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Bovine Tuberculosis Research Projects Funded by Defra

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Foreword

The information provided in this document is collated from Defra's Science Search Website (http://randd.defra.gov.uk/).

Note that the information was correct at the time of commissioning each research project. However, during the lifetime of a project, objectives and deliverables sometimes change based on initial findings or to better reflect policy needs.

Summary table

The table below provides a list of ongoing bTB research projects funded by Defra as of December 2014. Further information is available on the following pages and on Defra's Science Search Website (http://randd.defra.gov.uk/).

Project Code	Project title	Research Institution	Start date	End date	Total cost
SE3045	An exploration of factors that influence the expansion of the area affected by endemic bTB, Social Science insights into the factors involved in expansion of the area affected by endemic bTB	APHA	16/01/2012	31/01/2015	£602,910
SE3046	A study to examine the interactions between cattle and badgers	Institute of Zoology	01/04/2012	30/03/2015	£1,416,232
SE3126	Development of a farm assessment tool for targeting badger biosecurity measures	FERA	01/04/2011	30/04/2014	£339,372
SE3129	Badger Survey of England and Wales	FERA	01/10/2011	31/07/2013	£870,984
SE3130	Transmission modelling and cost-effectiveness analysis of cattle vaccination at a herd level	Imperial College London	01/11/2011	30/09/2014	£481,855
SE3131	Developing a surveillance system to report TB in cattle herds exposed to badger control in England	APHA	01/10/2013	30/09/2018	£474,358
SE3132	Estimating Badger Group Sizes in Different Landscapes in England and Wales	FERA	01/09/2012	31/07/2014	£363,494

Project Code	Project title	Research Institution	Start date	End date	Total cost
SE3133	A study to identify factors associated with the detection of new TB breakdowns via abattoir surveillance in GB	University of Cambridge	01/10/2013	30/09/2015	£323,596
SE3135	continuation of SE3121: Social Science study to accompany the Badger Vaccine Deployment Project	University of Gloucestershire	08/01/2014	31/03/2015	£40,375
SE3246	Development of an oral BCG vaccine for badgers - research component	APHA	01/01/2009	31/03/2015	£6,846,178
SE3247	Development of an oral BCG vaccine for badgers - regulatory component	APHA	01/04/2010	31/03/2015	£5,465,077
SE3261	Joint estimation of epidemiological and genetic processes for *Mycobacterium bovis* transmission dynamics in cattle and badgers (BBSRC co-funded project)	University of Glasgow	01/04/2014	31/03/2017	£99,135
SE3265	Field approaches to identifying *Mycobacterium bovis* infection in badger populations	FERA	01/04/2011	31/03/2015	£1,093,412
SE3266	Continued vaccine development: Improving BCG and developing non-sensitising vaccines for cattle	APHA	01/01/2012	31/03/2016	£7,159,452
SE3268	Antigen mining, DIVA assays and other diagnostic approaches	APHA	01/01/2012	31/03/2016	£3,026,750

Project Code	Project title	Research Institution	Start date	End date	Total cost
SE3269	A longitudinal model for the spread of bovine tuberculosis (BBSRC co-funded project)	University of Cambridge	01/09/2011	31/08/2014	£50,433
SE3270	Emida project: Mycobactdiagnosis (Development of novel diagnostic strategies for the ante-mortem immunodiagnosis of bovine tuberculosis and Johne's Disease)	APHA and other EU partners; INRA, CVI-WUR, Enfer Scientific (SME), FLI, AFBI, IZSLER, IZSVE-Verona, MRI, UCD/CVRL	01/04/2012	31/03/2015	£827,104
SE3273	Systems for sample collection (blood and urine) from unanaesthetised badgers for diagnostic purposes	APHA	01/10/2011	03/01/2014	£197,683
SE3274	Review and preliminary assessment of alternative badger control methods		19/08/2013	30/11/2014	£99,157
SE3277	Evaluation of the potential for GnRH and BCG vaccines to contribute to the management of bTB in badgers (Fertility control in badgers)	FERA	01/09/2011	31/03/2015	£387,568
SE3280	Optimisation of sampling strategies for improving sensitivity of *M. bovis* detection by PCR	University of Warwick	09/01/2012	18/10/2014	£467,353
SE3281	Research and development towards novel field-based approaches to the diagnosis of bovine tuberculosis in badgers.	APHA	02/04/2012	31/03/2015	£801,145
SE3284	A study to design risk based bTB surveillance regimes in England and Wales	University of Warwick	01/09/2012	31/08/2014	£273,636

Project Code	Project title	Research Institution	Start date	End date	Total cost
SE3285	The development of quantitative risk-based surveillance strategies for bTB in England & Wales	University of Glasgow	01/09/2012	31/08/2014	£290,857
SE3286	LGC technical development of bovine TB testing and participation in the bovine TB ring trial	LGC Limited	20/05/2014	31/07/2014	£109,981
SE3287	Field trial design to test and validate the performance of the CattleBCG vaccine and associated DIVA diagnostic test in England and Wales	Triveritas	10/03/2014	31/08/2014	£198,000
SE3288	Qualitative Risk assessment for CattleBCG vaccine: risks to public health	APHA	01/10/2013	31/07/2014	£156,325
SE3289	Study to comparatively assess methods to detect *M. bovis* from badger faeces	APHA	01/06/2014	31/12/2014	£146,858
SE3290	Development and testing of Operational Models of Bovine Tuberculosis in British Cattle and Badgers	University of Glasgow	01/09/2014	30/04/2015	£329,006

An exploration of factors that influence the expansion of the area affected by endemic bTB, Social Science insights into the factors involved in expansion of the area affected by endemic bTB (SE3045)

Although pre-movement testing for bTB has had a significant impact on the long distance spread of the disease, especially on transmission to areas with relatively low incidence (Mitchell et al. 2008, Defra 2010), the role of local spread in the expansion of bTB-affected areas is not well understood. Despite the net annual benefit of pre-movement testing for bTB being predicted to reach tens of millions of pounds by 2015, illustrating the benefits achievable by controlling risk factors, the predicted long term trend is for bTB to continue to increase its geographical extent in England and Wales (Defra 2010).

This proposal will further investigate and quantify known factors that are associated with local bTB spread, and also develop novel theories on other factors. The project will (through an integrated multidisciplinary approach) use a mixture of existing comprehensive datasets, such as Cattle Tracing System, VetNet, VeBus and land-use maps, and also gather new data and information from industry representatives through workshops and surveys.

To establish how far and fast areas of endemic bTB are spreading, the primary step will be to develop a robust measure of exactly what is meant by endemic status. Endemic not only denotes a high prevalence of disease but also requires the disease to persist in cattle populations (and the associated wildlife). We shall establish an operational mathematical measure of endemicity based on surveillance results, taking into account changes in bTB surveillance activities, and apply it to data from the last ten years. Knowing the genotype of the bacterium causing bTB will help distinguish overlapping endemic areas. The areas affected by endemic bTB at regular time points will then be mapped and measured for speed of expansion.

Once the locations of endemic bTB areas and their rates of expansion are defined, the risk factors aiding spread (including geographical features and farm management practices where the expansion is occurring) will be investigated. There is a substantial body of literature on risk factors for bTB herd breakdown and spread. To maximise the use of this previous work we shall mine the literature for factors that have been demonstrated to have a significant impact on bTB spread (e.g. cattle movements) and for other potential factors, and investigate the influence of these factors on the speed of bTB spread. Hypothesising that different risk factors will operate in areas depending on local infection pressure and farming contexts; we will explore risk factors for TB in the context of disease endemicity.

As part of the project, an interdisciplinary social research component will iteratively identify relevant farm practices that may act as risk factors in the spread of endemic bTB. It will involve intensive social research with farmers in areas vulnerable to the spread of endemic bTB leading to the identification of social risk factors that will be incorporated within a subsequent survey. Crucially, the social work package will draw on observation as well as

traditional oral forms of research (e.g. interviews and focus groups) to identify relevant farm practices. It will also involve farmers and other participants (e.g. vets) in meaningful ways, for example through the identification and agreement of risk factors.

Objective A1, Mapping the expansion of defined endemic fronts in England and Wales

- To identify and validate a meaningful and generally applicable mathematical method for detecting the endemic status of bTB in cattle for given places and times, and to use the method to map the expansion of the area affected by endemic bTB through time.

Objective A2, Evaluating risk factors that affect the rate of expansion of endemic areas

- To analyse the relationship between the rate of expansion of endemic areas and locally coexistent bTB risk factors.

Objective B1, Exploring Farm Practices and Risk Factors on the edge of endemic areas

- To iteratively identify relevant farm practices that may act as risk factors in the spread of endemic bTB.

Objective B2, The incorporation of social research data on bTB spread into the epidemiological analysis

- The incorporation of social research data on bTB spread into the epidemiological analysis

Objective C, Project Management

- To manage the project to Prince 2 principles including establishing a project board, communication plans, project reports and logistical activities related to other work packages.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18056&FromSearch=Y&Publisher=1&SearchText=se3045&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

A study to examine the interactions between cattle and badgers (SE3046)

Bovine tuberculosis (TB) is a cattle disease which has serious impacts on farmers and the farming industry; hence its control is a priority for Defra. One impediment to TB control is the fact that some populations of badgers are infected with TB's causative agent, *Mycobacterium bovis*.

Strong evidence shows that badgers transmit *M. bovis* to cattle, but the mechanism of interspecific transmission remains unknown. In principal such transmission might occur both through direct contact between host species, and through indirect contact caused by contamination of the environment. However, the relative importance of these two transmission mechanisms is unknown.

Both direct and indirect contact between badgers and cattle can occur in pastures where cattle graze and badgers forage, and inside farm buildings where cattle are housed, and

cattle feed stored, and which badgers visit. However, once again, the relative importance for *M. bovis* transmission of outdoor and indoor contacts is unknown.

Scientists' poor understanding of the most important route(s) of interspecific *M. bovis* transmission compromises the control of cattle TB. Were it known how and where badgers transmit infection to cattle, specific management could be implemented to reduce transmission. Lacking such information, guidelines on keeping badgers and cattle apart are necessarily based on judgement rather than evidence of cost-effectiveness, potentially discouraging farmers from implementing effective methods, and perhaps wasting resources on ineffective techniques.

We therefore propose to:

1. Quantify direct and indirect contact between badgers and cattle;

2. Describe the spatial distribution of badger-cattle contact, especially distinguishing indoor and outdoor contact;

3. Investigate risk factors for badger-cattle contact;

4. Assess how badger-cattle contact is influenced by management to restrict animal movement; and

5. Compare the likely cost-effectiveness of general approaches to reducing badger-cattle contact.

The project will be conducted at four sites in TB "hotspots" in Cornwall, representing a range of badger densities. A combination of GPS-collars (which monitor animal locations using the same mechanism as vehicle satellite navigation systems) and video surveillance will give an unbroken picture of badger and cattle movements both indoors and outdoors. Proximity sensors (which record events when tagged badgers come close to collared cattle) will provide additional information on contact. The resulting data will be analysed to provide indices of direct and indirect contact. These indices can be compared with environmental conditions, and with the characteristics of individual farms, cattle and badgers. Having observed patterns of badger-cattle contact over a full year, we shall explore the cost-effectiveness of management proposed to reduce such contact, by experimentally excluding badgers from farm buildings and/or excluding cattle from field margins.

The project's findings should help to inform policy development by:

- focusing management on the most likely form(s) of interspecific transmission, in the most likely location(s);

- helping to explain why certain previously-identified risk factors influence cattle TB risk; and hence

- allowing improvement of methods to limit *M. bovis* transmission between badgers and cattle.

Objectives:

1. Quantify opportunities for direct and indirect contact between cattle and badgers (within 24 months of project initiation).

2. Describe the spatial distribution of direct and indirect contact between badgers and cattle, especially distinguishing indoor and outdoor contact (within 24 months of project initiation).

3. Investigate risk factors for badger-cattle contact, including environmental conditions; farm type (dairy vs. beef); individual badger characteristics; and individual cattle characteristics (within 24 months of project initiation).

4. Assess how badger-cattle contact is influenced by management to restrict animal movement, such as fencing badgers out of farm buildings, and fencing cattle away from field margins (within 36 months of project initiation).

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18325&FromSearch=Y&Publisher=1&SearchText=SE3046&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Development of a farm assessment tool for targeting badger biosecurity measures (SE3126)

A previous study (SE3119) demonstrated the effectiveness of biosecurity measures that could be taken to exclude badgers from farm buildings. Results indicated that participating farmers were mostly unaware of the extent that badgers visited their farms, and as a consequence were unlikely to install biosecurity measures.

The aim of this project is to produce a simple tool that can be used by people without specialist ecological expertise (such as farmers, vets, Defra agency staff etc.) to assess a farm's likelihood of receiving visits from badgers. It is hoped that such a tool would improve take-up of biosecurity measures on farms that were shown to be at risk of badger visits. If a successful tool is developed, Defra will also explore other potential uses for it.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17685&FromSearch=Y&Publisher=1&SearchText=SE3126&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Badger Survey of England and Wales (SE3129)

Obtaining an up-to-date estimate of the current size of the badger population will help inform policy on badgers and will assist the UK in addressing its obligations under the Bern

Convention. The last National Badger Survey of Great Britain was completed in 1997 and was a follow-up to the original survey carried out in the mid-1980s. The 1990s survey revealed that badger numbers had increased substantially in the intervening decade. There is a commonly held perception that the badger population has continued to increase since then. Surveys of the badger population in Northern Ireland and Scotland were conducted in 2008. In order to provide updated estimates of the UK badger population, we need information on the size of the badger population in England and Wales. To address this need we propose to carry out a new survey of England and Wales in 2011-13.

Badgers in southern Britain tend to live in social groups within a shared territory. Within a territory, there tends to be one, distinct main sett. Badger main setts were used as a proxy for the presence of a badger social group in the previous two national surveys of Britain, and this approach has also been used in similar surveys in Ireland. The survey will require repeat surveys of the 1-km squares which comprised the previous surveys in the 1980s and 1990s. These squares are a representative sample of the landscape types in England and Wales. Repeating these original survey squares will provide the most powerful means to quantify any changes that have occurred since the last survey.

Each 1-km square will be completely resurveyed and all badger setts will be mapped. Surveys will be carried out by professional surveyors during the autumn, winter and spring (1st November to 31st March) when vegetation is at its lowest and badger setts are easier to find. Setts will be classed as main setts or other setts. The sett locations will be entered into a Geographical Information System overlaid with the Centre for Ecology and Hydrology's Land Cover Map in order to accurately assign habitat type / land class to sett locations and survey squares. This will provide a basis for exploring habitat and landscape determinants of badger sett location and density.

The density of main setts found in the sample squares will be used to derive estimates of the total abundance of social groups by extrapolating from sample data by land class group. This is the approach taken in previous surveys. Analysis of population change will be based on comparison of all three surveys in a single statistical model using the presence/absence and abundance of social groups as the main response variable. Estimates of social group abundance can be converted to an estimate of the number of badgers by multiplying by an appropriate mean badger social group size. These data are currently being collated as part of a Defra-funded project (SE3128). The GIS that we will develop will also be available as a tool to assess/predict regional badger populations.

Objectives:

1. To conduct a repeat field survey of badger setts in approximately 1700 1km squares that were surveyed in the 1980s and 90s

2. To produce estimates of the number of badger social groups in 2011-2013

3. To assess change in the number of social groups since the 1980s and the 1990s, if any

4. To produce estimates of the badger population of England and Wales, and of the UK

5. To build and make accessible a GIS for the estimation of badger populations at a regional scale

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18014&FromSearch=Y&Publisher=1&SearchText=SE3129&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Transmission modelling and cost-effectiveness analysis of cattle vaccination at a herd level (SE3130)

Vaccination has led to the eradication of the human disease smallpox and the World Health Assembly committed itself in 1988 to the eradication of polio using vaccines. Although this has yet to be achieved, the disease is remains endemic in only four countries (Nigeria, Pakistan, Afghanistan and India). Vaccines have also been used effectively (and stockpiled for use) to improve and protect animal health in both wild and agricultural populations, for health threats as diverse as tick infestations (de la Fuente et al. 2007), foot and mouth disease (Cox et al. 2010) and brucellosis (Herrera et al. 2008).

Each year the control of bovine tuberculosis (TB) in British cattle results in thousands of cattle being slaughtered. It has been recognized that cattle in many endemic areas face risks of infection with *Mycobacterium bovis* (the aetiological agent of bovine TB) both from other cattle and from wild badgers (*Meles meles*). A vaccine that effectively protects cattle from infection with *M. bovis* from both sources could become one of the most important tools used to limit the spread of bovine TB to British cattle.

It is anticipated that the *Mycobacterium bovis* bacille Calmette–Guérin (BCG) vaccine will be licensed for use in cattle in 2012. However, it cannot be used in the field immediately upon licensing due to the use of the tuberculin skin test to identify so-called TB reactor cattle. Vaccinated cattle would be identified as skin-test reactor cattle and a change to EU legislation would be required in order for British cattle to be tested with an alternative test that would be able to distinguish between vaccinated cattle and truly infected cattle. Thus, field use of a cattle vaccine is not anticipated until 2015 at the earliest.

A model of disease transmission and cattle vaccination will be developed to characterize the effect at the individual-herd level of various vaccination strategies. The model will also include cattle testing and disease control. Thus, the model will be able to characterize the required investment (including the investments required to carry out vaccination and cattle testing) associated with particular TB control strategies. The project team will use cost-effectiveness analysis to indicate which vaccination strategies would be likely to achieve the greatest level of impact per unit of investment.

A wide-ranging sensitivity analysis of the model and cost effectiveness analysis will be undertaken in which alternative model structures are examined as well as different values for assumed parameters. For example:

- Because the level of protection conferred by the use of the BCG vaccine in cattle is not yet known, this project will examine what is judged to be a plausible range of these values.

- The default model's structure will include use of the “Differentiating Infected from Vaccinated Animals” (DIVA) test alongside conventional testing using the tuberculin skin test. However, the project team will also consider the use of a non-sensitizing vaccine, that is one with which there is no interference with the conventional tuberculin skin test and therefore the use of the DIVA test is not required.

- Revaccination with BCG will be considered for various between-vaccine intervals as well as the use of a booster vaccine.

The project team will liaise regularly with Defra (and/or Animal Health Veterinary Laboratories Agency, as appropriate) colleagues to ensure that their policy questions are addressed as well as possible. The results of this work will be used to inform other Defra-funded work. In particular, the results will be used in a larger model being developed by the Veterinary Laboratories Agency (VLA) to examine TB control at a national level.

Thus the project is designed to deliver not just policy-relevant research, but policy-ready research.

This project aims to develop and promote understanding of herd-level models of cattle vaccination to demonstrate the likely effectiveness and cost-effectiveness of various testing and control strategies. The project has three objectives:

Objective 1 – To develop models of cattle herds including the key individual-animal and herd factors affecting vaccination success including births, aging, cattle trading with other herds (movements on and off) and cattle slaughter. This objective will be the focus of roughly the first 15-18 months of the project.

Objective 2 – To explore the effectiveness and cost-effectiveness of various vaccination strategies implemented in silico using the herd models developed. This objective will be the focus of months 16 to 26 of the project.

Objective 3 – To promote understanding of the herd modelling approach and cost-effectiveness analyses through presentations and workshops. This objective will be the focus of the last 6 months of the project.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18035&FromSearch=Y&Publisher=1&SearchText=SE3130&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Developing a surveillance system to report TB in cattle herds exposed to badger control in England (SE3131)

The aim of this project is to monitor bovine tuberculosis (bTB) incidence in cattle herds located within and just outside areas where badger control licenses will be issued (intervention areas) and compare to that of herds located within matched “comparison areas”, plus regional/national trends. As far as is possible within the design of the badger

control policy, the purpose is to: a) monitor the effect of the intervention on bovine tuberculosis and b) identify important changes that could affect the badger control policy as early as possible. Given the pre-determined design of the control policy, this project will act as a surveillance activity rather than being hypothesis-driven research.

In the first year, badger control licenses will be granted by Natural England (NE) to two pilot areas, with up to ten further areas licensed in each of the following three years. As each new area is selected, NE will share boundary location data, enabling us to identify herds located in each intervention area and its 2km “buffer” region.

We will first establish historical (three-years preceding intervention) bTB frequency measures in herds located within intervention areas so that changes over time can be assessed. Several bTB frequency measures will be analysed: e.g. number of new herd breakdowns per 100 herds tested, proportion of herds under movement restrictions due to a bTB breakdown etc.

Secondly, as bTB incidence may change over time for reasons unrelated to intervention, areas without intervention will be selected as comparison areas. Comparison areas most similar to intervention areas in features such as historical bTB incidence, cattle demography and geographical location will be selected. This is to reduce the risk that differences in incidence between intervention and comparison areas are due to factors other than badger control. However, it will not be possible to completely remove this risk or that of random variation and therefore cautious interpretation of study results will be required. Once comparison areas have been selected, reports will be compiled for each intervention: comparison couplet describing their historical bTB incidence and other features (e.g. cattle herd demography) that may influence the interpretation of future results.

Six-monthly and annual monitoring reports will examine different measures of bTB in cattle herds through simple descriptive statistics and graphs and more in-depth analyses where the effects of some factors (other than badger control) that could cause a difference between intervention and comparison areas, are adjusted for using regression techniques.

Any observable change in incidence resulting from the badger cull is likely to occur slowly over time and effects may not be observed in the early years of the study. However the effect of culling is anticipated to increase over time therefore the surveillance methods developed herein should be used for longer term monitoring.

Objectives:

Work package A – Couplet identification and characterisation

- Develop a project protocol document
- Establish baseline disease frequency data for cattle located in intervention areas
- Identify areas with no badger control measures to serve as comparison areas to those with the intervention.

Work package B – Monitoring bTB incidence in cattle herds

- Monitor measures of bTB incidence in cattle herds located in intervention areas relative to those in comparison areas.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18287&FromSearch=Y&Publisher=1&SearchText=SE3131&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Estimating Badger Group Sizes in Different Landscapes in England and Wales (SE3132)

This project aims to generate estimates of typical badger social group size in different landscapes, to be used in conjunction with sett abundance estimates from a national badger sett survey, to produce estimates of badger abundance in England and Wales.

A Defra (Project SE3129) funded badger survey of England and Wales (BSEW) is currently underway, aimed at up providing an updated assessment of the badger population status in the two countries. This survey is using a well-established methodology focussing on surveying plots of land for main setts. Within a badger group territory there is often a larger “main” sett, which tends to be occupied most or all of the time, with other, smaller setts being used more sporadically. Main setts are considered a good proxy for the presence of a badger social group, and have been used as such in several large-scale surveys (Cresswell et al, 1990; Feore et al, 1994; Smal et al, 1995; Wilson et al, 1997).

A drawback of previous national surveys has been the limited available data on typical group sizes, causing difficulties in translating the estimated abundance of social groups into meaningful estimates of badger abundance. This is particularly problematic as it is known from long-term intensive studies that badger population size can vary due to changes in numbers of social groups, or changes in group sizes, or both (e.g. Rogers et al 1997). Additionally, badger social group size may vary over time and space. Relatively small bias in estimates of group size could lead to significant error if applied to estimates of social group abundance at national or regional scales. Therefore, ideally this variability in group size would be incorporated into analyses of the BSEW survey results, in order to produce estimates of badger population size for England and Wales that are as accurate as possible, in addition to estimates of social group abundance.

In this study, we propose to estimate mean (or median, as appropriate) badger social group size by landscape type, in a way that will complement the BSEW results. We propose to collect data on group size from a sample of social groups in each of the different landscape types from which the BSEW 1-km survey squares are drawn. We aim to use hair traps to snag or “capture” badger hairs from locations at and near setts. The traps will be visited on a number of days during a sampling period of pre-determined length at each sett, and hairs collected on each visit. The hairs will be genotyped to give an individual genetic fingerprint for each badger from which hairs were trapped. A number of mathematical population estimation techniques based on rarefaction and capture-recapture approaches have been developed in recent years for use on indirectly sampled genetic data such as this. We will apply these as appropriate, given the nature of the data collected, to estimate group size at all setts sampled. These data will analysed to produce typical group size estimates for the different landscape types.

The estimates of typical group size can then be used to multiply with the estimate number of social groups in each landscape type generated from the BSEW. Hence, variability in

badger group size will be properly incorporated into the national population assessment, providing estimates with minimum bias at a national and regional level.

Objectives:

1. Produce contemporary estimates of typical badger social group size in 6 Land Class Groups which form the basis of the sample stratification for the BSEW.

2. Quantify the variability in badger social group sizes between and within Land Class Groups.

3. Use these group size estimates as multipliers for the BSEW. That is, for each Land Class Group, multiply the estimated number of social groups by the typical group size (mean or median, as appropriate) to give an estimate of badger abundance, with confidence intervals.

4. Provide a baseline of typical group sizes across different landscapes, which can act as a baseline for future surveys.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18396&FromSearch=Y&Publisher=1&SearchText=SE3132&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

A study to identify factors associated with the detection of new TB breakdowns via abattoir surveillance in GB (SE3133)

Bovine tuberculosis (bTB) currently costs the UK taxpayer around £100 million per year in surveillance testing and compensation. Despite this, the incidence of bTB breakdowns (i.e. herds in which movement restrictions are enforced due to suspected bTB infection) has increased dramatically over the past 30 years. Detection of some cases in slaughterhouses is important, in particular in areas where testing is only conducted on farms every four years. However, in the past couple of years the proportion of bTB breakdowns disclosed by slaughterhouse surveillance (as opposed to routine skin testing) has increased significantly. The reasons for this are not at all clear, and elucidating why these cases are being missed will provide important information, not only for policy makers trying to control the spread of the disease, but also to help improve our knowledge about the biological mechanisms involved in bTB spread, and how these interact with the routine testing structure.

This project aims to tackle these issues in various ways. Although herds are tested routinely, it is not necessarily the case that individual animals are routinely tested. One possible reason why more cases are being disclosed at the SLH is if a higher proportion of infected animals are not being tested due to trade movements. We will determine what proportion of the increase in slaughterhouse detection might be due to differences in the degree to which animals are skin tested during routine surveillance and pre-movement skin testing.

Specific objectives include:

1. Collation and assessment of the utility of all available slaughterhouse data on bTB diagnoses, from a range of different sources, including the TB50 system, the TB System and the TB Laboratory Management System. We will also assess the utility of lesion data collected by the Food Standards Agency.

2. Identification of factors associated with detecting bTB by finding visible lesions in slaughterhouses using a detailed and longitudinal statistical framework. We will do this by linking all available current and historic cattle movement and testing datasets in order to extract demographic information, testing history and location/movement information of individual cattle. Dependent on the output of Objective 1, we will also combine this with more detailed slaughterhouse surveillance information.

3. Assessment of the degree of change in the level of undisclosed infection in the cattle population over time, using a longitudinal statistical transmission modelling approach. We will use this model to identify factors associated with the observed increase in the detection of infected cattle in slaughterhouses. We will account for the geographical and temporal variations in background incidence in these analyses, as this may drive many of the underlying patterns. If possible, we will evaluate the performance of some individual slaughterhouses over time.

4. We will use data that are available from slaughterhouses in order to determine risk factors for detecting bTB in other farmed livestock species, such as sheep, goats and pigs.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18922&FromSearch=Y&Publisher=1&SearchText=SE3133&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Continuation of SE3121: Social Science study to accompany the Badger Vaccine Deployment Project (SE3135)

The study aims to assess farmer confidence in badger vaccines in the BVDP area (Stroud) and four non-BVDP areas; the latter also includes areas of high bTB incidence.

Objectives:

The aim of the project is to examine and establish levels of farmer confidence in the use of vaccination before, during and after vaccine deployment. The research will also examine those factors that have contributed to the degree of success achieved, as well as the motivators and barriers that might influence farmers' future use of bTB vaccines as well as other preventative measures for bTB.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19368&FromSearch=Y&Publisher=1&SearchText=SE3135&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Development of an oral BCG vaccine for badgers - research component (SE3246)

There was an estimated 225% increase in the number of bovine tuberculosis (TB) incidents in Great Britain between the years 1996 and 2006. This adversely affects animal health and welfare, and is a cause of considerable economic loss to farmers and Government. Although transmission of *Mycobacterium bovis* between cattle is an important factor in spread of the disease, the Eurasian badger (*Meles meles*) represents an additional wildlife source of recurrent *M. bovis* infection to cattle in both the UK and Ireland. Following the Ministerial statement of 7 July 2008 that badgers would not be culled as part of control measures for bTB, the vaccination of badgers against TB has come to the fore as a possible means to reduce and control bovine TB alongside other control measures.

It has long been recognised that delivery of vaccine in oral bait holds the best prospect for vaccinating badgers over a wide geographical area and has proved highly successful for mass vaccination of other wildlife species against rabies. In the short to medium term, the human TB vaccine, *M. bovis* Bacille Calmette-Guérin (BCG), represents the best available option for vaccinating badgers against TB. BCG vaccine has the advantage of a long history of safety and protection in a variety of animal species including badgers, but generally doesn't work unless the vaccine is live. This is a challenge in the case of oral delivery as live BCG by itself is killed by stomach acid. Therefore, the success of an oral BCG vaccine for badgers will depend in large part on the ability to maintain live vaccine for prolonged periods in bait and subsequently when consumed by badgers.

The other critical component of the oral vaccine for badgers is the bait itself. The success of any badger vaccination strategy will be dependent on both the number of badgers consuming the bait and the effectiveness of the oral vaccine once consumed. At its most simplistic, the intention is to use the most attractive and palatable bait possible. However, numerous other considerations must be taken into account including, the properties of the bait (such as cost, shelf-life, compatibility with the vaccine formulation, ease of manufacture, handleability), factors affecting attractiveness of bait to badgers (such as age of badger, geographical location, weather, season, access to alternative foodstuffs), and method of bait deployment (including quantity, duration, location, frequency).

The overall aim of this project is to complete the research and development on the vaccine formulation and bait already started by the Veterinary Laboratories Agency and Central Science Laboratory for oral delivery of live BCG vaccine to badgers. The two components (vaccine formulation and bait) must be compatible with each other such that: (1) the vaccine stays alive for an appropriate time in the bait; and (2) the bait containing the vaccine is highly palatable to badgers.

Experimental work on the vaccine formulation will focus on determining how long BCG vaccine remains alive in different formulations in and out of bait, generating data on the

oral vaccine suitable for licensing purposes, such as production methods and quality control measures, and identifying the best way to place the vaccine into bait.

Experimental work on the bait will use captive badgers to identify palatable baits and then evaluate the most promising of these in populations of wild badgers in order to assess palatability, identify the optimal strategy for bait deployment, quantify the uptake rate of the bait in different badger populations around the country, and estimate the risk of cattle exposure to bait. No vaccine will be present in the bait for these studies. On the basis of this work, recommendations can then be made to Defra on the optimal baiting strategy for badgers.

Before any oral vaccine for TB in badgers can be licensed, further work will be required to evaluate the effectiveness of the oral vaccine in badgers, as well as conducting regulatory studies to determine if the oral vaccine is safe; first to captive badgers, then to wild badgers. These studies are subject to successful completion of the objectives of this project, which are:

1. Determine most suitable formulation for incorporating live BCG vaccine into bait

2. Determine most suitable bait for oral vaccine delivery to badgers

3. Determine optimal strategy for deployment of bait to wild badgers

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=16212&FromSearch=Y&Publisher=1&SearchText=SE3246&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Development of an oral BCG vaccine for badgers - regulatory component (SE3247)

There was an estimated 225% increase in the number of bovine tuberculosis (TB) incidents in Great Britain between the years 1996 and 2006. This adversely affects animal health and welfare, and is a cause of considerable economic loss to farmers and Government. Although transmission of *Mycobacterium bovis* between cattle is an important factor in spread of the disease, the Eurasian badger (*Meles meles*) represents an additional wildlife source of recurrent *M. bovis* infection to cattle in both the UK and Ireland. Following the Ministerial statement of 7 July 2008 that badgers would not be culled as part of control measures for bTB, the vaccination of badgers against TB has come to the fore as a possible means to reduce and control bovine TB alongside other control measures.

It has long been recognised that delivery of vaccine in oral bait holds the best prospect for vaccinating badgers over a wide geographical area and has proved highly successful for mass vaccination of other wildlife species against rabies. In the short to medium term, *M. bovis* Bacille Calmette-Guérin (BCG) vaccine represents the best available option for vaccination of badgers against TB. Options for the formulation of BCG in bait will be explored under a complementary research project. This project will focus on experimental work using the oral vaccine in order to generate data for the licensing of the vaccine.

There are defined steps to achieve this as follows, and each of these requirements form part of this project:

- Experimental safety study on captive badgers performed to Good Laboratory Practice (GLP) accreditation.
- A field safety study on wild badgers performed to Good Clinical Practice (GCP).
- Demonstration of the effectiveness of the vaccine to badgers in experimental infection studies.

Another important element to this project and related to the safety of the vaccine, is assessment of the likelihood and consequences of exposure of non-target species to the oral bait vaccine. EU Directive 92/18/EEC requires that the potential risk to unvaccinated animals of the target or any other potentially exposed species shall be evaluated. The Veterinary Medicines Directorate (VMD) will be responsible for evaluating the application to obtain a National Marketing Authorisation for the oral badger vaccine. The most significant non-target species from the perspective of TB control is cattle. There is a need to quantify and determine the risks associated with exposure of cattle to the badger bait vaccine in advance of decisions over whether to vaccinate badgers orally as part of TB control policy, and make recommendations about precautions that can be taken to reduce the risk. The first element of this process will be addressed in the complementary research project, where the palatability of various candidate baits to cattle is being evaluated in a natural grazing situation using camera surveillance. In this project, we will evaluate uptake in the higher risk situation where baits are deployed at setts to which cattle have access, and then move towards an evaluation of the means of reducing this exposure risk.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=16213&FromSearch=Y&Publisher=1&SearchText=SE3247&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Joint estimation of epidemiological and genetic processes for Mycobacterium bovis transmission dynamics in cattle and badgers (SE3261)

The control and eradication of infectious diseases can be difficult for pathogens that are able to persist in multiple host species. This is the case for bovine tuberculosis (bTB), a disease primarily of cattle but also found in a number of wildlife species; in Britain and Ireland the most important of these is the Eurasian badger (*Meles meles*). While Ireland has had a persistent bTB problem in cattle, by the 1970's bTB had been almost eradicated from GB but since then there has been a dramatically re-emerging disease in cattle. Bovine TB is a zoonosis with implications for both humans and animal health, though chronic cases of either in Britain and Ireland are few. Control of bTB also places a severe strain on individual farmers, the farming industry and government, with a projected cost in England & Wales alone of over £1bn over the next decade. While it has long been

suspected that badgers are involved, research efforts to date have not determined the extent to which badgers are responsible for bTB in cattle.

One of the most important developments in epidemiology of the last few decades has been the increased use of “genetic fingerprinting” to identify patterns of disease spread. Until recently, this has largely been done using only a small number of selected regions in the genome. While this kind of “genetic fingerprinting” has been useful and shows that cattle and badgers in the same region are usually infected by the same bTB strain, the fingerprints are far from unique: many cattle and many badgers share the same type, making it impossible to determine who infected whom. In this project we will take advantage of novel technology making it feasible and affordable to sequence the entire *M. bovis* genome for large numbers of samples Because the bacterium occasionally makes mistakes while replicating its genome, new mutations constantly arise which are not seen using traditional fingerprinting methods but with the new technology creating a much more unique and discriminatory genetic fingerprint of transmission. Using samples collected over decades from cattle and badgers in GB and NI we will sequence the genomes of hundreds of isolates to genetically track the spread of the pathogen and to test whether it is predominantly maintained in cattle, in badgers or both. The unique opportunity exploited in this proposal is the availability of extraordinarily dense sampling of cattle and badger infection together with entire life histories of individual cattle, including movement to other farms and whether it became infected with bTB at some point in its life. This creates an exceptional resource, allowing us to compare our very detailed understanding of contacts between cattle and between herds with the genetic fingerprint information. Based on this information we will use mathematical models linked directly to statistical inference methods to simulate how the infection may have spread through cattle populations in Britain and Ireland and how it may have genetically changed in the process. This will be done under various different assumptions about the multiple possible sources and mechanisms of infection. By comparing our simulated results to the actual observations (e.g. the number of infected cattle and the type of bTB they carry, etc.), we will gain unprecedented insight into the drivers for the spread of the disease and what may prevent its current control.

Objectives:

1. Quantify the spatial scale at which *M. bovis* circulates locally in cattle and badgers - using the concept of a social network community to organise the clade structure of the inferred phylogeny. Characteristic spatial scales for local circulation of *M. bovis* will be identified and a sampling strategy to capture the spatial patterns will be determined.

2. Quantify transmission arising from cattle only epidemics using genetic and epidemiological data. An estimate will be made for the role of unobserved infections in cattle for the within-herd persistence of *M. bovis*. Different models of *M. bovis* mutation rates will be compared. Parameter distributions will be inferred for use as priors for cattle to cattle transmission in High Incidence Areas (HIA's).

3. Characterise the badger-cattle interaction. Using data from Woodchester Park and the Northern Ireland TVR study, genetic diversity of *M. bovis* in sympatric cattle and badger populations will be compared. The relationships between badger and cattle isolates will be characterised in terms of spatio-temporal network and building on (2) and other work, a herd/social-group scale model of badger-cattle interactions will be developed.

4. An integrated multi-scale modelling framework combining genetic and epidemiological date will be developed and used to predict the spatial spread and persistence of bTB in the combined two-host system.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=16912&FromSearch=Y&Publisher=1&SearchText=SE3261&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Field approaches to identifying Mycobacterium bovis infection in badger populations (SE3265)

The selective removal of individual and/or groups of infected badgers is an attractive proposition as part of a policy to control TB in cattle. This project will involve the investigation of sampling protocols that would be required for sett-based and trap-side identification of *M. bovis* infection in badger populations.

Development of any effective protocol would have several characteristics. It would depend on confidence:

- that it was possible to obtain suitable samples for testing
- that the samples were sufficiently consistent in the results they provided
- that the test used was consistent under a range of field conditions
- that levels of sensitivity and specificity at individual/group levels were appropriate to the management approach.

Each of these presents a major practical challenge, but we can make practical progress on some of these issues and gain important insights into others using data from the Woodchester Park study, because of the availability of detailed ecological and epidemiological information on the resident badger population.

The proposed work will make best use of existing and contemporary data to explore sampling approaches related to selective control of *M. bovis* infection in badger populations.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17686&FromSearch=Y&Publisher=1&SearchText=SE3265&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Continued vaccine development: Improving BCG and developing non-sensitising vaccines for cattle (SE3266)

The 1997 “Krebs” report recommended the development of TB vaccines for cattle and badgers, and a diagnostic test to differentiate infected from vaccinated cattle. Since then total investment by Defra in cattle vaccine development has now reached around £18m.

Vaccination of cattle against TB could help reduce the number of TB herd breakdowns and their severity by reducing the rate and the consequences of infection from cattle or badgers to susceptible cattle and its onward transmission. Therefore alongside other control measures, vaccination has the potential to make a significant contribution to the control and eventual eradication of bovine TB.

However, the primary candidate vaccine against TB in cattle, BCG, is not 100% effective and a proportion of vaccinated animals may still become infected. Recent research also indicates that the duration of BCG induced immunity is between 1 and 2 years for cattle.

Under current trade rules, vaccination with BCG would also have an impact on the ability to trade live cattle and cattle products, such as milk. This is because the current BCG-based vaccine under development in cattle can interfere with the primary diagnostic test, the tuberculin skin test, producing false-positive results in vaccinated (but uninfected animals). Under current rules animals testing positive to this test would have to be slaughtered or re-tested, they would not be eligible for trade and their herds of origin would lose their officially TB free (OTF) status.

Therefore, in parallel to the vaccine, a diagnostic test has been developed to differentiate infected from vaccinated animals (“DIVA” test), that could be used alongside the tuberculin skin test for cattle that remain sensitised to the skin test (for most animals sensitisation is transient and will disappear within 6 - 9 months of vaccination).

This project builds and extends on significant progress made over the last decade in the development of TB vaccines for cattle and addresses key policy requirements that arise from the short duration of immunity induced in cattle by BCG.

First, we will determine whether annual revaccination of cattle with BCG can maintain sufficient levels of protection over time. We will also test if re-vaccination with BCG results in re-sensitisation of vaccinated animals to the tuberculin skin test and whether the DIVA test can still be used in the face of a number of rounds of BCG vaccination and tuberculin skin testing. We will also investigate whether novel vaccination regimes can be developed to improve and extend BCG-induced protection against *M. bovis* infection and whether the lead subunit vaccine candidates identified in SE3224 can be used to boost and extend the duration of BCG-induced immunity without sensitising animals to the tuberculin skin test.

Our second main objective is to develop vaccines that do not sensitise cattle to the tuberculin test. This would allow the continuation of existing tuberculin skin test-based test and slaughter strategies alongside vaccination and is the approach to vaccine development favoured by stakeholders and the EU.

The expected outcomes of this project will be to optimise the way BCG vaccination should be applied in cattle, to improve its efficacy and to develop vaccines that, whilst effective, do not sensitise vaccinated animals to the tuberculin skin test.

Objective 1:

- Development of vaccination strategies that improve the duration of immunity of BCG in cattle

Objective 2:

- Development of vaccines that do not sensitise cattle to the single intradermal comparative tuberculin test (non-sensitising vaccines)

Objective 3:

- Identification of biomarkers that identify cattle protected against *M. bovis* infection

Objective 4:

- Screening novel vaccine candidates in mice to prioritise vaccines for testing in the bovine challenge model

Objective 5:

- Co-ordinating collaboration with the human TB vaccine programme

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17687&FromSearch=Y&Publisher=1&SearchText=SE3266&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Antigen mining, DIVA assays and other diagnostic approaches (SE3268)

The incidence of bovine tuberculosis in GB had been increasing since 1988 despite the use of a control strategy based on tuberculin skin testing and slaughter of animals that react positively to the test and remains high. The development of vaccination strategies for cattle is an important part of Defra's research into future control strategies. In order to allow cattle vaccination to become a viable control policy option, diagnostic tests are required that can differentiate between infected and vaccinated cattle, so-called DIVA tests (Differentiation of Infected and Vaccinated Animals). This is also an important consideration to obtain legal approval from the EU to allow cattle vaccination This project is therefore aimed at the continued development and optimisation of such reagents and approaches.

The prototype DIVA test based on Interferon-gamma testing and using 3 different antigens (ESAT-6, CFP-10, Rv3615c) needs further improvement to achieve test sensitivity levels comparable with those achieved with tuberculin. Further, an alternative DIVA test format

based on skin testing would have a number of advantages compared to blood tests including potentially lower costs and easier acceptance by stakeholders and legislators including the EU. In project SE3233 we have provided proof of principle that the same antigens used in the Interferon-gamma DIVA test can also function as skin test antigens, although more antigens will be needed to improve signal strength and sensitivity.

Building on these previous projects, we therefore propose to continue the multi-track approach taken in SE3233 in this proposal. The applied research part of the project is focused at validation of antigens for tests like the DIVA Interferon-gamma, the DIVA skin test as well as tests using novel biomarkers identified in project SE3233. The basic research approach will focus on the discovery of novel biomarker signatures that could support diagnosis and a risk-based approach to TB control, alone or in combination with vaccination by comparing test-positive visibly lesioned animals with those presenting without lestions (VL versus NVL). Although this project is aimed at DIVA tests, its focus is wider by addressing novel diagnostic approaches and antigen mining for subunit vaccination which directly feeds into the associated proposal of developing vaccines that do not sensitise tuberculin skin test activity.

Outcomes of this project will be further insights into diagnostic strategies that underpin policy development for the use of cattle vaccination including the development of non-sensitising vaccines. Specifically, this will include development of further improved diagnostic reagents that allow the differentiation of vaccinated and infected cattle so that test sensitivities approaching that of tuberculin can be reached. These strategies could be based on an improved version of the interferon-gamma assay, a multiplex blood test or, indeed, a DIVA skin test. In addition, antigens identified during this antigen mining operation will also be assessed for their suitability as potential subunit vaccine candidates. This project will furthermore provide a better understanding on disease progression and latency and a test that can discriminate between active and latent TB would support and underpin a risk-based approach to the control of this disease.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17854&FromSearch=Y&Publisher=1&SearchText=SE3268&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

A longitudinal model for the spread of bovine tuberculosis (SE3269)

Bovine tuberculosis (bTB) is an important disease of cattle and badgers with substantial socio-economic impact in the UK, currently costing the exchequer over £100 million per year in surveillance and compensation and also resulting in costly movement and trade restrictions for farmers. Despite intensive controls, disease incidence is still increasing. Currently herds are monitored for the disease through slaughterhouse surveillance and through regular skin testing. The frequency of routine testing for an individual herd is based on localised incidence of the disease, which acts as a proxy for risk of infection, but does not account for individual herd-level characteristics or cattle movements. Recent bTB research has focussed on examining potential underlying causes for this, including environmental contamination (e.g. re-infection from local wildlife reservoirs), insensitivity to

the surveillance test and the impact of large-scale cattle movements. It is the purpose of this proposal to extend our recent work identifying markers for the persistence of infection in individual herds into a dynamic longitudinal framework in order to quantify the mechanisms of transmission in the GB national herd and to test the utility of our results as an aid to risk-based surveillance.

The dynamics of transmission of bTB infection can be represented by a model with transmission driven by chance processes, with an observation process that is governed by an imperfect test procedure (or slaughterhouse identification of visible lesions), leading to partially hidden infection. Herds that contain one or more reactors are classified as breakdowns, which then have movement restrictions and more rigorous testing imposed until the herd tests clear. Testing and cattle movement information is available through several large national datasets. Recent mathematical modelling approaches have been developed using these data and, while these will provide useful information on population-level parameters, they average out some detailed information available at the individual herd level. Also, they were not designed to predict disease recurrence at the individual-herd level. Here we propose to build a dynamic, statistical, individual-herd level model, based on continuous surveillance data, which we will fit to the data using a likelihood-based approach.

The main methodological challenge will be to deal with the hidden states (infection) and the movement of animals between the herds. Recent advances in statistical methodology, such as “data-augmented” and “reversible-jump” Markov chain Monte Carlo allow the joint distribution of the observed and hidden states to be estimated simultaneously along with key infection related parameters. We will explore an exciting alternative called “sequential filtering”. The main challenge is that these statistical techniques are computationally intensive, especially given the large scale (approx. 130,000 premises) and long time frame (6+ years) of the datasets. However, advances in computer processing technology, such as architectures for running algorithms in parallel on graphics cards, provide an exciting and cost-effective way to approach this problem. The focus here is on bTB, but these sorts of models and the estimation issues that we will address are relevant to a wide range of infectious disease systems, and the methodology developed in this project would be applicable to a range of disease systems.

It is the aim of this project to elicit information about the hidden states of the system from the test observations using robust statistical methodology, in a way that allows us to identify high-risk herds based on the past history of infection, as well as on localised incidence and connectedness to other premises. This information would have a practical use in terms of targeting specific herds with more stringent or more regular testing.

The main aim of this project is to develop a longitudinal statistical model to explore the mechanisms of spread of bovine tuberculosis (bTB) in the GB national herd and thus to identify improved control policies. The model will take full advantage of the rich level of longitudinal data available, including the Cattle Tracing System, which includes information on animal movements throughout the whole of the GB cattle network. Specific objectives are:

- To develop likelihood-based transmission models for monitoring hidden bTB infections in individual herds over time that incorporate explicit information about the timing and results of both the surveillance tests (SICCT/gamma-IFN/slaughterhouse surveillance) and cattle movements, in order to understand better the mechanisms of persistence and transmission of the disease in the GB national herd.

- To extend these models to incorporate explicit localised spatio-temporal structure by using random effect terms to account for heterogeneity in transmission due to localised mechanisms and persistence of the pathogen (e.g. wildlife/environmental reservoirs/fomites spread).

- To develop fitting mechanisms for these models that can be made computationally tractable as well as cost-effective. This will most likely involves parallel processing, either by using multi-core CPUs, or alternatively through the use graphics processing unit (GPU) technologies.

- To explore whether the model could be used for surveillance, at either the individual herd or parish level. This would help to identify individual herds or regions that may be at a high-risk of harbouring undisclosed infection in order to better inform the targeting of control measures.

- To explore ways in which the parameter estimates and model predictions could be updated as new data becomes available, without having to refit the entire system.

- To incorporate all of the above into a user-friendly interface such that the model could be used for ongoing surveillance by Animal Health, the Veterinary Laboratories Agency or Defra. This would be done using open-source software such as R (possibly using a web-based front-end).

- To explore whether the methodologies could be extended into epidemic situations for real-time model fitting/prediction.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17882&FromSearch=Y&Publisher=1&SearchText=SE3269&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Emida project: Mycobactdiagnosis (Development of novel diagnostic strategies for the ante-mortem immunodiagnosis of bovine tuberculosis and Johne's Disease) (SE3270)

Mycobacterial infections of livestock such as bovine tuberculosis (bTB) or Johne\'s disease (JD) exact an enormous cost on European agriculture. bTB and JD are chronic inflammatory diseases caused by *Mycobacterium bovis* (MB) and *M. avium paratuberculosis* (MAP), respectively. Detection and slaughter of MB infected animals is required under EU law but JD control relies on voluntary cooperation. Both diseases can affect multiple domestic animals and wildlife. Current diagnostic tests are based on immune responses to bovine, avian and johnin tuberculin (aka PPD), reagents which have specificity, sensitivity and standardisation constraints. Due to cross reactivity, use of PPD reagents in MB skin testing elicits immune responses that may confound subsequent

immunological detection of both diseases. Furthermore, the demonstration that JD infection interferes with bTB diagnosis in dually infected herds further increases concerns about the performance of PPD-based diagnostic tests. In addition, the absence of adequate diagnostic tools for early detection of latent MAP and MB infected livestock severely hampers disease control.

We propose an integrated approach to the development of improved diagnostic tests based on assay platforms (skin testing, serology, and defined MB and MAP antigens) that can be applied across both diseases and adapted to multiple host species whilst exploiting differences in the immunobiology of bTB and JD. Our aim is to improve the diagnosis of bTB and JD per se and to generate tools that are not compromised in sensitivity or specificity by co-infection or testing regimes.

Rather than working in isolation on either bTB or JD, as has been common in the past, our consortium bridges both fields in a multi-disciplinary approach. Together, we have experience in the biology of the diseases; experimental MB, MAP and MB/MAP infection models (cattle and goats), cellular immunology, bioinformatics, antigen mining, lipid and protein biochemistry, test development and exploitation.

The consortium will generate sera collections from MB, MAP infected and MAP vaccinated animals and will have access to samples from live animals to validate and develop antigens and tests. Ours is a multi-pronged approach; validating prioritised MB and MAP antigens and platforms, platform development and antigen discovery combined with a basic research arm.

Work packages:

- WP1: To validate performances in relation to sensitivity and specificity of already available MB and MAP peptides, proteins, and lipid antigens on existing platforms (skin test, IFN-gamma release assay (IGRA), serological assays).

- WP2: Development of multiplex assays to diagnose TB and JD. The aim is to develop (i) serology multiplex systems using Luminex platform in conjunction with MB and MAP antigens tested in WP1; (ii) to develop a combined serology and cytokine multiplex based on IFNgamma and other cytokines. The latter approach will also encompass the use of non-peptide glycolipid and lipopeptide ligands.

- WP3: Antigen discovery. Additional potential MB or MAP specific antigens will be identified by the following approaches: (i) Antigen mining using bioinformatics to predict MB and MAP peptides recognised by CD4 T cells, with promising peptides then chemically synthesised and tested; (ii) Preparation of MB and MAP glycolipids, (ii) MAP protein antigens by comparative proteomics with candidates to be expressed as recombinant proteins. Antigens from all three approaches to be tested (serology and IGRA).

- WP4: Improved understanding of T cell immunity. (i) Characterisation of T cells recognising non-proteinaceous antigens. We will functionally characterise T cells that recognise glycolipid and lipopeptide antigens by defining the T cell subset(s) recognising these antigens, their restriction elements, cytokine profiles and ability to kill or lyse MB or MAP infected target cells; (ii) Identify host biomarkers of latency using a caprine sub-clinical MAP infection model: expression of modulatory cytokines, eicosanoid, local immunity or effector/memory T cell balance.

Outputs: (i) Diagnostic tests that improve the performance of TB and JD diagnosis; (ii) development of novel serological and cytokine multiplexes; (iii) discovery of novel MB and MAP antigens; (iv) characterisation of non-conventional T cell populations; (v) biomarkers associated with latency to detect animals at different disease stages to facilitate a risk-based approach to disease control.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17883&FromSearch=Y&Publisher=1&SearchText=SE3270&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Systems for sample collection (blood and urine) from unanaesthetised badgers for diagnostic purposes (SE3273)

Bovine tuberculosis (bTB) continues to represent a major animal health problem in Great Britain, with more than 25,000 cattle slaughtered in 2009 as a result of positive diagnosis. Badgers act as a wildlife reservoir of the causative agent of bTB, *Mycobacterium bovis*, and are implicated in the transmission of disease to cattle. Experience from Great Britain and elsewhere demonstrates that the elimination of disease in domestic livestock may prove problematic where such a wildlife reservoir of infection exists. Consequently, the control of infection in badger populations in regions of high bTB incidence is a priority.

The Department for Environment, Food and Rural Affairs (Defra) has emphasised the need to identify practical tools with which to detect TB infection in free-living badgers, at both the individual and group levels. The diagnosis of TB in live badgers represents a considerable scientific and methodological challenge, and to date no technique has been identified which can be applied to a cage-trapped wild animal without the need for anaesthesia. Such techniques would potentially permit the application of a “trap-side” testing approach, thus facilitating a broader range of management options than are currently available for the sustainable control of TB infection in badgers and cattle. Even if accurate, “trap-side” tests can be developed a significant limitation to their use will be the collection of the diagnostic sample itself. Currently, samples are taken from badgers following induction of anaesthesia by injection under Veterinary supervision and under the authority of Home Office A(SP)A licences and is not practical on a large scale in the field.

The work proposed in this project is for the development of new systems for collecting blood and urine samples from conscious trapped badgers without recourse to anaesthesia. This work will complement a separate project (SE3281) that will evaluate a range of novel diagnostic approaches for TB in wild badgers using the types of sample that could obtained using the methods proposed.

The work proposed in this project is for the development of new systems for collecting blood and urine samples from conscious trapped badgers without recourse to anaesthesia. Specific objectives of the project:

1. Develop methods for capillary blood collection from unanaesthetised badgers

2. Modify cage-traps to collect urine

3. Compare sensitivity of diagnostic assays between samples collected from anaesthetised and unanaesthetised badgers.The work proposed in this project is for the development of new systems for collecting blood and urine samples from conscious trapped badgers without recourse to anaesthesia.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17888&FromSearch=Y&Publisher=1&SearchText=SE3273&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Review and preliminary assessment of alternative badger control methods (SE3274)

Management of rural badger populations in areas with high incidence of bovine TB in cattle is part of Defra's long-term strategy towards reducing, and eventually eliminating, bovine TB from England. This study has been commissioned to assess the feasibility of alternative control methods, including those targeting the individual animal and sett-level controls.

Objectives:

- Literature review

To update an existing literature review of potential control methods, to explore which are currently the most feasible and humane methods for control of badgers.

- Nitrogen-filled foam

The research involved addressing whether nitrogen-filled foam is a feasible control option for use as a sett level control. No animals were exposed to nitrogen-filled foam during this work.

- Carbon monoxide

The planned research involves preliminary tests to investigate the potential use of carbon monoxide in a sett environment. These preliminary tests will not involve the use of either live badgers or active setts. Management of rural badger populations in areas with high incidence of bovine TB in cattle is part of Defra's long-term strategy towards reducing, and eventually eliminating, bovine TB from England. This study has been commissioned to assess the feasibility of alternative control methods, including those targeting the individual animal and sett-level controls.

Evaluation of the potential for GnRH and BCG vaccines to contribute to the management of bTB in badgers (Fertility control in badgers) (SE3277)

Overabundant wildlife conflict with human and biodiversity interests around the world. As human populations expand and economies grow the scale of these conflicts increase and new threats emerge, particularly with respect to biodiversity and zoonotic disease. In the UK alone, the costs of wildlife damage and its mitigation exceed £500 million per annum. There is a need to develop humane, economically viable and environmentally sustainable methods to better resolve these conflicts. Worldwide, efforts have been made for many years to manage wildlife populations by reducing fertility rather than increasing mortality, however, only recently have fertility control technologies (FCT) begun to emerge that offer potential for contributing to conflict resolution. This has culminated in the recent registration of the single-shot injectable immunocontraceptive vaccine GonaCon in the USA. Previous Defra projects (WM0406 & WM0408) have been at the forefront of international efforts to evaluate the potential of these emerging technologies and have succeeded in demonstrating the effectiveness of these tools in individual animals of a number of species including the European badger.

It is increasingly recognised that fertility control and disease vaccination can complement each other with respect to wildlife disease management. The concept is that vaccination increases the level of immunity in the population, such that the number of infectious animals is insufficient for a disease to persist, whilst fertility control reduces the number of new susceptible animals entering the population, hence maintaining the raised level of immunity for long enough for the disease to disappear. There is thus potential for fertility control to work synergistically with BCG vaccination to reduce bovine tuberculosis (bTB) in badgers and hence contribute to controlling the disease in cattle. Project WM0408 provided some encouraging preliminary results regarding the generation of immune responses to GnRH in free-living badgers individuals that would be expected to render them infertile. The onset and longevity of induced infertility and the population level consequences of such infertility remain to be established in badgers. These questions are the subject of a separate project that will examine GonaCon use in free-living urban badgers along with the development of an improved diagnostic tool to evaluate the immune response of badgers to Gonacon treatment. However, there are two further key issues that must be addressed to evaluate the potential of the combined badger vaccination approach to controlling bTB and which are reflected in this project as a collaboration between complementary expertise and facilities at FERA and APHA.

Firstly, delayed implantation of the embryo in the uterus is a particular characteristic of badger reproductive physiology. Depending on the associated hormonal control of implantation and pregnancy maintenance it may be that there is a delay in the realisation of infertility in this species via the use of GonaCon. Gaining this understanding will require regular observation and sampling of particular individuals at relatively short intervals that are not feasible for free-living animals. Hence, here we will undertake a captive breeding study to evaluate whether delayed implantation delays the realisation of reduced fertility in badger sows that will also evaluate the level of immune response that is sufficient to induce infertility in this species. .

Once proof of concept is established in this experimental paradigm then the study will proceed to examining the second issue. This reflects the potential interaction between the GonaCon and BCG vaccines. Because of the complexities of immune system function these interactions could be positive, neutral or negative. The only way of resolving this uncertainty is a captive study involving combined vaccination followed by challenge with *M. bovis*. This will be done using an established vaccination/challenge model for Vaccine Efficacy Study (VES) in captive badgers.

The study thus has two objectives, firstly to evaluate the consequences of delayed implantation (including levels of serological markers) for fertility control of badgers by injection with a single-shot GnRH immunocontraceptive vaccine.

Then, if further review of all relevant information considers it appropriate, to evaluate potential interactions between GonaCon and BCG protection against experimental challenge with *M. bovis*.

These studies are essential to the evaluation of the feasibility of a novel combined fertility control and disease vaccination approach to the intractable and contentious issue of mitigation of bTB in badgers and disease breakdowns in cattle.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17952&FromSearch=Y&Publisher=1&SearchText=SE3277&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Optimisation of sampling strategies for improving sensitivity of M. bovis detection by PCR (SE3280)

The proposed research aims to identify an optimal regime for badger faeces sampling, maximising the sensitivity of a previously validated PCR test for *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB). Previous work by this team demonstrated that a quantitative real-time PCR test identified a proportion of putative positive setts at Woodchester Park when compared to FERA data for the social groups tested. Of 11 social groups covered by PCR testing and FERA trapping, nine were found to be infected using several diagnostic methods on samples taken from trapped animals. Of these nine, the previous PCR ring trial identified two with a third putative positive at one laboratory. The level of positivity identified by PCR was approximately 22% which is in line with conventional culture based methods although less than STATPAK which identified 78% of positive setts over four trapping episodes in 2009, and routinely identifies approximately 50% (Fiona Stuart, pers. comm.). Badger latrine sampling for the PCR ring trial was carried out in July and August 2008 on a single day for each social group, which was a suboptimal strategy for maximising test sensitivity but was adequate for validating the reproducibility of the test.

Further work will consist of intensive latrine sampling at periods of peak badger activity, combined with sampling over a one year period matching FERA trapping studies to provide a definitive data set for modelling optimal sampling regimes. The PCR test will also

be used on faeces samples collected from trapped badgers, allowing exact comparisons with shedding rates estimated by cultivation at FERA.

Heterogeneity of *M. bovis* distribution with in single droppings will be investigated using material from captive badger populations at APHA which have tested positive by culture. This will allow modification of sampling strategy, with multiple samples from single droppings or homogenisation methods used, if heterogeneity in spatial distribution is shown.

These data sets will allow an improved sampling strategy to be calculated using computer simulations and will also demonstrate PCR test sensitivity in direct comparison with conventional testing methods at FERA.

Accurate investigation of PCR test sensitivity combined with matched comparisons with conventional trapping based testing will elucidate the utility of the PCR test as a practical non-invasive management tool for identifying badger social groups containing infectious animals.

The main aim is to improve sensitivity of the PCR test for *M. bovis* by optimising latrine sampling strategies. The proposed research will also allow a rigorous comparison of the PCR test with conventional trapping and diagnostics, elucidating the utility of the PCR test as a non-invasive tool to identify infectious badger social groups:

Objectives:

1. Cross-sectional sampling over a 12 month period matched to live trapping periods.

2. Intense longitudinal sampling of the same territories above every 2 days over 10 days in peak badger activity periods- September/October 2011 and February/March 2012.

3. To identify the distribution of *M. bovis* within droppings to improve detection probability if high special heterogeneity is observed.

4. FERA will retain subsamples of faeces from trapped badgers which will be screened using the PCR assay for comparison with culture.

5. Simulated sub-sampling of data sets to identify optimal sampling strategy, in terms of time of year, numbers of samples, and regularity of repeat sampling.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18036&FromSearch=Y&Publisher=1&SearchText=SE3280&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Research and development towards novel field-based approaches to the diagnosis of bovine tuberculosis in badgers (SE3281)

The Department for Environment, Food and Rural Affairs (Defra) has emphasised the need to identify practical tools with which to detect bovine tuberculosis (bTB) infection in free-living badgers, at both the individual and group levels. The diagnosis of tuberculosis in live badgers represents a considerable scientific and methodological challenge, and to date no technique has been identified which can be applied to a cage-trapped wild animal without the need for anaesthesia. The work proposed in the current project will evaluate a range of novel diagnostic approaches for bTB in wild badgers, with the emphasis on assays which may be readily transferred to an in-field test.

Currently, the only methods available for the rapid diagnosis of bTB in badgers, which could potentially be applied in-field, are based on the detection of antibody responses to infection. However, these methods lack sufficient sensitivity to detect an adequate proportion of infected individuals. Diagnostic assays based on the detection of earlier stages of the immune response to infection are more sensitive but currently require specialised laboratory facilities and technical expertise. Thus the development of more sensitive methods for detecting antibody responses to infection is crucial. The current project will evaluate two novel serological assays, which have shown potential for the diagnosis of bTB in wildlife. Furthermore, both of these assays have the potential to be transferred to portable in-field platforms, and evaluation of these technologies is included within this project.

An alternative approach to the identification of bTB infection in badgers, which we propose to assess within the current project, is to attempt to identify products from the breakdown of *Mycobacterium bovis* (the causative agent of bTB) by the host, using urine samples. We propose to assess the sensitivity of a nucleic acid amplification based test for the presence of short fragments of *M. bovis* DNA, which we have demonstrated to be present in the urine of infected badgers even when the bacterium itself cannot be cultured. This approach would potentially permit evaluation of the infection status of badgers using samples which might be obtained from live animals.

Finally, we propose the development of a novel technique to detect TB on the basis of the detection of mycobacterial lipids in faeces from experimental and wild badgers and to develop a simplified protocol for its use in the field. In contrast to the other methods to be developed within this project, this approach would not require trapping of wild badgers, and would thus enable the identification of infection at the sett or social group level.

Each of these approaches forms a distinct objective within the proposed project. Work will be undertaken in collaboration with relevant experts from both academic and commercial organisations. The research is underpinned by on-going projects at both APHA and FERA, providing access to unique sample sets which permit the evaluation of the diagnostic potential of each approach. This provides significant cost savings within the project, as a minimal amount of additional work is required to provide access to diagnostic samples.

This project is organised around three modules that take a comprehensive approach to improving diagnosis of tuberculosis in badgers. The first module is broken down into two

separate objectives. The second and third modules each constitute a distinct objective. Each objective within the project is comprised of a series of work-packages that outline the scientific approach to be taken.

The first module focuses on evaluation and development of improved serodiagnostic tests for TB in badgers. We have been involved in the development and evaluation of a large number of serological tests for TB in badgers over the last 15 years and published widely on the subject (reviewed in Chambers et al. 2009). Serodiagnosis of badger TB offers the prospect of simple, rapid, and affordable testing but as outlined above, has consistently lacked sensitivity at the individual animal level unless the animal is heavily infected with *M. bovis*. The inclusion of multiple antigenic targets has given modest improvement to sensitivity but the repertoire of conventional protein antigens recognised by infected badgers appears rather limited (Greenwald et al. 2003). Whilst accepting that badgers at an early stage of infection may simply have very little circulating antibody directed to *M. bovis*, or that antibody may be present but with low affinity or avidity, the greatest promise for improved serodiagnosis lies with improved technology and more accurate testing platforms.

In this proposal we focus on two technologies and collaborations for which we already have some working experience and proof of principle data (IDEXX and Vantix). In both cases the ultimate aim of the work is a robust, affordable and field-able test of enhanced sensitivity. Additionally, we intend to evaluate a commercially available lateral flow device (the dual path platform, or DPP) which is currently planned by the manufacturers to replace the Brock TB Stat-Pak.

The second module moves to a novel PCR-based approach for detection of TB infection in badgers. Despite the reservations expressed by an expert panel assembled by Defra in July 2010 to consider bovine TB and the use of PCR, they concluded that “more research is needed to improve infected sett identification using PCR. However, it cannot be guaranteed that such research will result in a practical test or one that is low cost”. In recognition of the fact that conventional approaches for TB diagnosis using PCR rely on amplification of a relatively large genomic target that is predicated on the organism being present in the diagnostic sample itself, we wish to explore a promising PCR-based approach: the detection of transrenal mycobacterial DNA in urine. We already have proof of principle that this approach can be used to detect TB infection in badgers even when *M. bovis* could not be cultured from the urine itself. In this project we shall optimise and evaluate the method further to determine if it could be used diagnostically.

The first two modules require diagnostic samples obtained from live, trapped badgers. These include serum, whole-blood and urine as those most practicably obtained from animals without recourse to anaesthesia. It is intended that this project will run in parallel with another APHA-led project funded through Defra’s Bovine TB Research Requirements Call 2011-2012 - R4: Developing a Minimally Invasive Method of Obtaining Blood from an Unanaesthetised Trapped Badger (SE3273). Throughout this proposal, emphasis is given to those approaches most likely to be field-able; that is, practical and cost-effective for deployment in the field.

The third module is focused on a novel method for detecting infection remotely; that is, without requiring badgers to be caught and sampled. This approach relies on the testing of faecal samples obtained from badger latrines. However, unlike previous attempts to develop a PCR-based approach to detect *M. bovis* genomic DNA in the sample, we wish to build on proof of principle data generated in collaboration with Professor David Minnikin

(University of Birmingham) that mycobacterial lipid can be detected in faeces from tuberculous badgers, even when the organism itself cannot be isolated by culture.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18064&FromSearch=Y&Publisher=1&SearchText=SE3281&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

A study to design risk based bTB surveillance regimes in England and Wales (SE3284)

Bovine tuberculosis (bTB) is an increasing problem for the national cattle industry; testing and control pose a significant financial burden to individual farmers and national government. Issues surrounding the control of bTB are highly emotive due to the potential role of badgers (and other wildlife) in the maintenance and transmission of infection, requiring that control be based on fully justified scientific rationale.

Testing of cattle in England and Wales (Scotland has recently been declared officially bTB-free) serves two main purposes, to inform about the levels of infection nationwide and to detect and control infection. Working closely with Defra and APHA we will define various goals to be optimized by any future testing policy; for example, these could include rapid progress to bTB-free status for a minimum cost, or maximum reduction in prevalence for a fixed cost.

Current testing policy is based on the recent history of infection in a local parish, together with pre- and, in Scotland, post-movement testing to prevent spread to new areas. This project will utilise cutting edge mathematical models and statistics to determine an optimal policy of testing. We propose to develop an intuitive “traffic light” system for prioritizing surveillance testing, where premises are classed as red, amber or green depending on their priority. This protocol will be based upon a wide range of statistical information, which has been shown to correlate with risk of infection; examples include herd size, breed types, age of cattle, location, bTB history and time since previous test. A detailed mathematical model will then be used to determine how these correlates should be combined to achieve the desired objective.

Our final goal is to present a simple, workable system for prioritizing herd-level testing, together with a simple (graphical) tool that can rapidly calculate and display prioritization. In addition, it is important that any testing protocol has an accompanying methodology that allows the global and regional prevalence of bTB to be estimated.

Our main aim is to develop a systematic, evidence-based prioritization scheme for bTB surveillance testing that optimizes a set of prescribed goals. This is achieved through six main objectives:

1. Review and assess the statistical risk of a herd suffering a breakdown or persistent infection based on farm, movement, geographical and test covariates

2. Build upon an existing national model of bovine TB transmission to include a more refined parameterisation of local spread and multiple test types.

3. Define measures of efficiency for surveillance and control systems, such that the merits of different protocols can be assessed.

4. Develop an optimized “traffic light” system for prioritising testing based on known statistical correlates and transmission risks (objective 1), and determine how the test results can be used to accurately reflect prevalence.

5. Predict the cost and impact of implementing “traffic-light” prioritisation using the spatial model (objective 2), and using the efficiency measure (defined in objective 3) compare this to the current programme.

6. Recommend a workable system for prioritizing herd-level testing and a simple tool (with graphical capability) that can rapidly calculate and display prioritization. This utilizes the results of objectives 3-5.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18323&FromSearch=Y&Publisher=1&SearchText=SE3284&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

The development of quantitative risk-based surveillance strategies for bTB in England & Wales (SE3285)

In recent work, a research group led by Kao identified risk-based surveillance strategies for bovine Tuberculosis (bTB) in Scotland, using a combination of statistical models and a simulation model for freedom from infection, to identify a series of possible strategies to reduce the number of regular herd tests for bTB in Scotland. The current proposal will be based on this work where appropriate, but because of the large endemic, “high risk areas” in England and Wales, we shall also consider different approaches for risk-based surveillance compared to Scotland, that will be guided by the identification of (i) areas where herds are at high risk of breakdown (HRAs), (ii) areas where herds are at a low risk of breakdown (LRAs), and (iii) areas that are perceived as LRAs, but are “transitional”, i.e. at a high risk of becoming HRAs in the near future (TAs). Depending on the type of area, different strategies will be adopted, according to the following criteria:

i. Reduction in testing - directly reduction of the amount of testing done, is a strategy aimed directly at reducing costs. Ideally, this will be a “dominant” strategy, whereby testing is reduced but with minimal risk of an increase in incidence of breakdowns. This strategy would be most similar to the recommendations for Scotland.

ii. Improvement in ascertainment - this aims to more quickly identify herd breakdowns, most importantly to identify when potential TAs might become HRAs.

iii. Onward risk reduction - this aims partly to identify herd breakdowns more quickly as in (ii) above, however the aim is also to specifically target herds that potentially move many cattle onto other herds, thus increasing the total number of breakdowns

in the national herd. This is more likely to be important in HRAs, though onward transmission must also be a consideration in other regions.

We shall adopt a four step approach to this project, using approaches and analyses based on existing work, either published or currently undergoing scientific peer review.

A. Identify HRAs, LRAs and TAs.

B. Develop statistical models to identify risk factors for breakdowns within each type of area.

C. Define risk-based surveillance strategies based on combinations of the criteria (i) to (iii).

D. A within-herd model of transmission will be fit to the available disease notification, demographic and livestock movement data, to identify the potential for these breakdowns to be the source of onward transmission under risk-based strategies in (C); i.e. if and when the new strategy allows some potential breakdowns to persist longer before identification, what is the risk of onward transmission?

E. Based on the best, analytically sound recommendations based on A to D, we shall consult with our partners with policy, farming, and veterinary experience, to determine their feasibility.

We shall therefore provide a comprehensive analysis of all three risk situations (HRA, LRA and TA) where the three aims of testing reduction, improved ascertainment and reduced onward risk will be balanced against the practicality of implementation and relevance to policy requirements.

Objectives:

1. An assessment of the strengths and weaknesses of different components of surveillance, including active and passive (slaughterhouse) surveillance and how to make optimal use of them in herds, or groups of herds, of different risk levels.

2. Definitions of subsets of the cattle population or types of herds based on risk of disease. This should include a description of the characteristics or parameters relating to a given population that are important in determining which surveillance regime should be applied to it.

3. Outlines of surveillance schemes optimised for different types of risk which can then be targeted with optimised surveillance regimes.

4. An estimation of the impact of the surveillance options proposed on the bovine tuberculosis epidemic.

5. An assessment of whether annual or biennial herd testing is justified in low incidence areas of England that are currently on 4-yearly testing.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18324&FromSearch=Y&Publisher=1&SearchText=SE3285&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

LGC technical development of bovine TB testing and participation in the bovine TB ring trial (SE3286)

Within this project LGC will rapidly explore 4 distinct DNA extraction methods, two different DNA analysis methods and the targeting of 3 potential mycobacteria sequence. These various options will be applied initially to BCG DNA and cells spiked into bTB-free badger faeces, as a bio-safe analogue to the bTB equivalent. The outcome of these studies will then identify the preferred approach to be taken forward by LGC. This will then be applied to bTB cells and DNA provided by the APHA laboratory. The nature of this short timescale requires considerable parallel processing and availability of biosafety trained staff but LGC will focus such staff to give this project priority.

It is planned that this project will have established the methodology to be applied to the forthcoming ring trial involving c.500 samples.

Objective 1 - Audit. LGC will conduct an audit of existing procedures that exist today in UK Academia, Government, Industry for the detection of bovine TB in badgers. The findings from this audit will be reviewed/considered to refine the best practice to be incorporated into the study below.

Objective 2 - Design and Preparation. Orders for all required initial materials will then be placed.

Objective 3 -DNA Extraction. Methods Development & Testing Validation. The objective will be to establish an SOP that can then be applied to the ring trial samples.

Objective 4 - DNA Detection. Methods Assessment & Testing Validation. The objective is to establish an SOP that can then be applied to the ring trial samples.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=18565&FromSearch=Y&Publisher=1&SearchText=SE3286&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Field trial design to test and validate the performance of the CattleBCG vaccine and associated DIVA diagnostic test in England and Wales (SE3287)

Tuberculosis (TB) in cattle is an infectious disease that presents an ongoing challenge to cattle farmers in affected regions e.g. parts of western England and Wales. Development

of a cattle vaccine against TB is a high priority for the Department for Environment, Food and Rural Affairs (Defra) and the Welsh government and funding has been made available for a major study of the disease.

The project is to design field trials of a vaccine to protect cattle against TB and of a new cattle TB diagnostic test to show both can be authorised for use in the UK. After authorisation the vaccine and new test could be used in conjunction with other control measures to reduce and hopefully eradicate cattle TB in the UK.

A potential cattle vaccine has been identified following substantial scientific work (initial results suggest it is likely to provide protection to approximately 60% of vaccinated cattle) but its safety and effectiveness in actual field use must be established.

The current test to diagnose TB in cattle unfortunately cannot differentiate between vaccinated cattle and infected cattle, so trials need to be designed to show if a new diagnostic test will work any better under field conditions. The new test must accurately differentiate vaccinated cattle from those naturally infected with TB to help control the disease. Having an effective test is essential for an overall disease control programme.

Prospective designs for field trials will be produced by a team of experts considering how the vaccine and the new diagnostic test could work, and the social and economic implications in the control of this complex disease. Experts in computer modelling, statistical analyses and conducting field trials to meet regulatory and international trade requirements will work with veterinary practitioners and farmers in TB regions to design the trials.

The objective of this project is to find the most appropriate and cost effective designs for complex field trials in cattle to satisfy both scientific requirements and to address problems to trading and farming caused by the uncertainty in the current TB tests. Subsequent results of field trials will be expected to:

1. support the authorisation of a new cattle TB vaccine by showing its efficacy and safety;

2. validate a new TB diagnostic test;

3. help understand how TB vaccination and testing would be acceptable to farmers, vets, and consumers;

4. assess the likely impact of the vaccine and new test on the UK TB disease situation and the control programme;

5. inform the work, costs and benefits of vaccine deployment in the UK.

TB controls have cost English taxpayers £500 million in the last 10 years, £1 billion is the estimated cost in England over the next decade if no further action is taken. Current control measures cause major difficulties for affected farmers. If well designed field trials are successful in meeting the objectives of the project this will save taxpayers money, support UK farming, and provide a more effective government control strategy leading to TB eradication from British cattle.

The licensing of cattle BCG is a major Defra policy priority. This project is to design a field trial that will estimate the efficacy of the vaccine and its associated diagnostics which is required to provide evidence to lift the ban on vaccination throughout the EU.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19116&FromSearch=Y&Publisher=1&SearchText=SE3287&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Qualitative Risk assessment for CattleBCG vaccine: risks to public health (SE3288)

As part of a disease control strategy to reduce the prevalence, incidence and spread of bovine TB in England & Wales a cattle vaccine is required. APHA have developed a BCG vaccine for use in cattle (CattleBCG). The vaccine has shown to provide a good degree of protection to cattle in both experimental and natural settings. In order to gain approval from the UK Veterinary Medicines Directorate (VMD) for a new Marketing Authorisation (MA) for CattleBCG, a risk assessment is required. The risk assessment is to assess the public health risks of CattleBCG being present in the food chain, in particular within milk and beef products. Additionally, data gaps and areas of significant uncertainty will be identified, highlighting areas for possible future research. A qualitative assessment will be carried out in the first instance with quantitative models produced where data allows. A full description of data and methodology used within the risk assessment will be provided within the final report.

The aim is to produce a qualitative risk assessment to provide an estimation of the risks to human health from the proposed use of the CattleBCG vaccine. To develop a QRA to:

1. Assess the risk of infection with the CattleBCG *M. bovis* strain due the consumption of milk and milk products.

2. Assess the risk of infection with the CattleBCG *M. bovis* strain due the consumption of beef products.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19172&FromSearch=Y&Publisher=1&SearchText=SE3288&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Study to comparatively assess methods to detect M. bovis from badger faeces (SE3289)

Over the last 7 years Defra has invested in the development of diagnostic tests to detect *M. bovis* in badger faeces. These tests have the potential to allow identification of TB infected badger setts without the need for trapping animals. The use of such tests could underpin future disease control strategies and policies.

Potentially useful PCR tests have been developed by Warwick University and Cepheid (in collaboration with BadgerCare). Such tests measure the amount of *M. bovis* DNA in a given sample. Queen's University Belfast has developed a lateral flow device (LFD) test, similar to a pregnancy test and which detects *M. bovis* bacteria present in a given sample.

In December 2013, the Diagnostic Programme Advisory Group (DPAG) of the Defra bTB Science Advisory Body reviewed the research undertaken in this area to date. DPAG's judgement was that it was not currently possible to definitively say which method was most suitable for routine deployment. They advised that an inter-laboratory comparative study of the currently available methods be undertaken, to identify the best performing diagnostic test for *M. bovis* identification in badger faeces. Once identified this test could then be taken forward for further development, validation and potentially commercialisation

The inter-laboratory comparative study will involve preparation of a panel of badger faecal samples of known TB status (consisting of a panel of negative, natural putative positive and spiked positive faecal samples). This will be prepared by APHA and distributed to participants as a laboratory comparative study. The panel will consist of 570 samples containing a mixture of TB negative, putative TB positive samples and spiked positive samples. It is planned to make 20 sets of samples (1 will used for stability and homogeneity testing) even though at present approximately 6 sets are required for currently known study participants. This will allow other sample sets to be used to test future diagnostic tests.

Participants will test the samples and subsequently submit their results for analysis. The analysis of the results will be undertaken by Royal Veterinary College staff. APHA will also carry out audits of the participating laboratories during testing to ensure adherence to submitted testing protocols

The results of this study will provide essential data to judge the impact, success and practicality of the use of such diagnostic approaches for the identification of *M. bovis* in badger setts. This project proposal has been prepared to bring together all the work associated with this laboratory comparative study, at the request of Defra. It has been previously planned to include the work as an additional work package of an existing project run by the APHA National Wildlife Management Centre - SE3265. As a result, some of the design and preparatory work required has already been completed. This explains why some of the milestones of the project have already been completed. However, they have been included in this proposal for clarity and completeness. The intention is to consolidate all past and future project spend on badger ring trial associated activities into this project.

http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=19296&FromSearch=Y&Publisher=1&SearchText=SE3289&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description

Development and testing of Operational Models of Bovine Tuberculosis in British Cattle and Badgers (SE3290)

The bovine Tuberculosis modelling initiative brings together internationally recognised academic experts in the development and interpretation of mathematical and simulations models of bovine Tuberculosis (bTB) transmission and control in cattle and badgers. The aim of this consortium is to develop and test an operational modelling framework of bTB transmission and control. This project will be based on the best available evidence regarding the epidemiology of bTB, emphasizing robustness over complexity, so as to enhance the long term viability of the framework being developed.

For the purposes of this project, we define the following:

i. Operational: useable by appropriately trained and educated/experienced staff within the APHA, with capacity of further adaptation and refinement by the initiative core members or other appropriately qualified individuals or groups.

ii. Modelling framework: a set of interlinked, consistently developed modelling modules that can be robustly used for addressing various policy relevant questions, at various scales.

iii. Robust: scientifically sound and founded on well-tested and clearly described principles (epidemiological, mathematical, statistical, demographical and ecological, as appropriate)

The initial phases of this project will emphasize the development of the framework to consider cattle-based controls, including variation in testing schedules in order to reduce spread due to cattle and/or reduce testing burden. In order to ensure forward compatibility, a parallel stream of development of models to understand badger bTB infection dynamics will be undertaken, with the aim of future incorporation of approaches that consider both simultaneously.

The overall aim of this proposal is to develop an appropriate suite of predictive models (a “modelling framework”) for bTB transmission and control across multiple scales relevant to disease control in GB. Upon completion, this framework will primarily be used by APHA epidemiologists, to inform and support policy development and policy impact assessment. This aim has four underlying themes:

1. to integrate our understanding of existing models of bTB transmission in cattle and badgers, into a consistently parameterized framework, where these are appropriate to our objectives,

2. based on identified gaps in the available approaches, to develop new models where knowledge is sufficient,

3. to advise on the potential vulnerabilities in model prediction, including socio-economic and behavioural contexts, and

4. to advise on new research that may need to be commissioned, in order to fill gaps in knowledge and/or advise where modelling approaches may be inappropriate.

These tasks will be embedded in the development of the operational framework to inform policy development support by APHA staff. These models will be based on sound epidemiological modelling principles with an emphasis on scientific and technical robustness. The easiest route to informing policy would be a predictive model for the control of bTB with a high level of granularity that explicitly includes local information and explicit cattle and badgers at the individual-animal level. Such an approach would have the advantage of being able to consider the full complexities of all likely disease control scenarios. While various models, both peer reviewed and in development or unpublished, incorporate some of these elements, to our knowledge, a robustly parameterised model including all these elements does not exist. Many data needed for such an approach are not available. Further, it is our view that even were perfect data to exist, a scientifically robust model of this type is likely to be beyond the scope of current approaches and therefore also beyond the remit of this group.

We note that as the demands on model outputs change (short term, medium term and long term predictability) appropriate caution will have to be exercised in the interpretation of outputs. In particular, longer term considerations of the extent and severity of the bTB epidemic can only be viewed as, at best, qualitative indicators, due to the uncertainties in the badger-cattle interaction, and the impact that human behaviour will have on the outcome.

Our specific Objectives include:

O.1 Development of within-herd model of bTB in cattle

O.2 Development of between-herd models

O.2.a Historical models

O.2.b Predictive models

O.3 Development of local badger models

O.3.a Historical models

O.3.b Predictive models

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