification of therapeutic agents for clinical studies would have the potential to prevent the damaging neurological sequelae in surviving human infants as well as to decrease mortality rates.
What species and approximate numbers of animals do you expect to use over what period of time?	We would anticipate using both mouse and rat neonates, approximately 2000 of each over the course of the project time of 5 years.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Initial pilot experiments will be conducted to set up scientific endpoints to earlier recognise ill animals, and prevent animals from dying unexpectedly. As this is a fast progressing infection, a small proportion of animals may die unexpectedly during this initial phase of the project. Following the pilot, we anticipate that in further studies the animals may feel unwell to the extent that they might experience a change in body temperature, weight loss of up to 10% or changing feeding patterns. All animals will be humanely culled before they become severely ill (i.e. lose more than 10% of body weight or stop feeding for more than a day) at the end of any experiment to enable collection- of blood and tissue samples to measure bacterial load.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	NBM has been studied using both in vitro and animal models. Cell culture models can only simulate specific stages of the infection process, and in order to mimic the human disease as closely as possible, to dissect the different stages of disease pathogenesis and to investigate the effects of potential treatments on disease outcome, it is necessary to look at the physiology of the whole organism during infection.
2. Reduction Explain how you will assure the use of minimum numbers of animals	Infection experiments usually require one to two litters of pups for one treatment. We will determine if there is cross-infection within a litter as assigning random treatment to individual littermates would decrease sample size. Furthermore use of bioluminescence imaging enables collection of data from the same animal through the experiment, rather than culling animals at each timepoint.

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.	Rat and mouse models of NBM associated with E. coil Ki infection have been described. The mouse model uses an inbred strain, which enables the benefit of genetically modified animals to address questions on the host-pathogen relationship. The rat model is long established in the preclinical field, however it uses outbred rats, and we would wish to investigate inbred animal strains to allow the use of smaller groups of animals. As it is hard to distinguish symptoms of illness in neonates such as ruffled hair used in adult animals, we will use the pilot study to determine endpoints that minimise animal suffering. As an example, whole animal imaging is noninvasive and may predict disease progression earlier than weight loss.