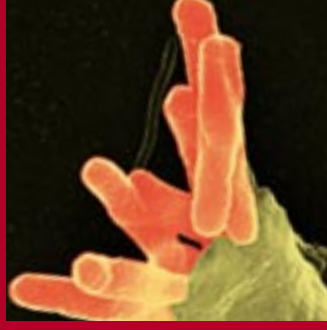

The Society for General Microbiology Independent Overview of Bovine Tuberculosis Research in the United Kingdom; a Defra Sponsored Review

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Front cover: Coloured SEM of a macrophage (green) engulfing cells of *Mycobacterium bovis* (orange). *Science Photo Library*

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SUMMARY OF RECOMMENDATIONS

General points

We identify three priority areas in which we believe new or additional investment in research could impact on the control effort. Related recommendations are developed further under the specific points below.

1 Diagnosis. We recommend further work on the development of simple screening tests applicable to cattle, badgers and contaminated environments. Critically, such tests should support high throughput and be focussed on sensitivity. Availability of such tests would allow more resource-intensive analyses with higher specificity to be deployed in confirmatory investigations that would lead to statutory intervention.

2 Farming practices. The evidence base related to relevant farming practices is weak. We have insufficient information regarding the levels of understanding that farm workers and managers have regarding key issues affecting the control of bovine tuberculosis (bTB) and how this and other factors translate into relevant farm-level and economic actions.

3 Existing resources. We recommend that several extant and developing sources of information should be fully exploited, monitored and analysed to extract maximum benefit. Most important in this regard is the continued analysis of the impact of the Randomized Badger Culling Trial (RBCT), where the net effects of interventions on bovine disease have yet to be resolved. Further, where they are relevant, maximum use should be made of the results of studies concerned with human TB to progress our understanding of bTB. Finally, there is a strong case for a comprehensive and authoritative description of the histories of the bTB epidemics in the UK and Ireland, and a comparison with historic bTB within these islands.

In addition to these points, we strongly endorse current efforts being made towards vaccination of badgers and cattle and comment on some specific aspects of the work.

A key aspect of our remit was to advise on the potential application of new technologies. In this regard, we note that newly established high-throughput genome sequencing technologies render much more extensive work in this area economically feasible. We identify several valuable applications of such data below.

Finally, we were asked to comment on aspects of the bTB problem which, though important, could not be resolved with currently available investigational tools. It would be of paramount value to determine the relative importance of specific routes of transmission including cattle to cattle, badger to badger, badger to cattle and cattle to badger, both in general and in specific breakdowns. We are not convinced that this can be achieved to more than a very limited degree at present. Nevertheless, some progress can be made in terms of the risk within each category.

Specific points

Diagnosis

1 Considerable investment is being made by other agencies in developing tests that directly detect the presence of *Mycobacterium tuberculosis* (Mtb), the agent of human tuberculosis (hTB). Any highly sensitive methods developed in this field are likely to be adaptable to bTB and we advise a watching brief in this area.

2 Evoked cytokine tests such as the gamma interferon (g-IFN) test detect infection at an earlier stage than the tuberculin skin test (TST). Thus a significant number of TST-negative, g-IFN-positive animals will be identified by the current testing regimen. These animals are an important resource for the study of certain key aspects of the natural history of infection, and the predictive capacity of evoked cytokine tests based on different antigen/cytokine combinations.

3 We recommend continued investment in development and evaluation of evoked cytokine tests both independently and in support of point 2.

4 It appears likely that the present TST-based screening programme is insufficiently sensitive to detect many infected bovines before onward transmission occurs. Although the g-IFN test is more sensitive, both logistic and economic reasons currently preclude its application as a first-line screen. A simple cheap test is therefore needed for screening. Provided that its sensitivity is close to that achieved by g-IFN, its specificity could be considerably lower. We recommend that epidemiological modelling be used to define target sensitivity and specificity levels for screening and confirmatory tests; this would give researchers targets to aim for, and provide a standard for assessing alternative approaches to large-scale screening. We discuss the technical approaches that might be deployed to achieve this.

5 The ability to reliably monitor environmental contamination with *Mycobacterium bovis* (Mb) could be extremely valuable in assessing reservoirs and sources of infection (e.g. badger setts and the farm environment). If validated, we support continued development of the established molecular approach. However, as highlighted in general recommendation 1, a sensitive primary screening test backed up by a specific confirmatory test would be the ideal analytical tool.

6 Mb strain AN5 should be subjected to genomic sequencing. This will allow recognition of whether AN5 PPD lacks important *in vivo* expressed antigens which should be included in immunodiagnostic reagents.

Pathogenesis

7 A full understanding of pathogenesis informs and underpins an understanding of the immune response, and hence will enhance opportunities in both vaccine development and diagnosis. Strategic research into the pathogenicity of Mb is therefore likely to contribute in the medium to long-term to new approaches to control of the disease.

8 It is widely implied in the literature that the pathogenicity of Mb essentially parallels that of Mtb, although the evidence base for this assumption is in some respects thin. Pathogenicity attributes, in appropriate *in vivo* and *in vitro* models, of representative bovine and badger isolates of Mb should be assessed to determine the validity of assumptions about the pathogenicity of Mb derived from studies of Mtb. The same is true of the bovine immune response, and how it relates to the research in mice and humans, which represents almost all the work done in this area.

9 A productive aspect of new research into the pathogenicity of Mb could be to focus on initial events in Mb infection in an appropriate model. Adhesion mechanisms and the role of antibodies at mucosal surfaces appear to have been relatively neglected in research into both Mtb and Mb and could be appropriate foci for future study.

10 The genome sequences of a cross-section of Mb isolates from cattle, badgers and environmental sources should be determined by next-generation sequencing technologies. This will allow a number of important investigations:

(a) The evaluation of variation in pathogenicity-associated genes, enabling inferences to be made about their likely role in virulence;

(b) An assessment of the hypothesis that the current epidemic is due to the emergence of a badger-adapted ecotype.

Environment and epidemiology

11 There is an urgent need to limit the spread of bTB through integrating the knowledge currently available to develop improved control strategies. For example, modelling studies might be encouraged to explore new approaches to better control the spread of bTB, such as zoning.

12 There remains a requirement to enhance the coordination of the many different research projects investigating different facets of the bTB problem. This should result in various sources of data being more widely available to researchers. This might prove particularly rewarding for modelling projects.

13 There do not appear to be sufficient detailed descriptive statistics available for general research purposes into bTB and this indicates the need for further in-depth longitudinal study of affected herds.

14 Further investigation is needed into animal behaviour and farming practices that increase the likelihood of indirect or (especially) direct contact between badgers and cattle, both while cattle are at pasture and while they are housed on farm premises. The aim would be to identify high-risk situations towards which appropriate control measures could be targeted.

15 Research is needed into the development of accurate, cost-effective diagnostic tools for use on live badgers. The absence of such tools is the most important single impediment to progress in various important issues relating to the epidemiology of bTB in badgers, the spatial correlation between bTB in cattle and badgers, and the after-effects of culling.

16 The Independent Scientific Group report claims that cattle measures are sufficient to control TB. We are not convinced of this and are concerned that TB may be self-sustaining in badgers. We therefore recommend investigation of TB in badgers in areas where there are no cattle (or at least no cattle TB) to address this point.

17 The cessation of the badger road-kill study means that the level of TB in badgers outside endemic areas is largely unknown. Some ongoing knowledge of this is important for understanding the role of badgers in the infection cycle, and for identifying new hot-spots.

18 It is imperative that provision be made for continued analysis of data on cattle bTB incidence in and around proactively culled and control RBCT areas, until such time as herd breakdown rates in and around the proactive areas return to baseline levels. A thorough cost–benefit analysis of proactive culling should then be undertaken.

19 It is likely that movement of badgers can be restricted by geographical features. We recommend studies aimed at determining what these features are, how effective they are, and where they map. This would allow smaller and larger areas to be identified that would be effectively isolated, enabling different control measures to be tested or implemented.

20 Genomic sequencing studies should be directed at identifying new variable markers with different rates of change for tracking strains. These would be additional to those markers used in established typing schemes and could provide a much more detailed understanding of transmission routes.

21 The presence of environmental mycobacteria has major implications for diagnosis in cattle, but is also important in human populations in development of disease, and efficacy of vaccination. It would help further understanding of the influence of exposure to these non-tuberculous mycobacteria to describe geographically where there are high levels of relevant species (including *Mycobacterium paratuberculosis*), for instance through skin test data or through sampling, and to consider whether different control strategies could or should be implemented in different areas based on this.

Vaccination

22 There are many questions surrounding the likely efficacy and mechanisms of action of BCG in badgers or cattle. These questions require addressing. The most important of these concerns the impact of exposure to environmental mycobacteria on the efficacy of BCG. However, the results from these studies should not hold back work to vaccinate badgers and cattle and should proceed alongside trials.

23 In the case of badger vaccination, adequate attention should be given to development of optimum bait deployment procedures.

24 A future bTB vaccine which complements or replaces BCG is likely to arise from the programme to devise an improved Mtb vaccine for use in humans. It is clear that Defra scientists have close links

and collaborate with researchers developing human vaccines against Mtb. There are high-value opportunities for the testing of human candidate vaccines in cattle (or badgers).

25 It seems unlikely that a stand-alone subunit vaccine for use in badgers or cattle will be identified. However, this position should be reviewed if an effective human stand-alone subunit vaccine becomes available.

26 Although the BBSRC programmes do support Defra vaccine work, linkage between BBSRC and Defra projects is not apparent in the documentation available to us. Linkage of all bTB research programmes could be improved.

Economic and social

27 Research on economic and social dimensions of bTB was, until recently, limited to assessing the costs of breakdowns and of control measures, with one study looking at public acceptance of control measures involving culling badgers. Farming practices – a critical element in prevention, control and recovery – have been largely ignored as a researchable issue. Current research is exploring a wider set of social and economic impacts of bTB breakdown, and is beginning to assess farmer behaviour from a multidisciplinary perspective (specifically, behaviour in response to movement restrictions). This is a welcome development that should be built on in future studies.

28 If policies are designed to inform and influence farmers' husbandry practices, an evidence base is needed. This should include studies of farmers' actual management/husbandry practices following an outbreak on their farm and in the vicinity of their farm, and the sources of advice and information they used (if any) in making changes.

29 This research should also include work to understand farmers' attitudes towards husbandry practices to reduce risk of badger–cattle contact, and their underlying motivations for changing or not changing their husbandry practices in response to the bTB threat.

Management

30 It is likely that successful control will only result from the effective implementation of a combination of approaches. We recommend emphasis on development of a systems approach to the control of bTB in cattle. This means modelling the ways in which different combinations of control measures would interact, and considering the appropriateness of different combinations of control measures to specific areas defined in geographical or disease status terms. We note that in poorly controlled aspects of human infection, such as *Clostridium difficile*-associated disease, deployment of all measures for which some evidence of efficacy is available has, anecdotally, been associated with improved control.

31 It is likely that control measures will be most effective where they are tailored to specific local circumstances. This requires a significant amount of work in extracting and confirming relevant information from databases and from the locality relating to cattle, badgers, geography and farming practices. This is labour-intensive, but could be partially achieved through imaginative use of resources such as veterinary undergraduate students.

1 INTRODUCTION

Bovine tuberculosis (bTB) remains a major threat to the dairy and beef industries in the UK as well as to the wellbeing of the livestock and wild animals that may become infected. The threat to human health remains effectively contained by the measures controlling the sale of milk and its pasteurization.

In 2007, over 25,000 cattle were slaughtered following positive diagnostic tests. This contrasts with just over 5,000 in 1997, the year in which the independent scientific review group, commissioned by Defra's forerunner MAFF, published the report (Krebs *et al.*, 1997) that led to the Randomized Badger Culling Trial (RBCT). Notwithstanding the insights gained from the RBCT, it is clear that the need for greater understanding and better control of bTB is urgent.

Ten years after publication of the Krebs Report, Defra has approached the Society for General Microbiology (SGM) with a view to assessing the current status of research relevant to the problem of bTB in the UK. The SGM (http//:www.sgm.ac.uk) is the largest learned society for microbiology in Europe and encompasses all subspecialties including veterinary and medical microbiology (see Appendix 3 for further information). Following assurances regarding the independent nature of our enquiries and the published report, the SGM Council convened a group of eight experts covering a range of relevant specialties. Our work has specifically been directed towards assessing relevant research and offering our opinion on the most advantageous areas for future investment with the best prospects for translation into improved disease control.

The Krebs Report led to the establishment of the Independent Scientific Group (ISG) that designed, implemented and analysed the RBCT. The ISG final report was published in June 2007 (Bourne, 2007); soon after, the Chief Scientific Advisor to the UK Government published a separate consideration of the RBCT conclusions (King, 2007). Apparent discrepancies between these two analyses of the RBCT led to much public debate concerning the best policy for bTB control. The House of Commons Committee on Environment, Food and Rural Affairs then published its review of the ISG's report (EFRA Committee, 2008). These three reports sit amongst a plethora of public discussions and publications debating policy.

Our remit is not directly concerned with policy but rather with the acquisition of knowledge and insight that may inform policy. We are a group of active researchers in fields broadly relevant to bTB. We have applied our expertise to published work, documents provided by Defra and discussions with a number of Defra-funded

researchers. Our efforts have been directed at considering the available research. We report here our opinions regarding this research and how future research investments may be made to best effect.

1.1 Specific brief

1.1.1 In the light of the Wilsmore and Taylor (Wilsmore & Taylor, 2008) review and including the independent review of the TB research programme in 2006 and the ISG's published papers on the RBCT and final report of 2007, to identify progress in the ongoing research programme and advise on whether the current balance is correct and whether the recommendations and objectives from the Krebs review (Krebs *et al.*, 1997) have been achieved.

1.1.2 Identify gaps remaining in the evidence base and ascertain whether they are relevant and important to policy development and possible to answer through further research. Provide recommendations on the timescale and scope of further research.

1.1.3 Identify areas of uncertainty that will not be answered by research and advise how these can best be handled/mitigated.

1.1.4 Provide recommendations on what direction the bTB research programme should take in the next 5–10 years (including economics, agricultural demographics and social science). This should include recommendations on what should be considered research priorities within areas, e.g. diagnostics, and overall research priorities. This should include consideration of what are the most urgent areas that should be examined and where there might be scope for the most progress within a realistic timescale.

1.2 Framework for this report

1.2.1 As described above, the SGM group was asked to take a fresh look at the problem of bTB. The ISG and other recent reporting groups have structured their reports around consideration of the role played by badgers in the bTB problem and the potential value of badger culling, whereas we have taken a systematic approach reflecting the microbiological background of the SGM. Accordingly, we have considered in sequence research concerned with: the diagnosis of bTB; the pathogenesis of disease; the environment and epidemiology; vaccines; economic and social issues; and, finally, management of farms and disease outbreaks.

1.2.2 In each of these six domains, where relevant, we have considered issues concerning the causal organism of bTB, the bacterium *Mycobacterium bovis* (Mb), the infected host, cattle, badgers and other wildlife, and environmental considerations.

1.2.3 In this manner, we have attempted a systematic and, within constraints of the time available and the expertise of the group, a comprehensive analysis of the research problem. Nonetheless, we recognize that further analysis in certain areas, notably mathematical modelling, would be desirable.

2 DIAGNOSIS

2.1 Current and recent research

2.1.1 The Krebs Report (Krebs *et al.*, 1997) recommended concentration on a vaccine-related diagnostic test. As we note below, there has been significant progress towards this aim. Development of improved tests applicable to badgers, blood tests suitable for monitoring a badger vaccination programme and the detection of environmental contamination with Mb were also highlighted. These are all areas in which substantial advances have been achieved.

2.1.2 The principal development has been the establishment and evaluation of evoked gamma interferon (g-IFN) tests applicable to cattle (2.3.6) and badgers (2.4.3). These tests assess cell-mediated immunity to specific antigens and have the potential to provide a much more accurate picture of the bTB problem. However, the need to draw and process a large number of blood samples remains a drawback of this approach.

2.1.3 The development of a DNA-directed test for detecting contamination of the environment with Mb is potentially extremely valuable. The requirement for this test to be properly validated before wider application is a priority recognized by Defra.

2.2 General points

2.2.1 Diagnosis is one of the critical steps in the control process. In the present framework for the management of bTB in the UK, any confirmed positive test result relating to bovines requires that the animals concerned be removed and that the herd be placed under restrictions until negative tests are obtained from the remaining animals. This action in response to a positive test is based on the premise that the animals concerned present a high risk for transmission to other members of the herd. While this may well be the only practicable response to a positive tuberculin skin test (TST), we note that the implications of positive test results obtained by newer diagnostic methods reflect different stages in the natural history of infection to those revealed by the TST (de la Rua-Domenech *et al.*, 2006). Thus, while the ability of these tests to detect infection with, or at least exposure to, Mb is generally well

validated, the degree to which a positive test identifies an animal that will develop active disease and will become infectious is presently unknown.

2.2.2 An ideal test or suite of tests would accurately recognize and explicitly distinguish exposure without infection, latent infection and active/progressive infection with and without infectivity. Indeed, it may become possible to recognize each of these states with existing technologies. However, we see two problems here. Firstly, a test that is more sensitive than the TST will lead to the recognition of more herd breakdowns. However, while for present practical purposes the cattle concerned must be considered at risk of becoming infectious, it is clearly possible that they may never do so. In the context of new tests, it is important to investigate the natural history of infection in animals that are TST-negative but positive by other methods and, providing infectious animals can be clearly recognized, to consider a framework for the management of positive animals that does not inevitably lead to slaughter. Secondly, it must be accepted that an animal that tests positive by a validated test has been exposed to Mb at some time, whether or not it becomes infectious. Thus careful monitoring of all the herds concerned would be appropriate, even if TST-positives are not found when the herd tests positive for the first time with the newly developed test.

2.2.3 The present TST- and abattoir-based screening tests for bTB have limited sensitivity as a means of detecting infected animals. There can be little doubt that, with the present testing regime, some subclinical infection can pass undetected. It is standard practice in communicable disease control to apply a high-sensitivity screening test that may have modest specificity in order to limit cost. This enables ascertainment of as many potential source cases as possible. These are then retested with a test that has greater specificity. The present pattern of testing for bTB is anomalous in this regard as the more sensitive but marginally less specific Bovigam test is not applied as the primary screen.

2.2.4 It should be possible to identify target sensitivity, specificity and cost for diagnostic tests to be used in different settings such as screening and confirmation in herds, individual animals, wildlife and environmental samples in order to achieve a satisfactory and amenable level of surveillance. Conventional TST testing has evidently not been successful in enabling bTB control in high-incidence areas in the current UK situation (although historically it was). Further test development should be targeted to meet the criteria indicated above. Achievement of these targets could simplify debate and increase confidence in the farming community.

2.3 Diagnosis of bTB in bovines

2.3.1 Detection of Mb: Detection of live Mb in samples from live or dead cattle is considered diagnostic for bTB. As is the case in human TB, it is well recognized that individuals may suffer from progressive infection without excreting live bacilli in any accessible sample material. The bacilli-positive animals are likely to be the easiest to diagnose using currently available methods, whereas the paucibacillary animals are likely to be the most difficult to detect by any method. Thus, while detection of live Mb provides 100% specificity regarding diagnosis, the approach offers little opportunity to achieve greater sensitivity.

2.3.2 Conventional microscopy and culture-based detection remain valuable confirmatory techniques. While various technical advances continue to be introduced in the laboratory culture of mycobacteria, some of which may achieve enhancements in numbers of bacilli recovered (Biketov *et al.*, 2000; Sweeney *et al.*, 2006), we do not attach priority to research in this area for the reasons outlined in 2.3.1.

2.3.3 As with Mtb, it has been difficult to achieve consistent sensitivity results with nucleic acid (DNA or RNA) amplification tests. However, there are several promising developments in the diagnosis of human TB in this area (Perkins & Cunningham, 2007). Ultimately, such tests may partially replace culture confirmation but again we do not attach priority to this area; this is partly due to the extensive efforts to produce economic and sensitive tests for Mtb and partly again for the reasons outlined in 2.3.1.

2.3.4 Antigen and antibody detection: In many infectious diseases, diagnosis may be achieved by detecting a highly specific component(s) of the causal organism that can be recognized with an antibody preparation or by detecting antibodies made by the infected individual against such components. While antigen tests are not used in bTB, there has been a considerable resurgence of interest in this field in human TB. Specific detection of such molecules as lipoarabinomannan in urine holds out some promise (Boehme *et al.*, 2005; Perkins & Cunningham, 2007). In this field, as in many others, we advise a watching brief on developments in human TB. At some point, it could be worth adapting tests developed for human investigations for use in cattle. It is possible, however, that these tests may be compromised by exposure to environmental mycobacteria, including *Mycobacterium paratuberculosis*.

2.3.5 Several tests for detection of antibodies against Mb in cattle are available. Without exception, these do not achieve sufficient sensitivity or specificity to be of value in the UK control programme. We consider it unlikely that a conventionally developed antibody test will be of value. However, new techniques to precisely map the B-lymphocyte response to the entire proteome of micro-organisms have opened up this field (Zhu *et al.*, 2006) and to date have not been tested for their potential in the context of diagnosis of bTB. These rely on high-throughput, large-scale *in vitro* expression of the proteome of an organism once its genome has been sequenced. By transferring these products to microarray chips, up to several thousand individual proteins can be screened using sera from infected/vaccinated animals to define the immunogenic determinants. It may be possible to perform this using expressed proteomes from Mtb, Mb and environmental mycobacteria to identify potentially useful and specific antigens for an antibody-based diagnostic test.

2.3.6 Tests of cell-mediated immunity: It has recently become possible to detect specific immune responses to antigens that do not elicit antibodies (T lymphocyte-stimulating antigens) in an amenable test format (e.g. Bovigam). These tests have made a considerable impact on the assessment of TB infection in both human and bovine contexts (Pai *et al.*, 2004; Vordermeier *et al.*, 2006). In general, the cells in the animal's blood are stimulated with T-cell antigens specific to Mb and the responses are measured by quantifying mediator proteins (cytokines) manufactured or released as a result. The Bovigam test uses Mb tuberculin as the antigen mix and g-IFN as the detected cytokine. There is considerable room for further development of these tests.

2.3.7 The mixture of antigens used in the test combined with the pattern of cytokines released provides numerous opportunities to establish relationships between the pathological stage of the disease and the host's reaction. In particular, it would be valuable to establish the capacity of such tests to recognize animals that have been exposed without established infection (self-cure), latently infected, infected with progressive disease and those that are currently both diseased and infectious. Most importantly, as is already the case in human TB, it will be possible to differentiate immunized animals from infected animals by using appropriate variants of these tests with so-called DIVA (Differentiated from Vaccinated) antigen mixes (Defra project SE3222).

2.3.8 We strongly advise maintenance of an active programme of research and development concerned with these evoked cytokine tests. The possibility that responses might be detectable from the cell populations present in milk could be worth exploration.

2.3.9 Biomarker studies: There has been much recent interest in the use of biomarkers for the diagnosis of specific disease states (Hodgetts *et al.*, 2007; Lalvani *et al.*, 2008; Perrin *et al.*, 2007). These have been particularly fruitful in cancer diagnosis. In such studies, detection of a specific molecule or combination thereof is shown to have a certain sensitivity and specificity for a particular diagnosis. In addition to the volatile organic compound studies undertaken at the Veterinary Laboratories Agency (VLA), we understand that mass spectrometry studies have been undertaken on various secretions from infected animals (Defra project SE3221; Pavlou *et al.*, 2004; Phillips *et al.*, 2007). We consider these studies useful but would not advise extension beyond the limits presently planned unless new developments provide a breakthrough in the robustness of a particular approach.

2.4 Diagnosis of bTB in badgers

2.4.1 The pathology and natural history of TB in badgers has many features that are distinct from the disease in bovines. Diagnosis is rendered difficult and costly due to the need to trap and anaesthetize animals prior to testing.

2.4.2 Diagnosis is currently based around two antibody tests, the Brock ELISA test and the Stat-Pak lateral immunodiffusion test, and the detection of live bacilli by culture. Although the antibody tests have low sensitivity, their specificity appears acceptable and they provide the mainstay of detecting subclinical TB in badgers (Chambers *et al.*, 2005, 2008).

2.4.3 A g-IFN test has recently been established with both mRNA and ELISA readouts. As expected, these tests show greater sensitivity than the antibody tests. Specificity is achieved by a comparative analysis of the responses to bovine and avian tuberculin (Dalley *et al.*, 2008; Sawyer *et al.*, 2007). These tests are more expensive than the antibody tests.

2.4.4 At present, there is no simple convenient and validated test to assess the infective status of a badger population associated with a particular location. As with bovines and humans, point of contact testing applicable to un-anaesthetized animals would be extremely valuable but does not appear achievable in the short or medium-term.

2.5 Strain definition in Mb

2.5.1 One of the most useful developments over recent years has been the development of tools to differentiate species and isolates of the Mtb complex at a genomic level. At a species level, this is based on conservation of SNPs and deletions, and it has transformed our understanding of the structure of the Mtb complex. In particular, it has become clear that the strains have evolved into host-adapted ecotypes (Brosch *et al.*, 2002; Smith *et al.*, 2003, 2006a, b), and some strains that were grouped into Mb can be usefully separated into different ecotypes or species – for example, an antelope ecotype, *Mycobacterium caprae*, found in goats, and *Mycobacterium pinnipedii*, found in seals. Indeed, one species long recognized as being separate (*Mycobacterium microti*) is more closely related to bovine Mb than to the antelope ecotype. It may be that there are further subdivisions into ecotypes, as this research depends on the identification of informative genomic differences as well as sampling of appropriate strains. Indeed, it has only recently become apparent that human Mtb strains are far more diverse than had been thought (Gagneux *et al.*, 2006; Gagneux & Small, 2007).

2.5.2 In the studies described above, Mb found in cattle and badgers group together, but this does not prove that they are the same organisms. Evidence that this was the case came from the identification of more rapidly evolving regions of the genome, in particular the Direct Repeat (DR) region, and minisatellites and microsatellites that allow variable number tandem repeat (VNTR) typing (Roring *et al.*, 2002). The DR region consists of a large number of small unique sequences flanked by common repeats, and occasional recombination events between the repeats lead to a loss of the intervening unique regions. Thus strains have evolved to carry different combinations of unique regions, detected through the spoligotyping technique (Aranaz *et al.*, 1996). A particular combination of unique regions is called a spoligotype. This technique has the advantage over VNTR typing that it is unidirectional (unique regions are never regained), whereas VNTR types can change in either direction. The spoligotype is therefore seen as the primary typing method, and VNTR typing subdivides each spoligotype.

2.5.3 Defra has funded a significant amount of research into the typing of the Mb isolates in the UK (projects SE3017, SE3020). This work has led to the following conclusions (2.5.4–6; summarized in Bourne, 2007; Wilsmore & Taylor, 2008):

2.5.4 There are a variety of strains that can be differentiated within the UK. The majority fall into two related spoligotypes (9 and 17), which suggests a clonal expansion of the pathogen.

2.5.5 There are geographical differences with particular spoligotypes being confined to particular regions, indicating that most spread is local in nature. Furthermore, there is congruence between Mb types isolated from cattle and badgers in the same geographical locality, indicating that these animals are being infected by the same strains, and that there is spread between cattle and badgers, although this does not indicate direction.

2.5.6 The 'modern' pattern of spoligotypes is very different from the historical (pre-1970) pattern. The first infected badger in the UK was found in 1971, and one tenable hypothesis is that there has been recent adaptation of Mb to survive and spread in badgers – a badger-adapted ecotype similar to ecotypes

described (with, in this case, cattle being a spillover population). This would explain a number of phenomena that differentiate the current epidemic from historic bTB infections, including:

- Why badgers are a major reservoir for infection now, but not before;
- Why traditional control methods, which were highly effective before, are no longer sufficient.

2.5.7 Addressing the hypothesis that Mb has recently become badger-adapted is possible, for example initiated through information gathered from sequencing additional genomes. If supported, this insight would also change the nature of the debate, which is currently fixed on a perspective that methods that worked before should still work now. It would also help different stakeholders come to terms with the need for different approaches in the current situation.

2.5.8 It may be that a combination of spoligotyping combined with an authoritative and detailed description of the historic and modern epidemics could also provide evidence for or against this hypothesis. A history of the epidemics could lead to valuable additional perspectives that are not apparent from the middle of the current epidemic.

2.5.9 Mention has been made above of the application of efficient and low-cost next-generation sequencing technologies. As well as throwing light on the evolution of the modern isolates, the application of high-throughput genome sequencing may identify other variable markers that would be useful in epidemiological studies. We recommend that this is investigated.

2.6 Post-mortem examinations

2.6.1 Diagnosis through post-mortem examinations depends predominantly on detection of visible lesions and therefore only readily detects relatively advanced disease. In cattle, such examinations provide evidence concerning the route of transmission (Neill *et al.*, 2001). Given the current view that the respiratory aerosol route is of overwhelming importance, post-mortems provide a means of confirming this pattern and of recognizing the frequency at which alternative routes, such as ingestion, were considered to have occurred.

2.6.2 Post-mortems carried out on badgers, notably on road kills, provide a means of detecting infection without active case ascertainment. While this is a crude instrument, such examinations, when positive for bTB, provide some additional indication of the geographical distribution of infection.

2.6.3 In both cattle and wildlife, samples taken at post-mortem may yield isolates of Mb. These are of great value in defining the distribution of different strains and can enable recognition of changes in their biological properties.

2.7 Detection of Mb in environmental samples

2.7.1 The development of specific DNA tests for the detection of environmental contamination by Mb opens up a significant opportunity. A validated and quantitative test of this type would provide the means to describe the geographical distribution of environmental contamination with the causal organism

of bTB. If test results from particular sample types can be correlated with the local prevalence and incidence of bTB in both cattle and badgers, this approach could provide a valuable tool in the control effort.

2.7.2 The results reported to date indicate a surprisingly high prevalence of sample positivity and level of contamination (Sweeney *et al.*, 2007; Young *et al.*, 2005). At 10^4–10^6 genome target copies per gram of sample this represents the highest DNA signal consistently recorded for a member of the Mtb complex in diagnostic samples. It has proved particularly challenging to achieve good sensitivity in the diagnosis of human TB by specific DNA detection. Available assays do not consistently outperform microscopy, which, at best, detects 10^3 target bacteria per gram, in most published studies. Problems with obtaining good DNA extraction and inhibition of amplification reactions are regularly encountered. These problems do not appear to have been encountered in the Mb study. However, the surprisingly high numbers of bacilli detected, combined with PCR's potential for giving misleading results, means that the data will need to be particularly solid if they are to be used for policy decisions.

2.7.3 Immunofluorescence detection and culture results following immunomagnetic capture enrichment appear to corroborate the DNA detection results (Sweeney *et al.*, 2006, 2007).

2.7.4 If robust methods based on the work reported can be established and replicated in other laboratories then this approach could be exceptionally valuable. These issues appear to be addressed in a recently established validation study (Defra project SE3231).

2.7.5 Whether the bacterial cells responsible for positive DNA signals were capable of transmitting infection at the time of sampling, and how long these signals persist in the environment, remain open questions and reflect generic problems in molecular diagnostics as applied to infection (Barer & Harwood, 1999). Considerable work will be necessary to develop reliable interpretive criteria for the present assay. Such work should be undertaken following the outcome of the established validation study.

3 PATHOGENESIS

3.1 General points

3.1.1 Pathogenesis can be defined as the process by which disease is caused, and the pathogenicity of an organism as the capacity of that organism to cause disease. Microbial factors responsible for conferring pathogenicity on a particular organism are often, though not always, virulence factors, e.g. toxins or other molecules that are directly responsible for eliciting host damage or dysfunction. In addition, factors such as the ability of micro-organisms to acquire nutrients within host tissues are often necessary for pathogenicity, although they do not directly damage the host. A full understanding of pathogenicity requires that all of these factors are taken into account. It should be noted that pathogenesis is not simply pathology.

3.1.2 A detailed understanding of pathogenesis may be crucial for development of a comprehensive strategy aimed at controlling an infectious disease, particularly where that disease has defied control efforts to date. Pathogenesis is intimately linked to stimulation of responses in the host that most obviously include the immune response and the induction of potentially damaging changes such as inflammation. Often, virulence factors themselves stimulate immune responses which, if the host recovers, may be protective against future disease or be pivotal in diagnosis. Thus definition of virulence factors may provide leads towards both vaccine discovery and improved diagnostics. Though unlikely to be of practical value in bTB, the possibility of developing drugs that specifically interfere with interactions between host and pathogen should also not be neglected.

3.2 Recent research

3.2.1 A survey of reports and listings covering research on Mb over the last 20 years indicates that, compared to human TB and many other major bacterial diseases, research to investigate and define bTB pathogenesis has been a neglected area. As examples, none of the following include any more than passing reference to the pathogenesis of Mb infection, in either cattle, badgers or other experimental models: the Krebs Report (Krebs *et al.*, 1997); the final report of the ISG on Bovine Tuberculosis (Bourne, 2007); the Wilsmore and Taylor review 2005 (Wilsmore & Taylor, 2005); and its update in

2008. Consideration of research projects commissioned by Defra and formerly by MAFF over the past decade identifies rather few that have considered pathogenesis. When this has been done, the focus has tended to be on pathology and the immune response, rather than on the broader implications of pathogenic processes. The recommendations arising from the Krebs Report (Krebs *et al.*, 1997) and the final report of the ISG (Bourne, 2007) did not include any research into pathogenesis.

3.2.2 The funded projects that come closest to a consideration of pathogenesis are listed below, with brief comments.

SE3015 '*Mycobacterium bovis* pathogenesis' 2000–2004. This project did not address pathogenesis directly, being more concerned with diagnosis.

SE3027 'Pathogenesis and immunology of *Mycobacterium bovis* infection in cattle' 2002–2005. The aim of this project was to analyse the mechanisms of immunity to Mb in cattle. Cellular immune responses and the pathological response to virulent Mb and avirulent BCG and the potential impacts of prior exposure to environmental mycobacteria were analysed. There was little if any consideration of pathogenesis processes.

SE3202 'The development of animal models to test candidate vaccines for Mb infection in badgers' 1998–1999. Mouse intravenous and guinea pig aerosol challenge experiments were done with a view to developing models for testing vaccines. The resulting pathology was assessed. Lesion number and degree of mineralization in guinea pigs challenged by aerosol differed between controls and animals vaccinated with MPB83. The work sheds little light on pathogenesis of the mineralized lesion.

SE3013 'Pathogenesis and diagnosis of TB in cattle – complementary field studies' 2000–2005. The project focussed on pathological investigation, recording visible lesion findings in reactor cattle (200 were studied along with 200 in-contact cattle). Lesions were most commonly founding lymph nodes in the thorax with secondary involvement in the head region. Lesion distribution was consistent with aerosol transmission. Nasal mucus was generally culture-negative, suggesting that coughing to produce aerosol droplets from the lower respiratory tract is more important in transmission than nasal contact between animals. This study does provide some insight into the importance of the lung environment rather than airway mucosa in the early events in pathogenesis, and would be consistent with the alveolar macrophage being the first point of contact between bacteria and host in the initiation of infection. The report does not shed light on virulence factors involved in these processes.

SE3024 'Low dose Mb infection in cattle: disease dynamics and diagnostic strategies' 2002–2006. Intratracheal inoculation with graded doses indicated a potential minimal infectious dose as low as 1 colony forming unit of bacteria, i.e. in all probability a single cell. Animals inoculated with a range of doses all developed lesions on the same timescale – there was no reduction at smaller doses and hence it is likely that diagnostic tests will be effective for early detection even of animals infected with a low dose. Aerosol challenge methods were also developed.

SE3033 'Housing of naturally infected cattle (field reactors) at the VLA for immunological and bacteriological analysis' 2004–2007. Final report not yet available in the public domain. Again there

was a very low level of nasal shedding. The research is more concerned with diagnosis than with pathogenesis.

SE3226 'Development of tools to study immunopathology in badger tuberculosis' 2005–2006. A rare example of detailed scientific study in badgers. Some animals were obtained from vaccination/challenge experiments; others were naturally infected. The histopathology of lymph node lesions was not the same as in bovines, potentially implying that processes in pathogenesis are not identical and there may be risks in extrapolating.

3.3 A working definition of pathogenesis and its implications

3.3.1 A useful framework (Smith, 2000) for consideration of the pathogenesis of infectious disease, such that mechanisms can be dissected at a molecular level in every important respect, breaks down the process of pathogenesis into several components as follows:

Colonization of the host. This includes the initiation of infection at the crucial end point of the transmission process, and the establishment of infection on a host surface.

Invasion of host tissues. In some rare cases, this does not take place, e.g. in cholera, where the pathogen remains external to the cells or tissues and inflicts damage through the action of an exotoxin.

Multiplication of the pathogen upon or within the host. Other than in the special case of pathology mediated by exposure to an exotoxin, such as in staphylococcal food poisoning, multiplication of organisms in association with the host is an essential facet of the process of infection.

Resistance of the pathogen to host defence systems. Many potential pathogens 'fail' at this stage of the infectious process. Those that succeed in establishing an infection generally have specific mechanisms to avoid, subvert or disable host defence systems. Defence systems include both innate and acquired immune effectors.

Infliction of damage upon the host. A phenomenon of variable severity, ranging from extreme in the case of acute and lethal infections like cholera, to marginal as in some chronic infections that may border on a commensal relationship between host and micro-organism.

Transmission. It is helpful, especially in the case of infections involving an environmental phase (outside the host) in the life cycle of the pathogen, to include consideration of mechanisms of survival, persistence and fitness for initiation of an infection as an additional component. These might come under the heading of transmission and be considered as either the first or last step in the sequence of events involved in disease causation.

3.3.2 Consideration of pathogenesis under the above headings often creates a useful framework in which to identify mechanisms of pathogenesis and appreciate the overall state of understanding of a particular infection; it also underlines the multifactorial nature of the pathogenesis of many infections. Furthermore, the framework emphasizes the need to study pathogenesis in the appropriate context, i.e. the pathogen *in vivo* within host tissues and not the isolated pathogen growing in the laboratory,

where the expression of genes encoding virulence factors is often demonstrably different from that occurring *in vivo*.

3.3.3 The above ideas were originally formulated by Harry Smith (University of Birmingham) in the 1960s and expanded during subsequent decades (Smith, 1968, 1989). Since then it has become apparent that, firstly, there are situations where regulation and coordination of the expression of genes governing virulence is an additional 'layer' of complexity in defining pathogenicity at a molecular level. Where virulence is multifactorial, coordinated expression of virulence genes may be necessary to achieve a particular effect. Secondly, environmental influences on virulence gene expression may be the key to initiation of pathogenic processes. These are often regarded as stress responses in the pathogen consequent upon exposure to the *in vivo* environment.

3.3.4 Subsequently, a very constructive extension of the intellectual framework of pathogenicity research was contributed by Stanley Falkow, who has defined a series of 'molecular Koch's postulates' (Falkow, 1988, 2004). These are devised to allow for the identification of virulence genes. Candidate genes may, for example, be knocked out by molecular biological methods and the effect on pathogenesis assessed in a model or 'real' infection scenario. In parallel with Koch's ideas on aetiology of disease, confirmation of the role of a gene in such an experiment should be confirmed by complementation tests, looking for restoration of the phenotype. Such an approach is crucial in defining vaccine candidate genes, since immune responses to virulence factors are often, if not usually, capable of providing protective immunity against infection.

3.4 Current state of knowledge regarding pathogenesis

3.4.1 A general impression from the literature strongly suggests a widespread assumption that the basic pathogenic mechanisms in bTB are essentially the same as those in human TB (de la Rua-Domenech *et al.*, 2006; Pollock *et al.*, 2006). This is supported by extremely high genomic sequence conservation. However, one or more of the genomic differences that do exist between the Mtb and Mb lineages (Ernst *et al.*, 2007; Garnier *et al.*, 2003; Hewinson *et al.*, 2006) presumably underpin the differences in host range (de la Rua-Domenech, 2006) seen in the two species.

3.4.2 There is a considerable amount of pathogenesis research on Mtb, and (understandably) relatively little on Mb. It would seem an appropriate and efficient strategy to (a) use the information gathered from Mtb and apply to Mb and (b) focus novel research on Mb-specific aspects of biology. There is evidence that Defra has followed the latter approach. For example, the Functional Genomics programme has specifically looked at aspects that are different between Mtb and Mb. This led to work demonstrating a non-functioning glycolysis pathway in Mb in comparison to Mtb (Keating *et al.*, 2005), which explained long-standing physiological observations on Mb growth requirements.

3.4.3 One aspect that is unclear is how it can be established that pathogenesis is functionally similar in the two pathogens. Some research uses Mb BCG as a tool, and similarities between Mtb and BCG can (usually) be extrapolated as being also present in Mb. (The proviso is that it is known that BCG has

retained some characteristics lost from Mb, such as the ability to grow using glycerol as a carbon source.) However, there is overall little research to confirm similarities between Mtb and Mb pathogenesis, and it would be useful to invest in carefully selected experiments with this aim. A cost-effective approach for this might be to fund laboratories which carried out the original research with Mtb to repeat the experiments using Mb. We recognize it will not be simple to do this in all cases, and we also recognize that the pathogens behave somewhat differently in the main animal models used so that this is by no means a simple comparison. However, not to address this issue seems a bigger threat. This could be combined with research looking at Mb-specific issues.

3.4.4 Two other relevant aspects which might affect the research undertaken are the fact that (though difficult and expensive for a number of reasons) the natural host can be used in Mb research, and that normally in the UK we are only looking at transmission and development of disease over the first 2 years of life (though cattle in some herds are much older).

An examination of what is known of the processes outlined in 3.3.1 indicates the following:

3.4.5 Colonization of the host. Knowledge of, for example, adhesins responsible for initial interaction with host surfaces appears quite limited. There is a current revival of interest in the role of the heparin binding haemagglutinin HBHA in Mtb (Hougardy *et al.*, 2007; Menozzi *et al.*, 2006). Parallel work with Mb might be productive. Infection is clearly initiated at mucous surfaces, in either the respiratory or gastrointestinal tracts. Interactions with host epithelial cells and their mucous secretions do not appear to be well explored. The role of innate host defence mechanisms in initial interaction of Mb with the bovine host also appears to be a neglected field. Cassidy (2006) has pointed out that neutrophils, as well as activated gamma delta and NK cells, may be abundant in early lesions in experimentally infected calves, rodents and guinea pigs prior to development of cell-mediated immunity. There appears to have been very little investigation of the role of these cells in host defence. Their activity could explain the occurrence of disease-free individuals among groups who had certainly been exposed to infection alongside cases that did develop disease. As with a number of other areas, further developments may be expected from Mtb studies, and, at present, we recommend a watching brief in this area.

3.4.6 Invasion of host cells and tissues. There appear to be few definitive studies of the initial events at the mucosal surface, although there is substantial research into interactions with macrophages, including initial uptake into the cells, especially in the case of Mtb. Some interesting recent developments in cellular invasion have been published, for example the use of flow cytometry to assess invasion of epithelial cells by Mtb (Chapeton-Montes *et al.*, 2008). There appears to have been very little work done on this aspect of pathogenesis in Mb although *Mycobacterium avium* subsp. *paratuberculosis* has received some close attention in this regard (Alonso-Hearn *et al.*, 2008; Patel *et al.*, 2006). The slow growth of mycobacteria clearly imposes major technical challenges to this research.

3.4.7 Multiplication within the host. There has been a massive and productive research effort over decades to explore the long-recognized ability of pathogenic mycobacteria to multiply within macrophages. This research has mainly focussed on Mtb, although there has been some informative work with

different mycobacterial species, notably with *Mycobacterium marinum*, where complementation of gene knockouts with orthologues from Mtb has been informative (Xu *et al.*, 2007). Little similar research appears to have been done with Mb (an exception is with secretion antigen SA5K in strain BCG; Bottai *et al.*, 2006) but there appears to be some scope for further activity in this field.

3.4.8 Resistance to host defences. The interaction of mycobacteria with the immune system is clearly a major focus of past and current research and the literature is extensive (Russell, 2007). The chronic nature of 'successful' mycobacterial infection requires that interaction with the immune system in pathogenesis is considered against the background of pathogen persistence in stable equilibrium with the host. This contrasts with the scenario of pathogenesis in acute infections, where the successful outcome for the pathogen might be induction of acute host responses that will disseminate the pathogen immediately to new hosts – for example a diarrhoeal response to an enteric infection. One area where there have been intriguing developments leading to renewed interest is the role of B cells (Maglione *et al.*, 2007, 2008) and antibodies (Reljic & Ivanyi, 2006), especially in initial interaction with the host, which may determine whether or not infections become established at all. While these phenomena might be explored fruitfully in Mb research, we currently advise a watching brief on the Mtb work in this area. It has been noted that against the background of a long-term interest in intracellular persistence of mycobacteria within hosts, extracellular residence may have been neglected (Ernst *et al.*, 2007).

3.4.9 Infliction of host damage. It is clear that there is a strong element of immunopathology in the damage done to the host (Pollock *et al.*, 2006). This aspect of mycobacterial disease has been very well researched over many years. Classical toxin production by mycobacteria has not been a theme in pathogenesis and there is little evidence to suggest that this is an area for future investigation.

3.4.10 Gene regulation. There are some interesting reports of the influence of regulatory genes (transcription factors) of Mtb on expression of potential virulence genes (Frigui *et al.*, 2008; Honaker *et al.*, 2008; Hunt *et al.*, 2008) and this is an area worthy of further study in Mb. Functional genomics studies of Mtb have been fruitful (Rehren *et al.*, 2007) and have further potential in parallel studies of Mb.

3.5 Genomics and genetic manipulation

3.5.1 Genomics studies have contributed some of the most useful insights into the biology of Mb (Brosch *et al.*, 2007; Mostowy *et al.*, 2005; Smith *et al.*, 2006a). The losses of genes and gene regions in the evolution of Mb are clear and there is potential for these losses to contribute subtle differences to pathogenesis in different hosts. However, the exact roles in virulence of many of the genes lost in the Mb lineage remain ill-defined. A recent review (Ten Bokum *et al.*, 2008) suggests that counter-intuitively, gene losses from the Mtb lineage may lead to 'hypervirulence' due to the loss of mechanisms that moderate mycobacterial growth and damp down host immune responses. Such a scenario would be compatible with greater potential virulence of the Mb lineage that might correlate with its apparently broader host range, and might explain the apparent paradox that adaptation to its natural host species is being accomplished by mutational loss rather than gain of genetic information. Animal models for

studies of these subtle facets of pathogenesis are probably too crude to resolve these issues although transgenic approaches may allow specific points to be addressed. There appears to be much scope for new whole-genome sequencing of multiple isolates by 'next-generation' sequencing technology, which may reveal unsuspected differences between isolates with potential biological significance.

3.5.2 Together with next-generation genome sequencing (Loman & Pallen, 2008), which potentially has much to offer in terms of helping us focus on appropriate aspects of research, we would recommend that selected Functional Genomics technologies be considered. These include technologies that have been used effectively already with Mtb, such as the genome-wide essentiality TraSH studies, where a great deal of directly useful information could be generated, and the comparison with Mtb could be very valuable (Sassetti *et al.*, 2001, 2003). By focussing on technologies that have proved themselves to be particularly effective, the limited resources will be better used. This may again be usefully carried out by Mb biologists working in collaboration with the experts in each field rather than setting up each technology *de novo*. It will also require an element of horizon-scanning to identify and evaluate technologies that will make a difference. It may also indicate that certain aspects should be left until technology and costs catch up (as with next-generation sequencing).

3.5.3 Some of these studies are made more difficult by technical challenges that have yet to be resolved in Mb (as compared to Mtb). For example, the construction of defined mutants is more difficult than in either Mtb or BCG. Funding to overcome these technical hurdles would be a good investment, as it is unlikely that other people do this.

4 ENVIRONMENT AND EPIDEMIOLOGY

4.1 General points and current research

4.1.1 As part of this review, interviews were held with several scientists currently or previously involved with bTB projects in the British Isles. There was general agreement that there is an urgent need to limit the spread of bTB through integrating the knowledge we currently had available. This was as much a priority as any single area of deterministic based research that may be desirable but inevitably would result in delays. Our review indicated that, following the Krebs Report (Krebs *et al.*, 1997), a wide range of research, including epidemiological and environmental components, had been commissioned by a range of funding bodies from a variety of research institutes. Examples of these projects are listed below (proposed end date in parentheses):

SE3026 Bovine TB transmission in restocked herds: risk factors and dynamics. University of Warwick (2006)

SE3032 The long-term intensive ecological and epidemiological investigation of badger populations naturally infected with *Mycobacterium bovis*. CSL, York (2006)

SE3035 Estimating the badger density in Randomized Badger Control Trial proactive and control areas. CSL, York (2007)

SE3040 A preliminary analysis of existing data to provide evidence of a genetic basis for resistance of cattle to infection with *M. bovis* and for reactivity to currently used immunological diagnostic tests. EBRC, Edinburgh (2009)

SE3117 Cost–benefit analysis of badger control. CSL (2007)

SE3119 An experiment to assess the cost effectiveness of farm husbandry manipulations to reduce risks associated with farmyard contact between badgers and cattle. CSL, York (2009)

SE3229 Enhanced modelling and prediction of the spread of bovine tuberculosis in mainland Britain: impacts of cattle movements, climate and spoligotype. VLA (2007)

SE4202 An evaluation of biases in the AMLS and CTS databases. University of Oxford (2007)

BBE020925/1 Intra- and extra-cellular mechanisms affecting the persistence of *Mycobacterium bovis* in the environment: towards molecular surveillance of bovine TB. University of Warwick (2011)

BBE0183351 The interplay between host and pathogen genetics in the increasing incidence of bovine tuberculosis. Roslin Institute (EBRC), Edinburgh (2008)

BBSB08868 The biology of environmental *Mycobacterium bovis*, and its significance to the epidemiology of bovine tuberculosis. University of Warwick (2007)

Apparently there remains a need for enhanced coordination of this research and pooling of the current data resources to help optimize progress. Modelling is one of the tools that will help develop improved approaches to control but validated data for parameterization remain a limiting factor.

4.2 Descriptive data for cattle

4.2.1 Descriptive data for research into bTB in Great Britain can be compiled from two complementary sources (Mitchel *et al.*, 2008). The Cattle Tracing System (CTS) contains births, deaths and movements for all cattle in Great Britain since the year 2000. Systems to extract the required data have been developed and previously described (Gilbert *et al.*, 2005b; Mitchell *et al.*, 2005).

4.2.2 Data on bTB can be obtained from Defra's disease surveillance database VETNET, which records details of bTB tests and their results. This system also records details of the newly introduced Pre-Movement Testing results for the positive tests but not, unfortunately, the negative results which have created problems for project SE3229 as described by Mitchell *et al.* (2008). An evaluation of biases in these data was investigated in project SE4202.

4.2.3 Although these overview data are now available to researchers from Defra, it is not so clear where detailed descriptive statistics can be obtained for affected herds. It appears that such information is not readily available. Most current studies appear to be utilizing the results from the farms within the RBCT such as were reported by Rameirez-Villaescusa *et al.* (2008) and the recently awarded Defra-funded project entitled 'A county parish holding herd (CPHH) level spatial and temporal analysis of the Randomized Badger Culling Trial (RBCT) dataset'. Although using the available resource, such studies cannot be seen as fully representative of the situation elsewhere in Great Britain. It is clear that cattle movements are involved in an increasingly large number of outbreaks outwith south-west England with little evidence of wildlife vector involvement.

4.2.4 There would be a case for targeted surveillance to try to establish solid descriptive data describing longitudinal details of bTB outbreaks to assist all areas of bTB research; in particular future modelling exercises aimed at enhanced control would benefit from such data.

4.3 Descriptive data for badgers

4.3.1 Owing to the complex pathogenesis of bTB and the lack of adequate diagnostic tests (see section 2.4), estimating the prevalence of the disease in badgers is far from straightforward. Longitudinal data are

provided by a long-term epidemiological study, initiated in 1977 and still ongoing, of a population of about 30 social groups of badgers at Woodchester Park, Gloucestershire.

4.3.2 Members of this population (about 250–300 individuals at any one time) are trapped several times per year and live-tested for bTB infection by means of the ELISA test and by culturing samples of faeces, urine, sputum and wound exudates. By these diagnostic criteria, a large majority of badgers (perhaps 80%) never contract bTB, with only about 5% becoming infectious and 2–3% superinfectious (Wilkinson *et al.*, 2000).

4.3.3 Post-mortem examination of badger carcasses yielded by the RBCT paints a similar picture, classing 16% of badgers as infected, 6% as infectious and 1–2% as severely infectious (Bourne, 2007, paras 4.17 and 4.25; see also Jenkins *et al.*, 2008b). Similarly, data from road-killed badgers show an overall prevalence of 15% (Bourne, 2007, para. 4.21). Although the congruence between these different sets of results seems reassuring, none of the relevant diagnostic methods is 100% sensitive, meaning that prevalence is almost certainly underestimated. Indeed, a more intensive examination of 205 carcasses of badgers culled in the RBCT revealed almost twice the rate of infection as did the standard procedure (Bourne, 2007, para. 4.16). Since 2006, the Woodchester Park badgers have also been tested with g-IFN and Stat-Pak, and the data from these tests may lead to a refinement of prevalence estimates.

4.3.4 This uncertainty about prevalence levels underlines the inadequacy of currently available diagnostic tests (see section 2.4). In particular, the ability to assess the risk that badgers pose to cattle is limited by the fact that we do not know whether badgers with mild or undetectable pathology are able to transmit infection.

4.3.5 Both the Woodchester Park data and the RBCT data show that prevalence is higher in adults than in cubs, and higher in males than in females (Bourne, 2007, para. 4.17). There is also evidence that the disease tends to progress more rapidly in males than in females (Wilkinson *et al.*, 2000). Current research (Defra project SE3032) is examining other potential correlates of infection, such as body condition.

Spatial patterns

4.3.6 There is good evidence, both from the Woodchester Park study and from the RBCT, that infection is spatially clustered in undisturbed badger populations (Bourne, 2007, para. 4.18). For example, at Woodchester Park, infection has been persistently diagnosed in a cluster of contiguous social groups in the south-west part of the study area, while the rest of the population has remained relatively disease-free (Delahay *et al.*, 2000). Conversely, there is equally convincing evidence that an enhanced rate of movement between badger groups, caused either by culling or by natural dispersal events, results in an increase in the prevalence of bTB (Vicente *et al.*, 2007; Woodroffe *et al.*, 2006). In terms of managing the risk of disease transfer to cattle, the fact that bTB is spatially clustered in undisturbed badger populations suggests that control strategies could be targeted towards stable and persistent foci of infection. However, the lack of accurate, cost-effective diagnostic tools for use on live badgers means that foci of infection cannot at present be detected.

4.3.7 On a larger spatial scale, however, little is known about prevalence patterns. Data from a national

programme of testing of road-killed badgers, undertaken from 1972 to 1990, suggest a rough correlation, at a regional level, between the likelihood of infection in badgers and risk to cattle (Krebs *et al.*, 1997, Appendix 9). On the other hand, infection in badgers has been detected in areas where herd breakdowns are infrequent; and it has not been detected in badgers in some areas where herd breakdowns are relatively common (Krebs *et al.*, 1997, section 4.3). A second Road Traffic Accident Survey was carried out during the RBCT, from 2002 to 2005, but this was restricted to seven counties chosen to represent either high, or historically low but increasing, bTB risk to cattle. The results of this survey show substantial variation in prevalence between counties but, in some cases, estimates of prevalence are compromised by small sample sizes (Bourne, 2007, Table 4.10).

4.3.8 In short, it seems likely that bTB in badgers is widespread but its prevalence outside the RBCT areas is largely unknown. Consequently, it is impossible to compare the risk that badgers pose to cattle in the hotspot areas with the risk that they pose elsewhere in the UK. Unfortunately, although they give some crude information regarding the occurrence of bTB in badgers, previous experience suggests that road-kill surveys do not yield sufficient numbers of carcasses to provide the necessary data; and we lack the diagnostic tools to determine prevalence in live-trapped badgers.

4.3.9 Lack of knowledge of the prevalence of bTB in badgers outside the hotspot areas also means that we do not know whether the disease is self-sustaining in badgers. A modelling study suggests that bTB could persist for long periods of time in social groups containing a minimum of six adults and juveniles (White & Harris, 1995). The best empirical evidence is the detection of bTB in road-killed badgers in parts of the country where cattle breakdowns are absent or rare (see above). However, sample sizes are small, making this evidence essentially anecdotal in character. The persistence of bTB for over two decades in the Woodchester Park study area is not confirmatory, since this area is not immune from cattle breakdowns. The importance of resolving this issue is that if bTB is indeed self-sustaining in badgers, cattle-based control measures alone, as recommended by the ISG, will not eliminate the disease in either species. As noted above, however, we currently lack the means of obtaining meaningful prevalence estimates without culling large numbers of badgers.

Temporal patterns

4.3.10 Data from Woodchester Park suggest a significant degree of inter-annual variation in the prevalence of bTB in badgers. This does not correlate with changes in population density over the same time period but is associated with the degree of between-group movement of badgers (Vicente *et al.*, 2007). As regards longer-term temporal trends, data from MAFF-taken badgers suggest an increase in bTB prevalence throughout the 1980s and 1990s, while data from road-killed badgers suggest an increase during the 1990s only. There is some suggestion of a correlation, across years, between herd breakdown rates and bTB prevalence in road-killed badgers, but this correlation may be an artefact of sampling biases (Krebs *et al.*, 1997, section 4.3).

Population density of badgers

4.3.11 Badger population density has been estimated from two systematic national surveys carried out in the 1980s and 1990s. Based on numbers of main setts found in selected 1 km squares, these suggest that

the population density of badgers is highest in south and south-west England, and Wales (Wilson *et al.*, 1997), indicating a rough spatial correlation between badger population density and risk to cattle. However, the data do not allow comparisons to be made at finer spatial scales. Attempts to produce predictive models of badger population density, using habitat and other variables, have not been very successful (for references see Newton-Cross *et al.*, 2007).

4.3.12 As regards temporal trends, the UK badger population is estimated to have increased by 77% between the mid-1980s and mid-1990s, with increases being especially marked in the West Midlands region (Wilson *et al.*, 1997). No national survey of badgers has been undertaken since the 1990s but the general perception amongst farmers and naturalists is that badger populations in general are at least stable and are probably increasing.

4.3.13 We conclude that the Woodchester Park project and the RBCT have provided valuable epidemiological information, in particular about the prevalence of bTB in badgers and about the manner in which the disease is spatially distributed on a fine scale. Both studies also show that prevalence increases when between-group movements of badgers are especially frequent. At a regional level, there is some reason to suppose that the incidence of bTB in cattle is correlated with prevalence of the disease in badgers and with badger population density. However, we do not know (i) whether the same correlations are manifest at the level of individual counties or parishes; (ii) whether bTB is self-sustaining in badgers (though the balance of probability is that it is; or (iii) to what extent badgers with mild or undetectable pathology are able to transmit infection. The most important single impediment to addressing all of these issues is the lack of accurate, cost-effective diagnostic tools for use on live badgers.

4.4 Transmission (summarized in Fig. 1)

Transmission of bTB from cattle to cattle

4.4.1 Aerosol spread is still considered the most important route for cattle-to-cattle transmission but there is conflicting evidence on nasal shedding of Mb (for references see Wilsmore & Taylor, 2008). Conflicting results between Defra projects SE3015 and SE3033/SE3013 probably require resolution since there is limited evidence for indirect transmission through the environment.

4.4.2 Despite our incomplete understanding of how transmission occurs, it is widely accepted that increased cattle movements associated with increasing herd sizes is strongly associated with the spread of bTB. There is a clear need to explore new approaches to control the spread of bTB. Analysis of CTS data in relation to pre-movement testing has already yielded valuable results (Gilbert *et al.*, 2005b; Mitchel *et al.*, 2008). Modelling of the probable success of interventions such as zoning should aid improved decision support and therefore modelling would seem to be a promising area for research investment (if reliable data were available for parameterization).

4.4.3 Molecular epidemiology promises to significantly enhance our understanding of the transmission of bTB (Wilsmore & Taylor, 2008). This would seem to be a promising area for further research investment.

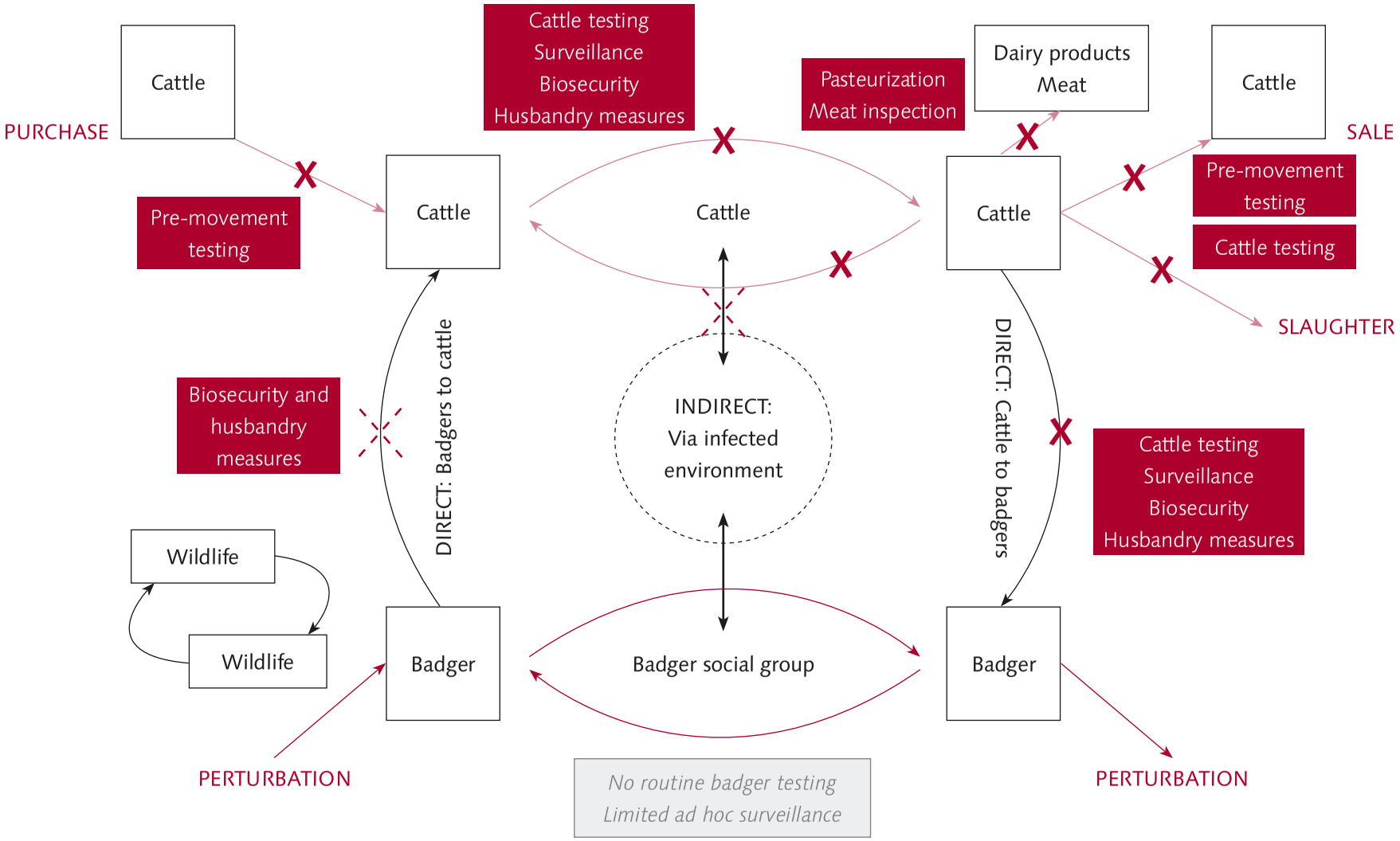

Fig. 1. Mb transmission with interventions *This diagram is derived from a Defra document*

Transmission of bTB from badgers to cattle

4.4.4 The Krebs Report concluded that 'evidence strongly supports the view that badgers are a cause of herd breakdowns' but that, owing to the indirect nature of the relevant evidence, this point could not be regarded as proven (Krebs *et al.*, 1997, p. 121). Since then, the case for badger-to-cattle transmission has been considerably strengthened, primarily by evidence provided by the 'Four Areas Trial' in Ireland and by the RBCT in England that culling of badgers affects the incidence of bTB in cattle (Bourne, 2007; Jenkins *et al.*, 2008a). However, the transmission mechanism remains poorly understood. It could be argued that the implementation of blanket methods of control such as culling or vaccination of badgers does not require that we first understand the transmission mechanism. However, knowing how badgers make contact with cattle would assist in the development of more precisely targeted methods of control.

4.4.5 Thinking to date has focussed on two issues, namely whether: (1) contact between badgers and cattle is direct (i.e. involves direct transmission of an aerosol produced by a badger) or indirect (i.e. transmission results from contamination of the environment with Mb by badgers); and (2) contact occurs while cattle are at pasture or as a consequence of visits by badgers to farm buildings. These possibilities, in combination, yield four potential, non-exclusive badger-to-cattle transmission scenarios. The relative importance of these four scenarios is unknown.

4.4.6 There are circumstantial reasons for supposing that direct transmission via aerosol contact may be more important than indirect transmission via contaminated feed or soil. In particular, infection in both cattle and badgers is most commonly associated with the respiratory tract; substantially smaller numbers of bacilli are required to infect cattle by this route; and Mb survives sufficiently well whilst airborne for aerosol transmission to be effective (for references see Wilsmore & Taylor, 2008, p. 29).

4.4.7 Observational studies suggest that direct contact between badgers and cattle, while the latter are grazing on pasture, is rare (for references see Wilsmore & Taylor, 2005). However, one case has been reported of badgers and cattle simultaneously feeding from a trough containing concentrated feed (Garnett *et al.*, 2002). Somewhat more convincing evidence of opportunities for direct transmission has been obtained from observations of badgers in or around farm buildings. Some farms have been shown to be highly attractive to badgers, which visit in order to consume stored feed, primarily in the form of grain or concentrated cattle feed (Garnett *et al.*, 2002, 2003; Tolhurst, 2006). In doing so, badgers sometimes come into sufficiently close proximity to housed cattle for respiratory contact to be possible. However, the evidence of such contact remains essentially anecdotal since only a small number of direct contacts between badgers and cattle have so far been observed.

4.4.8 Rather more attention has been given to the possibility of indirect transmission and, in particular, to the idea that cattle, while at pasture, become infected while grazing on grass that has been contaminated by tuberculous badgers. While early work suggested that cattle tend to avoid grass contaminated with badger excreta (Benham & Broom, 1991), a subsequent study shows that they do graze active badger latrines when the sward length in the rest of the field has been reduced (Hutchings & Harris, 1997). Cattle may also graze grass that has been contaminated by single deposits of urine or faeces, as happens

when badgers urinate or defecate away from latrines. In addition, one instance has been reported of a cattle feeding-trough becoming heavily contaminated with badger faeces and urine (Garnett *et al.*, 2002). However, since almost all cattle become infected via the respiratory route, these scenarios require a mechanism whereby Mb that is present on grass, soil or cattle feed is aerosolized and inhaled during olfactory investigation, rather than ingested during feeding. Such mechanisms have been proposed but it is unclear how realistic they are.

4.4.9 As noted above, badgers sometimes visit farm buildings to consume cattle feed, and when they do so the feed in question can become contaminated with faeces, urine or other exudates (Garnett *et al.*, 2002, 2003; Tolhurst, 2006). If stored cattle feed is contaminated in this way, bacilli could become aerosolized when the feed in question is transferred to feeding troughs.

4.4.10 Two lines of evidence suggest that badgers that are in the terminal stages of bTB infection, and that are consequently highly infectious, may be especially likely to frequent farm buildings and hence to come into direct or indirect contact with cattle. Firstly, there are anecdotal accounts of debilitated badgers taking up residence in farm buildings including, in at least one case, a cattle shed (Cheeseman & Mallinson, 1981; Garnett *et al.*, 2002). Secondly, badgers found dead in or close to farm buildings are more likely to have been infected with bTB than badgers killed by road traffic (Cheeseman & Mallinson, 1981). Such animals could act as 'superspreaders'. Although the ISG has expressed doubt about the relevance of this idea on the grounds that bTB only progresses to an advanced stage in a small proportion of badgers (Bourne, 2007; Jenkins *et al.*, 2008b), we feel that the 'superspreader' concept deserves further consideration, both through empirical work and by means of modelling studies.

4.4.11 Similarly, little attention has been given to the idea that some cattle may be more likely than others to come into contact with badger-related sources of infection. In a test carried out in New Zealand, dominant herd members were more likely to become infected after exposure to sedated tuberculous possums, presumably owing to a stronger investigative tendency on the part of these individuals (Sauter & Morris, 1995). If a correlation were found between dominance and infection in cattle in the UK, it would imply that individual cattle play an active role in exposing themselves to sources of infection such as badgers or badger excreta, rather than becoming infected purely passively via activities, such as grazing, that are common to all members of the herd.

4.4.12 We conclude that further investigation is needed into behaviours that result in indirect or (especially) direct contact between badgers and cattle, both while cattle are at pasture and while they are housed on farm premises. Such research would shed light on the relative plausibility of different transmission scenarios, the tendency of severely infected badgers to behave in ways that increase the probability of transmission, and the possibility that dominant and subordinate cattle differ in their tendency to display risk-prone behaviour. Ultimately, the results would enable control measures to be more precisely targeted towards high-risk situations. Given that transmission is in absolute terms a rare event and that direct observation or video surveillance are both highly labour-intensive, research is needed into the use of automatic monitoring systems, such as contact sensors, as a means of acquiring the requisite data.

Transmission of bTB from cattle to badgers

4.4.13 Woodroffe *et al.* (2006) showed that a delay in detecting and removing reactor cattle from herds during the 2001 foot-and-mouth disease epidemic resulted in a widespread increase in the prevalence of bTB in badgers. This suggests a significant level of transmission from cattle to badgers. Prior to this, little consideration had been given to either the likely frequency of cattle-to-badger transmission or the mechanism underlying it. In principle, however, it could, like badger-to-cattle transmission, be either direct or indirect; and it could occur either while cattle are at pasture or as a result of badger visits to farm buildings. For example, badgers often dig around in cowpats in search of invertebrate prey (Kruuk, 1989) and they also forage in and around cattle sheds (Garnett *et al.*, 2003). However, given that cattle, unlike badgers, very rarely shed Mb in faeces or urine, direct aerosol transmission seems most likely. Consequently, the type of behavioural research that we recommend in relation to badger-to-cattle transmission (see section 4.6.9) should also shed light on routes of cattle-to-badger transmission.

4.5 Environmental reservoirs of Mb

4.5.1 Until recently, little consideration has been given to the existence of environmental reservoirs of Mb infection, other than in the form of grass or cattle feed contaminated by badger excreta (see above, sections 4.6.5 and 4.6.8). However, recent work by Courtenay *et al.* (2006), using PCR to detect Mb in environmental samples, found Mb in samples taken from setts on 78% of farms, and in samples from latrines on 56% of farms. The farms in question were taken from both endemic and non-endemic areas. In addition, sampling of setts and latrines at Woodchester Park revealed a weak but significant correlation between the proportion of environmental samples testing positive for Mb and the intensity of infection in resident groups of badgers. These results suggest that environmental contamination, presumably originating from infectious badgers, may be widespread. Subsequent work using real-time PCR (Sweeney *et al.*, 2007) provides further evidence of the species identity and cellular origin of the signals, and suggests remarkably high densities of Mb cells (6.8×10^{4}–5.4×10^{6} cells per gram of soil) in environmental samples (see also section 2.7). If they can be confirmed and extended, these results have the following important potential implications: (1) environmental reservoirs of Mb could constitute sources of infection of cattle; (2) they could compromise the testing of cattle for bTB; (3) they could compromise the vaccination of cattle or badgers against bTB; and (4) screening for the presence of environmental Mb could provide a method of identifying likely animal sources of infection and hotspot contamination points on individual high-risk farms.

4.5.2 The most urgent research priority regarding this work is that the results be validated, and a project aimed at this is under way (Defra SE3231: VLA, Weybridge, and University of Warwick). This project will also investigate the viability of environmental Mb cells and examine the feasibility of using environmental sampling to detect hotspots of Mb contamination on farms with persistent breakdowns. An additional study (BBSRC project BBSB08868: University of Warwick) will quantify the turnover and viability of Mb cells in laboratory microcosms, and the spatial location of point sources of contamination on a sample of farms. If the ongoing validation study is successful then further research will be required to

assess the risk that environmental Mb poses to cattle. In addition, should the development of a PCR-based environmental screening tool prove feasible, research will be required into the development of optimal sampling regimes in order to minimize the cost of screening potentially very large numbers of sites.

4.6 Role of wildlife other than badgers

4.6.1 Two major Defra-funded investigations into the prevalence of bTB in wildlife other than badgers have recently been undertaken. The first of these (SE3029, University of Oxford; see Mathews *et al.*, 2006) involved culture of Mb from faeces, urine and tracheal aspirates taken from live animals (mostly rodents). The second (SE3010, CSL; see Delahay *et al.*, 2007) involved culture of Mb from tissue samples taken from carcasses of a wider variety of species including rodents, sciurids, carnivores and ungulates. The second study also involved a semiquantitative risk assessment taking into account not only the prevalence of TB in the wildlife species in question but also ecological factors affecting the potential for transmission of infection to cattle. Both studies suggest that the only wildlife species posing a potential risk to cattle, other than badgers, are deer, especially fallow deer *Dama dama*. Subsequent Defra-funded field, laboratory and modelling work (SE3036, CSL; SE3037, Risk Solutions) suggests that although red and fallow deer are likely to excrete Mb into the environment, there is at present only a low risk that they transmit bTB to cattle. Nevertheless, wild deer do pose a significant risk to cattle in other countries, especially when the deer in question occur at high densities (for references see Wilsmore & Taylor, 2008).

4.6.2 Although it is widely believed that deer are becoming more numerous and more widespread in the UK, the source of this perception is unclear since systematic data on nationwide population trends in most species of deer are lacking. Relevant information seems especially sparse in the case of fallow deer (Battersby, 2005). We therefore recommend that Defra liaise with relevant bodies, such as Natural England and the Tracking Mammals Partnership, to ensure that adequate monitoring systems are in place to provide reliable information about the distribution and numbers of deer, especially fallow deer, now and in the future.

4.7 Modelling

There will be an increasing need to use models to represent multiple causal links within the 'system' to help explain and predict many aspects of bTB. This necessary development was highlighted in the recent review by Pfeiffer (2007). To be more effective, there should be increased coordination of the different projects investigating all aspects of this disease. Of four modelling projects reviewed, only SE3026 generated any data, while the others (SE3117, SE3229, SE4202) all relied on pre-existing data. However, as highlighted above, there is a perceived need for improved summary data for bTB in cattle so some effort should be extended to generate such a resource.

5 VACCINES

5.1 Current and recent research

5.1.1 The Krebs Report (Krebs *et al.*, 1997) stated that 'In the long run, the best prospect for control of bovine TB is to develop a vaccine for cattle. This is a long-term (more than ten years) strategy and success cannot be guaranteed. However, targets and milestones can be identified to monitor and evaluate progress at five yearly intervals. We recommend that the development of a cattle vaccine and an associated diagnostic test to distinguish infected from vaccinated cattle should be a high priority for MAFF's long-term research strategy. A badger vaccine, although posing greater technical problems in terms of both development and delivery, should also be kept as an option. During the next five years much of the basic research required will be relevant to both badgers and cattle'.

5.1.2 Against this background, the Defra programme of research, supported by underpinning BBSRC projects, has the following overall aims:

- To demonstrate a near-term option for the vaccination of cattle;
- To demonstrate a near-term option for the vaccination of badgers;
- To identify candidate next-generation improved vaccines for use in cattle.

Where appropriate, we have also considered work outside of the Defra programme and which could support the aims outlined in the Krebs Report.

5.1.3 The Defra programme on vaccines includes work to devise vaccines for use in badgers or in cattle. Project SE3223 is a critical element of the programme and involves devising a formulation of BCG suitable for use in badgers. As part of this programme, the safety of the formulated vaccine in badgers will be determined. Project CB0116 will carry out more detailed efficacy testing of candidates in wild badgers. These projects build on project SE3228 (completed), which demonstrated the safety and immunogenicity of BCG in badgers. A more detailed study to investigate safety, immunogenicity and lack of shedding following the immunization of wild badgers with BCG is under way (CB0115).

5.1.4 The programme to devise a vaccine for use in cattle (project SE3224) involves looking at ways of improving the efficacy of BCG. This work builds on previous work to identify a subunit bTB vaccine. Whilst a subunit vaccine alone now appears to be unlikely to offer good levels of protection, an alternative approach being investigated in SE3224 involves a BCG prime-live vectored vaccine boost. Of the candidates being evaluated (MVA-Ag85 and Adeno85A), the Adeno85A vaccine looks most promising. There is some work on protein antigens, both from the viewpoint of exploiting them as subunit vaccines and as targets for correlates of protection studies. This project is running in parallel with a programme to identify adjuvants and diagnostics which are suitable for use in cattle.

5.1.5 Clearly one issue for the development of cattle vaccines is the demonstration that the vaccine limits transmission of bTB in a natural setting. A model to test this is being developed in project SE3227, where vaccinated neonates will be introduced into an experimental herd of reactor animals. The project plans to test the efficacy of BCG and BCG-primed and subunit-boosted animals. This ambitious project clearly underpins future cattle vaccine studies.

5.1.6 A critical theme which runs through the vaccines programme is the linkage with the diagnostics programme. The linkage between introduction of g-IFN tests and the use of vaccines in cattle is especially critical. In our opinion, the use of vaccines in cattle will be very difficult without the introduction of a g-IFN-based test based on antigens absent from the vaccine (see 2.3.7).

5.1.7 The development of a substantial population of vaccinated cattle will present a major challenge to the routine screening programme since the blood tests required for evoked g-IFN testing place different time demands on veterinary surgeons and must feed into a substantial sample-processing infrastructure. A DIVA antigen-based skin test might be considered as an alternative but this will require extensive evaluation. Moreover, we have outlined our concerns regarding use of screening tests with lower sensitivity than the g-IFN test. The major challenge remains to develop better screening tests that are suitable for application on the scale required, that have the highest possible sensitivity and include DIVA capability.

5.1.8 There is a significant body of work on bTB funded by the BBSRC. This work supports a greater understanding of the bovine immune system. These projects have a strong focus towards understanding the interaction of Mb with antigen presenting cells (APCs). Projects BBD0038061, JRE10834, BBSEI00000984, BBSEI00001188 and BBSEI00001211 will characterize the biology of bovine dendritic cells, the interactions of Mb with bovine APCs such as dendritic cells and macrophages and the subsequent interactions of APCs with other cell types. Project BBD0015361 is focussed on characterizing the interactions of APCs in the upper airways of cattle with recombinant bovine respiratory syncytial virus. The information gained in this project would also be relevant to the induction of mucosal responses by bTB vaccines.

5.1.9 Although the BBSRC programmes do support Defra work, linkage between BBSRC and Defra projects is not apparent in the documentation presented. However, Defra gets sight of all proposals funded by the BBSRC and there are collaborative links between IAH and VLA teams working on bTB.

5.2 Evidence that vaccines will work

The evidence is that BCG is of some value in humans, and the available evidence is that the immunization of badgers or cattle with BCG could have some merit, at least in reducing the degree of pathology and shedding. In cattle, various trials have revealed variability in the ability of BCG to protect against infection with bTB (Francis, 1947; Hewinson *et al.*, 2003; Mahmood *et al.*, 1988; Skinner *et al.*, 2001). The available evidence indicates that the BCG vaccine is most effective if given to calves under 6 weeks of age. The reduced efficacy of the vaccine in older animals likely reflects the prior exposure of animals to environmental mycobacteria (Buddle *et al.*, 2002; Fine, 1995; Stanford *et al.*, 1981). It is not clear whether BCG will be more effective in young badgers, but this seems likely. This could have implications for the effectiveness of any future badger immunization programme.

5.3 Potential value of BCG

5.3.1 The choices of vaccine that can be used to protect against bTB are: (1) a live vaccine; (2) a dead whole-cell vaccine; (3) a subunit vaccine (e.g. protein or peptide); or (4) a heterologous prime–boost strategy where a combination of these is given.

5.3.2 The most obvious vaccine to use is BCG, and this offers many advantages against which any alternative vaccines need to be compared. These include the facts that it is well characterized (genetically and biologically), safe in humans and safe in the animals in which it has been tested. BCG is likely to be acceptable in terms of environmental safety (an issue with badger vaccines), is the basis for all new human vaccine strategies, is currently being taken through an EU approval process for use in cattle, is partially effective, and can be a marker vaccine that combines with a diagnostic test. The approach being followed in human trials is for BCG to be used in combination with a second vaccine as a prime–boost strategy. This is partly because few vaccines show any improvement on BCG when used alone but do improve on BCG when used in combination, and also because any strategy not including BCG is unlikely to be approved on ethical grounds. While the ethical issues are different with cattle and badgers, the logic of using a vaccination strategy that includes BCG as the first line of attack is compelling. Other vaccine strategies may then be developed, while important lessons are learned through BCG trials.

5.3.3 Unlike a human vaccine, any cattle vaccine must be combined with a diagnostic test that can 'Differentiate Infected from Vaccinated Animals' (DIVA), and has been EU-approved. This is particularly difficult with a whole-cell vaccine such as BCG, which is antigenically complex and similar to the current approved PPD diagnostic antigen, but it means that the efficacy of a vaccination strategy has to be modelled in the context of the sensitivity and specificity of the DIVA test.

5.4 Questions and priorities for research on BCG

5.4.1 What is the evidence as to the safety and efficacy of BCG in cattle and badgers, both in experimental and natural infections?

5.4.2 What causes the variability? Perhaps the most concerning issue is the lack of protection seen in natural infections.

5.4.3 Is BCG likely to be effective in inducing protective immunity only in young badgers?

5.4.4 BCG is notoriously location-dependent in human TB control; do we know how effective BCG is in the UK, or in different parts of the UK?

5.4.5 There is some evidence for a genetic difference in protection. How does this relate to the breeds of cattle used in the UK?

5.4.6 There is evidence in human TB that this variation is partly due to differential exposure to environmental mycobacteria. The presence of environmental mycobacteria also has major implications for diagnosis in cattle.

- Can we describe geographically where there are high levels of relevant environmental mycobacteria, for instance through skin test data or through sampling?
- Could/should different control strategies be implemented in different areas based on this?
- Could vaccination of neonates avoid issues with vaccine efficacy, although diagnostic efficacy may still vary?

5.4.7 How effective does vaccination have to be? For example, several studies talk about a reduction in pathology rather than full protection.

5.4.8 Is the alternative diagnostic test effective?

5.5 Potential successors to BCG

5.5.1 Due to the fact that BCG has limited efficacy against pulmonary TB yet has an important protective role against paediatric disease, substantial efforts have been made to find a successor to BCG as a live attenuated vaccine for humans. From our discussions, it is clear that Defra scientists appreciate the value of close links with researchers developing human vaccines against Mtb and have initiated collaborative projects to apply knowledge in this area to bTB. For example, the MVA-Ag85 and Adeno85A candidate vaccines for use in humans are being evaluated in cattle.

5.5.2 However, the bTB vaccine programme needs to continue to take account of newly emerging human vaccine candidates and have the provision to test these vaccines in the Defra-funded programme. To date, these alternatives have not been fully evaluated in the context of resistance to Mb. Of particular interest is the BCG Hly/delta UreC construct (Grode *et al.*, 2005), which offers the potential to induce improved $CD8^+$ T-cell responses. Our understanding is that the BCG Hly/delta UreC vaccine is currently being tested in cattle as a stand-alone vaccine. It could also be included in future prime–boost scenarios and where possible in vaccination studies in captured badgers under laboratory conditions.

5.5.3 Some work has been performed in generating new attenuated strains of Mb which are immunogenic but not pathogenic, but not all of these have been protective. Recently, protective experimental vaccines

based on attenuated Mtb, by single or double mutation of two-component regulators (*phoP*) or bacterial genes which control host cell apoptosis and therefore T-cell priming (*secA2*), have also been generated (Hinchey *et al.*, 2007). These candidates have significant regulatory obstacles to their implementation in humans and are also unlikely to be accepted as candidates for bovine vaccination.

5.6 Correlates of protection

5.6.1 Developing immunological measurements which correlate with efficacy of vaccination is essential for any vaccine programme and particularly relevant to studies on resistance to mycobacteria. Currently, analysis of adaptive immune responses to mycobacteria in humans and animals (including cattle and to a lesser extent badgers) is dominated by assessment of g-IFN following restimulation of antigen specific T cells *in vitro*. Although g-IFN is necessary for resistance, there is growing evidence that measuring this (or any other) immune parameter alone is not a reliable marker of resistance to infection or vaccine efficacy.

5.6.2 Recent studies in other infections using mice, non-human primates and humans suggest that induction of multifunctional T cells is necessary for optimal protection (Darrah *et al.*, 2007; Mueller *et al.*, 2008; Seder *et al.*, 2008). The ability of multifunctional T cells to make several effector cytokines (e.g. g-IFN and TNF), migrate to different anatomical locations (determined by chemokine and other receptor expression), recruit and organize other cell populations (e.g. via chemokine secretion) and proliferate (reflected in their ability to secrete IL-2) must all be considered in assessing their potential in vaccine-mediated resistance. Work involving Defra-funded scientists (Wedlock *et al.*, 2007) has shown that g-IFN alone is not always a correlate of protection in vaccinated cattle and has started to address other cytokine parameters (e.g. IL-2) in this context but more work on the biology of multifunctional T cells in cattle is needed. This requires good multiparameter flow cytometry facilities and should address whether g-IFN$^+$/TNF$^+$/IL-2$^+$ multifunctional T cells are found in cattle following infection or vaccination, whether these correlate with the extent of protection and whether their frequency can be further enhanced by new vaccine candidates/regimens being developed. Such studies will provide the best possible picture of the diversity of the bovine T-cell response to vaccination.

5.6.3 If attempts to generate a vaccine for use in badgers are to be productive, considerable investment in understanding the nature of anti-mycobacterial immune responses and their relationship to disease pathogenesis is needed in this species. Defra-funded scientists are currently leaders in this field but progress is limited by the lack of diverse immunological reagents, particularly antibodies to key cytokines such as IL-4, IL-10, IL-13, etc., and the logistics of studying these animals in an experimental setting. Appropriate Category 3 animal facilities for badger research must be available and support to generate the reagents for detailed analysis of the cell-mediated immune response is needed. The response of badgers to mycobacteria is likely to be different in some or many aspects to that of humans or cattle, given the dynamics of their exposure, the presence of other ongoing diseases (such as silicosis and parasite infection) and suggestions that their T-cell responses may be directed to a different set of mycobacterial antigens. Basic studies defining the biology of CD4 and CD8 T-cell responses in infected

or vaccinated badgers are needed in order to identify correlates of vaccine-mediated protection in this species. Application of the multifunctional T-cell concept described above to badger vaccination would also be highly desirable but again will require much greater investment in developing immunological reagents in this species. Defra may wish to assess the real need for this in prioritizing limited research funds and to consider whether BCG and the current immunological readouts are good enough for achieving a licensed vaccine that is safe and efficacious.

5.6.4 There is also a need to develop a broader approach to identify other T-cell parameters which would most accurately predict vaccine efficacy in cattle. One approach to identify previously unknown markers involves generating mRNA signatures of peripheral blood T cells from vaccinated individuals re-stimulated *in vitro*. High-throughput microarray technology (e.g. Illumina) can identify some 40,000 transcripts in each sample in humans and this technology is now being applied to cattle (although it is unlikely to be feasible in the short-term in badgers). Defra scientists have begun to investigate this approach and such studies should be continued.

5.7 Alternative formulations and adjuvants

5.7.1 The focus of the Defra programme is to exploit BCG for use in badgers or cattle, to evaluate any promising human candidate Mtb vaccines in badgers or cattle and to identify protein antigens. Some of this work is ongoing. The programme needs to take account of the possible timescales for the licensing of alternatives to BCG. Such alternatives could take a decade or more to licence for use in humans.

5.7.2 However, there is a unique opportunity for the bTB programme to support efficacy testing of Mtb vaccine candidates for humans. Controlled efficacy studies in animals which are challenged with Mb could provide invaluable data on the efficacy of candidate human vaccines. Such challenge studies would not be permitted in humans, and corresponding efficacy data would be dependent on expensive and time-consuming clinical trials. Such studies in cattle also provide an important advantage over human trials in enabling access to infected tissues for analysis.

5.7.3 Vaccination changes the dynamics of granuloma formation and composition as well as the distribution of bacteria within and outside organized immune foci. The processes underlying these effects all provide important clues regarding the mechanism of vaccine-mediated protection which cannot be obtained in humans. Validating correlates of protection based on immune responses in peripheral blood with actual outcomes in infected tissues is also an important and unique goal for which this work would provide an opportunity.

5.7.4 The use of a subunit vaccine in cattle or badgers is certain to require formulation with a suitable adjuvant which promotes T-cell responses. The relative roles of $CD4^+$ and $CD8^+$ T cells in protective immunity are not known, but the evidence is that both types of response are required for protection.

5.7.5 Despite significant effort in the worldwide community, there has been a lack of success in identifying adjuvants which promote strong $CD8^+$ responses. There is some work in the Defra programme to

investigate adjuvants for use in cattle with subunit vaccines (project SE3224). In our opinion, the likelihood of an effective stand-alone subunit vaccine for Mtb or bTB is low.

5.7.6 For subunit vaccines, the focus is likely to be on the testing of adjuvants, which have been shown to work in humans, also work in badgers or cattle. However, the identification of suitable adjuvants is likely to be hampered in two ways. Firstly, the type of immune response that is required for protection of badgers or cattle is not known, though the working assumption is that cellular immunity will play an important role. Secondly, it is not known whether adjuvants that have been tested in mice or in humans will perform similarly in badgers or cattle.

5.7.7 However, work to identify immunogenic proteins is well justified from the viewpoint of developing correlates of protection. These proteins could well find their way into future versions of the g-IFN assays for diagnostics. As highlighted above, the development of appropriate diagnostic assays will be critical to the licensing and use of any future bTB vaccines.

5.7.8 The work to test vaccines such as Adeno85A and MVA-Ag85 builds on work to devise human vaccines for Mtb. It seems most likely that live or live vector-delivered vaccines will perform best as next-generation bTB vaccines.

5.7.9 Priming with one vaccine construct and subsequent boosting with another (so-called heterologous prime–boosting) is widely viewed as an essential tool to generate effective $CD4^+$ and $CD8^+$ T cells. In humans, the efficacy of BCG against paediatric TB means that the priming event will remain anchored by some form of improved BCG in infancy with later boosting using subunit vaccines delivered in adjuvant or via live viral vectors. Such heterologous prime–boost scenarios are also likely to be the most effective in cattle.

5.7.10 Research by Defra-funded scientists is leading the field in investigating prime–boost approaches in cattle and this should be continued to identify the optimal prime and boost combinations for resistance against Mb. Again, the ability to directly compare vaccine efficacy with immune responses both at the site of infection and in the peripheral blood is a valuable opportunity which is lacking in human vaccine trials. Unfortunately, the prime–boost approach is unlikely to be feasible in a badger vaccination programme given the logistics of vaccine delivery to this wild population.

5.8 Mucosal versus parenteral immunization

5.8.1 Many effective vaccines mimic the natural infection with respect to the route of infection. However, relatively little is known about the natural route of infection of cattle, and even less about the natural route of infection of badgers. In both cases, the aerosol route of infection seems most likely for Mb (see 4.6.3). Therefore, the development of mucosal immune responses might be important to prevent infection and transmission. In badgers, vaccines that prevent the transmission of disease are a priority. In cattle, vaccines that prevent infection would be more important, but prevention of transmission would be of value.

5.8.2 The development of orally delivered vaccines might go some way towards the induction of mucosal immune responses, and the Defra programme does include work towards an orally delivered BCG vaccine; this is actually the primary goal for practical/cost reasons. The programme of work should also consider other approaches. It may be worth investigating whether immunization with Adeno85A or MVA-Ag85 by a mucosal route (as part of a prime–boost approach) in cattle or in badgers is effective.

6 ECONOMIC, SOCIAL AND BEHAVIOURAL CONSIDERATIONS

6.1 Introduction

6.1.1 The Krebs Report (Krebs *et al.*, 1997) had little to say on economic and social considerations. The costs of breakdown and the impact on human welfare on affected farms are presented as given rather than the subject of future research (para. 7.2.2). There is one recommendation on economic research – that costs and benefits of different control strategies be investigated through integrating economic and transmission models (para. 7.7.3 f.).

6.1.2 The report also (section 5.7) refers to indications that farmers were not taking up MAFF advice on husbandry practices to minimize contact between cattle and badgers – advice that had been issued since 1986 and routinely given to farmers experiencing an outbreak. It recommended an industry-led study, comparing efficacy of 'proactive husbandry methods' designed to keep cattle away from badgers, and badgers away from cattle. This recommendation has been partly implemented through commissioning of Defra project SE3119 in 2005. The ISG (ibid. para. 5.7.2) also suggested that farmers' reluctance to follow MAFF advice was due to a mixture of 'logistical difficulties, perceived impracticalities, cost, conservatism and lack of convincing evidence that husbandry could have an effect'. But they made no recommendations that research be done to test these assumptions.

6.1.3 The Independent Husbandry Panel (Phillips *et al.*, 2001) subsequently identified several husbandry practices (based in part on advice in the MAFF leaflet '*TB in Cattle: Reducing the Risk*' MAFF, 1999) through which farmers could limit transmission, particularly in pastures:

- fencing off of badger setts;
- rotational grazing and other adjustments to grazing management, including avoiding grazing obvious badger latrines;
- vigilance in removing and disposing of dead badgers;
- fencing to keep badgers out of high-risk areas (buildings, silage pits);
- raising feeding troughs in pasture to put them out of reach of badgers;

- fencing off large badger latrines;
- repairing walls and other measures to reduce the number of badger crossing points at field boundaries;
- preventing entry to buildings where there is evidence of badger intrusion;
- avoiding scattering concentrates on the ground and raising licks out of reach (e.g. suspending them from trees);
- routine disinfection of equipment and buildings where reactor cattle are housed.

6.1.4 They recommended, as a high priority, research 'to quantify the extent and nature of badger visitation of farm buildings and food stores, including silage clamps, and to determine the extent of close contact between badgers and cattle in the field' in order to build an evidence base on which exclusion strategies could be developed.

6.2 Current and recent research

The current research portfolio includes four projects which will significantly take forward our understanding of behavioural and economic considerations.

6.2.1 SE3039 'Identification of changes in individual and global farmer behaviour relating to the movement and management of cattle in the UK with particular reference to the introduction of bTB control measures'. This project is trying to identify farmer behavioural responses to changes in the regulations on cattle movements, particularly relating to pre-movement testing. The two-track approach is, first, to analyse trends in movement at an industry level by applying network analysis to cattle movement data and then correlate points of change with changes in the regulatory environment; and second, to undertake interviews with individual farmers to explore the motivations and other factors that influence their behavioural response to specific bTB control measures. A strength of this project is its use of robust methodologies in network analysis and social psychology, and its consequent combination of quantitative and qualitative data and methods of analysis. This is the only Defra-funded study relating to bTB that focusses on behaviour and behaviour change among livestock farmers.

6.2.2 SE3117 'Cost–benefit analysis of badger control'. Although this is listed as current, it was due to be completed in May 2007. There is a final report on the initial 2 month phase, which was designed to establish the feasibility or otherwise of a cost–benefit model combining outputs from previous studies by CSL and by the University of Reading (SE3112: 'Assessment of the economic impacts of bTB and alternative control policies'). This initial work demonstrated that it is possible to incorporate outputs from the stochastic spatial simulation model of badger TB produced by CSL into a cost–benefit analysis model developed by the University of Reading. The resulting 'TB CBA meta-model' was then to be developed further and used to assess a wider range of control scenarios.

6.2.3 SE3119 'An experiment to assess the cost-effectiveness of farm husbandry manipulations to reduce risks associated with farmyard contact between badgers and cattle'. This builds on the findings of SE3029 and subjects 'two broad husbandry practices' to reduce contact between badgers and cattle in

the farmyard to cost–benefit and cost-effectiveness analysis. The earlier project (end December 2005) looked at contact in the farmyard, specifically feedstores. It concluded that electric fencing was highly effective at reducing badger entry to buildings in the farmyard. The researchers costed the practice on the basis of a single 'typical' farm. This pair of projects is a response to the recommendation of the Independent Husbandry Panel (Phillips *et al.*, 2001) that experimental work be done to test the efficacy of husbandry methods. There is no indication from the documentation on this project that farmer behaviour, or farmer response to these husbandry practices, is being addressed in the study.

6.2.4 SE3120 'Investigate the longer-term effects on farm businesses of a bTB breakdown'. This project is the first to consider, as a researchable issue, the social, health and well-being effects of a bTB breakdown – i.e. looking at the effect on the individuals involved as well as the farm business, at both individual and wider community level. Strong points in this study include the use of well-tested methods from other fields – to assess social well-being and mental health, and farmers' willingness to pay (through a choice experiment) for a range of vaccine scenarios.

6.2.5 Outside the TB research portfolio are two Defra-funded projects on biosecurity which include some consideration of the management of TB on cattle farms. SE4002 (Development of farm-specific biosecurity risk management strategies for cattle herds and sheep flocks) identified risk factors based on environmental and animal movement/contact indicators as a basis for risk assessment of individual holdings. Likelihood of contact with badgers was not included specifically in the analysis, though proximity of nature reserves to the farm was included and emerged as a significant risk factor. SE4003 (An integrated approach to biosecurity on UK cattle and sheep farms) is an interdisciplinary study which specifically includes research on farmer (and veterinarian) attitudes and motivations towards biosecurity measures.

6.3 Scope and reliability of available studies

6.3.1 Economic and social research in completed projects to date (as reviewed by Wilsmore & Taylor, 2008) covers three main areas:

- Costs to farmers of a TB breakdown: from a sample of 151 farms that have suffered a breakdown, costs range from £229 to £103,817, though for 90% the costs were less than £18,513 for dairy herds and less than £11,462 for beef herds. However, 79% suffered a net loss after compensation payments
- Cost–benefit analysis of specific control measures including a range of culling scenarios, and (more recently) specific husbandry measures;
- Public choices/attitudes/willingness to pay in respect of efforts to reduce the badger population, the overall conclusion from which was that the public thinks that a reduction in the badger population is something they are willing to put up with, but they would put a high price on avoiding a strategy that involves the deliberate killing of large numbers of badgers.

6.3.2 This research is broadly robust in terms of acceptable designs, methods, sampling and analysis and the findings can be considered broadly reliable – at least for the regions in which the research was conducted. Cost–benefit studies have become increasingly sophisticated in coverage of realistic scenarios in terms of patterns of outbreak, coverage of all relevant costs and benefits, and the approaches and models of different research teams are being integrated to give a much more robust understanding.

6.4 What are the gaps in this area and how seriously do these impede policy decisions?

6.4.1 The main gaps are in the range of husbandry measures being subjected to studies of efficacy, costs and benefits; and the lack of evidence on farmers' behaviour and its determinants. These gaps make it difficult to develop policy initiatives designed to influence farmers' behaviour and husbandry practices.

6.4.2 In particular, we have no research-based evidence for farmers' attitudes and behaviour in respect of badgers, husbandry recommendations for minimizing contact, and the allocation of costs (between the industry and government) of control measures.

6.4.3 Recent research – including studies commissioned by Defra – on other areas of farmer behaviour (adoption of recommended technologies, insurance against consequential losses, responses to policy change) suggests that understanding farmers' attitudes, the rationale for their behavioural decisions, and the influence of various organizations and individuals on their behaviour can inform both policy and communication strategies. We also know that farmers are motivated not only by the financial bottom line and that there are distinct behavioural categories of farmer who respond to different sets of motivations.

6.4.4 Current project SE3039 is making a start in this area, in the specific area of cattle movements. For the most part, we are left with repeated assumptions, such as those in the Krebs Report referred to above. Similar unsubstantiated suggestions are made in the final report of SE3029: 'Few farmers appear either aware of the problem [of farmyard contact] or willing to deal with it by investing in husbandry and biosecurity best-practice. This may partly be due to the perceived low quality of advice available'.

6.4.5 A similar comment occurs in the report of the Independent Husbandry Panel (Phillips *et al.*, 2001): farmers are 'unaware of the potential infection hazards within their buildings or that badgers may be visiting them frequently, creating a high risk situation'. They also report, though, that they received 'evidence that farmers can, and do, adjust their management practices to accommodate the restrictions and difficulties caused by *M. bovis*. They should be assisted to be flexible, in whatever ways are feasible'.

6.4.6 No research has been conducted on farmers' knowledge of bTB transmission and how this is related to their attitudes and responses. Research in this area should include studies that explore farmers' knowledge and compare it with scientific knowledge (and areas of uncertainty) thereby improving the evidence base for future policy implementation on control measures and husbandry recommendations.

6.5 Recommendations for future research

6.5.1 Research on economic and social dimensions of bTB was, until recently, limited to assessing the costs of breakdowns and of control measures, with one study looking at public acceptance of control measures involving culling badgers. Farmer behaviour – a critical element in prevention, control and recovery – was largely ignored as a researchable issue. Current research is exploring a wider set of social and economic impacts of bTB breakdown, and is beginning to assess farmer behaviour from a multidisciplinary perspective (specifically, behaviour in response to movement restrictions). This is a welcome development that should be built on in future studies.

6.5.2 Effective policy initiatives require an understanding of the changes farmers make in their management and husbandry practices following an outbreak on their farm or in the vicinity, their rationale for those changes, and their sources of advice, information and influence.

6.5.3 Researchers and policy makers have frequently made assumptions about farmers' knowledge of, and particularly their attitudes towards, husbandry practices to reduce the risk of badger–cattle contact, and their underlying motivations for adopting (or not) specific husbandry recommendations that are thought to reduce contact. If policies are designed to inform and influence farmers' husbandry practices, an evidence base is needed. This should include studies of farmers' actual management/husbandry practices following an outbreak on their farm and in the vicinity of their farm, and the sources of advice and information they used (if any) in making changes.

6.5.4 Farmers' behaviour in respect of bTB is likely to be influenced by their understanding of the mechanisms of bTB transmission and of the risks of a breakdown. Research in this area (see 4.6) would help inform both policy and communication strategies. This research should include work to understand farmers' attitudes towards husbandry practices to reduce risk of badger–cattle contact, and their underlying motivations for changing or not changing their husbandry practices in response to the bTB threat.

7 MANAGEMENT TOOLS

7.1 Recommendations of the Krebs Report and subsequent research

7.1.1 The Krebs Report made a number of recommendations for research, for example in areas such as diagnosis, epidemiology and vaccine development, that were seen as relevant to the development of new or existing management options in the medium to long-term. Research of this type is considered in previous sections of our report. Here, we consider recommendations that, while also containing a research aspect, were directly relevant to the immediate management of bTB in cattle.

7.1.2 Three major recommendations of this type were made, namely that: (i) a controlled trial be carried out of two culling methods (proactive and reactive culling) to investigate the impact of these on herd breakdown rates; (ii) more, and transparent, data on herd breakdowns should be collected to assess the correlates of local variation in risk, taking into account variables such as badger presence, husbandry practices, climate, landscape, etc.; and (iii) an experiment be carried out involving the manipulation of husbandry practices, with herd breakdown rate as dependent variable (Krebs *et al.*, 1997).

7.1.3 The first of these recommendations, that a trial of culling methods be undertaken, was fully implemented in the form of the RBCT (Bourne, 2007). Briefly, the RBCT showed that reactive culling failed to reduce, and may even have increased, the incidence of bTB in cattle. As a result of this finding, the reactive culling part of the trial was terminated and reactive culling is no longer regarded as a viable control option. With respect to proactive culling, the trial showed that while this did reduce cattle bTB within the culled areas, it increased it in a region, about 2 km wide, immediately surrounding each of the culled areas (the so-called 'edge effect'). As a result of this finding, the ISG concluded that proactive culling was not cost-effective (see also sections 7.2–7.5).

7.1.4 The second of the Krebs Report recommendations, concerning the collection of better data on herd breakdowns, was implemented in the form of a questionnaire survey. The initial intention was to collect detailed data on every breakdown that occurred within the RBCT trial areas, and on three times as many control farms, using a questionnaire called TB99. For logistical reasons, the survey was subsequently scaled down and a simplified version of the questionnaire (CCS2005) was introduced, but a substantial amount of data were nevertheless collected. Unfortunately, while the analysis of these

data implicates a variety of risk factors in this or that geographical region or in this or that part of the study, these were not consistent enough to provide a firm basis for management recommendations. The ISG concluded that while 'cattle movements, herd contacts, use of fertilizer, housing and feeding practices' can all impact on herd breakdown risk, 'there is no universal solution for farm management to reduce the risk of a herd becoming a breakdown' (Bourne, 2007, pp. 137–138).

7.1.5 The third recommendation of the Krebs Report, namely that an experiment should be carried out to determine the effect of different management practices, was not pursued, owing to the impracticability of carrying out a large-scale controlled experiment on commercial livestock farms (Bourne *et al.*, 2005). The fact that the TB99 and CCS2005 surveys have subsequently failed to identify consistently important risk factors (see above) provides a further argument against this type of approach.

7.1.6 The RBCT showed that in some circumstances, culling can lead to an increase in the rate of herd breakdowns (see above). This was attributed to culling-induced disturbance in the socio-spatial organization of badgers (the so-called 'perturbation effect'), which was thought to cause an increase in the incidence of bTB in badgers and hence an increase in the rate of transmission to cattle. A considerable amount of behavioural and genetic evidence has since accumulated that is consistent with this interpretation, insofar as it shows that territoriality is reduced, rate of between-group movements increases, incidence of bTB increases, and the degree of genetic mixing increases, in badger populations subjected to culling (e.g. Defra projects SE3032, SE3110). However, this evidence falls short of showing a causal link between these events and an increase in the incidence of bTB in cattle; and doubts remain as to whether, in some triplet areas, there was sufficient time for the proposed chain of events to have occurred between the onset of culling and the appearance of an increase in herd breakdown rate (Godfray *et al.*, 2004). Thus acceptance of the perturbation hypothesis relies to some extent on the absence of any more plausible explanation of the edge effect, rather than on the quality of the currently available evidence (King, 2007).

7.1.7 Several projects are relevant to the identification of risk factors for bTB incidence in cattle. Like the TB99 and CCS2005 surveys, projects that attempted to find ecological or husbandry-related correlates of herd breakdowns (SE3002, SE3004) have not succeeded in identifying risk factors of sufficient generality and importance to generate practical recommendations. By contrast, convincing evidence has emerged that restocking of herds with previously infected cattle was a significant cause of herd breakdowns in previously non-endemic areas following the 2001 foot-and-mouth disease epidemic (SE3026; see also Gilbert *et al.*, 2005a). One project (SE3230) is attempting to define and characterize 'problem' herds, i.e. herds where breakdowns are especially frequent, movement restrictions are prolonged and numbers of animals slaughtered are high. This is a worthy line of enquiry, since the bTB control programme tends to treat all breakdowns as equal, while it is clear that some are more serious and prolonged than others. Indeed, the inclusion of all breakdowns in surveys aimed at identifying risk factors may be a principle cause of the complexity and inconsistency of the results of these studies. If 'problem' herds can be satisfactorily identified, then a renewed search for risk factors, targeted specifically towards those cases, could be worthwhile.

7.1.8 Following the discovery that badgers sometimes frequent farm buildings in search of food, in the course of which they may make direct contract with cattle or contaminate cattle feed (Garnett *et al.*, 2002; see also section 4.6), research oriented towards biosecurity issues has been undertaken. One project (SE3029) has confirmed that some farms are highly attractive to badgers; that visits by badgers to farms are correlated with climatic factors related to the availability of alternative sources of food; and that standards of farm biosecurity are generally lax. This project also showed that electric fencing can effectively keep badgers away from localized resources such as stored cattle feed. A second project (SE3119) is looking more extensively at the phenomenon of badger visits to farm buildings; is attempting to identify the factors that make particular farms attractive to badgers; and is assessing the cost-effectiveness of biosecurity manipulations aimed at deterring badgers. Unfortunately, this study will not have the power to determine whether enhanced biosecurity reduces the risk of herd breakdown.

7.1.9 Two projects (SE3117, SE3229) have modelled the effects of pre-movement testing of cattle on herd breakdown rate and have concluded that it could be cost-effective. A third project (SE3039) is investigating the effects of pre-movement testing on cattle movement patterns and on farmers' own perception of changes in their behaviour consequent upon the introduction of pre-movement testing. It will be important to determine, in due course, whether pre-movement testing impacts on herd breakdown rate by specifically preventing the movement of infected animals or by altering the pattern of cattle movements in general.

7.1.10 To summarize, two of the three management-related recommendations of the Krebs Report were fully implemented and were well backed up by associated research projects. The RBCT itself, and research related to it, has provided invaluable information about the effects of two different culling regimes. The most important practical implications are that (i) reactive culling is unlikely to be effective in any circumstances; and (ii) if proactive culling is to be effective it needs to be done in a way that minimizes the 'edge effect'. The search for husbandry-related factors underlying local variation in risk of herd breakdown has been less successful and has not led to clear management recommendations. Krebs's suggestion that there be an experimental test of husbandry-related manipulations was (justifiably) shelved for practical reasons.

7.1.11 Additional research, not directly triggered by the Krebs Report, has focussed on farm biosecurity (in particular, the risk posed by visits by badgers to farm buildings) and on the likely impact, and practical effects, of pre-movement testing of cattle. Farm biosecurity and pre-movement testing have both emerged as important issues in the last few years and both figure prominently in the ISG Final Report (Bourne, 2007).

7.2 Current status of culling as a management option

7.2.1 Following the completion of the RBCT, the ISG concluded that 'badger culling is unlikely to contribute usefully to the control of cattle TB in Britain' (Bourne, 2007, p. 21). This view was based on the fact

that (a) reactive culling at best produced no effect on, and possibly even increased, the incidence of TB in cattle; and (b) proactive culling, although it had a positive effect (i.e. it reduced cattle TB) within the culled areas, had a negative effect (i.e. it increased cattle TB) in surrounding areas (the 'edge effect'). However, a report by the Chief Scientific Advisor, Sir David King, questioned the ISG's conclusion, suggesting that proactive culling could still be viable as a management option provided that it was carried out over a large area, was sustained for at least 4 years, and took maximal advantage of barriers to badger movement so as to minimize the perturbation effect (King, 2007).

7.2.2 Having considered these opposing viewpoints, the House of Commons Environment, Food and Rural Affairs Committee concluded that 'under certain well defined circumstances it is possible that (proactive) culling could make a contribution towards a reduction in incidence of cattle TB in hot spot areas' (EFRA Committee, 2008). Subsequently, the Secretary of State for Environment, Food and Rural Affairs has stated that licences to cull badgers for purposes of bTB control will not be issued to farmers in England, though he has also left open the possibility of revisiting this decision under exceptional circumstances or in the light of new evidence. The Minister for Rural Affairs in the National Assembly for Wales, by contrast, has recommended a 'targeted' cull of badgers in areas of high herd breakdown rate. At present, then, proactive culling of badgers remains a potential management option, though whether it will be implemented, and if so where and how, remains to be seen.

7.3 Recolonization

7.3.1 If badgers contribute significantly to the incidence of bTB in cattle, the overall cost-effectiveness of proactive culling will depend on the rate at which badgers recolonize the proactively culled areas and on the prevalence of bTB in the recolonizing population. For example, if prevalence increases as a consequence of culling, then the long-term effect of culling, once recolonization is complete, could be a higher rate of herd breakdowns than was experienced before culling began.

7.3.2 Although the Krebs Report explicitly recommended that research be undertaken into post-culling recolonization, this recommendation has not been pursued and we still know little about recolonization and nothing about its occurrence in areas comparable in size to the RBCT triplets. A study of two relatively small areas in Gloucestershire, from which five and six social groups of badgers, respectively, were removed in the late 1970s, suggests that it may take as long as a decade for badger populations to recover to pre-cull levels (Cheeseman *et al.*, 1993), in which case badger removal could continue to impact on cattle TB incidence for many years after the cessation of culling. The RBCT provided a unique opportunity to monitor the recolonization of relatively large proactively culled areas but regrettably this opportunity has been missed and it may now be too late to pursue it. However, we strongly recommend that any future culling operations should be accompanied by proper monitoring of population density and, if possible, bTB prevalence in the relevant badger populations before, during and after culling in order to (i) determine how effective culling has been in terms of reducing badger density and (ii) track the process of recolonization.

7.4 Continued analysis of post-RBCT data

If recolonization of culled areas is slow, then the effects of proactive culling, as carried out in the RBCT, can be expected to continue for some years. Indeed, the latest analysis of the RBCT data shows that the incidence of bTB in cattle within proactively culled areas has now declined to a level substantially below that recorded at the time of cessation of culling, while the 'edge effect' has disappeared (Jenkins *et al.*, 2008a). It is essential that provision continues to be made for periodic analysis of RBCT data relating to cattle bTB incidence in and around proactively culled areas and within corresponding control areas, until such time as an equilibrium situation is re-established (i.e. until there are no significant differences in herd breakdown rate between proactively culled, surrounding and control regions). Only then will it be possible to compare the overall costs and benefits of proactive culling.

7.5 Alternative culling regimes

So far, proactive culling has only been considered in the form of simultaneous removal, over a large area, of as many badgers as possible. However, it is possible to envisage other forms of less-intensive culling that would reduce badger populations more gradually and might, as a consequence, avoid major perturbation effects. The aim of such culling would be to reduce badger population density to a level at which the disease is no longer self-sustaining. We suggest that consideration be given to the modelling of alternative culling regimes, using existing information about badger behaviour and population dynamics.

7.6 Fertility control

7.6.1 The Krebs Report gave brief consideration to the possibility of using fertility control as a way of reducing badger population density and hence of controlling bTB in badgers (Krebs *et al.*, 1997, p. 86). The advantage of this approach is that it would be less detrimental than culling to badger welfare and hence would be more publicly acceptable. Also, from the point of view of their reproductive biology, badgers are in principle a good candidate for fertility control (Tuyttens & MacDonald, 1998). The use of chemosterilants, delivered orally and aimed at female badgers, was considered by Krebs *et al.* to be the most promising method of achieving fertility control. However, the risk of uptake by non-target species, including cattle, was acknowledged to be a serious obstacle to this approach.

7.6.2 Since the Krebs Report, interest in agents of fertility control has switched towards immunocontraception, which involves preventing reproduction by stimulating immune responses against gametes or reproductive hormones. Immunocontraceptive agents should in principle be longer lasting, cheaper and more species-specific than chemosterilants. However, despite considerable expenditure on the development of immunocontraceptives, especially in the USA, France and Australia, some very basic scientific, ecological and ethical issues remain to be resolved (e.g. Cooper & Larsen, 2006). In addition, immunosterilization is being developed primarily in the context of control of pest species, not control of disease. It could be inappropriate for the latter purpose because it would select against individuals that have the best immune systems (Nettles, 1997) and might also interfere with disease diagnosis (Tuyttens & MacDonald, 1998).

7.6.3 An additional problem is that high-density badger populations contain a considerable reserve of spare breeding capacity amongst females. This means that fertility control is unlikely to cause a substantial reduction in cub numbers unless the uptake rate of the agent in question, and its efficacy, are very high. For this reason and others, models suggest that fertility control alone is unlikely to constitute an effective means of controlling bTB prevalence, though it could conceivably have a role in combination with culling (Swinton *et al.*, 1997; White *et al.*, 1997). The results of a recent field trial of fertility control (by gonadectomy) in brushtail possums are no more encouraging, insofar as they showed no overall effect on the rate of transmission of bTB within the possum population (Ramsey *et al.*, 2006).

7.6.4 In conclusion, we see no reason to depart from the view of Krebs *et al.* (1997, p. 86) that fertility control has little potential value as a bTB management tool. Research in this area should not be given high priority.

7.7 Farm husbandry measures

7.7.1 Research has shown that visits by badgers to farm buildings could result in direct or indirect transmission of bTB to cattle (see sections 4.6 and 7.1.8 above). Although this evidence is circumstantial in nature, enough has been done to suggest that substantial numbers of farms are subject to visits by badgers. From the point of view of management, the implication is that enhanced biosecurity, for example by preventing badgers from accessing cattle sheds or feed stores, might reduce the risk of herd breakdowns.

7.7.2 A likely bar to getting farmers and vets to take biosecurity seriously is the absence of any direct evidence that it would reduce the risk of a herd breakdown (EFRA Committee, 2008, p. 46). In principle, the relevant evidence could be collected but this would require a trial on the scale of the RBCT. In addition, different farms are attractive to badgers for different reasons, making it impossible to identify a single biosecurity measure, or a limited set of measures, that could be manipulated in an experimental test.

7.7.3 We conclude, therefore, that, notwithstanding the desirability of assessing, in a formal scientific manner, the efficacy of enhancements to farm biosecurity, cost and other logistical considerations make this impractical. However, provided that Defra knows about any biosecurity measures that farmers implement, it should be possible, in the long term, to build up a case–control database that would enable efficacy to be assessed. This depends on Defra opening and maintaining an effective communication channel with farmers on biosecurity matters (see below and section 6).

7.7.4 One important finding from the relevant studies is that not all farms are attractive to badgers. Identifying the reasons why some farms are visited by badgers while others are not is an important research priority and is part of the remit of an ongoing project (SE3119). Depending on the outcome of that project, further research in this area may be justified. In addition, more research may be needed into the effectiveness and cost-effectiveness of different ways of deterring badgers, since electric fencing is unlikely be a practicable solution in many cases. Above all, however, Defra needs to find ways of persuading the farming community that a reasonable level of biosecurity makes sense, and this may be a subject for research as well as for policy decisions (see also section 6).

7.8 A systems approach to bTB control

Our assessment is that a single approach to the control of bTB is unlikely to be successful at this point in time, because, as is evident from our review, all of the approaches proposed have potentially significant limitations. For example, even new diagnostics based on g-IFN release are unlikely to be effective in identifying latently infected animals and will only be used periodically; the use of diagnostics in cattle will not eliminate environmental reservoirs of disease; vaccines for either badgers or cattle have limitations with respect to their likely efficacy and, in the case of badgers, the degree of coverage of the population; culling may only be effective in certain circumstances, for example where geographical barriers reduce the extent of the 'edge effect'; improved husbandry measures could have some impact on the frequency of disease, but the required husbandry procedures are poorly defined and their impact is unquantified. Consequently, a combination of approaches will be required to achieve significant control of bTB. This means that future models should consider how different control measures would interact if implemented together, and how appropriate different combinations of control measures would be to different areas defined either in terms of their geography or in terms of their disease status.

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9 RESEARCH PROJECTS FUNDED BY DEFRA CITED IN THE REPORT

CB0115 Field trial to assess the safety and efficacy of Bacille Calmette Guerin (BCG) vaccine administered parenterally to badgers. CSL, Aston Down/VLA. End 2010.

CB0116 Efficacy testing of BCG in badgers. VLA. End 2010.

SE3002 Ecological correlates of tuberculosis incidence in cattle. Dept of Biological Sciences, University of Warwick. End 2003.

SE3004 Multivariate analysis of risk factors affecting tuberculosis incidence in cattle herds – phase 1. VLA. End 2004.

SE3013 Pathogenesis and diagnosis of tuberculosis in cattle – complementary field studies. VLA. End 2005.

SE3015 *Mycobacterium bovis* pathogenesis. IAH, Compton. End 2004.

SE3017 Development and evaluation of strain typing methods for *Mycobacterium bovis*. VLA. End 2005.

SE3020 An integrated approach to the application of *Mycobacterium bovis* genotyping for the control of bovine tuberculosis in GB. VLA. End 2004.

SE3024 Low dose TB infection in cattle: disease dynamics and diagnostic strategies. VLA. End 2006.

SE3026 Bovine TB transmission in restocked herds: risk factors and dynamics. Department of Biological Sciences. University of Warwick. End 2006.

SE3027 Pathogenesis and immunology of *Mycobacterium bovis* infection in cattle. IAH. End 2005.

SE3029 An investigation of potential badger/cattle interactions including the extent of badger visitations to farm buildings and food stores, and how cattle husbandry methods may limit these. CSL, York. End 2005.

SE3032 The long-term intensive ecological and epidemiological investigation of badger populations naturally infected with *Mycobacterium bovis*. CSL, York. End 2006.

SE3033 Housing of naturally infected cattle (field reactors) at VLA for immunological and bacteriological analysis. End 2007.

SE3035 Estimating badger density in Randomized Badger Control Trial proactive and control areas. CSL, York. End 2007.

SE3039 Identification of changes in individual and global farmer behaviour relating to the movement and management of cattle in the UK with particular reference to the introduction of bTB control measures. University of Liverpool. End 2009.

SE3040 A preliminary analysis of existing data to provide evidence of a genetic basis for resistance of cattle to infection with *M. bovis* and for reactivity to currently used immunological diagnostic tests. Roslin Institute. End 2008.

SE3110 Molecular genetic analysis of badger social structure and bovine TB. CSL, York. End 2006.

SE3112 Assessment of the economic impacts of TB and alternative control policies. University of Reading. End 2004.

SE3117 Cost–Benefit analysis of badger control. CSL. End 2007.

SE3119 An experiment to assess the cost effectiveness of farm husbandry manipulations to reduce risks associated with farmyard contact between badgers and cattle. CSL, York. End 2009.

SE3120 Investigate the longer-term effects on farm businesses of a bTB breakdown. University of Exeter/ADAS. End 2007.

SE3202 The development of animal models to test candidate vaccines for *Mycobacterium bovis* infection in badgers. VLA. End 1999.

SE3220 Molecular and epidemiological characterization of the PPD diagnostic reagent. VLA. End 2007.

SE3221 Volatile organic compound analysis for the rapid diagnosis of disease: TB in badgers and cattle as proof of principle. VLA. End 2008.

SE3222 Development of improved diagnostic tests for the detection of bovine tuberculosis. VLA. End 2008.

SE3223 Development of an oral BCG vaccine bait formulation for badgers. VLA. End 2008.

SE3224 Continuation of the development of vaccines against bovine TB in cattle. VLA. End 2008.

SE3226 Development of tools to study immunopathology in badger tuberculosis. VLA. End 2006.

SE3227 Evaluation of the protection efficacy of vaccines against bovine TB in a natural transmission setting. VLA. End 2011.

SE3228 A safety study of BCG vaccine in wild badgers – preparatory work. VLA. End 2005.

SE3229 Enhanced modeling and prediction of the spread of bovine tuberculosis in mainland Britain: impacts of cattle movements, climate and spoligotype. VLA. End 2007.

SE3230 The problem of TB-herd characterisation, prediction and resolution. VLA. End 2009.

SE3231 Validation and epidemiological application of molecular methods for monitoring *M. bovis* survival and dissemination in the environment. VLA. End 2010.

SE4002 Development of farm-specific biosecurity risk management strategies for cattle herds and sheep flocks. RVC. End 2006.

SE4003 An integrated approach to biosecurity on UK cattle and sheep farms. SAC/RVC/RU. End 2008.

SE4202 An evaluation of biases in the AMLS and CTS databases. University of Oxford. End 2007.

More detailed information on Defra's bovine TB research and development programme is available at http://www.defra.gov.uk/animalh/tb/research/index.htm

10 RESEARCH PROJECTS FUNDED BY BBSRC CITED IN THE REPORT

BBC5088851 Proteomic signatures of bovine TB. University of Southampton. End 2008.

BBD0015361 Immune inductor and effector sites in the upper airways of cattle and influence of site of antigen expression on induction of mucosal immunity. IAH. End 2009.

BBD0038061 Do post-receptor binding events decide the fate of mycobacteria in bovine macrophages? IAH/Royal Veterinary College. End 2009.

BBE0183351 The interplay between host and pathogen genetics in the increasing incidence of bovine tuberculosis. Roslin Institute (EBRC) Edinburgh. End 2008.

BBE0184911 The molecular basis and impact on host response of phenotypic variation across *Mycobacterium bovis* molecular types. VLA. End 2010.

BBE020925 Intra- and extra-cellular mechanisms affecting the persistence of *Mycobacterium bovis* in the environment: towards molecular surveillance of bovine TB. University of Warwick. End 2011.

BBSB08868 The biology of environmental *Mycobacterium bovis*, and its significance to the epidemiology of bovine tuberculosis. University of Warwick. End 2007.

BBSEI00000984 Molecular analysis of the interaction of intracellular pathogens with bovine antigen presenting cells and host immune responses. IAH. End 2009.

BBSEI00001188 Investigation of innate immune interactions between gamma delta T cells and dendritic cells. IAH. End 2008.

BBSEI00001211 Antigen presenting cell populations in cattle. IAH. End 2009.

JRE10834 Influence of *Mycobacterium bovis* on the T cell stimulatory capacity of bovine antigen-presenting cells. IAH/University of Edinburgh. End 1999.

A full list of research projects on bovine tuberculosis funded by BBSRC is available at http://www.bbsrc.ac.uk/science/grants/index.html

Appendix 1

Documents made available to the review group and not listed in References

Bourne, F. J. and other authors (2007). Response to 'Tuberculosis in cattle and badgers: a report by the Chief Scientific Adviser'. Defra, London, 18 pp.

Defra (2005). Government strategic framework for the sustainable control of bovine tuberculosis (bTB) in Great Britain; a sub-strategy of the animal health and welfare strategy for Great Britain. Defra Publications, London, 69 pp.

Defra (2006). Review of the bovine TB research programme, 3–6 July 2006. Internal document, 450 pp.

Defra (2007). Options for vaccinating cattle against bovine tuberculosis. http://www.defra.gov.uk/animalh/tb/pdf/vaccine_cattle.pdf 79 pp.

Defra (2007). Options for the use of badger vaccines for the control of bovine TB. http://www.defra.gov.uk/animalh/tb/pdf/vaccine_badger.pdf 62 pp.

Defra (2008). Defra bTB research and development programme; project summary booklet. Internal document, 123 pp.

Defra Science Advisory Council (2005). Independent review of research on bovine tuberculosis (bTB). 13 pp.

Godfray, C. J. and other authors (2004). Independent scientific review of the randomized badger culling trial and associated epidemiological research. Report to Mr Ben Bradshaw MP, Parliamentary Under Secretary, Defra. 79 pp.

Appendix 2
Abbreviations

APCs	Antigen Presenting Cells
BBSRC	Biotechnology and Biological Sciences Research Council
BCG	Bacillus Calmette-Guérin (vaccine against human tuberculosis)
bTB	Bovine Tuberculosis
CSL	Central Science Laboratory (Defra)
CTS	Cattle Tracing System
Defra	Department for Environment, Food and Rural Affairs
DIVA	Differentiated Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
DR	Direct Repeat (region of genomes)
EFRA	House of Commons Select Committee on Environment, Food and Rural Affairs
ELISA	Enzyme-Linked ImmunoSorbent Assay
g-IFN	Gamma Interferon
HBHA	Heparin Binding Haemagglutinin
IAH	Institute of Animal Health
IL-2	Interleukin-2
ISG	Independent Scientific Group
MAFF	Ministry of Agriculture, Fisheries and Food (forerunner to Defra)
Mb	*Mycobacterium bovis*
mRNA	messenger Ribonucleic Acid
Mtb	*Mycobacterium tuberculosis*
PCR	Polymerase Chain Reaction
PPD	Purified Protein Derivative or Mantoux test for tuberculosis (also known as TST)
RBCT	Randomized Badger Culling Trial (the 'Krebs Trial')
RNA	Ribonucleic Acid

SGM	Society for General Microbiology
SNP	Single Nucleotide Polymorphism
TB	Tuberculosis
TNF	Tumour Necrosis Factor
TraSH	Transposon Site Hybridization
TST	Tuberculin Skin Test
VETNET	Defra disease surveillance database
VLA	Veterinary Laboratories Agency
VNTR	Variable Number Tandem Repeat

Appendix 3

About the Society for General Microbiology

'to advance the art and science of microbiology'

The Society for General Microbiology (SGM) was founded in 1944/1945 and is now the largest microbiological society in Europe. It has over 4500 individual members of whom 75% are resident in the UK. The remainder are located in more than 60 countries throughout the world. Almost all full members are qualified to doctoral or higher level; there are 1000 postgraduate student members. More than 500 schools and a number of companies are corporate members.

The Society provides a common meeting ground for scientists working in academic centres and in a number of fields with applications in microbiology (medicine, dentistry, veterinary medicine, pharmaceuticals, numerous industries, agriculture, food and beverages, the environment and education). The majority of Society members are employees of universities, research institutes, health services, government agencies and small to multinational companies.

The science of microbiology covers a great diversity of life forms: disease-related molecular structures such as prions and viruses, archaea, bacteria, fungi, protozoa and algae. Microbes are of crucial importance in a number of processes affecting all life on Earth: the cause and control of disease, fertility of soils and aquatic environments, fermentation, biodegradation of waste materials and dead biomass, bioprocessing steps in drug and antibiotic production, and molecular biotechnology.

The Society's objective is to advance the art and science of microbiology. It does this by:

- Organizing regular scientific meetings at centres throughout the UK and abroad, where microbiologists meet to hear and discuss the latest research findings. The largest meetings last 4 days and involve up to 1400 participants.
- Publishing four major international learned journals: *Microbiology*, *Journal of General Virology*, *Journal of Medical Microbiology* and *International Journal of Systematic and Evolutionary Microbiology*. The journals are available on-line through HighWire Press (http://www.sgmjournals.org).
- Representing the science and profession of microbiology to government and the media. The Society is represented on a number of biological and biomedical committees and organizations, in the UK and internationally, thereby exerting influence on science policy and education, regulatory affairs and international collaboration.
- Promoting microbiology as a career for young people, by increasing awareness of microbiology in schools and aiding the development of teaching resources. The Society also provides grants for young scientists to attend scientific meetings and training courses.

- Keeping members informed of current developments in professional and scientific matters in microbiology, through publication of the magazine *Microbiology Today* and other means.

The Society is a Charity registered in England and Wales (No. 264017) and in Scotland (No SC039250), and a Company Limited by Guarantee, registered in England (No. 1039582). It is governed by a Council drawn and elected from the membership. The Society employs a staff of 30 at its headquarters.

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Appendix 4
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