Bovine TB: Second Report of the Independent Scientific Group on Cattle TB

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Chairman's Introduction

1.0.1 It is now over a year since the Independent Scientific Group (ISG) published its first report "Towards a sustainable policy to control TB in cattle". Many challenges have been faced and much has been achieved since then. This report provides an update on progress (up to and including 30 November 1999), sets out the processes we and the Ministry of Agriculture, Fisheries and Food (MAFF) have gone through to develop and implement the programme recommended, and outlines key issues for the future.

1.0.2 From the outset, the ISG, encouraged by Ministers, has seen the logic of taking a holistic view in the search for a sustainable base to underpin future cattle TB control policies. An over-arching objective of our work is to control TB in such a way as to allow the harmonious co-existence of cattle and wildlife, specifically badgers, since Ministers have made clear that the elimination of badgers is not an option for future policy. We recognise the need to understand the epidemiology of TB in cattle and wildlife better, and the programme of research we have recommended is designed to achieve this. There is a widespread misconception that the research strategy of the Group is concerned only with the field trial. Our first report made clear that a combination of research measures was essential if we were to understand more clearly the epidemiology of the disease in all the species implicated and develop sustainable control policies.

1.0.3 The Group has therefore developed a wide-ranging epidemiological investigation into TB in both cattle and wildlife, of which the randomised field trial - a central plank of the Krebs proposals - is just one part. The approach of the ISG has been to identify the major questions that need to be addressed and how answers can best be obtained. Major questions relate to factors influencing the prevalence and persistence of the disease in cattle and wildlife, risk factors contributing to the development of the disease in cattle, transmission routes between and within species, the use of effective diagnostic techniques and the effectiveness and economic value of potential control options.

1.0.4 Given the lack of investigative tools for studying the epidemiology of TB in wildlife, the field trial is currently the only way we can collect much of the essential epidemiological information and relate the underlying pattern of TB infection within the wildlife population to the incidence of TB in cattle. It is also

the only way of quantifying the contribution of wildlife to cattle TB and of determining if culling badgers is an effective method of controlling TB in cattle. It is essential to find an effective and acceptable way to control cattle TB in the long term. Failure to complete our work successfully could leave the whole issue in limbo for a further extensive period.

1.0.5 An extensive package of research has been put in place by the Government on our advice. Many of its components are interlinked, and need to be run in parallel with the field trial. In addition to the studies mentioned above, work is ongoing to develop new technologies to improve disease diagnosis in cattle and wildlife and to develop effective vaccines, to track the movement of tubercle bacilli between and within species more accurately and to gain more information on the ecology of wildlife hosts.

1.0.6 An important component of the research is a comprehensive risk assessment. A great deal of attention has been paid to the design and the implementation of a new TB risk factor questionnaire, known as TB99, which, along with other studies, will dovetail with the field trial and enhance the data that are yielded to answer important questions.

1.0.7 Existing TB data, collected in the past primarily to aid on-farm management of incidents, will be evaluated and analysed to provide additional information, particularly on risk factors and the effectiveness of diagnosis.

1.0.8 Factors influencing the persistence of TB in cattle herds and the contribution of cattle-to-cattle transmission to the disease need to be better understood. We are advising in this report that additional work be put in place as a matter of priority to better understand the pathogenesis of TB in cattle. Key questions are: how quickly does the disease develop in cattle, at what stage in the disease process is transmission to other animals likely to occur, how early in the disease process can accurate diagnosis be made, and how effective is the tuberculin skin test in situations of increased disease risk?

1.0.9 It is to be expected that a considerable amount of time and energy has been devoted to the randomised field trial given its scale and complexity. We are committed to the approach which is based on three treatments (proactive culling of badgers, reactive culling after a TB breakdown and no culling at all) applied to discrete areas assembled into “triplets”. Progress with this aspect of our work has been considerable, although not as rapid as initially hoped. Operating procedures have been devised and tested, new staff recruited and

trained, and auditors appointed. Six triplets have been identified; work has begun in all of them, though badger culling has so far only been undertaken in two.

1.0.10 It is disappointing and frustrating that circumstances have limited the number of triplets put in place. But, as the Government made clear in its response to the recent Agriculture Select Committee report, there have been unrealistic expectations about the rate at which our programme could be implemented, and we have maintained that it is vital to ensure all elements of the trial and the related research programme are carefully thought-through before initiating any action in the field. It would not have been practically possible to put all ten triplets in place in a short space of time. Nevertheless, we will identify all, and expect to have implemented the majority of, the remaining triplets by the end of 2000.

1.0.11 Difficulties with the course of the trial have undoubtedly served to fuel the fires of the pro- and anti- badger critics and this encourages interference with the work. At one extreme are those who are convinced that badgers are the prime source of TB in cattle and that the only acceptable and effective way to control cattle TB is to eliminate badgers from large tracts of the countryside. At the other are those who believe that the badger is the innocent victim, that culling badgers achieves nothing under any circumstances and should not be countenanced, and that the answers lie in improved cattle disease management and husbandry. Both of these views are based on presumptions coupled with selective interpretation of limited, and often conflicting, available data. We simply do not know enough about the dynamics of the disease at present to eliminate lines of enquiry. The ISG has consciously sought to stand back and take an objective view of the TB problem, and identify, through the best available science, a path to resolving the complex problems posed by bovine TB. If a lasting answer to those problems is the goal - as it must be - the outcome of our work should not be prejudged and our epidemiological investigation, including the trial, must be allowed to run its course without interference. All elements must be pursued; an incomplete picture will benefit no-one.

1.0.12 The ISG and MAFF have taken this message to various organisations and individuals over the last sixteen months, and of course to the Agriculture Select Committee, but it is clear we must extend our efforts in the future. We look forward to working with the Ministry in their continued efforts at improving

public understanding of the problem and the Government's strategy for addressing it. For the year ahead more generally we hope to see more research work initiated, as well as substantial progress in the enrolment of new trial areas. The fruits of some retrospective analyses of existing TB data should become available, and we aim to undertake an initial analysis of the information accumulated through TB99. Outside our immediate sphere we welcome the creation of the new TB Forum to address the continuing rise in TB incidence in cattle, and we will be playing our part in that initiative.

1.0.13 While the ISG is convinced of the need to maintain its independence, and has not been afraid to assert this, we have necessarily worked in close partnership with MAFF across the broad range of our activities. I owe gratitude to Ministers and MAFF senior management for their support and vocal endorsement. The ISG support team has made a major contribution to our work and especially the advisors from the Central Science Laboratory (CSL), the Veterinary Laboratories Agency (VLA) and the Chief Scientist's Group. The skills and professionalism of the MAFF Wildlife Unit, tasked with translating our designs into the field, continue to impress - their teams do an excellent job in very difficult circumstances. We are grateful to the Veterinary Field Service for piloting and implementing TB99 and to the VLA Investigation Centres for their support and constructive inputs. My continued appreciation goes to members of the ISG who give patiently and generously of their time in pursuit of a difficult goal, remaining committed to the approach that we are taking.

1.0.14 Lastly, a word about the layout of this report. We have moved away from the format used in our first publication towards the one we adopted for our response to the Agriculture Select Committee. We identify the major questions relating to the epidemiology and pathogenesis of TB in cattle and wildlife, and then comment on how these can be best answered. Other issues relating to development of future policy options are highlighted and we move on to describe progress being made, problems that have been encountered and lessons learned. The extensive appendices add to the information provided in the report. It is certainly my hope that the report is accessible and readable, and that it gets across the breadth and depth of our work.

John Bourne December 1999

2 - Understanding the Epidemiology of Cattle TB

2.0.1 Bovine tuberculosis (caused by the organism *Mycobacterium bovis*, abbreviated *M. bovis*) is a disease which poses a potential human health risk. It causes suffering to cattle, thousands of which are being slaughtered each year (over 6,000 in 1998), and both financial hardship and emotional distress to the farmers whose herds are affected. Despite 25 years of attempted TB control involving the removal of badgers, incidence of the disease is increasing and a new concerted approach is required. Little is known about the dynamics of the disease. In particular, there are no quantitative data to show the contribution badgers make to TB in cattle, nor whether badger culling is effective in controlling cattle TB.

2.0.2 Over the course of the year we have developed our assessment of the Krebs recommendations. We continue to align ourselves with his findings but have recommended an extended and strategically broad-based and interactive programme of research directed towards understanding the epidemiology of TB in cattle and wildlife, including consideration of factors related to cattle-to-cattle transmission which may be at play in influencing the control of the disease.

2.0.3 We emphasise that there is probably no single solution to the problem of TB in cattle. For future Government policy to have a firm scientific base, a comprehensive and objective approach must be made not only to obtain definitive information on the quantitative contribution of cattle, badgers and other wildlife species to the incidence of TB in cattle, but also to understand how the TB bacillus spreads within and between cattle, badgers and other wildlife populations.

2.0.4 Notwithstanding the long history of TB in cattle and its management, there are insufficient data available to assess the quantitative significance of factors such as husbandry, farming techniques, and exposure to reservoirs of infection in wildlife on the development and maintenance of TB in affected cattle herds. We also need to determine disease prevalence and infectivity in badgers and other wildlife and factors which influence the maintenance of infection in wildlife populations.

2.0.5 In short, we need to understand the epidemiology of TB in cattle and wildlife better. This is essential if the Government is to achieve the objective of

controlling cattle TB while ensuring the co-existence of TB-free cattle with wildlife.

2.1 Questions to be addressed

2.1.1 In light of the above, we have reviewed the questions set out in paragraphs 1.6 to 1.8 of our original report, and have redefined and expanded them.

2.1.2 We conclude that formulation and evaluation of policy options demands basic information that will provide answers to the following key questions:-

A. What are the origins of infection? What proportion of TB cases in cattle is caused by:

- other cattle
- badgers
- other wildlife species.

B. What risk factors predispose cattle herds to TB outbreaks?

Various factors have been proposed as contributors to herd incidents, but no rigorous analysis has ever been carried out to identify and then assess the quantitative significance of these factors.

Such risk factors might include:

- farm husbandry (eg. livestock management, nutrition and health programmes, stocking density, source and purchases of stock, land management and cropping practises).
- TB incidents in nearby herds
- climate
- geographical features
- exposure to infection in badgers and other wildlife
- different strains of *M. bovis*.

C. What is the pattern and epidemiology of TB infection in badgers and other wildlife?

The distribution of TB infection in badgers and other wildlife at both national and local level is not known, and nor are the factors that influence the

maintenance of infection within species and how and why this may vary between sites.

Factors affecting TB in badgers and other wildlife might include:

- abundance and social group structure
- population dynamics
- prevalence of infection
- severity of disease
- different strains of *M. bovis*.

D. What are the possible and most probable routes of transmission of infection to, within and between cattle herds - urine, faeces, respiratory discharge?

2.2 Addressing the questions

2.2.1 The Group recognises that the only way it can advise Ministers on the development of a science base capable of underpinning a sustainable long-term TB control policy is on the basis of a multi-faceted investigation. We believe this must include:

i. a questionnaire based epidemiological survey
ii. the field trial
iii. studies into cattle pathogenesis
iv. a road traffic accident survey
v. evaluation and analysis of existing TB data, and
vi. other related research.

2.2.2 The majority of these elements are now in place, including programmes aimed at wildlife ecology, ecological consequences of badger removal, estimation of badger population density and social group structure, vaccine development and molecular epidemiology. They are all interlinked and need to be pursued in parallel.

3 Questionnaire-based Epidemiological Survey (TB99)

3.0.1 It is our view that an objective and comprehensive assessment of the factors which predispose herds to a TB breakdown and their relative

importance can best be achieved by surveying a large number of cattle farms using a structured questionnaire to collect carefully specified data which are then subjected to appropriate analysis. We have considered the value of other approaches (for example, on-farm experiments) to provide useful complementary data but have dismissed them at this stage since risk factors and their contributions remain so uncertain, and because of the difficulty of controlling the many possible variables involved.

3.1 The development of TB99

3.1.1 For many years MAFF has collected information from all farms experiencing a TB breakdown, using a structured form referred to as TB49. This was designed solely to aid incident management and not to gather epidemiological data. However, the form attracted criticism because it was used in an attempt to ascribe the cause of the TB outbreak, based on a subjective assessment.

3.1.2 Professor Krebs proposed that the TB49 form be re-designed. We strongly endorsed this recommendation, and a working group was established at the outset to develop a questionnaire which would secure data on a range of potential risk factors. In parallel, a research assistant was appointed to work with the Veterinary Laboratories Agency on the design of a database to manage the information to be collected.

3.1.3 In designing the new questionnaire, designated TB99, the working group was guided by a number of key, although sometimes competing, principles:

a. the need for objectivity
b. the need to be comprehensive
c. the need to provide data amenable to statistical analysis
d. the need for practicability.

3.1.4 Applying these principles required extensive consultation with research workers in various fields, farming and conservation interests, and others in this country and overseas. Designing a questionnaire that was sufficiently comprehensive but which was amenable to analysis and would not over-burden farmers proved challenging. As part of the development process, MAFF staff piloted a draft version of TB99 on-farm at the end of 1998. In parallel MAFF consulted publicly on the version being piloted. Both actions proved extremely valuable and the feedback was largely positive. The working

group took full account of the pilot exercise and of the views of respondents to the consultation in further refining the questionnaire. MAFF has implemented a national training programme for staff administering the final form to maximise objectivity.

3.1.5 On each farm to which it is applied TB99 collects data on a wide range of potential risks factors relating to the 12 month period prior to the herd breakdown. These include cattle herd composition and health, cattle movements, the type of farm enterprise (including land type/use, soil type, presence of other domestic species), husbandry factors (such as grazing practices, fertiliser use, effluent management, water sources, housing/bedding arrangements, supplementary feeding practices, steps taken to avoid contact between cattle and wildlife) and the presence of potential wildlife sources of TB infection. These data will be analysed to identify and rank the factors which, individually and in combination, appear to predispose farms to TB breakdowns (see Section 8.1).

3.1.6 TB99 was seen by some as too detailed, and by others, as too limited in scope. We feel that, at this stage, we have got the balance about right. TB99 meets the key objective of scanning as wide as practicable and reasonable a range of factors which could influence TB in cattle. Failure to meet this requirement would have risked missing key factors by prejudging their relevance. At the same time, because of the need to be concise and user-friendly, the Group accepts that more detailed studies may be necessary in the future if the analyses of TB99 identifies factors that show significant associations with risk of TB in cattle.

3.2 Application of the questionnaire

3.2.1 TB99 was formally launched by MAFF in April 1999, following a brief second pilot phase. The questionnaire is completed by trained MAFF State Veterinary Service staff through one or more personal interviews with the farmers concerned. At the time of going to press over 500 questionnaires had been completed.

3.2.2. The questionnaire is now being used on all farms in Great Britain (within and outside trial areas) on which a confirmed TB breakdown occurs and is also being applied retrospectively to all breakdowns which occurred since the beginning of 1999. In trial areas, every breakdown (whether confirmed or not) triggers a TB99 survey. There are two slightly different

versions of the TB99 questionnaire. One, ‘the case form’, is used on the breakdown farm. In addition, in trial areas, for each such breakdown a ‘control form’ is applied to each of three comparable herds (including, where possible, one contiguous herd) from within the same trial area. The control form collects information on comparable farms (over a comparable time-period) where no outbreak has occurred and is identical to the ‘case’ form except that it omits questions included for incident management purposes. On-line proof copies of both the case and control forms are available on MAFF’s TB Website (www.maff.gov.uk/tb/krebs/c9_pdf.htm). The selection of control farms has been limited to trial areas because, at this time, these are the only areas for which detailed badger epidemiological data will be available.

3.2.3 We are grateful to all those farmers who have given valuable time and provided detailed information to help us with this work. Our thanks go also to the State Veterinary Service staff who have been involved in administering the form, and have provided essential feedback.

3.3 Reviewing progress

3.3.1 The Group recognises that the questionnaire is a substantial document and understands concern about its complexity. Given its importance to our objectives, we plan to subject TB99 to a thorough review programme, both in the field and as data are audited and entered into the database, and, with MAFF, to continue to monitor the implementation of the new form. Reports from MAFF on the early experiences suggest the questionnaire has been generally well received. Preliminary analysis will help evaluate the robustness of data and identify changes which could improve its quality and coverage.

3.4 Complementary studies

3.4.1 TB99 is being complemented by other studies into possible risk factors. The Veterinary Laboratories Agency, the Royal Veterinary College and the Universities of Cambridge and Warwick are each undertaking their own multifactorial analyses of TB data.

3.4.2 The Group has considered the case for complementing TB99 with the introduction of an additional ‘five year’ questionnaire (either as a stand-alone questionnaire or as an adjunct to TB99). The proposed five year questionnaire was to be applied on a sample of farms located in trial areas with a view to analysing the differences between management practices on those farms in

the previous 12 months compared with a similar 12 month period five years earlier and, where significant differences were detected, assessing whether these could be associated with an increase or reduction in the risk of a TB outbreak. However, after careful thought we have concluded that the resource implications involved in obtaining a sufficiently large sized sample, taken together with concerns about the reliability of historical data, outweighed the potential benefits. We are nevertheless considering further the desirability and viability of a straightforward questionnaire which would be completed by farmers on enrolment into the trial, so that potentially valuable data are not lost.

4 The Field Trial

4.0.1 The field trial is the most contentious component of our work. It is, however, essential to future disease control policies that we obtain accurate data on the prevalence of TB in badgers and are able to relate the underlying pattern of TB infection within badger populations to the incidence of TB in cattle. It is also necessary to study the distribution of TB in badger populations, and how this is affected by variables such as population density, social group size and structure, disposition of badger territories, and past badger removal operations. Unfortunately there is at present no reliable diagnostic test for TB infection in badgers that can be used in the live animal, and accurate diagnosis can therefore only be made at post mortem. Only by carrying out the field trial can essential epidemiological data be obtained, and, critically, the field trial is the only means by which we can answer two key questions: (a) what is the quantitative contribution of badgers to TB in cattle; and (b) is culling effective in controlling the disease in cattle, and if so, in what circumstances?

4.0.2 The trial will evaluate the effects of badger culling on the incidence of TB in cattle by comparing three treatments applied in trial areas enrolled as 'triplets':- proactive culling (initial removal of as many badgers as feasible consistent with welfare constraints and thereafter maintaining numbers at a low level); reactive culling (removal of badger social groups with access to a farm in response to an outbreak of TB on that farm); and survey only (no culling).

4.0.3 If badgers cause a substantial proportion of herd breakdowns in the trial areas, then the areas subjected to the proactive strategy should be the first to

show reduced TB incidence in cattle. The reactive strategy is designed to determine the effect on TB incidence in cattle of removing badgers located around TB breakdown farms, which are more likely to be infected with *M. bovis*, while leaving other social groups relatively undisturbed. The survey-only areas are also vital to the trial as they establish the effects of no culling and allow comparison with the two culling strategies.

4.0.4 The trial is one of the largest scientific field exercises ever undertaken and hence has needed meticulous planning and an ordered approach to maximise its effectiveness. In addition to designing the trial to generate the rigorous scientific data needed, and constructing the intellectual framework within which those data will be analysed, our approach has involved careful consideration and review of operational factors, and of the welfare, ethical and environmental implications of the work.

4.1 Trial design

4.1.1 The design of the trial required the specification of the method of treatment allocation, the number of farms to be enrolled and the timescale of the trial.

Treatment allocation

4.1.2 In theory, the three strategies could be allocated individually to farms enrolled in the trial, but many badger social groups may have territories overlapping more than one farm and, thus, potentially be allocated to more than one treatment. To avoid this and, furthermore, to reduce the interference between different strategies, all of the farms in relatively large trial areas (roughly 100 km^2) are assigned the same treatment.

4.1.3 Trial areas are grouped into triplets (with each of the three treatments being allocated to one area within each triplet). As far as possible the similarity of trial areas within a triplet is maximised, but, as explained in our first report, precise matching is not always feasible in practice; nor do we consider it essential.

4.1.4 Treatments are assigned randomly to each triplet to avoid any biases that might lead to inherent differences between the three areas. The randomisation procedure is conducted at the latest possible stage so that neither the level of consent given by occupiers, nor the determination of area

boundaries, nor the intensity of work across the areas concerned, are influenced in any way.

Statistical power

4.1.5 An important feature of the planning of the trial was to ensure that the size of the trial was appropriate for its intended purpose. The statistical power calculations for the trial, originally presented in the Krebs report and adopted by the ISG, were based on the simple but reasonable assumption that the variability of numbers of observed cattle TB breakdowns is essentially that found in the Poisson distribution, the statistical distribution governing the count of events occurring totally at random.

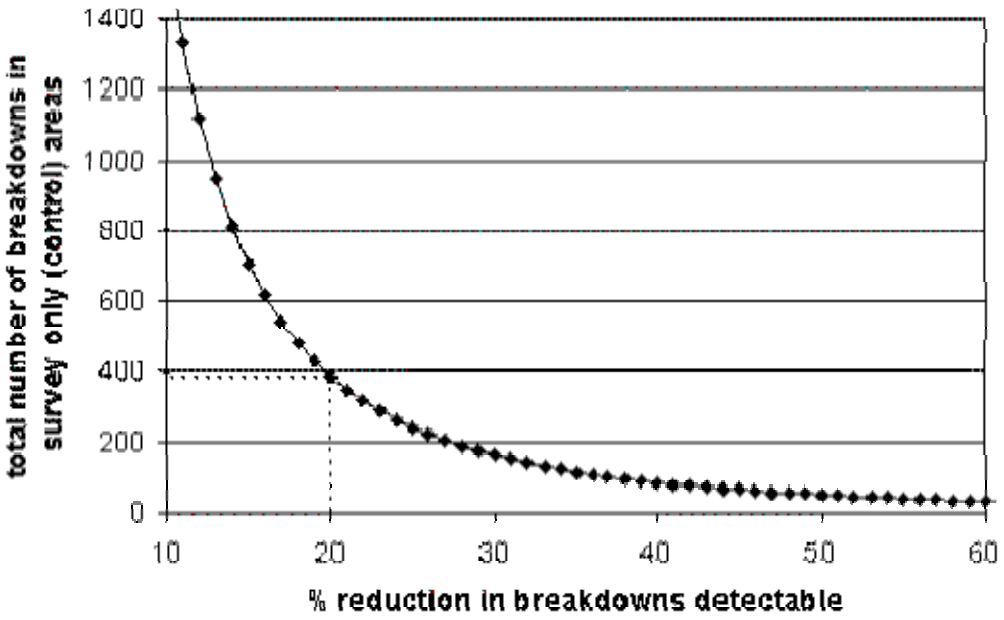
4.1.6 Based on the historical incidence of TB in cattle across Great Britain between 1992 and 1996 inclusive, the Krebs report recommended that a minimum of 30 100km^2 areas should be included in the trial. It also illustrated that the expansion of the total trial area beyond 3000 km^2 would yield diminishing returns in terms of the number of repeat and contiguous breakdowns that would be covered.

4.1.7 The statistical power is the probability of detecting a reduction in incidence of TB in cattle, if it exists. It depends primarily on the total number of TB breakdowns (i.e. the cumulative incidence) in the survey-only (control) areas and the percentage reduction in the breakdown rate in the treatment (proactive and reactive) areas. It was suggested in the Krebs report that if all 10 triplets were implemented immediately, and incidence of TB in cattle remained at the level observed over the previous five years, that a reduction as low as 20% in the trial areas subject to reactive culling (deemed likely to be the less effective of the two badger removal treatments) should be detectable within five years. For logistical reasons, it was not possible to implement all the triplets simultaneously. Based on the original calculations of the Krebs team this would have the effect of extending the timeframe necessary for the trial to deliver the "target" referred to above. However, the disease situation since Professor Krebs reported has not been static.

4.1.8 Figure 4a below illustrates the total number of breakdowns required for the trial to have a 90% probability of detecting a given percentage reduction. In trial design terms it is accepted that 100% probability is extremely difficult to achieve, if not impossible. The minimum probability for scientific trials is generally accepted to be 80%. With 90% probability, the statistical power for

the trial exceeds this. Working from the Krebs reference to an average 20% reduction in TB incidence, 385 breakdowns in the survey-only areas would be required to yield a 90% chance of detecting such an effect on the incidence rate of TB in cattle.

Figure 4a



4.1.9 To translate the total number of breakdowns into a timetable for the trial requires the consideration of 'triplet years'. Each triplet year represents one year's observation in a triplet once initial proactive culling is complete. (On the Krebs team's assumptions, 50 triplet years would have accumulated by the end of five years - 10 triplets implemented simultaneously, multiplied by five years' observation). Table 4b maps out the implementation timetable for the trial and projects the accumulation of triplet years over the calendar years ahead.

Table 4b

Calendar year	1998	1999	2000	2001	2002	2003	2004	2005
Triplets subject to initial proactive culling	1	1	5	3				

Triplet years accrued in calendar year		1	2	7	10	10	10	10
Total triplet years accrued by calendar year end		1	3	10	20	30	40	50

[Due to rounding, triplet years have been slightly underestimated.]

4.1.10 To calculate when a given reduction in the incidence rate of TB in cattle might be detectable, the total number of TB breakdowns required is divided by the average breakdown rates per survey-only area per year. This results in the number of triplet years necessary. Thus, if the average breakdown rate per survey-only area were 8 breakdowns per area per year, then 48 triplet years (= 385/8) would be required to yield a 90% chance of detecting a 20% reduction in the incidence rate of TB in cattle. From table 4b it can be seen that 48 triplet years should have accrued by the end of 2005.

4.1.11 Higher TB incidence in the trial areas (but the same ratio of incidence rates between treatments) would reduce the number of triplet years required to detect a difference. For comparison, if the average breakdown rate per survey-only area were 12 breakdowns per area per year, then 32 triplet years (= 385/12) would be required to yield a 90% chance of detecting a 20% reduction in the incidence rate of TB in cattle. 32 triplet years should have accumulated by early 2004. The tables at Appendix B show the three year and one year TB incidence figures on the basis of which Triplets B (Devon/Cornwall) and C (East Cornwall) were selected.

4.1.12 Obviously, if a consistent statistically significant effect of culling were to be observed earlier than either of these points, valid results could be obtained sooner than originally suggested. However, in order to evaluate the consistency of the ratios of breakdown rates between triplets, the minimum coverage necessary would be for all 10 triplets to have been observed for at least a year, and preferably two (to allow for cattle testing timetables, and any

lag in the impact of culling). From Table 4b, it can be seen that on the projected timeline for the trial, the earliest this could occur would be the end of 2002.

Assumptions

4.1.13 The assumption that TB breakdowns occur totally at random will tend to underestimate the variability to be encountered in practice. For example, there is some evidence of clustering of TB breakdowns in space and time, though this is likely in part to be an artefact of the testing regimes used. The final analysis of variation will be based on the observed consistency of the ratios of breakdown rates (for example, the ratio within a triplet of the TB incidence in the proactive area divided by the TB incidence in the survey-only area) between triplets, adjusted for herd and cattle numbers and possibly other features. The amount of variability encountered will only become apparent as the trial progresses.

4.1.14 While 10 is the minimum number of triplets advisable for effective error control, the viability of the trial does not, as such, depend on the validity of the original power calculations. These were formulated in terms of detecting the presence of a 20% or greater reduction in breakdown rates and in terms of specific assumptions about the variability to be analysed. An equivalent and equally satisfactory formulation, would be the estimation of the ratio of breakdown rates with appropriate confidence limits.

4.1.15 Non-compliance with the trial through interference with culling operations, denial of access for survey or culling teams (particularly in the proactive and reactive areas) and illegal killing of badgers (especially in the survey-only area) would all reduce the differences between treatment areas. Depending on the circumstances, such factors could serve to mask the true effect of culling treatments. The statistical power of the trial is sufficient to deal with non-compliance of all these types, but if significant problems were experienced the duration of the trial might have to be extended. This would be in no-one's interests: it would delay the accumulation of the scientific data required to inform policy, and could result in more badgers having to be culled than would otherwise have needed to be the case.

4.2 Welfare and ethical issues

4.2.1 In designing and implementing the trial we have given as much weight as possible to animal welfare considerations.

Capture methods

4.2.2 We remain committed to the use of humane methods to capture badgers. Cage traps, designed specifically for the capture of badgers, are a widely accepted form of capture and allow easy identification and release of non-target animals. On our recommendation, preliminary trials have been carried out to assess alternative capture methods, such as padded leg-cuffs, but these investigations are not at a stage which would lead us to contemplate their use in the trial.

4.2.3 Standard operating procedures stipulate that traps should be set as late in the day, and then checked as early in the day, as possible, to minimise the period in which trapped badgers are held in captivity, and to limit any non-target capture. Trap visit times are kept under continual review.

4.2.4 Procedures have been formulated for humane despatch of trapped badgers by shooting, and checks have been established to ensure that instant death has occurred. A record is kept of any injuries observed during post mortem examinations. Non-target species are released except on the rare occasions when they are severely injured, in which case they are despatched humanely.

4.2.5 Humane capture and despatch operations have been subject to internal checks and external audit by an independent expert on animal welfare. The auditor's final report will be published.

The closed season

4.2.6 As described in our first report, in designing the trial we moved away from Professor Krebs's original recommendation and adopted a closed season to minimise the risk of capturing females with dependent cubs below ground.

4.2.7 The closed season (1 February - 30 April) was selected on the basis of the experience that, once cubs are old enough to appear above ground regularly (usually by late April), they can be easily captured and despatched humanely. Changes to working practices for reactive culling operations have

led to the suspension of trapping over weekends (whereas proactive operations require continuous capture over a set period). For the months of May and June, when cubs might still be expected to be dependent, this raised the possibility that cubs could be left unattended if their mothers were captured late in the first week of trapping. We therefore recommended that for those particular months reactive operations should include continuous trapping over weekends.

Ethical issues

4.2.8 The ISG has considered the ethical questions posed by the field trial, and principally whether the culling of a protected wildlife species can be justified either for scientific purposes or for the benefit of another (non-protected) animal. We explored these issues at length in drawing up our first report, and have kept them under review since, discussing them with key individuals, including the independent welfare auditor.

4.2.9 The broad-based Government decision to proceed with the trial was based on a lengthy public consultation and, crucially, on the ISG's design for the trial which incorporated the extensive welfare provisions referred to above. The trial is aimed at producing conclusive results as quickly and efficiently as possible and we are satisfied that it withstands an ethical examination.

4.2.10 Until the trial has been completed it is impossible to say how many badgers will be killed. Using the assumption that average badger density is around 5/km^2 the Krebs report suggested that some 12,500 might be taken during a five-year trial. However, experience from culling operations to date suggests the Krebs figure might prove an over-estimate. Trial culling operations will be conducted over less than 1% of Great Britain's surface area and no more than 4% of the South West land mass (specifically, the area covered by the State Veterinary Service's West Region). On this basis we consider that it will therefore not have a significant effect on the national badger population, estimated during the last national badger survey, co-ordinated by Bristol University, to be in the order of 310,000.

4.3 Challenges faced and lessons learned

4.3.1 In taking the trial forward we have continually assessed our approach and modified it where this has become necessary. Over the last 16 months

we have met a number of challenges, some of them foreseen, others unexpected. Most have been of a practical or logistical nature, and have often required some fine tuning of the prescribed operating procedures. One of the challenges, that of communicating our approach, is covered in Chapter 10. In a few cases a re-evaluation of procedures has been required. This section records some of the main obstacles we have encountered and the steps taken to address them.

Wildlife Unit (WLU) management issues

4.3.2 MAFF appreciated that the trial could not be undertaken without a marked increase in the number of staff working in the Ministry's Wildlife Unit. Accordingly, a recruitment exercise for 60 additional field staff was launched in December 1998. The aim was to have the new recruits trained and in post before the end of the following closed season (30 April 1999). This target slipped, mainly for logistical reasons, including the need for extended security checks on candidates and the level of detail required in the training programme. While recognising the problems involved, the Group regrets the consequential delay, which has itself caused some slippage in the overall trial timetable, and has recommended that the administrative aspects of appointment procedures are streamlined wherever possible. We welcome current plans to strengthen the WLU's management structure and administrative support services.

4.3.3 The sheer scale of the trial requires a heavy human resource input, and the flexibility and goodwill of staff are key factors to the success of the trial. In addition, the requirements of the Working Time Regulations 1998 have had to be taken into account in devising work plans, and, in part, necessitated our amending the trial programme to minimise where possible the need for weekend working. The logistics of staff working away from home for extended periods have had to be carefully planned and co-ordinated. We re-emphasise our gratitude to Wildlife Unit staff (and their families) for their forbearance, and for the energy, initiative and professionalism they have demonstrated in the field.

Terrain and weather

4.3.4 Recognised from the outset as factors which could not be managed, terrain and weather have significantly influenced progress on the ground. In particular, surveying work has often been hampered by unyielding geography

and inclement conditions. Initial forecasts of staff resources were based on surveying work carried out during the old badger removal operations, which tended almost exclusively to be farm-based, and did not require all related badger setts to be mapped out and classified. By contrast, trial operations take the WLU into all manners of terrain at all times of the year.

4.3.5 The recruitment of additional staff and their flexible deployment, along with some modification of operating procedures to maximise efficiency has helped to improve progress, and to ensure that surveying and trapping timetables have meshed.

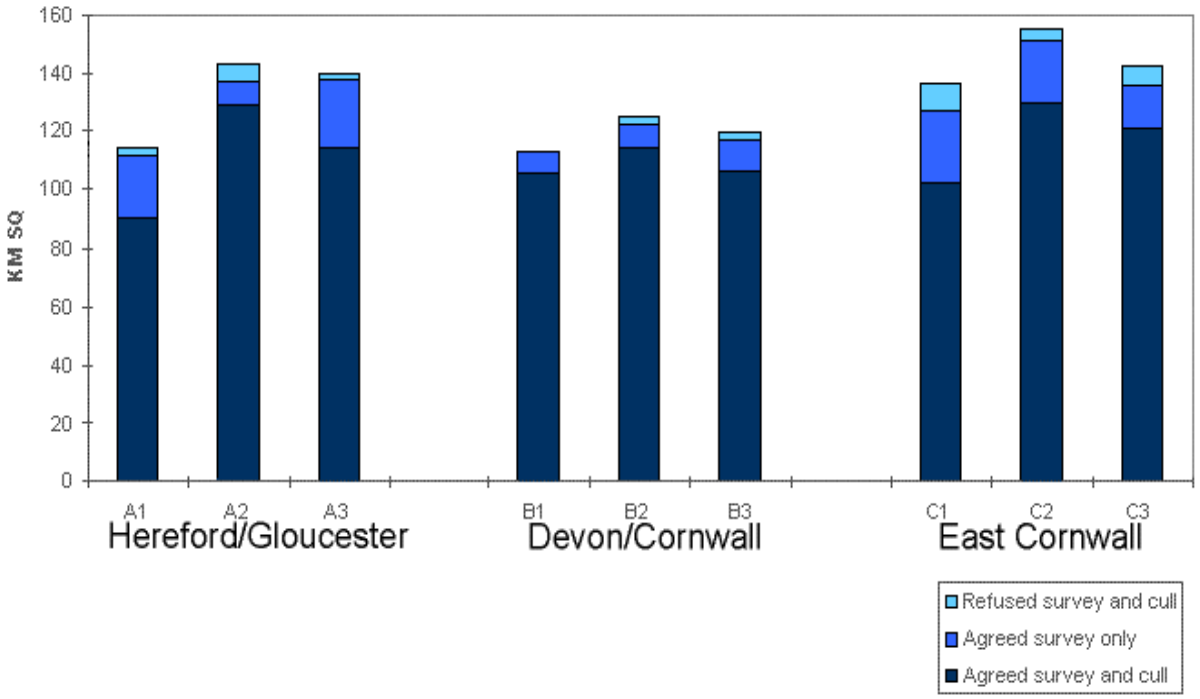
Participation in the trial

4.3.6 Maximising participation in the trial, which is entirely voluntary, depends in part on the effectiveness with which the aims are communicated to occupiers of land in trial areas. Great care has been taken to explain that the trial is essential to establishing a sustainable future cattle TB control policy, vital not only to the protection of public and animal health and the agricultural economy, but also to enable cattle and badgers to co-exist. Land occupiers are invited to give consent for two levels of trial operations on their land: surveying and culling. If, for whatever reason, occupiers are reluctant or feel unable to give permission for badger culling, they are asked if they would be prepared to allow surveying only to take place.

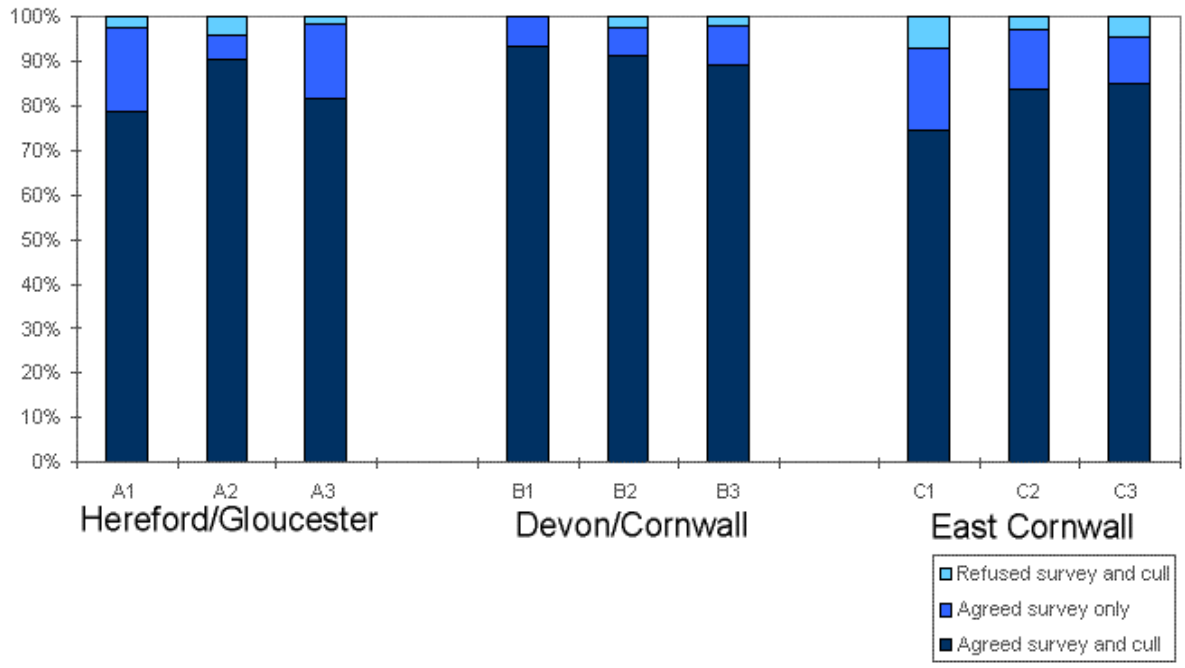
4.3.7 The response to date from land occupiers has been very positive. By way of illustration, Figure 4c shows the levels of consent for the three triplets in which surveying has been completed. The graphs demonstrate convincingly that allegations that the trial has not been supported in rural areas are entirely unfounded.

Figure 4c

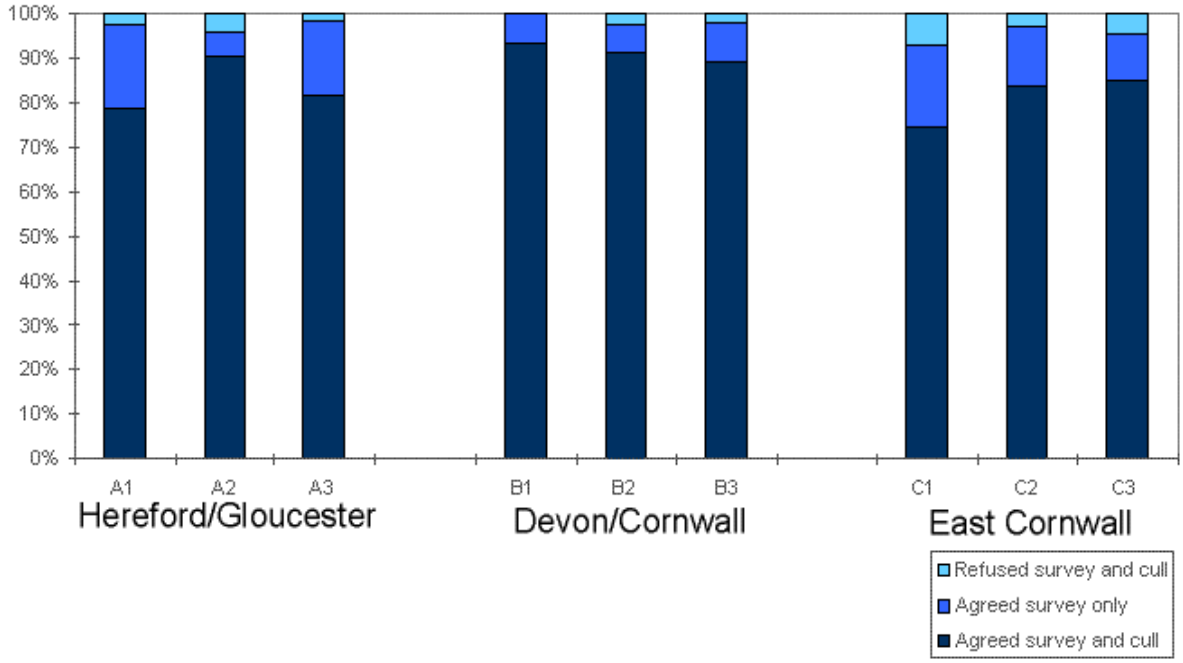
Level of co-operation - results of visits to trial areas and inner buffer zones (total available area for which permission for trial operations was sought



Level of co-operation - results of visits to trial areas and inner buffer zones (percentage of available area)



Level of co-operation - results of visits to trial areas and inner buffer zones (percentage of all occupiers visited)



Interference with the trial

4.3.8 We have always recognised that the culling of badgers would be highly emotive and that the trial was likely, throughout its duration, to give rise to opposition and be subject to interference in a variety of forms.

4.3.9 Interference has had a number of effects. The obstruction of staff and a limited amount of damage to equipment required work in some trial areas to be rescheduled, with some consequent delays. In other cases working patterns had to be adjusted.

4.3.10 Initially, it was MAFF's policy to publicise, as early as possible, details of triplets and this was a laudable approach given the aims of open government. Maps of the first two triplets were made public. However, releasing triplet details gave a significant advantage to those seeking to intimidate Wildlife Unit staff and disrupt the trial. Consequently, on grounds of staff safety and landowner privacy only the most basic information about the location of triplets is now published before initial proactive culling in the areas concerned is complete. We have recommended that detailed maps are not made available until after the trial has finished.

4.3.11 The Group regards the health and safety of those involved in the trial as paramount and supports the risk assessment procedures followed by MAFF to protect its employees. In respect of field work a key factor has been

to obtain and act on advice both from MAFF's own security advisers and the police concerning the timing and location of operations and this will continue in planning future work.

4.3.12 Paragraph 4.1.15 explains the consequence of interference, both from protesters and from land occupiers who take unlawful action against badgers: in short, it will take longer for the trial to show its true effect. This will delay the introduction of a sustainable policy to control TB, and could well lead to more badgers having to be culled than would otherwise have been the case.

Health and Safety Executive prohibition on badger post mortem examinations

4.3.13 In August 1999 the Health and Safety Executive (HSE) served a notice on the Veterinary Laboratories Agency (VLA) prohibiting the post mortem examination of badgers from high TB risk areas until new safety facilities had been put in place. In the interim badgers could only be post mortemed in Category III safety cabinets - with a consequent significant reduction in throughput. Effectively, post mortem examinations of badgers captured in the trial were put on hold.

4.3.14 We had always envisaged that laboratory capacity within the VLA - the only organisation with the regional structure capable of meeting the needs of the trial - could become overstretched during peak periods of trapping operations. Accordingly we recognised that it might become necessary to freeze badger carcases for subsequent examination, and we recommended that various studies into the effects of freezing the organism *M. bovis*, in cattle and badger tissues, should be put in place. Some of this work is already complete; other aspects will follow. Any diagnostic correction factors developed can be applied retrospectively.

4.3.15 The VLA is on course to have the requisite new post mortem facilities installed by early 2000. Meanwhile, badger carcases from trial operations are being freeze stored, pending examination during the 2000 closed season. Although the HSE prohibition has been a frustration, its impact on trial operations is likely to be minimal.

4.4 Field trial procedures and processes

4.4.1 Standard operating procedures (SOPs) for all aspects of the trial and related work have been developed with MAFF; indeed, a major feature of our work has been to provide advice on procedures and monitor feedback to make further improvements. SOPs are kept under constant review and continue to evolve. Throughout the process we have attached importance to working closely with staff on the ground to witness procedures being applied. In this way ISG members have been better able to assess the need to make procedural changes to take account of existing expertise, practical issues and feedback from WLU staff.

4.4.2 Together with MAFF we have given an undertaking to publish a summary of the operating procedures. This summary is included at Appendix A of this report. Once again, in drawing up such a document we have had to balance our desire for openness with the need not to disclose details which could jeopardise staff safety or the further conduct of the trial.

4.4.3 A number of key areas of our work call for more detailed or comprehensive explanation, not least to demonstrate the rigour with which the trial is being carried out. The following sections deal with these.

4.5 Trial and treatment areas

4.5.1 The selection and development of trial and treatment areas is a detailed and often lengthy process involving various stages, each of which must be signed off by the ISG before the next one can begin. Figure 4d explains the process in visual form, using a fictitious area.

Identification of trial areas

4.5.2 For the effects of badger culling on TB incidence to be detected most clearly, the trial needs to take place in areas where the number of new TB outbreaks is likely to be the highest. Our analyses indicate that past history of herd breakdowns in a given area is the best available indicator of the risk of future ones. For this reason, potential trial areas, grouped into triplets, have been selected first and foremost on the basis of the number of herds which have had confirmed breakdowns over the most recent three year period for which records are complete, and with a particular focus on the disease situation in the immediately preceding year. This emphasis on up-to-date and

recent TB data is important, as it allows the trial to take account of new areas where the disease is emerging.

4.5.3 Using specifically designed software, point centres for possible trial areas are identified, and, from these, circular trial areas are developed. Once approved by the Group these candidate areas are tailored to fit geographical boundaries. Proposed trial areas, as they are then known, are submitted to the ISG for ratification. Full details of this selection process are set out in our first report.

4.5.4 Given the objective of locating trial areas in known TB hotspots, and the fact that MAFF's TB control policies in the last 25 years have also been focused on such hotspots, the Group recognises that many of the trial areas identified so far include areas which have been subject to badger removal operations in the past. These have undoubtedly had some effect in disturbing the local badger populations which we are assessing. It is our expectation, however, that by continuing our current triplet selection policy the trial will encompass areas where resident badger populations have not been the subject of culling operations by MAFF in the recent past.

Trial and treatment area boundaries

4.5.5 Following the triplet selection process, proposed trial areas are developed into finalised trial areas, with inner and outer buffer zones, within which MAFF will conduct operations. While the key characteristics within and between individual trial areas which comprise a triplet must be maintained, final adjustments are made to the trial area boundary to reflect, *inter alia*, the position of farm boundaries, rivers, and settlements on the fine scale maps used by MAFF's Wildlife Unit.

4.5.6 The boundaries of treatment areas (which are those in which trapping takes place in the proactive and reactive areas) can only be prescribed once putative badger social group territories have been delineated (see paragraph 4.6.5 below). This process effectively extends the operational areas into inner buffer zones, since badger social groups are not constrained by trial area boundaries. Any sett outside a trial area boundary which is associated with a social group of badgers whose territory falls within the trial area must be subject to the same treatment (proactive or reactive culling) once allocated.

4.5.7 Figure 4e explains the relationship between the trial area and its buffer zones, and the treatment area.

Figure 4d

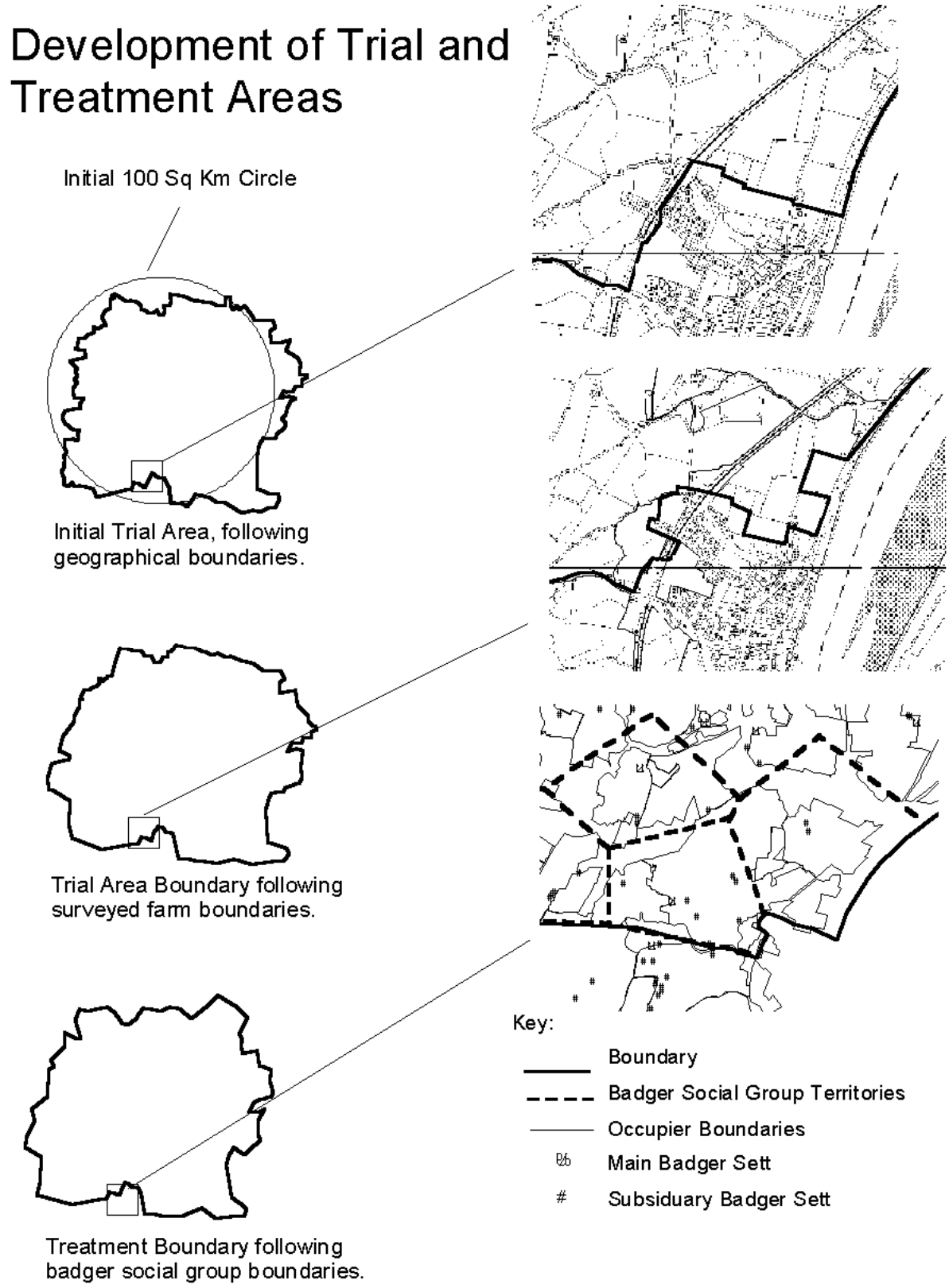
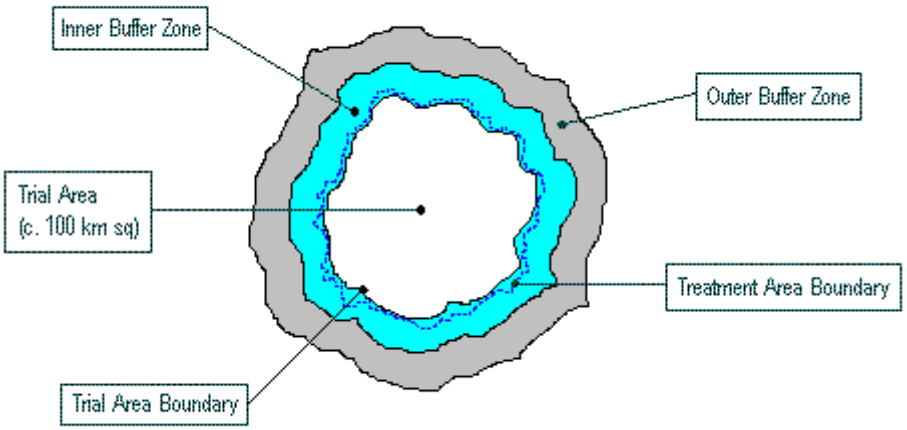


Figure 4e



4 The Field Trial

4.6 Surveying

4.6.1 The surveying of trial areas and buffer zones for badger activity represents a crucial part of the trial and a major commitment of resources. We place considerable importance on achieving as complete a picture as possible of badger activity and distribution in trial areas, irrespective of whether they will be the subject of culling operations. In addition, we stand to learn a great deal about badger population dynamics generally through this aspect of the work.

4.6.2 WLU staff carry out designated surveys and assist in carrying out internal audits of the classification of setts into 'major' and 'minor' within the territory of a badger social group. The area surveyed includes all available land in the trial area and inner buffer zone and that in the outer buffer zone necessary for the purposes of social group territory delineation (see below).

4.6.3 Following claims that large scale illegal killing of badgers was being carried out in trial areas, the ISG recommended the introduction of additional spot checks on badger activity and for signs of interference. To date these ad hoc, unannounced surveys have revealed no evidence of illegal interference of setts.

Territory delineation

4.6.4 Both the reactive and proactive culling treatments are designed to be applied to social groups of badgers. This objective depends upon allocating setts to social groups by delineating likely territories or home ranges from field signs. The design of a standard operating procedure that will allow WLU staff to meet this objective has demanded careful thought, experience with survey maps, and many discussions with WLU staff.

4.6.5 The allocation of setts to social groups is a three-stage process. A first attempt is made to locate possible territory borders by drawing Dirichlet tessellations around all identified main setts. Some setts may be re-classified as main or other setts, in consultation with survey teams, at this stage. In the second stage, hypothetical borders are modified according to locations of field signs (especially border latrines) and geographical features such as rivers and major roads. Finally, all setts falling within a notional territory are allocated one or more social groups with high, medium or low confidence, according to their proximity to main setts, territory borders, and unsurveyed areas.

4.6.6 Notional territories are used to define culling areas. All setts falling within a focal area (proactive trial area or breakdown land in a reactive area) are subjected to culling, as are any setts linked to them with high or medium confidence.

4.6.7 The process of allocating badger setts to social groups is kept under close review, and is subject to external audit. Data on badger genetics should also help to ascertain the accuracy of the delineations. (see paragraph 7.5.2).

4.7 Culling operations and post mortem investigations

Randomisation

4.7.1 Once surveying is complete the three treatments (proactive; reactive; survey-only) are randomly assigned to the areas within the triplet (see paragraph 4.1.4).

Treatments

4.7.2 In survey-only (no cull) areas, further action involves spot checks of badger activity and scheduled biennial re-surveys. Similar re-surveys take place in the other treatment areas.

4.7.3 In reactive areas, culling of badgers is triggered by confirmed incidents of TB in cattle. Only badgers from social groups with access to land inhabited and grazed by the herd(s) in question are trapped. Reactive operations are not activated until initial proactive culling is complete in the triplet, from which point triplet years (see paragraph 4.1.9) are also calculated.

4.7.4 In proactive areas, as many badgers as possible are removed, subject to the welfare constraints imposed by the trial's defined procedures. These areas are subject to regular follow-up culls to maintain numbers at a low level.

Trapping operations

4.7.5 Trapping is undertaken on a saturation basis, but with cage traps being sited only around badger setts which are active. Because, as is explained in Section [ ] there is at present no validated observational technique for estimating badger numbers, a working assumption has to be made about the badger population in a treatment area for the sole purpose of determining the number of traps to be deployed.

4.7.6 A period of pre-baiting of traps, the length of which will vary according to uptake, then takes place. The trapping phase itself will also vary according to circumstances. For initial proactive culls the default period is 12 nights of trapping, but the trial design requires that proactive culling should end before then if it is believed the maximum available number of badgers in a treatment area has been caught and before immigration from outside that area commences. To assess when immigration is likely to occur, procedures have been put in place to monitor and compare capture rates at the centre and the outer region of the treatment area.

4.7.7 The locations of cages are documented carefully to ensure that all are checked after they had been set to trap. As a further safeguard, the same teams are responsible for locating, setting and checking the same traps, wherever possible, to minimise the chance of any trap being overlooked. Signs of badger activity and other trapping details are recorded on a daily basis, as is evidence of interference; the latter information may be of benefit to the police, and can also be taken into account in the eventual statistical analysis of the effectiveness of the operation.

4.7.8 Throughout trapping operations welfare considerations are always to the fore (see Section 4.2). In no area is this more important than for the despatch

of animals captured. This is undertaken only by trained and qualified MAFF staff, in accordance with procedures which have been thoroughly reviewed throughout the year in close liaison with the internal and external auditors. Additional on-site checks were also introduced following media coverage of the Putford proactive cull to reassure animal welfare organisations and the public at large that procedures were being properly followed.

Heart blood sampling and submission of carcases for post mortem examination

4.7.9 Blood samples for research work into developing a reliable live test for TB in badgers are taken from badgers once they are dead. Samples are also taken for a serum bank which may be of future scientific value.

4.7.10 Carcases are submitted for post mortem examination on a blind basis, that is details of the location of capture are not provided to the laboratory undertaking the examination. This is partly to protect the confidentiality of the information, but also to ensure that the post mortem investigation is in no way influenced by the carcase's provenance.

Protocol for badger post mortems

4.7.11 Answers to critical questions on the prevalence of TB in badgers, its possible links with badger density, social group size and structure and particularly their relationship to TB incidence in cattle, depend on an accurate diagnosis of TB in badgers.

4.7.12 In contrast to the situation with cattle, there is no live test for TB in badgers. Diagnosis can currently only be achieved by carrying out a full post mortem examination.

4.7.13 The ISG has recommended that every badger culled during the course of the trial is subjected to a rigorous post mortem procedure. Diagnosis of TB is based on a number of criteria, which include morbid pathological assessment of disease state of lymph nodes and viscera, demonstration by histological staining of acid fast bacilli, and laboratory culture of selected lymphoid tissues and visceral and skin lesions.

4.7.14 All infected badgers are potentially capable of excreting tubercle bacilli and thus of transmitting disease to other susceptible animals. Available

knowledge of TB pathogenesis in cattle and observations based on previous post mortem examination of badgers suggests that only a limited number of infected animals transmit the disease, that this transmission process may be intermittent or cyclical, and likely to feature either early or late in the disease process.

4.7.15 We have recommended that a quantitative assessment of tubercle bacilli in the respiratory tract and the kidney (hypothesised to be a major infection source) be made on all badgers at post mortem by culturing a respiratory tract lavage and either urine or a bladder lavage.

Operational evaluations

4.7.16 Post cull evaluations, based on field surveys and analyses of trapping data, are undertaken to assess the efficacy of culling operations. From these, we are satisfied that operations to date have been effective and in line with our expectations.

4.8 Auditing

4.8.1 Our first report emphasised the importance of the appointment of external auditors for certain aspects of our work.

4.8.2 In September 1998 the Ministry advertised for an external independent auditor of field procedures. The contractor was required to assess (on a sample basis) the effectiveness of surveying, social group delineation and badger culling carried out by MAFF Wildlife Unit staff in accordance with the prescribed standard operating procedures. The specification also required an assessment of and report on the relevant standard operating procedures.

4.8.3 A contractor was selected in November 1998 and preparatory work on an audit programme began immediately. In the interests of obtaining the most comprehensive and detailed audit the contractor was given unrestricted access to trial staff and documents

4.8.4 In pursuit of our aim to ensure the highest standards in all trial operations, another independent auditor was selected to evaluate the welfare aspects of the trial's despatch procedures, and began work in May 1999.

4.8.5 Both external auditors have submitted interim reports. Final reports will be published by MAFF subject to any changes necessary to safeguard confidentiality.

4.8.6 In accordance with the recommendations of the ISG's first report, internal audit measures have been put in place to monitor compliance with standard operating procedures. As a further check in this area, members of the Group have accompanied Wildlife Unit staff at various times to observe, at first hand, compliance with surveying, trapping and despatch procedures and supervised exercises to delineate social group territories.

4.8.7 Paragraphs 8.2.14and 8.2.15 in Chapter 8 outline the planned audit of the trial's design and arrangements for checking the data which are gathered.

4.9 Summary of progress

4.9.1 To date, the ISG has approved six triplets of trial areas for enrolment into the trial. The first five have been announced (and the sixth is due to be named shortly):

Triplet A - Gloucester/Hereford
Triplet B - Devon/Cornwall
Triplet C - East Cornwall
Triplet D - Herefordshire
Triplet E - North Wiltshire

4.9.2 Surveying in Triplets A, B and C is complete, and has begun in D and E. Triplets B and C have been the subjects of proactive trapping operations, and, in the case of B, also of reactive culling. Following significant levels of interference in Gloucestershire and Herefordshire, operations there have been delayed; we expect to return to those areas before the end of the trial.

Operations in the Devon/Cornwall triplet

4.9.3 Surveying in this triplet began in August 1998 and was finished by mid-November last year. The siting and pre-baiting of traps was completed by the end of November. Trapping started on 2 December and was complete by 13 December 1998. 238 badgers were caught.

4.9.4 The first batch of reactive culls in the Hartland area of the triplet was carried out in May/June 1999. To date 68 badgers have been caught.

4.9.5 At the time of going to press, reactive culling operations are in progress in the Hartland area. The follow-up proactive cull in Putford is also being taken forward.

Operations in the East Cornwall triplet.

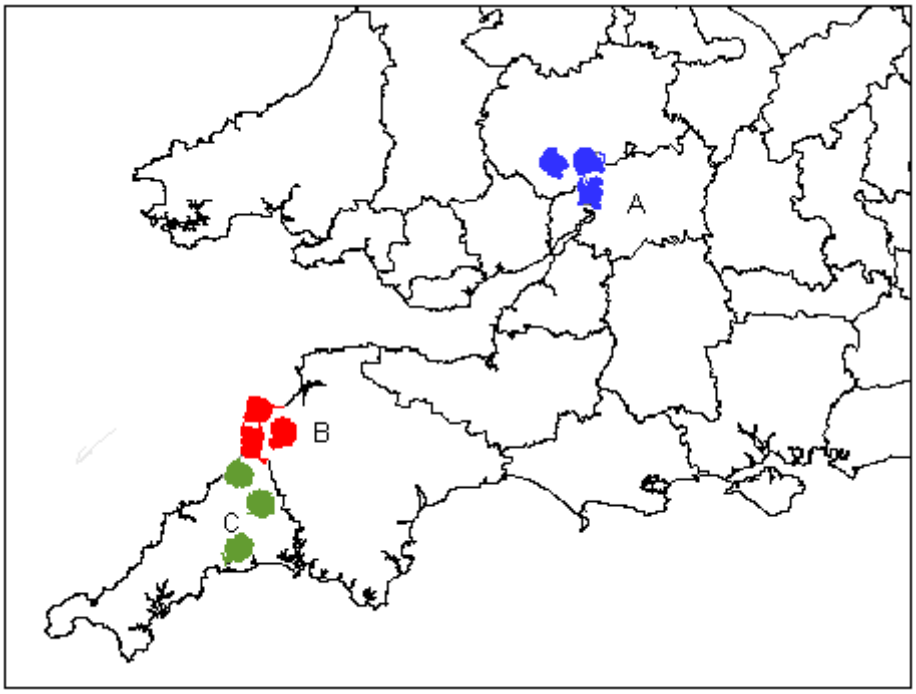
4.9.6 Surveying in this triplet began in April 1999. Dense woodland terrain slowed down the normal rate of progress, and surveying was completed slightly later than expected at the end of September. The siting and pre-baiting of traps was completed by the middle of October. Trapping started on 19 October and was complete by 29 October 1999. 246 badgers were caught.

4.9.7 Reactive and follow-up operations in this year will not take place until after the 2000 closed season.

4.9.8 Further details regarding each of these triplets can be found in Appendix B, in line with our recommendations on data release (see Section 8.3).

4.9.9 The location of triplets A, B and C are given in the map below. The locations of other triplets will not be released until initial proactive culling has been completed (see paragraph 4.3.10).

Figure 4f



5 Cattle Pathogenesis and Diagnosis

5.0.1 The Group attaches a high priority to determining the relative importance of cattle-to-cattle transmission of TB. We consider that this issue has not been adequately addressed in the past and may be of far greater practical significance than has been appreciated. The assumption underlying the long-established controls for cattle TB is that cattle to cattle transmission is of critical importance: that is why movement restrictions are imposed immediately when reactors are found in a herd.

5.0.2 In the absence of any external source of infection, the slaughter of infected cattle identified by regular tuberculin testing, coupled with movement restrictions, could be expected to control the disease. This has happened in many countries, and in parts of Great Britain, but in other regions of this country the disease has persisted. This has been interpreted as evidence for the existence of a wildlife reservoir of infection in these regions. Irrespective of the source of infection the increased risk of infection places greater demands on the testing programme. There is therefore a need to consider whether or not control of the disease is constrained by limitations in current testing procedures. We need to know more about the dynamics of the disease in

cattle and its temporal relationship to diagnosis, transmission routes, and the effectiveness of early diagnosis.

5.0.3 The analyses of existing information to identify risk factors will go some way towards identifying the role of cattle-to-cattle transmission but these analyses will be much strengthened by data from the TB99 epidemiological questionnaire and from the trial when these become available.

5.0.4 Some aspects of disease pathogenesis in cattle are being covered in the molecular epidemiology and vaccine programmes but further work is needed. The ISG has prepared a paper which reviews the current state of knowledge on pathogenesis and diagnosis of TB in cattle, and raises a number of questions which will determine future research funding priorities. This can be found at Appendix C. The ISG is holding scientific discussions with experts who can contribute to this work to discuss and design experimental protocols.

5.0.5 The Group welcomes MAFF's support for this work and, in particular, the immediate provision of funding to initiate studies in this area. The research requirements were published in November 1999.

6 The Road Traffic Accident Survey

6.0.1 Future policy options may necessitate the kind of information on TB prevalence in badgers which is otherwise only available for trial areas. In the absence of a reliable test or range of tests to diagnose the infection in live badgers (and the unlikely prospect of developing such tests in the short to medium term), the ISG supports the Krebs team's recommendation that a road traffic accident (RTA) survey of badger carcases be re-introduced. However, we have recognised that the value of the RTA survey to provide prevalence data requires validation and so have recommended that initially it be instituted in the seven counties within and around the areas where the field trial is most likely to take place. This will allow the survey's findings to be considered alongside TB prevalence data from badgers culled in the trial.

6.0.2 The RTA survey is the only element of the programme we have recommended on which little progress has been made so far. This has been disappointing, although we accept the resource constraints facing MAFF, and we recognise the contradictions that have understandably emerged in trying

to construct a survey which meets both the Ministry's surveillance needs and our own data gathering requirements.

6.0.3 During lengthy consideration of the possible ways forward, however, it has emerged that a considerable amount of data were already being generated by the informal, ad hoc collection of badger carcases by a number of the State Veterinary Service's divisional offices. In 1998, some 1200 carcases were collected and examined - a similar sample size to that envisaged for the seven counties survey. MAFF agreed with the ISG that this ad hoc work was of limited value and could be put to better use.

6.0.4 Plans have now been drawn up, at least for the interim, to re-direct this informal effort and structure it to provide prevalence data. These plans were due to be implemented this autumn, but the recent HSE prohibition notice on the post mortem examination of badgers at the Veterinary Laboratories Agency (VLA) forced a further postponement. (Owing to the relatively poor quality of RTA carcases we would not recommend freezing them for future post mortem examination; in any event, this would encroach on storage space designated for badgers culled during trial operations). The VLA expect enhanced post mortem facilities will be available in the new year, and we would expect the RTA survey to be implemented after that time, within existing MAFF resources.

6.0.5 The findings from the survey will be kept under review by the Group. If it becomes apparent that information needs are not being met we will ask Ministers to re-consider the introduction of the RTA investigation originally envisaged.

7 The Wider Research Programme

7.0.1 A broad programme of research projects has been put in place complementing the other measures discussed earlier in this report. A variety of research institutions is involved in the programme, and a wide range of expertise in the various areas is being tapped, at both national and international level. Some work has been commissioned directly by MAFF from its own agencies, but the largest spend is on projects which have been put out to open competition - in line with current MAFF policy and the recommendations of the Krebs Review team.

7.0.2 The projects are summarised at Appendix F, which is correct at the time of going to press, but will be supplemented in the near future in the light of our recommendations on further research into cattle pathogenesis and diagnosis. The rest of this chapter provides details on key areas of research.

7.1 Evaluation and analysis of previously collected data

7.1.1 MAFF holds a large body of data on TB in both cattle and badgers, including information on incident management (through the old TB49 form), cattle testing, badger removal operations, post mortem examinations, road traffic accident carcase surveys and strain typing. In addition, data from the Central Science Laboratory's Woodchester Park research facility are available. There are also other relevant data, including farm census, Geographical Information Systems (GIS) and meteorological data. The Group has held from the outset that thorough evaluation and analysis of these data could help answer many of the questions posed, identify areas which merit closer investigation, help to inform the production of guidance leaflets, and complement other ongoing research activity (including the risk analysis work deriving from the TB99 epidemiological questionnaire). It is our hope and expectation that the first wave of results from these analyses will be completed by the end of 2000 (see Chapter 8 on data handling).

7.2 Vaccine development

7.2.1 The ISG recognises the need for effective vaccines and strongly supports the implementation of the vaccine research programme, focusing on cattle vaccines, while retaining an interest in badger vaccines. There are intrinsic advantages in working on developing a cattle vaccine. Studies on cattle immunology and immuno-genetics are well advanced, immunological reagents and research tools have been developed and can be rapidly applied to *M. bovis*, and potential vaccine candidates can be evaluated in the target species. Cattle vaccine development can be expected to provide a launch pad for possible badger vaccines. The various vaccine projects will also benefit from developments in molecular epidemiology and notably the sequencing of the *M. bovis* genome.

7.2.2 We supported MAFF’s decision to appoint an independent programme co-ordinator, and are satisfied that the research work is both adequately funded and progressing along the right lines. Through the programme co-ordinator, Dr Jo Colston, Head of Mycobacterial Research at the National

Institute of Medical Research, we are assured that the programme is benefiting from the best cross-fertilisation of ideas and understanding available in both animal and human medicine at national and international level. Dr Colston will be producing regular reports for MAFF; the Group will consider these and provide any necessary comment and analysis.

7.2.3 The Group also supports the Ministry's intention to establish an official vaccine steering group to chart progress from the development of candidate vaccines up to the time of marketing the finished product. The steering group will look at issues such as intellectual property, regulatory aspects (at both UK and EU level), the trade framework, industry partnership and marketing, in parallel to the research work. The ISG will be represented on the vaccine steering group.

7.2.4 Although the vaccine programme is being undertaken by leading experts in the field, we would caution that success cannot be guaranteed, even in the long term. Despite a heavy research effort only limited progress has been made in developing a new human vaccine and there would be a number of practical problems to address with the use of both cattle and badger vaccines. It may well be possible to overcome these in the course of time, but it is our view that a vaccine would be unlikely to provide the whole solution to the problems posed by TB. A combination of measures, built around the epidemiological information our studies produce, will probably prove necessary to manage the disease in both cattle and wildlife.

7.3 TB in wildlife species other than badgers

7.3.1 The Group accepts that little is known about the role of badgers in the dynamics of *M. bovis* infection in cattle in Britain. The same holds true for the role of other wildlife species and two parallel studies, one in trial areas, are being funded to address this gap in knowledge. These will involve surveying multiple sites to collect data on the ecology of potential *M. bovis* reservoirs, investigation of the prevalence of *M. bovis* in wildlife species and degree of excretion in target species through post mortem examination (including bacteriological culture) and attempted diagnosis in the live trapped animal. Quantification of the relative risks to cattle will involve modelling and risk analysis through the integration of ecological and epidemiological data.

7.4 The ecological consequences of badger removal

7.4.1 The ISG recognises the necessity of carrying out an ecological survey to understand the consequences of badger removal. Before the trial began the Central Science Laboratory (CSL) was commissioned to produce an Environmental Impact Assessment, based on existing literature relevant to the area of badger removal. The executive summary of their report was published in the Group's first report.

7.4.2 Evaluation of badger culling as a potential means of TB control demands a detailed investigation of the ecological consequences which may arise from the implementation of such a policy. The Group therefore advised that CSL be commissioned to carry out a full study in trial areas.

7.4.3 The ecological consequences project began in February 1999; the coverage and emerging findings from this research project will be kept under review by both MAFF and the ISG.

7.5 Badger ecology

7.5.1 If badger control were to form part of cattle TB control policy in the future, we believe that accurate methods for assessing badger numbers which do not involve capture of the animals would be essential. At present there is no available observational procedure which allows accurate estimates of badger populations in a given area. The ISG has fostered two separate projects to develop observational techniques for estimating population size.

7.5.2 The trial itself will allow us to test theories concerning how social group structure and perturbation affect the distribution, prevalence and severity of TB infection. An initial genetic analysis, using material from badgers trapped in the Putford proactive cull, has been carried out and we have recommended to MAFF that this work be extended. Elsewhere, outside the trial, a study has been underway for several years into a badger population perturbed by past removals; we will be cross-referencing findings from that project, together with data from CSL's Woodchester Park facility, with our own observations from the proactive and reactive treatment areas.

7.5.3 Despite previous extensive badger research there remains much we do not know about aspects of badger ecology and behaviour relevant to TB control. The field trial and its linked studies - including the ecological consequences of badger removal - offer opportunities to extend our

knowledge in this area. The Group has recommended that these opportunities be exploited.

7.6 Molecular epidemiology

7.6.1 Scientific advances in the field of molecular biology over the last decade have produced investigative tools that could not have been envisaged even 25 years ago. For the purposes of our work, developments in genetic fingerprinting and in the strain typing of *M. bovis* will allow the genetic relationships of tubercle bacilli from and within different animal populations - cattle, badgers and others - to be examined, complementing the epidemiological study in determining potential routes of transmission. These technologies will also be used in the ongoing work on vaccine development and on improving diagnostic tests, and in our studies into the pathogenesis of the disease in cattle.

7.6.2 Under the broad heading of molecular epidemiology we have endorsed a number of research projects. In addition to the vaccines and diagnostic work in cattle, research focused on the development of an accurate test for TB in live badgers, genetic analysis of badgers from trial areas, strain typing and the joint MAFF/Wellcome Foundation funded sequencing of the *M. bovis* genome are all in progress at various centres of excellence in the UK and abroad. The genome sequencing project is to be completed in 2000, and this should pave the way for further advances in understanding the epidemiology and pathogenesis of tuberculosis.

7.6.3 Although these technologies may be described as "cutting edge", current molecular typing methodologies are not yet sufficiently discriminatory to answer questions on, for example, transmission, with the precision that we require. We also recognise that when more discriminatory techniques are available there will still be a need for comprehensive epidemiological data to supplement molecular investigations. The Group highlights the importance of maintaining the current research levels in molecular epidemiology studies, and to encourage the involvement of research workers with expertise in population genetics and molecular genetics, and possibly other disciplines, in the analysis and interpretation of data.

7.7 Economic evaluation of policy options

7.7.1 TB in cattle has potentially severe economic consequences, both in terms of the loss of productivity of the animals affected and the (private and public) costs of veterinary testing and other measures that have to be instituted to control its spread. It is clearly important to know the likely magnitude of these impacts - on the incomes of farmers, on the agricultural economy and on public expenditure - at different levels of disease incidence, as this information is an essential part of the framework Ministers will employ in deciding ultimately on a long term control policy. Should such a policy involve some control of the badger population in particular areas, then a further consideration will be the economic weighting that might be attached to badgers and other wildlife. In keeping with our aim to establish the widest possible rigorous information framework on which TB control policy can be based, we believe this more elusive area of economic valuation could usefully be explored.

7.7.2 To provide the information base that will allow an appropriate evaluation of the economic dimensions of possible control policies we therefore recommend that specific research is undertaken in the following three areas:-

- farm-level effects of bovine TB and its control.
- the wider economic effects of TB in the agriculture sector.
- the "ecological economics" dimension.

Results from these studies must be available by the time the Group is ready to prepare and examine possible sustainable TB control policies. We recommend, therefore, that these studies are commissioned in the year 2000.

7.7.3 A more detailed explanation of the economic studies proposed by the Group is at Appendix D.

7.8 Husbandry factors

7.8.1 In its report earlier this year the Agriculture Select Committee concluded that insufficient attention had been paid to the possible benefits of different farm management practices, and recommended among other things that MAFF institute an assessment, via an independent panel, of the available knowledge in this area.

7.8.2 The Group accepts that, logically, husbandry is likely to play a part in the overall TB picture. Common sense would dictate that farm management will

have an impact on disease control: this is the philosophy behind the recent publication by MAFF of simple guidance leaflets on TB. On balance we support the Select Committee's recommendation that this area be reviewed, although we have some doubts as to the prospect of any new solutions for TB control emerging at this stage. The scientific literature on husbandry and TB is limited and far from rigorous, and the acceptance of anecdotal evidence is inconsistent with the scientific approach the ISG is trying to establish in its studies of the problem. We are, however, ready to play our part in reviewing the panel's findings; MAFF has advertised for experts to take this exercise forward.

7.8.3 It has been suggested that on-farm experiments could be put in place now to measure husbandry effects. Given the multitude of possible variables involved, it remains the ISG's view that the only practical way to tackle the wider issue of what predisposes certain farms to outbreaks of the disease is through comprehensive risk analysis, using a questionnaire-based epidemiological survey (see Chapter 3.0).

7.9 Field trial collateral research

7.9.1 The Group emphasises the importance of maximising the research opportunities provided by the field trial, subject to constraints on MAFF resources and to data confidentiality considerations. We have therefore recommended a number of research projects (covered elsewhere in this report) which draw directly on or benefit directly from, the work on the trial itself. In addition, measures have been put in place to bank serum and genetic material from the badgers caught, to encourage further studies in the future from interested scientists as resources and new technologies become available.

7.9.2 A number of specific requests have been put to the Group for consideration and, subject to protecting the confidentiality of the farms concerned, we have been pleased to give our support for them. For example, one of these was for material to be used in a project looking at wildlife reservoirs of *M. paratuberculosis* (to come from badgers from farms known to have had incidents of Johne's disease in cattle).

7.9.3 Each request for access to trial material (which must include details of the work being or about to be undertaken, and the relevance of or need for

the material concerned) is examined by MAFF and the ISG on a case by case basis.

8 Data Handling

8.0.1 A considerable body of data will be generated by the field trial and related research. This chapter sets out what is or will become available, how the data will be analysed and when we recommend they should be released.

8.1 Data availability

8.1.1 Section 7.1.1 outlines the existing TB data that are available. Not all of these are in a user-friendly format, and part of the ongoing research effort is to computerise existing paper records, and render other datasets more accessible.

8.1.2 Data from the field trial are provided to the ISG as soon as they become available, and are regularly updated. As a Group, however, we are deliberately not informed of the incidence of TB in cattle in the trial areas, once selected, in order that our deliberations are not unconsciously influenced by this information.

8.1.3 Of the research projects which have been put in place we will have a direct input into the multivariate analyses of risk factors. These will link with the outcomes of our own analytical work. Similarly, we would expect to direct the analysis of any retrospective husbandry information deriving from the trial areas. With other research work the ISG will play its part in ongoing review procedures, and obviously draw on the results of the various projects when presenting advice to Ministers.

8.2 Analysis

8.2.1 The Group has drawn up a rolling data analysis programme, to take account of existing and future data.

Existing data

8.2.2 Our priorities in this area are to consider:

- cattle testing data (matched, as appropriate, against farm census data) - beginning with a spatial/temporal descriptive analysis of data from the last five, and then ten years;
- BRO data - although badger removal operations, carried out over several decades, were not the subject of scientific controls, it should be possible at least in part to assess their impact on badger populations, the extent of TB infection in badgers, and should also provide a picture of repeat breakdown rates;
- TB49 data (in conjunction with other data sources) - looking for pointers on husbandry and other relevant factors. Much will depend here on the strength of the data the old incident management forms can provide.

Further studies will assess, *inter alia*, the Woodchester Park data on badger populations, the strain typing database, and the findings from the multifactorial analyses of risk factors.

Trial data

8.2.3 Various elements of the composite trial database will be analysed on an ongoing basis. However, the first comprehensive evaluation will not take place until a sufficiently large sample of data is available (see paragraph 8.2.8 below).

TB99

8.2.4 The use of the TB99 questionnaire for all TB outbreaks since the beginning of 1999 presents, for the first time, an opportunity to establish a comprehensive descriptive database on cattle TB in Great Britain. Initial analyses of the 1999 data by spring 2000 will serve to test the robustness of the data being collected and to help identify where changes to the form could improve its quality and coverage.

8.2.5 The use of both the TB99 case and control forms within field trial areas is crucial. It provides a unique opportunity simultaneously to analyse the data contained therein alongside the information collected during the culling trial on the distribution and the pattern of TB in badgers. In addition, a comparison of TB99 data for case and control farms may identify husbandry and/or farm management practices associated with risk of infection. It will also be possible to undertake further multifactorial analyses by examining TB99 data alongside

other datasets (for example, meteorological data) to help determine which factors seem to be associated with TB in cattle.

Working practice

8.2.6 The analytical work will initially be carried out by two research assistants, reporting jointly to the ISG through the Group's and MAFF's epidemiological advisors. A composite trial database, including the TB99 epidemiological survey, has been developed by the Veterinary Laboratories Agency, working with the MAFF Wildlife Unit, and building on design work carried out by a contracted spatial epidemiologist during the early part of this year. The research assistants will further develop the database, linking it as appropriate to other datasets.

8.2.7 The ISG has consistently emphasised the importance of blind analysis of the impact of trial strategies on TB incidence in cattle and the absolute need for data confidentiality. The practical considerations of this requirement have been carefully considered. The epidemiologists, supported by their research assistants, will be the only individuals with access to all the relevant data.

8.2.8 After 100 TB incidents have occurred in trial areas (or one year from the end of the second proactive cull) - in other words, November 2000 if not before - a code should be assigned to each of the treatments in the trial areas implemented, and the data presented to the Group. The ISG, directed by its statisticians, will then undertake an initial blind analysis. A similar analysis will be repeated at six-monthly intervals thereafter until the end of the trial.

8.2.9 In the interim, discussions of trial operations data will exclude any cross reference to TB breakdowns in the areas concerned.

8.2.10 The final analysis of the very extensive data that will be available at the end of the trial will, among other aspects, examine the following:

- the overall comparison of the effects of culling on breakdown rates, providing a quantitative assessment of the contribution of the badger to TB in cattle;
- the nature of the differences between and within triplets, and of differences between individual farms within the trial areas including data collected from TB99;

- the significance of prevalence of infection and disease state of badgers.

A synthesis of these and other data, combined with the conclusions from economic and ecological analyses, will form the basis of our advice to Ministers on possible control options.

Timescales

8.2.11 We anticipate that the first analyses of existing TB data (as set out in paragraph 8.2.2 above) should be complete by the close of 2000, and this should help inform the Government's efforts to strengthen existing TB controls and to advise the farming community on appropriate husbandry practices.

8.2.12 The Group intends that TB99 data arising from the trial areas should be analysed to the same timescale as the trial data (November 2000, if not before). Major revisions of the questionnaire should not be contemplated before then, although MAFF may of course wish to amend the layout of the questionnaire for operational reasons in the light of the interim examination of the 1999 TB data next spring.

8.2.13 Section 4.1 on the design of the trial explains when the trial might deliver final results. The Group understands the enormous pressure from interested parties, and particularly the farming community and conservation bodies, for data from the trial and related studies to be generated, analysed and published as soon as possible. Although it is unlikely, we do not discount the possibility that significant effects from the trial or wider research programme might become apparent before the scheduled end of the trial. However, we emphasise that if future TB control policy is to be put on a sound scientific basis, accurate and comprehensive quantitative data must be gathered and the subsequent analyses carefully carried out. We also emphasise that the integrity of the trial must not be jeopardised at any stage by premature disclosure of data - see paragraph 8.3.5.

Auditing

8.2.14 We have considered the audit of data collected and analysed in the trial, which we referred to in our first report. The ISG intends to carry out its own audit of data (in connection, for example, with the application of TB99) and this will supplement MAFF's own internal quality checks. However, we

plan to return to this area - and the possible need for additional, external audit - during the course of next year.

8.2.15 An analysis of the power of the trial was originally conducted to determine the extent of trial activities necessary to generate sufficient data to allow dependable conclusions to be drawn. The Agriculture Select Committee could find no flaws in this design, but recommended that the original data and accompanying analysis be verified by an independent expert. We accept this recommendation and MAFF has the appointment of this auditor in hand.

8.3 Data release

8.3.1 Our objective throughout has been to be as open as possible about the trial within the constraints imposed by participant confidentiality, safeguarding staff security and protecting the integrity of the trial. This is reflected in the number of meetings and other communications initiatives we have been involved in during the last 18 months.

8.3.2 As far as possible, given the need to protect personal and commercial information, and resource constraints, we recommend that MAFF make existing TB data available to the wider research community. We welcome the publication of monthly incidence statistics on the Ministry's TB Website, and the Government's more general efforts to improve understanding about its TB research and control strategy.

Data to be released

8.3.3 The ISG has given very careful consideration to the timing and nature of the release of trial data and operational information. We have concluded that for each triplet the following background and operational details will be routinely released:

a. the rough location of trial areas and the treatments allocated to them
b. the aggregated history of herd breakdowns across the triplet
c. the percentage of the area for which permission for survey and culling was given
d. the number of traps used
e. the number of badgers and non-target species trapped
f. aggregated data on past badger removal operations (including numbers of badgers caught and TB prevalence).

However, in order not to assist those seeking to disrupt the trial and, particularly, for reasons of staff safety, the above information will not normally be released until after completion of initial proactive culling. Those details applying to the first two proactive culls are set out in Appendix B. Hereafter triplet details will be published on MAFF's TB Website.

8.3.4 Trial data relating to reactive culling and follow-up treatments in proactive areas will be published in the Group's annual reports. Data from reactive areas will be aggregated to protect landowner privacy.

Risks associated with premature disclosure

8.3.5 It is a fundamental principle of scientific trials that data must not be released prematurely since that could compromise the subsequent integrity of the trials. The Group is concerned that no data should be released from the field trial and related research which could discourage farmers' willingness to participate in the trial, or encourage either illegal killing of badgers or interference with trial operations. For this reason, we have recommended that a narrow band of data covering the prevalence of TB in badgers caught in the trial, locations of capture, and the number and location of cattle TB breakdowns in trial areas following the selection of trial areas, should not be disclosed. The Group has asserted and reinforced the need for total confidentiality of these data within the Ministry. They will be kept confidential until such time as they can be safely released with a considered analysis by the Group.

Other data

8.3.6 The ISG will consider with MAFF any specific and targeted trial-related data requests not falling into the categories in paragraphs 8.3.2 and 8.3.3 above.

Research projects in trial areas

8.3.7 A number of the research projects commissioned by MAFF on the Group's advice involve work in trial areas, or with trial-related data or material. Mindful of the risks associated with unguarded or premature release of sensitive details, we have recommended to MAFF that specific confidentiality clauses be inserted in the contracts of the relevant contractors. These oblige the researchers involved to obtain the permission of both MAFF and the ISG

before disclosing any such details ahead of the completion of their projects and normal publication of their work. MAFF has accepted this in principle, and we welcome, with our thanks, the understanding and constructive response of the research groups involved.

10 Communicating our approach

10.0.1 From an early stage we recognised the need for clear presentation of our scientific approach. The ISG's objective, set out by Ministers, is to provide the basis for policy options that will allow TB in cattle to be controlled in a way that allows for cattle and wildlife (particularly badgers) to co-exist in the agricultural landscape. The achievement of this objective has major implications for a wide range of interested parties and the dissemination of accurate and accessible information about the work programme underpinning it is a major factor in ensuring our philosophy is widely understood.

10.0.2 It is disappointing that media coverage, much of it misleading, has focused almost entirely on the field trial component. The broad based epidemiological research programme developed as a consequence of our objective approach, and its ultimate aim, have been largely ignored. Fixation with the trial, undoubtedly fuelled by pressure groups, has had the unfortunate effect of continually placing badger culling centre stage. This imbalance needs to be redressed vigorously, and we look forward to working with MAFF to achieve this.

10.0.3 The Group applauds Ministers' attendance at meetings in newly designated triplets to explain the Government's strategy on controlling TB in cattle. Such attendance indicates not only the importance of the strategy but Ministers' commitment to it. The Group certainly attaches emphasis to its members attending meetings and conferences and will continue to join in presentations to and open debate with interested parties. That said, we recognise that no matter how logical, scientific and compelling are the arguments for our research strategy, certain organisations and individuals, by virtue of their own interests, are unable to accept it. In such cases, the best that can be done is to ensure that members of those organisations, and not simply their representatives, have accurate information about our work and underlying philosophy.

10.0.4 The TB section of the MAFF Website is an important source of information on TB in cattle and badgers. It is designed to provide current and historical information on TB control policies in Great Britain and to illustrate developments and action taken to control the disease. It provides up to date statistics on the incidence of the disease in cattle. The Group recognises the further potential of the Website to communicate its approach and will work with MAFF to ensure that this is tapped.

10.0.5 Group members have met with many organisations and individuals; Appendix H lists the meetings and conferences in which the ISG has participated.

11 Looking ahead

11.0.1 The Group has worked to a demanding schedule over the last 18 months. The full Group convenes monthly, and sometimes more frequently, with sub-groups taking forward specific issues. Between meetings a great deal of preparatory work is done by members, the Secretariat and by MAFF and its agencies. Developments with the trial in particular have necessitated frequent, ad hoc attention. In addition, Group members have, with Ministers and officials, been taking the message of our approach out to interested parties and the wider public.

11.0.2 There is little sign of a lull in the near future, as we continue to advise on the implementation of the field trial and the wider research programme. However, with much of the programme in place, and TB99 being implemented successfully, we will be able to concentrate more on data analysis and the strategic direction of the programme for future years.

11.0.3 The trial establishment phase (by which we mean the enrolment of all the triplets required and the completion of initial proactive culling operations in all of them) will continue into 2001. We expect that by the end of 2000 seven triplets will have been subject to proactive culling operations, and the remainder enrolled into the trial.

11.0.4 Our overwhelming concern is to ensure that a scientifically rigorous understanding of the disease is established enabling us to advise on the development of sound policy options as quickly as possible. We will keep the research programme under constant review, and be on the alert for new technological developments which could facilitate our studies. We will

continue to monitor progress with the trial, working on triplet selection and data analysis, as well as advising Government on any design or operational amendments which may be necessary.

12 Summary of the Group's Approach

12.0.1 This report details the programme we have put in place over the last 16 months and explains the philosophy we have adopted. Set out below is a summary of our approach, which we intend to use as both the platform and framework for our future annual reports.

The ISG’s role

12.0.2 The Independent Scientific Group on cattle TB was set up following the acceptance by Ministers of the recommendations contained in the Krebs Report (1997). From this it was clear that the problem of TB in cattle was extremely complex, still poorly understood and that previous policies to control the disease had been inadequate. It recognised that substantial further work was necessary if an informative framework was to be established that would be adequate to underpin an effective policy to control the disease in the future.

12.0.3 The role of the ISG is to provide the scientific base for such a policy. From the outset we have adopted a holistic approach, recognising that sustainable control policies could only be achieved through a better understanding of the epidemiology of TB in cattle and wildlife reservoirs. Implicit in our approach is the recognition that the widespread elimination of badgers from large tracts of the countryside would not be politically or socially acceptable, hence we have sought to explore a much wider consideration of the problem and its possible solution(s).

Epidemiology and pathogenesis

12.0.4 We considered at length the TB problem and the approaches that might be taken at some length and put forward initial proposals, which were approved by Ministers in August 1998. Since then we have, with MAFF, put in place a major research programme on the epidemiology and pathogenesis of the disease. This is designed to advance our understanding of the determinants and dynamics of its persistence and its transmission between and within animal populations, and to explore explicitly the link between TB in

badgers and the occurrence of TB breakdowns in cattle herds. Our working approach is built around identifying the major epidemiological questions that needed to be answered and then considering how answers can best be found.

TB diagnosis

12.0.5 The cornerstone of an epidemiological study is accumulation of accurate data on the prevalence and distribution of infection and appropriate analysis and interpretation of the data. But above all there is a need for accurate and sensitive disease diagnosis. There is currently no reliable TB diagnostic test, or range of tests, that can be used in live wildlife. A reliable diagnosis can only be made by post mortem examination and bacteriological culture of body tissues. Thus, regrettably, accurate data on TB prevalence in wildlife - essential to answer critical questions on the maintenance of TB in wildlife and its potential for transmission to cattle - can only be obtained by killing samples of wildlife populations, including badgers.

12.0.6 The accurate diagnosis of TB in cattle is also essential if we are to understand the epidemiology of the disease and put in place effective controls. Current methods of diagnosing TB in cattle have been highly effective in controlling the disease in many countries and in parts of Great Britain. However, given the increased risk of infection in those areas of Great Britain where TB has remained a problem there is a need to question whether cattle TB control in this situation is constrained by limitations in current diagnostic procedures, and to consider the impact this may be having on persistence of the disease and cattle to cattle transmission. A clearer understanding of the dynamics of the disease in cattle and how this relates to and influences diagnosis and disease transmission and prevalence is essential. There is a need for greater effort in this area.

12.0.7 The comprehensive package of research now in place includes therefore, as a crucial component, the use of modern technologies to develop new and improved diagnostic tests for cattle and wildlife. These techniques are also being used to develop molecular tools to discriminate more clearly between the different TB strains, to track more accurately the precise movement of TB organisms between and within species, to understand genetic typing of badger populations and to develop effective vaccines.

Risk factors

12.0.8 A major component of our research initiative is the implementation of a detailed on farm epidemiological survey, collecting information using a carefully designed questionnaire (TB99). This questionnaire is to be administered to all farms experiencing TB breakdowns and, for comparison within trial areas to comparable nearby farms in which no infection has recently been detected. This study is designed to evaluate risk factors which may predispose herds to infection and includes questions relating to herd characteristics, husbandry, land use, and exposure to external and internal sources of infection. The questionnaire has been devised and piloted, staff have been trained in its use and it is currently being applied to breakdown farms within and outside of the trial areas. The questionnaire is comprehensive and demands a high degree of involvement from farmers, which we have consistently received, and for which we are most grateful. We are conscious of criticisms that it is too detailed (though some by contrast believe it could usefully be even more detailed). However, we considered the information needs extremely carefully and recognise that, in the current poor state of understanding of the epidemiology of TB, there is no escape from the need to assemble a database from farms that will allow full analysis of the possible risk factors and characteristics which may explain the occurrence of herd breakdowns, together with other appropriate datasets (including geographical and climatic features). We plan to use the data collected and experience gained in the implementation of TB99 thus far to review its design next year.

The field trial

12.0.9 The randomised field trial is but one part (albeit important) of our holistic approach. It is also the most contentious. There are major epidemiological questions relating to the role of the badger in cattle TB that must be answered, and policy options to be explored if we are to develop a sustainable control policy. These can only be addressed if we have the information to be derived from the trial. Past policies on badger control have not provided quantitative information on the role of the badger in the continued high incidence of herd breakdowns in the “hot-spot” regions. Also it has left open the question whether selective badger culling, improves, worsens or has no effect on the situation. Only by carrying out the randomised field trial in well defined areas can we answer these questions once and for all.

12.0.10 As well as demanding rigorous operational procedures we continue to place great weight on welfare issues in the conduct of the field trial. Culling operations are carried out by specially trained Government personnel and use cage traps only and these are widely considered as a humane form of capture. Standard operating procedures for field work are clearly defined and constantly reviewed and subjected to internal and external audit in order to assure that the highest standards of welfare are met.

Data analysis

12.0.11 Both the randomised field trials and the TB99 investigation will have to be implemented for a number of years before they can generate sufficient information to allow us to offer dependable advice for farmers and Ministers. In an attempt to provide some advice more rapidly we have initiated a full evaluation and in depth analysis of existing data held by MAFF and its agencies.

Interim measures

12.0.12 Realistic and effective proposals, particularly for the short term, for the better management of cattle TB may come from organisations and individuals from outside the ISG and MAFF. We have consistently taken the opportunity to consult widely, and we welcome the creation of the new "TB Forum" to solicit constructive input from interest groups and stake holders. We also welcomed the Agriculture Select Committee's review of TB control policy and were reassured by their firm support for our activities contained in its report of April 1999.

12.0.13 We are aware that cattle TB incidence continues to rise especially in the south west but also with the emergence of new areas, and this creates a pressure for us to provide answers as quickly as possible. Extreme factions who see badgers as either the villains of the piece or innocent victims have heightened emotions and encouraged increased resistance to the work we are doing. If a lasting answer to the TB problem is the goal - as it must be - the outcome of our work must not be prejudged and our epidemiological investigation, including the randomised field trial, must be allowed to run its course without interference. All elements of the programme, many of which are interlinked, must be run in parallel and to completion. This programme has been carefully planned as a comprehensive and integrated approach to the

problem and any lessening or diversion of the effort will result only in an incomplete picture which will benefit no-one in the longer term.

Evaluating progress

12.0.14 Given the scale and complexity of the programme of research that we have proposed some problems in implementation are perhaps inevitable. However, the Road Traffic Accident survey is the only element of the programme we have recommended on which almost no progress has been made so far. This is disappointing since the survey is necessary to yield valuable information. Of greater concern is the delay in getting all the field work in place. Slippage in the programme has allowed only two proactive culls to be completed to date. We accept that it would not have been logistically possible to put all the triplets in place in a short space of time but illegal interference with the field work causes further unwarranted delay, practical concern and frustration in pursuit of the study's objectives. Nevertheless, by the end of 2000 we will expect all of the planned triplets to have been enrolled and the majority of these to have been proactively culled.

12.0.15 We will continue to monitor the progress of the programme of work now in place and advise on the commissioning of other work as opportunity or need arises. During the next few months we shall finalise triplet selection, and by this time next year, would expect to be in a position to carry out an initial analysis of data.

Appendix A - Summary of Standard Operating Procedures

1. Introduction

1.1 Standard operating procedures are prescribed for each stage of the trial. They are reviewed by the Independent Scientific Group in the light of experience to improve and streamline practices where possible. For reasons of staff safety and security the full text of procedures has not been published but the following summary sets out the main features of how the trial is being conducted. Other procedures, mainly relating to administration, have also been prescribed but are not summarised in this appendix.

1.2 The standard operating procedures relating to surveying, trapping, social group territory delineation and humane despatch have been subject to independent audit.

2. Triplet selection

2.1 The centre point of candidate trial areas (grouped into threes to form triplets) are identified by the ISG on the basis of specific criteria. These criteria include surface area, a minimum number of cattle holdings and, in particular, the immediate and past history of cattle TB breakdowns, for which there are reliable, current data.

3. Delineation of trial area boundaries

3.1 The centre points (described in paragraph 2) are developed into trial areas and buffer zones in three stages, as follows:

- candidate trial areas of 100 km^2 are mapped drawing a circle of radius 5.64 km about the centre point.
- proposed trial areas are mapped by adjusting candidate trial area boundaries to take account of natural features and known farm boundaries. Once proposed trial areas have been ratified by the ISG a list of occupiers of land in those areas is drawn up and used as the basis for visiting and enrolling participants in the trial.
- trial areas are formed by adapting proposed trial area boundaries to take account of survey information and are subject to ratification by the ISG.

In delineating boundaries care is taken to maintain, as far as possible, the circular shape of trial areas and the balance of key characteristics used to determine centre points.

3.2 Buffer zones are defined as follows:

- Inner buffer zone: an area extending 1 km from a trial area boundary which may not overlap any other buffer zones.
- Outer buffer zone: an area extending 1 km beyond an inner buffer zone boundary which may touch or overlap other outer buffer zones.

4. Visiting occupiers of land

4.1 Visits to occupiers of land are made to explain and request participation in the trial. The importance of the trial to the strategy to combat TB in cattle is emphasised and its voluntary and confidential nature explained. Formal consent to participation is sought by signature to a standard agreement which incorporates a map recording the boundaries of the occupier's land. Occupiers are asked to signify if they are required to obtain consent from a third party. Participation may extend to culling and/or surveying and also auditing and research projects associated with the trial being conducted on an occupier's land.

5. Surveying for badger activity

5.1 Surveys are undertaken to identify and record the location of main and subsidiary setts and other signs of badger activity including latrines, boundaries and visible runs. Such information is used to delineate badger social group territories, where present, finalise the boundaries of treatment areas and identify areas where traps may be sited. Surveys may also provide information on illegal interference with badgers and/or setts.

5.2 Types of surveys undertaken are as follows:

- initial - full surveys, undertaken prior to randomisation, of all three treatment areas in a triplet.
- post cull - a survey to assist in assessing the effectiveness of operations (particularly in proactive areas).
- reactive - limited surveys in response to confirmed cattle TB breakdowns. The aim of such surveys is to record all signs of badger activity and identify social groups using the breakdown premises.
- follow-up - restricted to proactive treatment areas. Follow up surveys are of setts only and are normally undertaken 5-9 months after completion of culling and annually thereafter. The aim is to identify badger activity and sites for placing cage traps.
- at 3 years - full surveys of samples of each treatment area (2 years after the initial survey in Year 1). Such surveys are carried out independently of earlier surveys.
- at 5 years - as for 3 year survey, but 4 years after the initial survey in year 1.
- spot surveys of setts to check for illegal interference.

6. Social group territory delineation

6.1 Badger social group territories are delineated to identify proactive and reactive treatment areas and so that trapped badgers may be attributed to social groups for analysis purposes. It is recognised that in some cases social group territories cannot be fully delineated; this may be because of the lack of survey data or because clear social group structures and territories may not exist due to low population densities or distribution of populations resulting from past badger removal operations.

6.2 The first step in the delineation process is to plot main setts on maps and construct hypothetical territories using Dirichlet tessellations. Secondly, tessellated boundaries are adjusted to take account of topographic features and field signs recorded during the surveying stage of the trial. Finally, setts are categorised according to the certainty with which they can be allocated to territories.

7. Randomisation

7.1 In the presence of two members of the ISG and an independent person, treatments are (by the role of a die) allocated at random to the three trial areas within a triplet. The allocation of treatments is only disclosed, on ISG advice, when surveying in the triplet concerned has ceased and culling operations are ready to begin. Trial participants are notified by letter of the treatment which has been allocated to their land.

8. Trapping

8.1 Pre-baiting is undertaken prior to trapping to familiarise badgers with bait and to maximise trapping efficiency. Trapping of badgers is by cage trap only and a closed season operates from February to April, to reduce the chance of catching lactating sows with dependent cubs. The maximum trapping period is 12 days but may be shortened if, for example, badger activity ceases. Trapping would be suspended if, due to bad weather, there was a risk that setts could be flooded or trapped badgers would suffer extreme exposure.

8.2. Standard procedures require traps to be checked as early as possible during the day and set to trap as late as possible. These provisions seek to minimise the period for which trapped badgers remain in cages and to reduce catches of diurnal non-target species. These procedures may be varied in the interests of staff safety.

9. Humane despatch procedures

9.1 The despatch of badgers may only be undertaken by fully trained MAFF staff in accordance with strict procedures. Cage trapped badgers are despatched by shooting to the head. Immediately after shooting, checks are undertaken to ensure that the badger is dead and confirmation that such checks have been completed is indicated by the application of a coloured marker to the carcase. If there is any doubt that a badger is dead a second shot is administered immediately.

9.2 Non-target species are released unless injured to an extent that would make it inhumane to do so. For smaller species despatch is by one or more sharp blows to the head. Veterinary advice would normally be sought on the despatch of other species.

10. Submission of carcases for post-mortem examination

10.1 Procedures set out how carcases must be bagged, labelled and transported to the place of post-mortem. Particular attention is paid to health and safety issues to avoid possible aerosol transmission of bacteria from carcases.

11. Blood samples from despatched badgers

11.1 Blood samples are taken from despatched badgers for use in the development of blood-based diagnostic tests for *M. bovis* infection in live badgers, and for the establishment of a serum bank. Samples are taken only after the badger has been despatched and checks completed that it is dead.

12. Post-mortem procedures

12.1 Post-mortem examination of badgers is undertaken to determine *M. bovis* infection at the time of death. This involves examination for the presence or absence of suspected tuberculous lesions and the collection of a standard selection of lymph nodes and, where applicable, collection of suspicious lesions. Infection with *M. bovis* is determined by bacteriological examination of the specimens collected at post-mortem examination. Tubercle bacilli are quantitated in the respiratory tract and urine. Other material may be collected for collateral research projects approved by the ISG.

12.2 A check is made for cage related injuries which, if present, must be recorded for analysis.

13. Mycobacteria - cultural examination of badger tissues

13.1 Mycobacteria are slow growing organisms requiring specialized media for their cultivation. The medium must contain substances sufficient to suppress the growth of contaminants but allow the growth of Mycobacteria.

13.2 Culture tubes are examined weekly for up to 6 weeks for evidence of growth of Mycobacteria. Where the culture demonstrates growth suspected to be Mycobacteria, a further set of culture tubes, containing typing media for confirmation of *M. bovis*, are inoculated and incubated. These cultures are examined until sufficient growth occurs to enable a differential reading for identification to be made, usually about 3 weeks. A known positive culture of *M. bovis* is inoculated onto each new batch of media and an uninoculated tube used as a negative culture to act as controls.

14. Spoligotyping

14.1 Spoligotyping ("spacer oligonucleotide typing") is based on the detection of DNA polymorphisms present at one particular chromosomal locus, the “Direct Repeat” (DR) region, which is uniquely present in Mycobacterium tuberculosis complex bacteria. Spoligotyping represents the first polymerase chain reaction (PCR)-based fingerprinting technique for Mycobacterium bovis to be widely accepted and is the only currently available technique which allows cost effective fingerprinting of every *M. bovis* isolate from GB.

14.2 The DR region in *M. bovis* BCG consists of directly repeated sequences of 36 base pairs, which are interspersed by non-repetitive DNA spacers, each 35 to 41 base pairs in length. In *M. bovis* BCG there are 49 copies of the DR sequence whereas in other strains of *M. bovis* the number of DR elements vary significantly and this variation forms the basis for strain typing using spoligotyping.

14.3 Spacer sequences are initially amplified by PCR using primers targeted to the DR sequence. The presence or absence of spacers within the DR region is detected by hybridisation of the PCR product to a set of immobilised oligonucleotides each corresponding to one of 43 potential unique spacer

DNA sequences within the DR and visualised using a chemiluminescence system. The variation in spacers is used to obtain different hybridisation patterns of the amplified DNA.

14.4 The value of spoligotyping for strain differentiation of *M. bovis* has been assessed critically in several studies and it is now recommended that spoligotyping be used for rapid screening of isolates, and followed up by a more discriminatory technique such as PGRS-RFLP if further discrimination is required. A further advantage of spoligotyping over the RFLP techniques is that, being PCR-based, it can potentially allow for simultaneous diagnosis and typing from clinical samples.

15. Identification of controls

15.1 Confirmed TB incidents (i.e. "case herds") in trial areas are subject to epidemiological investigations (using questionnaire TB 99) involving the selection of three control herds in the same trial area. One control herd will be contiguous to the case herd and the other two will be selected at random.

15.2 Data on potential risk factors is collected for individual reactor cattle in case herds. Procedures are prescribed for the collection of equivalent information for individual cattle in control herds and for the selection of those animals. Rules on sampling, defining which herds are eligible for selection as contiguous and non-contiguous herds and for managing farmer refusals are also prescribed.

16. Notification of TB herd breakdowns

16.1 Standard procedures are laid down so that breakdowns in reactive areas trigger reactive culling. All breakdowns in trial areas are recorded on a central database with account being taken of cattle movements into and out of those areas.

Bovine TB: APPENDIX B - Characteristics and Operational Data from Triplets Subject to Initial Proactive Culling Operations: Devon/Cornwall and East Cornwall Triplets

Table B i: Key data for Devon/Cornwall Triplet

1.Triplet name	Devon/Cornwall		
2.Trial area	Hartland B1	Putford B2	Bude B3
3.Number of cattle holdings in trial area	91	151	145
4. Historical incidence of TB in cattle in herds in trial area			
confirmed breakdowns: 3 year (1995-97)	30	35	36
12 month (1997)	11	23	12
annual incidence: 3 year (1995-97)*	0.1099	0.0773	0.0828
12 month (1997)*	0.1209	0.1523	0.0828
5.Total surface area (trial area and inner buffer zone) (km^2)†	119	143	130
6.Total area for which permission for trial operations was sought (trial area and inner buffer zone) (km^2)	114	125	117
7.Number of land occupiers visited in trial area and inner buffer zone	164	270	232
8.Treatment	Reactive	Proactive	Survey only

9.Number of traps	To date: 230	Initial: 700	Not applicable
10.Number of badgers caught.	68 to date	Initial: 238 Follow up: in progress	Not applicable
11. Non target species: Caught:	100	87	Not applicable
Found dead in trap or despatched:	5	18	Not applicable
12.Aggregated data on badger removal operations in localised areas under the 'interim strategy' 1986-1997.			
- Total number of badgers caught	396	441	423
- Percentage of badgers caught found to be infected with TB	32%	20%	33%

* - Number of breakdowns divided by total number of herds (per annum), expressed as a decimal figure.
† - Some of this surface area will automatically be unsuitable for trial operations (including, for example, settlements, airfields, roads, rivers, lakes, quarries etc.)

Appendix B - Characteristics and Operational Data from Triplets Subject to Initial Proactive Culling Operations: Devon/Cornwall and East Cornwall Triplets

Table B i: Key data for East Cornwall Triplet

1.Triplet name	East Cornwall

2.Trial area	Otterham C1	Launceston C2	Lanreath C3
3.Number of cattle holdings in trial area	151	180	107
4. Historical incidence of TB in cattle in herds in trial area			
confirmed breakdowns: 3 year (1995-97)	21	16	14
12 month (1997)	7	9	5
annual incidence: 3 year (1995-97)*	0.0464	0.0296	0.0436
12 month (1997)*	0.0066	0.05	0.0467
5.Total surface area (trial area and inner buffer zone) (km^2)†	145	157	151
6.Total area for which permission for trial operations was sought (trial area and inner buffer zone) (km^2)	137	155	140
7.Number of land occupiers visited in trial area and inner buffer zone	259	315	237
8.Treatment	Reactive	Survey only	Proactive
9.Number of traps	---	Not applicable	Initial: 800
10.Number of badgers caught.	---	Not applicable	Initial: 246
11. Non target species: Caught:	---	Not applicable	40

Found dead in trap or despatched:	---	Not applicable	0
12.Aggregated data on badger removal operations in localised areas under the 'interim strategy' 1986-1997.			
- Total number of badgers caught	185	358	348
- Percentage of badgers caught found to be infected with TB	24%	24%	20%

* - Number of breakdowns divided by total number of herds (per annum), expressed as a decimal figure.
† - Some of this surface area will automatically be unsuitable for trial operations (including, for example, settlements, airfields, roads, rivers, lakes, quarries etc.)

APPENDIX C - Pathogenesis and Diagnosis of Infections with *M. bovis* in Cattle

The following key points are apparent from a review of the literature on the pathogenesis and diagnosis of bovine tuberculosis:

- The infective dose of *M. bovis* has a profound influence on the kinetics of infection and severity of disease in cattle.
- Comparison of the pathology in naturally and experimentally infected cattle suggests that most natural infections are initiated by a low infective dose of *M. bovis*.
- Available information on the kinetics of bacterial excretion, although limited, indicates that there is an early period of sustained bacterial excretion in the first few weeks of infection followed by more intermittent excretion. However, there is a lack of quantitative date on this aspect of the disease.
- Observations on the numbers of reactor cattle per herd indicate that many reactor cattle do not transmit infection to in-contact animals: The basis

of the variability in infectivity is unclear as is the route by which animals become infected.

- The currently used tuberculin test is highly specific (>99%) but its sensitivity at standard interpretation appears to be only about 90%. This implies that up to 10% of herds containing a single infected animal remain undetected and that the test is unlikely to detect all infected animals in multiple reactor herds.
- Because a positive skin test result is often based on measuring small differences in skin thickness, the test is likely to be subject to error on the part of the operator. The interpretation of the test is also complicated by non-specific responses induced by other species of mycobacteria, which although controlled for by the use of *M. avium* antigen in the test, probably limit its sensitivity.
- There is also a lack of reliable experimental data on the kinetics of skin test responses and the period of non-reactivity to a second skin test in infected cattle. The possible influence of multiple skin tests, as occurs in protracted breakdown incidents, on the sensitivity and specificity of subsequent tests also needs to be examined.
- While these limitations have not prevented the eradication of TB in many parts of the UK, they may be a significant constraint to control of the disease in areas of the country where TB has persisted.
- An alternative in vitro diagnostic test, based on measurement of interferon-gamma (IFN-γ) production in cultured blood, gives similar sensitivity to the skin test but has inferior specificity. However, the two tests detect slightly different populations of diseased cattle.

  Experimental studies using recombinant *M. bovis*-specific antigens in an IFN-γ test have produced promising results, suggesting that development of a more specific IFN-γ test may be possible.

  Four areas of high priority for research, which could potentially lead to improved herd testing protocols and also provide a stronger scientific basis for conveying advice to farmers on management of TB incidents, are identified:

- The analysis of risk factors in herds that suffer recurrent breakdowns.
- The development of improved diagnostic tests.
- Pathogenesis of *M. bovis* infections in cattle, focusing particularly on aspects relating to transmission of infection and diagnosis.
- Mathematical modelling of *M. bovis* transmission in cattle.

Introduction

1. Current control measures for bovine tuberculosis (TB) aim to detect and remove infected cattle and to prevent spread of infection both within and between farm premises. While these measures have been effective in eradicating TB in a large part of the UK and in other countries, the disease has persisted in certain regions of the country and recently has shown an alarming increase in incidence, despite an intensive programme of testing. This has been interpreted as evidence for the existence of a wildlife reservoir of infection in these regions. Irrespective of the source of infection, the increased risk of infection places greater demands on the testing programme. There is, therefore, a need to consider whether or not control of the disease is constrained by limitations in current testing procedures and whether there are areas of research that could lead to improved efficacy of the control measures.

2. This document assesses available data on the efficacy and scientific basis of current cattle testing procedures, and reviews those aspects of the pathogenesis of TB in cattle that relate to diagnosis and transmission of infection between cattle. Because of possible differences in the epidemiology of the disease and the performance of TB testing in different parts of the world, discussion of the natural disease is focused on data obtained in the British Isles. Several areas of research considered relevant to development of improved disease control measures are identified.

Pathogenesis

The natural disease

3. The endemic state of bovine tuberculosis in the early part of this century resulted in a variety of pathological syndromes some of which were associated with clinical disease (Stamp, 1944; Francis, 1947). Herd testing has dramatically reduced the prevalence of infection and also the incidence of cases with severe pathological changes. The latter is presumably due to the early identification and removal of most infected animals, but may also be a consequence of a reduced intensity of bacterial challenge.

4. In the vast majority of infected cattle identified by tuberculin testing, pathological changes are confined to the respiratory tract and associated lymph nodes, and in many of these only a few small lesions are found at post-

mortem examination. Disseminated disease is rare: Costello *et al* (1997), in a study of infected animals in depopulated herds in Ireland, found disseminated lesions in only 4 out of 353 tuberculous animals.

Experimental infections in cattle

5. There are few recent experimental studies of the pathogenesis of bovine tuberculosis and none that have examined the disease process in any detail. Two studies in which animals were infected experimentally with different doses of *M. bovis* have provided evidence that the infective dose has a profound influence on the severity of disease (Neill *et al*. 1988, Buddle *et al*. 1994). Animals that received high doses of *M. bovis* ($5x10^5$-10^6 cfu) intratracheally or intranasally developed multiple diffuse lesions and, in some cases, clinical disease, whereas the severity of disease varied at lower doses ($5x10^2$-10^4 cfu), from diffuse lesions in some animals to no visible lesions in others. An additional group of calves given 10^2 cfu, in the study by Neill *et al*. 1988, did not develop any lesions and remained skin test-negative (although *M. bovis* was isolated from a nasal mucus sample in one animal 100 days after infection).

6. The study by Neill *et al* (1988) also monitored nasal shedding of *M. bovis* on a weekly basis. Infections established with high (~10^6) and low (~10^4) doses of *M. bovis* were both characterised by an early period when bacterial excretion was consistently detected, often at high levels, followed in those animals that did not develop severe disease, by intermittent excretion. The peak levels of excretion generally occurred later (4-7 weeks compared to 3-4 weeks) and were more variable in animals infected with a low dose of organisms than in those infected with a high dose. However, the extent of the variation in level of bacterial excretion between animals and at different stages of infection is unclear, since bacterial recovery was quantified arbitrarily (scored 1-3).

7. The experimentally infected animals that develop limited pathology and survive to become intermittent excretors of *M. bovis* appear most similar to the majority of field cases of TB, suggesting that natural infection is usually initiated by a low dose of *M. bovis*. The variability in the severity of disease and level of bacterial excretion following experimental infection with low doses of organisms also indicates that there is variation in the inherent susceptibility of cattle to infection.

8. It is unclear whether or not cattle can completely resolve infections with *M. bovis* and, if so, whether such animals are detected by TB testing procedures. In the experiments carried out by Neill *et al* (1988), it was not possible to conclude whether the animals that received ~10^2 cfu of *M. bovis* failed to become infected or developed a transient infection that gave no residual lesions or skin test reactivity. Further immunological analyses of animals infected with low doses of organism might help to answer this question.

Cattle to cattle transmission of *M. bovis*

9. The data cited above on nasal excretion of *M. bovis* indicate that those animals that develop fulminant disease are likely to be an important source of infection for in-contact animals. However, most naturally infected, tuberculin-positive cattle exhibit limited pathology, and nasal excretion of *M. bovis* can only be detected in a small percentage of these animals at slaughter (Neill *et al*, 1988). The experimental data suggest that a period of more sustained excretion may have occurred at an earlier stage of infection, in such animals.

10. Two studies conducted in Ireland have examined transmission of infection from naturally infected reactor cattle to in-contact controls. In the first of these studies, a total of 22 reactors were either housed or grazed (individually or in small groups) with 29 susceptible cattle for periods ranging from 4 to 7 months. None of the in-contact animals became tuberculin-positive or had evidence of infection at post-mortem examination (O'Reilly and Costello, 1988). The second study involved 10 pairs of reactor cattle each housed with one susceptible animal for 12 months (Costello *et al*, 1998). At the end of this period one of the in-contact animals had gross TB lesions, and *M. bovis* was cultured from the lymph nodes of a further 3 animals.

11. In contrast to the studies with naturally infected cattle, workers in N. Ireland observed that most susceptible cattle became infected when housed together with infected animals during the period immediately following experimental infection (Neill *et al*, 1989; Cassidy *et al*, 1999b). Transmission of infection was less consistent when calves were introduced into groups of infected animals several months after initiation of infection. While these findings suggest that cattle may be more infective during the early stages of infection, the numbers of animals involved in the experiments were too small to draw firm conclusions. Further studies involving larger numbers of animals are required to address this issue and to investigate the influence of infective dose on subsequent transmissability of infection.

12. Herd breakdowns frequently involve only 1 or 2 reactor animals. In the Republic of Ireland, 47% of the 18,607 herd breakdowns that occurred between 1982 and 1989 involved one reactor (Griffin and Dolan, 1995). Denny and Wilesmith (1999) also reported that 55% of the herds with confirmed infection, over a 9 year period in N. Ireland, had only one animal affected. Similarly, in 46% of the 1288 herd breakdowns that occurred in Great Britain in 1997 and 1998, a single reactor was detected at the disclosing test, and a further 12% involved two reactors (R. Clifton-Hadley, personal communication) Since reactors may have been infected at any time since the previous test, these figures would indicate that many reactor cattle present a low risk of infection to in-contact animals. However, transmission clearly does occur in some cases. The likelihood of transmission may relate to the level of bacterial excretion in the period immediately following infection, which in turn is likely to reflect the dose with which the initial animal was infected and its inherent susceptibility to infection. In animals with more long-standing infection, secondary factors such as intercurrent disease, metabolic stress or reproductive status might alter immune competence and result in periods of bacterial excretion. These aspects of the pathogenesis/ epidemiology of bovine TB are poorly understood.

13. A recent case control study carried out in Northern Ireland (Denny and Wilesmith, 1999) identified farms contiguous with those that have suffered a TB breakdown as being at increased risk of infection. On most of the farms in the study there was opportunity for nose-to-nose contact between cattle on contiguous farms. While these data might suggest that spread between contiguous farms is an important cause of herd breakdowns, this is somewhat at odds with the observation that there is very limited transmission between cattle within many affected farms. An alternative explanation is that the cattle on these farms are exposed to a common external source of infection, such as wildlife.

Routes of transmission between cattle

14. The way in which *M. bovis* is transmitted between cattle is also poorly understood. In naturally infected cattle, tuberculous lesions are most commonly detected in retropharyngeal, bronchial and mediastinal lymph nodes, ie. the lymph nodes draining the respiratory tract. This has been taken as evidence that infection occurs by inhalation. Because lung lesions are found in only a small proportion of cases (usually <15%), and in 20-30% of

cases lesions are confined to the head lymph nodes, it is considered that in many cases infection is probably initiated in the upper respiratory tract. This view has been reinforced by the recent observation that *M. bovis* can be isolated from nasal mucosa and palatine tonsil of some reactor cattle (Cassidy *et al*, 1999a).

15. Experiments carried out earlier in this century indicated that much larger doses of *M. bovis* are required to infect cattle via the oral route than intranasally (Chaussé, 1913). Moreover, animals experimentally infected by the oral route invariably had tuberculous lesions in the gut and associated lymph nodes (McFadyean, 1910; Francis, 1947). By contrast, infection by the intranasal or intratracheal route results in pathology confined mainly to the respiratory tract (Neill *et al*, 1988; Buddle *et al*, 1994). While these data argue that infection is more likely to occur by inhalation, they do not exclude the possibility that ingestion of small numbers of organisms may occasionally result in animals being infected via the buccal cavity. It is conceivable that infected sputum ingested with contaminated food could adhere to the oral or buccal mucosa and lead to infection of the drainage lymph nodes. Although such a route of infection may be inefficient, it could be significant in the field if there is more frequent exposure to infection by ingestion than inhalation.

16. An alternative way of investigating how transmission occurs between cattle is to determine whether direct contact is required and, if not, over what distance infection can be transmitted between animals sharing a common air space. These questions are clearly amenable to experimental investigation but have not been addressed to-date. More reliable information on transmission using this type of approach would not only improve our understanding of the epidemiology of the disease but also provide a basis for giving practical advice to farmers.

Diagnosis

Introduction

17. In view of the difficulties encountered in culturing *M. bovis* from many naturally infected cattle, diagnosis in the live animal cannot rely on assays that detect the organisms or their constituent antigens. Diagnosis must, therefore, be based on detection of a specific immune response to *M. bovis*. Most immunologically based diagnostic tests rely on the detection of an antibody response. However, antibody responses to *M. bovis* cross-react

extensively with other species of mycobacteria to which cattle are exposed. Furthermore, the onset and magnitude of antibody responses to *M. bovis* varies between animals, so that some animals only produce detectable antibody many months after infection. Studies of antibody responses to PPD in reactor cattle have revealed a much higher frequency of antibody detection in animals with disseminated lesions or pulmonary lesions than in those in which lesions were restricted to the lymph nodes (Ritacco *et al*, 1991). Even when isolated *M. bovis*-specific antigens have been used in antibody detection assays the sensitivity of the assays for detection of infected animals has been poor (Wood and Rothel, 1994).

18. A number of investigators have observed a rapid anamnestic antibody response to the MSP70 *M. bovis* protein in infected cattle following tuberculin testing, including animals with very low levels of antibody at the time of testing (Harboe *et al*, 1990; Wood and Rothel, 1994; Lightbody *et al*, 1998). This prompted the idea that detection of antibody to MSP70 could be used as an auxiliary test to improve detection of infected cattle. However, a recent trial of this assay in Ireland (Yearsley *et al*, 1998) has yielded disappointing results, only 31% of reactor animals with confirmed infection being detected.

19. Diagnostic tests for TB , therefore, have to rely on detection of cell-mediated immune responses to the organism. Infection with *M. bovis* stimulates a complex array of cellular immune responses, a dominant component of which is a Type 1 CD4+ T cell response. The tuberculin test currently used to diagnose bovine TB is an in vivo assay of this response, although the test was developed long before its immunological basis was understood.

The tuberculin test

20. Various versions of the tuberculin test have been used since the early part of this century. The single comparative tuberculin test was adopted for general use in the UK in 1947. At that time, the test involved the intradermal injection at separate sites of purified protein derivative (PPD) prepared from M. tuberculosis and *M. avium*. *M. bovis* PPD was substituted for the M. tuberculosis PPD in 1975, when it was shown to offer superior sensitivity and specificity. PPD is prepared from the supernatants of 10 week liquid cultures of mycobacteria. Proteins in filtered culture supernatants are precipitated with trichloroacetic acid. Re-dissolved precipitates are tested for antigenic potency in guinea pigs, along with a reference standard preparation. The precise

protein concentration of the working dilution of PPD varies from batch to batch but is in the region of 1mg/ml. One hundred microlitres of PPD are administered intradermally. The sites of inoculation are examined for increased skin thickness 72 hours later, as evidence of a cellular immune response.

21. The frequency of herd testing varies according to the annual incidence of herd breakdowns at the parish level, ranging from four-yearly testing in regions where TB is uncommon to annual testing in areas with a high incidence of TB.

22. The interpretation of the tuberculin test is complicated by immunological cross-reactivity of *M. bovis* with other environmental mycobacteria species. It is for this reason that *M. avium* is included in the test, and interpretation of the results is, in most instances, based on detecting an increase in skin thickness in response to bovine PPD that is greater than that observed in response to avian PPD. Monaghan *et al* (1994) states that 8-12% of cattle in Ireland and the UK respond to avian PPD. However most confirmed reactor cattle exhibit responses to *M. avium* as a consequence of the cross-reactivity of the immune responses induced by *M. bovis* (Lesslie *et al*, 1975c, see below).

23. Two different levels of interpretation of the tuberculin test are used in the UK, standard and severe. Standard interpretation is applied to herds with no recent history of TB, while severe interpretation is applied to herds that have had recent confirmed cases of TB, and can be applied retrospectively (at the discretion of the MAFF Divisional Veterinary Manager) to reinterpret a previous test read at standard interpretation, if confirmed reactors are revealed.

24. The criteria for interpreting the test, which have remained unchanged since the use of *M. bovis* tuberculin was introduced in 1975, are as follows:

Table C i

Level of interpretation Applied	Reaction to avian PPD	Classification of response to bovine PPD	
		Reactor	Inconclusive
Standard	-	5mm or more	1-4mm
	+	5mm or more	1-4mm greater than the

		greater than the avian reaction	avian reaction
Severe	-	1mm or more	-
	+	3mm or more greater than the avian reaction	1-2mm, even if up to 2mm less than the avian reaction

The above classification appears to have been arrived at empirically.

25. There is only one report on the detailed skin test reactions observed in infected cattle using the current version of the tuberculin test (Lesslie *et al*, 1975c). The study, which was part of the initial field trials of *M. bovis* PPD, examined skin reactions in 58 cattle confirmed as infected with *M. bovis* by postmortem examination, out of a total of 88 animals that had been slaughtered. Fifty-two of these animals had given positive skin test reactions and 27 inconclusive reactions, at standard interpretation, and a further 9 animals were identified as high risk in-contacts. The skin test reactions to *M. bovis* and *M. avium* PPD in the 58 animals were as follows:

Response to *M. avium* PPD (mm)	Response to *M. bovis* PPD (mm) in excess of response to *M. avium*									
	-2	-1	0	1	2	3	4	5	6	>6
0								1		1
1								1		2
2							2		1	7
3					1	1	1	1		4
4			1	2	1				1	1
5		1			1					3
6	1			1	2		3			2
7	1			1	1				1	2
>7	1								2	6
	Inconclusive					Reactor				

	Severe interpretation

From Lesslie *et al*, 1975

All but two of the animals had reactions of at least 1mm, and 50% of them had reactions of greater than 4mm to *M. avium* PPD; 43 (74%) would have been considered as reactors and 15 (26%) as inconclusives by the criteria currently used for severe interpretation. Of these inconclusives, 5 (8%) had reactions to bovine PPD that were equal to or less than the reactions to avian PPD (which were 5 mm or more in these 5 animals). Unfortunately no comparable data is presented in this study on the reactions in reactors in which infection was not confirmed.

26. Two points are apparent from these data. First , detection of many infected animals relies on measuring small changes in skin thickness, which is likely to be open to error on the part of the operator. Second, it is clear that "non-specific" responses induced by exposure to other species of mycobacteria significantly complicate the interpretation of the test.

Sensitivity

27. The sensitivity of the tuberculin test is defined as the percentage of truly infected animals identified by the test. There are limited reliable data on the sensitivity of the test in the UK or Ireland. This is principally because of the difficulty in obtaining post-mortem data on tuberculin-negative animals, in order to determine the incidence of undetected infected animals. The frequently quoted figures of 77-95% (mean 86%) cited by Monaghan *et al* (1994), based on a number of previous studies of the efficacy of the test, are of questionable value. Several of these studies were carried out before 1975 when the use of bovine PPD was introduced. The sensitivity values reported in the more recent studies ranged from 90% to 95%. However, these studies were based on postmortem examination of small numbers of animals which were predominantly tuberculin-positive. The sensitivity of the test will also be influenced by whether it is read at standard or severe interpretation, and many of the published studies do not indicate the breakdown of reactors into these categories.

28. A recent study by Costello *et al* (1997) in Ireland, which involved post-mortem examination of 2528 cattle from 47 herds selected for depopulation

following large single outbreaks or chronic infection in the herds, provided an opportunity to examine the sensitivity of the test: 317 of the 353 cattle found to be infected gave a positive reaction in the tuberculin test at standard interpretation, indicating a sensitivity of 90%.

29. These limited data indicate that the sensitivity of the tuberculin test, at standard interpretation, may be in the region of 90%. The failure to detect 10% of infected animals would have potentially important implications both for initial detection of infected herds and identification of all infected animals in herds with multiple reactors. One in 10 herds with single infected animals would remain undetected; based on figures for single reactor herds in Great Britain in 1998, this translates into approximately 38 undetected breakdowns. In herds suffering a breakdown, the likelihood that infected animals remain undetected by tuberculin testing would increase as the number of reactor (infected) animals increases, although the sensitivity of the test in this situation is liked to be higher because of application of severe interpretation. Nevertheless, failure to detect all infected animals may contribute to the prolonged nature of some multiple reactor breakdown incidents.

Specificity

30. The specificity of the test is defined as the percentage of truly uninfected animals that are correctly identified (ie. poor specificity results in false positive reactions). Values for specificity can be obtained most reliably by determining the incidence of reactors in TB-free populations of cattle. Such studies indicate that the specificity, at standard interpretation, is well in excess of 99% (Lesslie, *et al*, 1975b; Neill *et al*, 1994). Given the crude nature of the tuberculin test and the requirement to distinguish specific from non-specific responses using two different antigen preparations, the test gives a remarkably high level of specificity.

Re-testing protocol

31. Herds in which reactor animals are confirmed as infected are re-tested at 60 day intervals until they have had two consecutive clear tests based on severe interpretation, and are re-tested again 6-months later at standard interpretation. Herds in which infection is not confirmed in reactor animals are retested after 60 days and, if clear, after a further 6 months, both at standard interpretation. Herds in which inconclusive responses are detected in the absence of reactors are re-tested after 42 days. There appears to be two

reasons for the interval of 60 days following the initial test that reveals reactor animals. Firstly, a delay is required to enable detection of infected animals which at the initial test are in the early stages of infection and have not yet developed tuberculin reactivity. A 30-50 day period is quoted as the time required to develop tuberculin reactivity. However, this interval is based on old data derived from studies using an early version of the skin test (Vallée and Panisset, 1920; Francis 1947).

32. The second reason for the 60 day interval relates to evidence that tuberculin testing results in a period of non-reactivity to subsequent re-testing. Loss of reactivity within a few days of initial testing was described by Kerr *et al* in 1946. A study by Radunz and Lepper (1985),which is often quoted in relation to this 60 day interval, examined tuberculin reactivity upon re-testing in cattle inoculated with killed *M. bovis* in paraffin oil; immunised animals were non-reactive when re-tested 4 and 7 days after the initial test but had regained their reactivity by 60 days. More recently Doherty *et al* (1995) confirmed that naturally infected reactor cattle had decreased skin reactivity when re-tested after an interval of 7 days. Hence, although there is clearly a period of reduced reactivity in infected animals, following an initial skin test, the precise duration of the depressed reactivity has not been determined.

33. Skin testing of experimentally infected cattle has been shown to result in an anamnestic antibody response to the *M. bovis* antigen MPB70, 7-10 days after administration of PPD (Harboe *et al*, 1990; Wood and Rothel, 1994; Lightbody *et al*, 1998). Lightbody *et al* (1998) observed that this response was associated with a marked shift in the immunoglobulin isotype of the antibody from IgG2 to IgG1. As discussed below, an enhanced interferon-g response is also observed 3-6 weeks after skin testing in infected animals (Rothel *et al*, 1992). The observation that a single dose of PPD can stimulate these responses and transiently depress skin test reactivity in infected animals, raises questions about the possible consequences of repeated tuberculin testing. Given that herds that suffer protracted breakdown incidents may be tested 4 or 5 times in one year, and therefore receive 4 or 5 doses of both avian and bovine PPD, it is conceivable that immunological priming could alter the sensitivity or specificity of subsequent tests, or the course of disease in animals that subsequently become infected. This issue has not been addressed experimentally.

Detection of infected animals by post-mortem examination

34. The detection of characteristic pathological changes in reactor cattle at post-mortem examination and/or microbiological culture of *M. bovis* from tissue samples, are required to confirm that tuberculin-positive results are indeed due to *M. bovis*. Such findings have also been used to assess the specificity of the test. Detection of gross pathological lesions at routine meat inspection of carcases is also employed to screen for infected animals in slaughterhouses. 35. Two variables can influence the proportion of reactor cattle identified as infected, namely the numbers of truly false positive cases and the ability to detect those animals that are infected. If the rate of detection of false positives is constant over time and in different regions, the proportion of truly infected cattle in reactors identified by standard interpretation of the tuberculin test should increase as the incidence of TB increases. On the other hand, this may be offset by an increase in the proportion of false positives detected by severe interpretation of the test. Even in groups of animals identified by severe interpretation, the proportion of truly infected animals might be higher in groups made up of multiple reactors from a small number of herds than in groups comprising one or two reactors from many herds. Any analyses of the proportion of reactors that are confirmed as infected need to take these variables into consideration.

36. The disease caused by *M. bovis* in cattle varies in severity, with many natural cases having only a few, small, grossly detectable lesions at post-mortem examination. Moreover, available data indicate the likelihood of confirming infection by microbiological culture from tissue samples, in the absence of grossly visible lesions, is very low. It therefore follows that the proportion of infected animals detected will depend on the thoroughness of the post-mortem examination.

37. Overall, infection with *M. bovis* is confirmed in between 40% and 50% of reactor cattle in Great Britain. The following are examples of the rates of confirmed infection in published surveys in the UK and Ireland:

Table C iii

No. of reactors	No. with confirmed infection	Country/Region	Publication

293	84 (29%)	Ireland	O'Reilly and McClancey 1975
425	165 39%	SW England	Wilesmith 1982
153	74 (48%)	N Ireland	Neill *et al*1996
783	321 (41%)	Ireland	Costello *et al* 1997

Neill *et al* (1994b), cited above, found a higher proportion of infected cattle in animals identified by standard interpretation of the tuberculin test than in animals identified by severe interpretation: 49/74 (66%) compared to 25/79 (32%).

38. A study by McIlroy and colleagues (1986) has provided evidence that a more rigorous post-mortem examination can reveal a higher proportion of infected animals. The lungs and associated lymph nodes of randomly selected reactor cattle were cut into 5 mm slices for visual inspection, and samples from lung and a range of lymph nodes, as well as nasal and tracheal mucus, were subjected to microbiological culture. The rates of confirmed infection were as follows:

Table C iv

Reactor category	No. of reactors	No. with visible lesions/confirmed infection
Standard	39	33 (84.6%)
Severe	16	4 (25.0%)
Total	55	37 (67.3%)

As well as revealing a higher rate of confirmed infection than in previous studies using standard post-mortem protocols, this study also detected a higher proportion of infected animals in reactors identified by standard interpretation than in those identified by severe interpretation.

39. The distribution of lesions and the results of culture of tissues from these animals were as follows:

Table C v

Reactors with visible lesions	Lesions present in		Culture of *M. bovis*	
	Lymph nodes[1]	Lungs[2]	Lesions	Nasal and/or Tracheal mucus
37	37	27 (73%)	37	7 (19%)

[1] Retropharyngeal, bronchial or mediastinal
[2] 78% of affected lungs had only 1 or 2 lesions
32% of lung lesions <5mm in diameter

The frequency of detection of lung lesions in this group of animals is much higher than in animals subjected to routine abattoir post-mortem examination, which usually reveals lung lesions in less than 15% of confirmed cases. However, lesions were still detected more frequently in the lymph nodes. Thus, as with confirmation by abattoir examination, which relies on detecting lymph node lesions to confirm infection in about 90% of cases, inspection of the lymph nodes associated with the respiratory tract proved to be the most rewarding means of confirming infection.

40. The results of this study imply that a proportion of reactors that remain unconfirmed by current abattoir examination are infected with *M. bovis*. Since 40-50% of herd breakdowns involve only one reactor, this would suggest that some currently unconfirmed breakdowns are due to *M. bovis*.

41. Additional evidence that a proportion of unconfirmed incidents are associated with *M. bovis* was presented by Wilesmith and Williams (1987) who showed that in the South West of England the incidence of unconfirmed incidents was significantly higher in parishes with a history of confirmed incidents than in those with no recent confirmed incidents:

TB history of parishes (1972-83)	Unconfirmed incidents/tests	Incidence of unconfirmed incidents/tests	Relative risk of two types of parish
Parishes on annual testing			

- Those with no confirmed incidents	29/1,298	2.23%)	1.89
- Those with confirmed incidents	171/4,054	4.22%)	
Parishes with 2-yearly testing			
- Those with no confirmed incidents	105/15,311	0.68%)	2.56
- Those with confirmed incidents	67/3,841	1.74%)	

These authors concluded that a significant proportion of unconfirmed incidents in areas at risk of TB are likely to be due to exposure to TB. These data, taken together with the postmortem analyses of reactor cattle, suggest that some infected animals remain undetected, both at the level of testing and at post-mortem examination.

Development of improved/alternative diagnostic tests

42. The main shortcomings of the tuberculin test, in addition to the limitation in sensitivity, are the difficulty of standardising reading of the test, because it is applied by a large number of field operators, and the requirement for two farm visits per test. The latter significantly increases the cost of the test. The apparent requirement for a minimum interval of 60 days between tests is also a disadvantage in some circumstances. Attempts have, therefore, been made to develop an alternative in vitro test.

43. Type 1 CD4+ T cell responses can be detected by measuring the proliferation of blood lymphocytes cultured for 4-5 days in vitro with *M. bovis* antigens. However, because this assay requires separation of lymphocytes from whole blood and the use of radioisotopes to measure proliferation, it is unsuitable for use as a routine diagnostic test. An alternative test, involving culture of whole blood with *M. bovis* antigen and measurement of interferon-g (IFN-γ) production by responding T lymphocytes after 16-24 hours, has been

developed in Australia (Wood *et al*, 1991; Wood *et al*, 1992). Since the test uses the same antigen (*M. bovis* PPD) that is employed in skin testing, it also requires a parallel assay using avian PPD to distinguish specific from non-specific reactions.

44. Results of field trials of the IFN-γ test conducted in Australia (Wood *et al*, 1992), the Republic of Ireland (Monaghan *et al*, 1997) and N. Ireland (Neill *et al*, 1994) can be summarised as follows:

Table Cvii

Trial	Tuberculin test		IFN-γ -test		
	Sensitivity	Specificity	Interpretation[1]	Sensitivity	Specificity
Australia	68.1	96.7%	a	81.8%	99.1%
R. of Ireland	nd	nd	a b	87.7% 84.2%	90.6% 96.2%
N. Ireland	83.1%[2]	100%	b	84.3%	99.6%

[1] The criteria for interpretation are described in Monaghan *et al*, 1997
[2]Severe interpretation
nd - Not determined; the animals used to evaluate sensitivity in this study were all tuberculin-positive

45. As for the tuberculin test, values for sensitivity and specificity of the IFN-γ test depend on how it is read, gains in sensitivity being achieved at the expense of losses in specificity (Wood *et al*, 1991; Monaghan *et al*, 1997). Since Australia uses a different version of the tuberculin test, which gives lower sensitivity than in the UK, and because of potential differences in background responses to PPDs, the comparison of the two tests in the Australian trial has little relevance to the UK situation. Moreover, the Irish trial did not include a comparison of the two tests because the animals used to evaluate sensitivity of the IFN- test were all tuberculin positive.

46. The animals used to assess sensitivity of the tests in the N. Ireland trial were also a biased population, in that, in addition to reactors, animals that gave inconclusive results in the previous tuberculin test and animals considered to be at risk of infection were selected for inclusion. Hence, the

values obtained for sensitivity were not considered by the authors to be accurate in absolute terms. However, they provided a valid comparison of the two tests (Neill *et al*, 1994). Overall, the findings indicate that the IFN-γ test can, depending on the criteria used for interpretation, give a level of sensitivity similar to that achieved by the tuberculin test in the UK and Ireland. However its specificity is inferior.

47. Although in the N. Ireland trial, the tuberculin and IFN-γ tests detected similar numbers of diseased animals, the positive populations were slightly different, in that a small number of the IFN-γ animals gave a negative skin test reaction and vice versa (Neill *et al*, 1994a, 1994b). Infection was confirmed in 14 out of 39 skin test-negative IFN-γ-positive animals that were available for slaughter. Of particular interest was the observation that 12 of the 14 diseased animals had given an inconclusive tuberculin reaction at a previous test.

48. Because of the numbers of false positives that the IFN-γ test would generate, it cannot be considered in its current form as an alternative to the tuberculin test. However, there may be circumstances where its use in conjunction with the tuberculin test could be justified.

49. In cattle experimentally infected with *M. bovis* and tested at 14 day intervals using the IFN-γg assay, animals infected with 5 x 105 cfu *M. bovis* developed positive reactions at day 14, whereas animals infected with 5 x 10^2 cfu did not give positive reactions until day 28 (Buddle *et al*, 1994).

50. Rothel *et al* (1992) showed that reactivity in the IFN-γ test is depressed in some infected animals for 7 days following skin testing with *M. bovis* PPD. However, there was a subsequent enhancement of the response peaking at 3-5 weeks after skin testing (Rothel *et al*, 1992). The relative response to *M. bovis* and *M. avium* PPD was unaffected. Tuberculin testing with *M. bovis* PPD did not elicit an IFN-γ response in uninfected animals and did not result in enhancement of the IFN-γ response to either avian or bovine PPD in *M. avium*-infected cattle. Reactivity in the IFN-γ test was shown to be unaltered 10 days after skin testing experimentally infected cattle with both bovine and avian PPD (Buddle *et al*, 1994). These findings indicate that the IFN-γ test could be used in testing protocols in conjunction with the tuberculin test, providing it is not applied within 7 days following skin testing.

Improving the specificity of diagnostic tests

51. The cross-reactivity with *M. bovis* of cell-mediated immune responses induced by other species of mycobacteria complicates the interpretation of the tuberculin test. It is presumably also responsible for at least some of the false positive reactions in the IFN-γ test, although there are no published data on this. Direct evidence that cattle are exposed to a variety of Mycobacteria species was presented by Pollock and Anderson (1997a) who reported that 11 different species were represented in 279 isolates of mycobacteria from cattle tissues:

Table C viii

Species	% of isolates
M. avium *M. intracellulare* *M. scrofulaceum*	46%
M. terrae complex	39%
M. kansasii	8%
M. fortuitum	5%
M. gordonae *M. marinum* *M. xenopi*	1%
M. chelonae *M. phlei*	1%

52. Attempts to overcome the problem of detecting non-specific responses in the diagnostic tests have focussed on identification of antigens that are largely specific to *M. bovis* and stimulate strong immune responses in infected cattle. A number of protein antigens have been identified, based on their ability to stimulate T cell proliferative responses in experimentally infected animals. The peptide sequences within these proteins recognised by T cells have been identified, by screening panels of synthetic peptides.

53. The responses of small groups of reactor cattle to these proteins have been examined using either T cell proliferation or IFN-γ assays. Table C ix below is a summary of the findings:

Table C ix

Antigen	Assay	No. of reactors tested	No. positive	Publication
MPB 70	T cell proliferation	14	12	Pollock *et al* 1994
19 kDa	T cell proliferation	14	7	Pollock *et al* 1994
MPB 57	T cell proliferation	14	4	Pollock *et al* 1994
38 kDa[1]	T cell proliferation	14	12	Pollock *et al* 1994
Esat 6	IFN-γ	14	12	Pollock & Anderson 1997a
Esat 6	IFN-γ	19	18	Pollock & Anderson 1997b
MPB 64	T cell proliferation	18	6	Vordermeier *et al* 1999a
MPB 83	T cell proliferation	18	9	Vordermeier *et al* 1999a
Esar 6	T cell proliferation	18	12	Vordermeier *et al* 1999a

[1] A panel of synthetic peptides was used to detect T cell proliferation to this antigen.

A majority of the reactor animals responded to several of these antigens. However, no one protein detected all reactors.

54. Where different antigens were compared in the same study, it was clear that there was variation between animals in the antigen specificity of the responses, suggesting that by using a combination of antigens it might be

possible to increase the level of sensitivity. Vordermeier *et al* (1999b) have compared a cocktail of 3 antigens (MPB 64, MPB 83 and Esat 6) and a mixture of 7 immunodominant peptides from these antigens, with PPD in both T cell proliferative and IFN-γ assays. However, the protein and peptide antigen mixtures were less sensitive than PPD in both assays: in the IFN-γ test, 27 of 29 tuberculin reactors (93%) were detected with PPD, whereas only 72.4% and 65.2% were detected with the protein and peptide mixtures, respectively. These levels of sensitivity were not significantly enhanced when only the reactors with confirmed infected were considered. These findings indicate that additional *M. bovis* proteins need to be incorporated into the assay. Nevertheless, the results of these studies offer promise for the development of a test with improved specificity, which can be standardised under laboratory conditions. Such a test would be of value for use initially in follow-up screening of breakdown herds to ensure that all infected animals are detected.

Research Priorities

Risk factors in herds that suffer recurrent breakdowns

55. The observation that a majority of herd breakdowns involve only one or two reactor animals indicates that many naturally infected cattle have a limited capacity to transmit infections. However a small proportion of herds suffer recurrent TB incidents involving multiple reactors. These breakdowns cause particular distress and financial hardship to the farmers involved. While the recurrent nature of these breakdowns may be due to repeated introduction of infection from a common source (eg, badgers), the possibility that infection is persisting in the herds needs to be considered. The latter would imply that transmission between cattle is occurring in these herds and that infected animals are not being detected by the tuberculin test.

56. An analysis of a range of risk factors should be undertaken to determine whether or not there are peculiarities in the make-up, management and health status of these herds, that might account for their recurrent TB problem. A detailed analysis of the test history of these farms should also be undertaken to assess the tuberculin reactions of reactors at previous tests. The parameters that should be examined include:

- Herd type and size
- Severity of disease in reactor cattle

- Genotype of *M. bovis* strains
- TB test history including number of reactors at first test, evidence of previous inconclusive reactors and background responses to *M. avium*
- Intercurrent disease

Development of an improved diagnostic test

57. The development of a diagnostic test that detects only those components of the immune response that are specific for *M. bovis* would be a significant step forward. The results obtained in studies with recombinant *M. bovis* antigens have highlighted the possibility of using a mixture of defined antigens in an IFN-γ assay. The utility of such an assay will depend on whether or not most (preferably all) naturally infected cattle respond to one or more of the antigens and whether or not the responses are truly *M. bovis*-specific. If sufficiently sensitive and specific, the assay would be particularly useful for follow-up testing in breakdown herds.

Pathogenesis of the disease in cattle

58. As discussed in the preceding sections, there are several aspects of the pathogenesis of *M. bovis* infections in cattle, relating to transmission of infection and the application of diagnostic tests, that are poorly understood. Current testing procedures are based on incomplete knowledge of the kinetics of infections in cattle, and there is relatively little information on how *M. bovis* is transmitted between cattle. The experimental work that has been carried out on cattle has mostly been with high infectious doses of *M. bovis*, which usually produce fulminant disease. Future work should include the use of a low dose experimental model that results in a spectrum of disease similar to that observed in the field. A clearer understanding of how and at what stage of infection transmission of *M. bovis* occurs between cattle would provide a stronger scientific basis for giving advice to farmers on how to limit spread of infection. More accurate knowledge of the kinetics of the immune responses detected by diagnostic tests could lead to modifications in testing protocols that improve their efficiency in detecting infected animals.

59. The following questions need to be addressed:

- How does infectious dose affect the kinetics and quantity of bacterial excretion?

- At what stage(s) of infection are animals infective for in-contact controls?
- During the infective stage(s), is contact required for transmission of infection and, if not, over what distance can infection be transmitted?
- What are the kinetics of development of reactivity in the tuberculin test and other laboratory-based assays in animals infected with different doses of *M. bovis*?
- How long following an initial tuberculin test do animals remain non reactive to a second test?
- Does repeated tuberculin testing alter the sensitivity or specificity of the test, or the kinetics of disease in infected animals?

Mathematical modelling of *M. bovis* transmission in cattle

60. A reliable mathematical model of the transmission dynamics of bovine tuberculosis would be useful for investigating the consequences of shortcomings in current diagnostic procedures and for exploring how these procedures might be improved. Some of the information required to develop an accurate model would need to be obtained from the proposed studies of pathogenesis outlined above. However, in the short term, an exploratory model would be of value to identify those parameters that need to be examined in more detail in experimental and epidemiological studies.

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APPENDIX D - Economic Studies to Inform the ISG's Work

The Group has recognised from the start that, if it is to offer recommendations on sustainable policies for the control of bovine TB, its proposals must be built on the kind of dependable technical data that the trial and other scientific enquiries currently underway are designed to produce. But they must be sustainable also in the wider sense of being convincing to those involved in the commercial operations of cattle farming; defensible in terms of the financial costs and benefits accruing to the public purse; and consistent with the general perceptions of a society that voices strong concern over aspects of the rural environment.

This implies that by the time we are in a position to construct and evaluate alternative TB control policies we will need appropriate information relating to the economic and financial aspect of the disease and its control. This means studies relating more to the operational farm/sector/countryside level than the more narrowly focused 'scientific' (i.e. natural science) and technical levels of the already established research programmes. These latter studies will allow us to answer questions about proposed policy approaches along the lines of "does it work?", "how will it work?" or "what is necessary to ensure it will work?". The economic studies should be designed to answer questions about "is it worth it?", "to whom is it worth it?", "is this the best way of doing it?" and "supposing we don't do it, what then?". To give useful advice to Ministers we need to have considered some of these non-technical aspects of a control policy. This is not to say that the final choice will be on economic grounds, but only that the economic dimensions are an integral part of the complex issues involved. In the end, regardless of the formal information framework and policy analyses we may be able to present, there will be still a number of political judgements that only Ministers can make.

There are three main types of economic studies that we need to pursue, each adding an essential dimension to our ability to understand and construct what might be "sustainable".

Farm-level effects of bovine TB and its control

A TB breakdown has a potentially severe impact on the profitability of the affected farm business. There will be a direct loss of revenue from reduced milk or animal sales, loss of quality assurance or other price premia, disruption to breeding programmes and the general operation of the farming system, and from movement restrictions imposed until the herd is declared clear. (Even at 100% of the financial value of reactor cattle, compensation will almost always fall well short of those losses.) The magnitude of these revenue losses obviously depends on many individual farm factors (size and productivity of herd, length of time before clear tests, type of farming system, etc).

In addition there will be additional expenditures exacerbating the financial damage to the farm business - unplanned feed and husbandry costs associated with the forced holding of animals, costs of conducting further TB tests, ultimate acquisition of replacement animals, etc. Added to those are an array of possible avoidance costs that may be incurred by farms in response to the threat of a breakdown when it is known there is a TB risk in the area; like all insurance premiums these are real costs, even though an actual event may not occur.

In simple terms the sum of these financial effects on a farm business - "the farm level costs of a TB breakdown" - are by the same token the benefits to be gained by avoiding a breakdown. This is therefore essential information in evaluating the economic implications of any proposed control policy predicted to reduce the number of breakdowns, for it provides a first (although limited) estimate of the benefits to be set against the cost of the control measures. In the context of a political concern over the impacts of TB on farmers' incomes, it is a prime area of information need.

We know very little about this aspect. The National Farmers' Union study which suggests a TB breakdown costs an average of around £36,000 per farm does not have a reliable provenance in terms of research methodology (self-selected sample, restricted information framework, unverifiable responses). An independent and rigorous survey of, say, 150 breakdown farms structured to reflect size and type of farm and breakdown history, collecting conventional farm management data on costs and revenues of the breakdown event is essential to fill the void and provide a basic data set for economic analysis and any further modelling of policies.

Economics effects of TB in the agricultural sector

From an economic standpoint it is not the personal incomes of farmers that is the primary issue of cattle TB. Rather, it is the implications of TB incidents on the wider agricultural economy (the 'national farm') and its cattle livestock sector. If private and public resources are to be employed via some policy action specifically to control cattle TB, what are the aggregate private and public gains that are expected to arise as a result of that?

To answer this key question requires a rather wider and more intricate economic study. A framework needs establishing to identify all the negative effects in the UK economy of the existence of TB in its cattle herd (at current, projected and policy-controlled levels perhaps) and then to estimate the economic valuations they carry. The range of these effects is broad, not always obvious and covers direct and indirect impacts - from human health threats and consumer concerns over food safety at one level to the adjustments in farm production and resource use at the other, but including also public sector costs of disease monitoring, testing and the other veterinary reactions, coupled with any wider implications to the cattle economy due to trade effects and constraints on regional development or responding to changing market demands. This could develop into a fairly detailed conceptual and empirical modelling exercise, whose purpose is to allow a 'full' economic assessment of the benefits of TB control policies. The presence of price distortions due to agricultural policy interventions will need to be accounted for in an attempt to identify the real economic value of the components considered.

A second component is a detailing of the economic costs of those actual and potential intervention measures (culling badgers, vaccinating cattle, doing nothing, no-cattle areas, continuing research expenditures) that might underpin a sustainable policy for TB control. MAFF data will be the prime source of information for the public sector expenditures, the farm survey study should enable aggregate estimates of the private producer costs, and wider explorations will be necessary to include the more tangential and indirect implications.

Such a study lies at the core of any economic evaluation of public policies for TB control, for identifying the 'best' one or assessing the cost-effectiveness of a restricted set that are in some sense most acceptable/appropriate. There are standard economic methodologies for putting together the cost and

benefit streams in making these assessments. It is the structured research on compiling the detailed database to underlie the final evaluations that is lacking.

The ecological economics dimension

The studies suggested above reflect the conventional micro and macro-orientations (firm level, sector level) of economics research. They are based on viewing the costs and benefits of cattle production, and its associated disease management actions, in the context of agricultural products which have a market value and resources which have a market value (perhaps distorted by policy or other interventions, but nevertheless having a recordable base of data). However, in the particular case of bovine TB there is this difficult element of the possible role of wildlife as a disease vector, and hence their control/elimination as part of a control policy. Wildlife are the classic 'public goods', things which have an economic value which is not reflected in a market price because they are not bought and sold by groups/individuals. They are genuine economic commodities (because people attach value to them and are prepared to incur financial or other costs to preserve/protect/gain more of them) and so must be included in any comprehensive economic evaluation; but they are not commercial commodities, and so an everyday market system does not throw up the data that reflects their valuation relative to everything else. (The fact that they do not enter into the accountant's calculations does not mean they can be excluded from the economist's.) It is desirable to have some idea of an economic weighting that can be attached (as a cost) to the badgers that are killed, or (as a benefit) to those that are not killed, as a consequence of particular disease control options that are being assessed. Without this the economic characterisation of any policy will be incomplete and inconsistent, giving no indication of how 'ecological' values and 'monetary' values are to be considered together.

Although difficult, the implicit valuation of non-market goods is not impossible and economic methodology has developed substantially over the past 10-15 years in discerning the values that society places on things - environmental features, access, countryside amenity, etc - equivalent to that which it would express through a market if such a thing existed. These methods generally are based on the concept of "willingness to pay" (which is precisely what a market records) but derived through techniques of preferences expressed and

revealed by an appropriately structured socio-economic inquiry. Given the emotive and special interest prominence attached by some people to the badger as a wildlife species, while others range from being less frantically concerned to being totally indifferent, it would seem important to attempt to quantify the overall economic value that might be attached to the badger as a resource that is 'used up' (i.e. killed) in a TB control policy. This is a conceptually and empirically difficult study, for which there is no direct precedent to act as a model and so it would involve original research into the methodology of 'the economics of wildlife' - an area that will gain increasing importance in rural resource use in the future. However, we think it to be an important component in the broad research prospectus that the ISG is fostering. The aim is not to reduce the whole evaluation or selection of policies to an accountants formula. Rather, it is to broaden the base of the analysis of any proposed control policy; and to ensure the information base contains estimates of value that are not reflected in market price/cost data, as well as those from more conventional accounting sources.

All three research areas listed need to have delivered results by the time the Group is in a position to prepare and examine possible sustainable TB control policies.

APPENDIX E - Possible Action outside Trial Areas - Advice to Ministers

BACKGROUND

1.The Group is pleased to respond to the Minister's invitation to take stock and consider whether any steps might be taken outside of the trial areas that might control the rising incidence of TB in cattle.

2.The Group has considered whether the trial should be:

a. run as an isolated approach in trial areas alone but combined with a pre emptive culling strategy applied outside those areas (i.e. a combined strategy);
b. replaced by pre-emptive culling in areas of high incidence of TB in badgers (i.e. a replacement strategy.)

3.The Group is implementing a strategy based on the conclusions of Krebs. This strategy recognises that while available scientific evidence suggests

badgers are involved in cattle TB incidents, it is not presently possible to quantify their involvement or to evaluate the benefit of badger culling. In such circumstances, it is difficult to defend the routine culling of badgers as a disease control policy.

4.The Group has, therefore, advised Ministers to put in place an integrated and open-minded approach to consider what contribution the badger makes, along with other wildlife and cattle, to TB in cattle. The emphasis is on securing a clear understanding of the epidemiology of TB in cattle and other possible wildlife reservoirs in order that future cattle TB control policies can be rationally based.

PRE-EMPTIVE CULLING OUTSIDE TRIAL AREAS

5.In summary, pre-emptive culling outside trial areas would be inconsistent with the objectives and methodology of the trial programme the Group has established. If pre-emptive culling outside currently identified trial areas were to take place in areas of high cattle TB incidence, it would seriously compromise the scientific approach adopted by the Group and effectively nullify the whole trial programme. In order to meet its objectives the trial needs to be located in areas of high TB incidence in cattle; however, the majority of these trial areas have yet to be identified. Any intervention to cull badgers in the potential trial areas would, therefore, render them useless for trial purposes and hence would undermine the trial programme. In addition, it would send a message to farmers inside and outside trial areas that the badger was 'culpable', thereby encouraging many (including those within the "survey only" areas of the trial) to take action into their own hands e.g. by illegally killing badgers. Widespread illegal culling within triplet areas could compromise the integrity of the trial.

6.Furthermore, pre-emptively culling badgers anywhere outside the trial would be inconsistent with the open-mindedness and objectivity that Ministers insisted the Group take in approaching this complex problem, since the philosophy of the trial is that it is currently impossible to assess whether the badger is, or is not, a significant factor in the cattle TB problem. While we recognise the serious dilemma that Government faces as a result of the rising incidence of cattle TB, and that Ministers might wish to adopt the "precautionary principle" and anticipate the possible trial findings on the extent of the badger's contribution, we believe that this would be premature, possibly

ineffective and in the longer run damaging. We are unanimous in considering this undesirable.

PRE-EMPTIVE CULLING IN AREAS OF HIGH INCIDENCE OF TB IN BADGERS

7.The Group has considered in depth whether a pre-emptive culling strategy could be applied as an alternative to the trial in areas of high incidence of TB in badgers i.e. a replacement strategy.

8.It is important to appreciate at the outset that there is a major conceptual difference between the replacement strategy approach and that of the Group. Proponents of the replacement strategy make the assumption that badgers are the principal source of infection for cattle, that they contribute considerably to outbreaks of TB in cattle, and that culling badgers is a cost effective means of controlling TB in cattle. This conflicts both with the conclusions of the Krebs report (on which Government policy is based) and with the guiding presumption of the trial programme, namely that the extent to which badgers contribute to TB in cattle remains to be determined. It is possible that the badger removal operations of the past 25 years have been based on a false assumption.

THE REPLACEMENT STRATEGY AS A SCIENTIFIC INVESTIGATION

9.Advocates of the replacement strategy have suggested that it differs little from that of the ISG, although they generally accept that it does not have the same degree of scientific rigour (rigour which, they argue, is unachievable in a field experiment).

10.The Group does not agree that scientific rigour cannot be achieved in a field trial. In addition, it does not accept the assertion that the differences between the replacement strategy and the trial are minor. There are of course some features that are, or could be, common to both approaches. For example:

a. accumulation of data on badger density, social group structure, TB prevalence in badgers (but only in areas of assumed high badger TB prevalence where it is suggested pre-emptive culling should be targeted).

b. risk analysis could be carried out through the use of an epidemiological questionnaire.
c. an ecological impact assessment could be undertaken.
d. collateral research (e.g. investigation of TB infection in other wildlife species, validation of observational methods for estimating badger density etc.) could be carried out in pre-emptive cull areas.
e. the use of cage-traps only for catching badgers.
f. a no-cull period to reduce cubs' deaths in setts.

11.However, as well as the conceptual difference highlighted at paragraph 8 above - concerning the presumed role of the badger - there are other major differences. These relate to the method of selection of triplet areas in the trial and the pre-emptive culling areas proposed as part of the replacement strategy, and the central importance, in the Group's proposals, of the adoption of controls and randomisation. These features are key to the avoidance of misleading conclusions and possible wrong policy choices subsequently.

12.The Group identifies triplets for the trial areas on the basis of specific criteria. These include surface area, a minimum number of holdings and in particular the immediate and past history of cattle TB breakdowns, for which there are reliable, current data. It is anticipated that ten triplets will be enrolled into the trial.

13.By contrast, the replacement strategy proposes that pre-emptive culling should centre on "areas of high badger TB incidence", the assumption being there are "foci" of high and low TB prevalence in badgers. However, it is unclear how such areas are to be identified given the data that are available. Post-mortem data from Road Traffic Accident (RTA) surveys and badger removal operations are the only source of information on prevalence of disease in badgers. These data are limited in both space and time, and are subject to possibly significant bias. Since the RTA survey was suspended in 1990, data on current TB prevalence in badgers are not available. TB in cattle has spread considerably (and unpredictably) in this time, and it is possible that the distribution of TB in badgers has changed in a similar manner. Consequently, there is no rigorous methodology that could be applied to the selection of areas for pre-emptive culling.

14.The proposed replacement strategy raises other fundamental questions that are left unanswered. What TB prevalence would be sufficient to trigger a

cull? What prevalence would define the edge of the culling area? Where would the culling stop (in space and time)?

15.The absence in the proposed replacement strategy of comparable "survey-only" (i.e. no culling) control areas eliminates the scientific rigour necessary to quantify the contribution of badgers to cattle TB, or properly to evaluate different culling strategies as future policy options. It is claimed that the "next" identified pre-emptive areas would provide a contemporary control, but these would not be randomised and could not provide a control over time. At best the outcome would be that while a crude, qualitative assessment of the contribution of badgers to TB in cattle might be made, the quantitative effects could not be determined. Hence the proposal amounts essentially to a speculative "clearance strategy", not a structured trial designed to resolve the outstanding questions that have characterised the badger and bovine TB problem for the past 25 years.

WHAT DOES THE KREBS/BOURNE STRATEGY PROVIDE WHICH THE REPLACEMENT STRATEGY DOES NOT?

16.The Krebs/Bourne approach yields a number of benefits, including:

a. a scientifically based approach to understand the role of various factors in the development of TB which will provide Government with the information to consider a greater range of policy options for the future.
b. an approach with reflects current Government guidelines on basing policy on sound science.
c. an objective assessment of the contribution of cattle, badgers and other wildlife to cattle TB incidents with the aim of controlling TB in cattle, not conditioned by the prior assumptions concerning the role of the badger.
d. an assessment of reactive culling as a possible future policy option.
e. an economic assessment of the costs and benefits of alternative approaches, including badger removal measures.
f. collation of scientific data from outside trial areas in later stages of the study.

LOGISTICS AND COST

17.The proposed Krebs/Bourne trial will cover 3,000 sq. km of the South West of England, plus buffer zones, comprising about 10% of the south West (31,210 sq. km in total). Proactive culling will take place in a third of the trial area and reactive culling will also cover a third of the trial area. However with an assumed breakdown rate of less than 30% of the herds in the reactive treatment areas badgers will be culled from not more than 4% of the total South West land mass in the proactive and reactive trial areas.

18.The field programme envisaged for the trial will employ 60 MAFF Wildlife Unit (WLU) staff for the first two and a half years of the trial in order to complete the proactive cull (ten triplets) and concurrently carry out the reactive culls. The final two and a half years will require a lower intensity of culling in the trial areas, but studies outwith the trial areas during this period will involve WLU staff in collecting data from TB breakdown and control farms in addition to trial area incidents.

19.By contrast under the proposed replacement strategy, 12% of the land area of the South West would be pre-emptively culled. A one-off cull with no repeat surveying after the initial pre-trapping survey is outlined in the proposal. It is logistically possible that the goal of completing two proactive culls per year and culling 12% of the South West land mass over a five year period is not unrealistic using the same complement of WLU staff used in the trial. The costs of the proposed replacement strategy fieldwork would not, however, be less than the field trial, and may be **more** because of the culling intensity over the full five years.

20.Were the replacement strategy proposal to be accepted and implemented concurrently with the field trial, the competition for resources could seriously compromise the field trial and the proposed extension of investigations outside the trial areas. It would also take time for staff to come on stream; the training requirements for surveying, trapping and humanely disposing of badgers are considerable.

21.It is impossible to be precise about the number of badgers that would be killed, but on a straightforward projection based on the area covered, the replacement strategy approach would result in up to three times as many badgers being culled as in the field trial.

CONSIDERATION OF THE REPLACEMENT STRATEGY PROPOSAL AS A POLICY OPTION

22.In view of the acceptance that the replacement strategy proposal would not have scientific rigour, and therefore would not offer the opportunity to resolve the long-standing questions over the nature and quantitative significance of badger involvement in cattle TB, the Group has considered its likely impact simply as an attempted disease control policy. In summary:

a. the replacement strategy proposal is based on the twin assumptions that the badger is a major source of infection for cattle, and that the culling of badgers is the most cost effective way of dealing with this. This questions Professor Krebs's conclusions and the Government's decision to implement his recommendations;
b. a pre-emptive cull as proposed in the replacement strategy without any attempt to build in scientific rigour would repeat the omissions of the past. It could be designed to provide more high quality data than earlier large-scale culls (e.g. Thornbury) and might also provide an indication whether badgers contribute to TB in cattle. However the central question - what is the quantitative significance of the badger? - would not be answered. As a result of this, the development of future policy options and the economic evaluation of their potential merits would be severely constrained;
c. because the pre-emptive cull would be focused on specific areas it is inevitable that large numbers of farmers would feel left out and that their own problem was not being addressed. Even if the assumption on the role of badgers were to be proved correct, the replacement strategy approach is very limited in scale and would likely do little to reduce TB incidence across the wider South West region in either the short term or over the longer term. The resulting demands would be for an even more widespread cull (a reactive strategy?). It is not clear where culling would end - at a point in time or at Land's End? This emphasis could encourage farmers more widely to take the law into their own hands;
d. consideration must be given to the influence on cattle TB incidence of repopulation of pre-emptively culled areas. Proponents of the replacement strategy assume that these areas would be repopulated by relatively TB-free badgers, but there is no evidence for this and it must be open to question. A one-off pre-emptive cull would be likely to remove up to 80% of the badger population. One cannot predict how long repopulation would take. It is likely to be shorter than the 10 years or so claimed following the Thornbury cull, where virtually all badgers

were eventually removed as a result of an intensive three-year gassing programme;

e. there is no evidence to support the claim made for the replacement strategy that fewer badgers would be culled than in the Krebs trial or that Government costs on TB control would be reduced; and
f. the message that would be given to conservation groups in the UK and Europe - if unrestricted badger culling was being allowed, at the same time that the Government accepted there was inadequate scientific evidence for involvement of badgers in cattle TB breakdowns - would invite very predictable reactions.

OTHER ISSUES

23.The Group has considered a number of other options including the use of TB 99 outside trial areas, the design of the RTA survey and proposals for cattle free zones and "firebreaks", put to the Agriculture Select Committee, and its views are as follows:

a. the new TB 99 questionnaire should be applied nationally as soon as practicable, but its primary benefit in the short term will derive from the detailed data gathered from breakdown farms and controls in the trial areas;
b. the new RTA survey, which is an integral part of the trial programme, should proceed on the basis recommended, so that its findings on the distribution, and possibly prevalence of TB in badgers can be validated. It would not be appropriate to extend it beyond the seven counties at this early stage, although the RTA survey's coverage could be regularly reviewed; and
c. because of the lack of quantitative data on the contribution of badgers and other wildlife to cattle TB breakdowns, it is not possible to evaluate the benefit of some of the recent proposals relating to cattle free zones and "firebreaks". However, the Group believes there would be merit in exploring the various "cattle-based" options with interested parties, to see if any practical benefits could be gained.

COMPLEMENTING THE TRIAL - FUTURE POLICY OPTIONS

24.It has been clear from the outset that in the short-term neither farmers nor environmentalists were likely to be appeased by the Krebs/Bourne proposals. It must remain a priority for Government to explain to all interested parties that

the trial is a carefully thought-through attempt to find sustainable answers to a difficult problem.

25.Planned, targeted meetings with farmers, conservationists and other pressure groups are necessary to get across the objectives of Government policy and the logic of the trial study. Some useful work has already been done. A more general public relations strategy incorporating these meetings, leaflets and publications, and broadcast media could be built up.

26.The precautionary principle could be adopted by the Government in respect of immediate implementation of measures to reduce cattle to cattle transmission, together with consideration of the "cattle" options that have been proposed. This would serve to boost the fourth element of the Government's five point TB research and control strategy:

a. protection of public health
b. vaccine development
c. research into transmission
d. strengthening cattle controls
e. the field trial

and provide an obvious platform for interested parties (industry groups, environmentalists, veterinarians, scientists, government officials, etc.) to meet and discuss and plan future options. We recommend such a forum be instigated, and would be happy to play an appropriate part in it.

CONCLUSION

27.The Group believes killing badgers outside trial treatment areas should not be sanctioned. It is possible, dependent upon available data, that as the trial continues this policy option could be reconsidered. In the meantime we advocate strengthening cattle controls together with methods for further improving understanding about, and co-operation with, the trial.

APPENDIX F - Summary of MAFF-funded TB research work

COMMITTED MAFF TB RESEARCH 1998 to 2004

	RESEARCH TITLE and contractor	START DATE	COMBINED COST 1998/1999 TO 2003/2004 INCLUSIVE
A	Molecular typing of *Mycobacterium bovis* **VLA**	01/01/97	81,443
B	An epidemiological study of a badger population naturally infected with *M. bovis* **VLA**	01/04/98	86,262
C	Longitudinal study of natural *Mycobacterium bovis* infection in badgers **VLA**	01/04/98	135,727
D	A spatial analysis using GIS risk factors associated with TB incidents in cattle herds in England and Wales **VLA**	01/01/99	173,308
E	Ecological correlates of tuberculosis incidence in cattle **Warwick Univ**	01/07/99	374,181
F	Multivariate analysis of risk factors affecting incidence of TB infection in cattle **Royal Veterinary College**	10/05/99	37,563
G	Multivariate analysis of risk factors affecting tuberculosis incidence in cattle herds - phase 1 **VLA**	01/04/99	137,479
H	Improved diagnostics for cattle **VLA**	01/04/99	511,347

I	Quantification of the risk of transmission of bovine TB from badgers to cattle withinlocalised areas **VLA**	01/04/99	167,504
J	Integrated modelling of *M. bovis* transmission in badgers and cattle **CSL**	01/04/99	890,769
K	Detection and enumeration of *M. bovis* from clinical and environmental samples **VLA**	01/04/99	297,046
L	The risk to cattle from *M. bovis* infection in wildlife species other than badgers **Oxford Univ**	01/05/99	960,052
M	The risk to cattle from wildlife species other than badgers in areas of high herd breakdown risk **CSL**	01/10/99	588,135
N	Development and evaluation of strain typing methods for *M. bovis* **VLA**	01/10/99	1,109,682
O	Understanding the route of TB transmission from badgers to cattle **Bristol Univ**	01/10/99	266,941
P	Assessment of the humaneness, efficacy, useability, and non-target risk of padded foot cuffs **CSL**	01/11/98	58,000
Q	Assessment of the validity of the current necropsy protocol to detect tuberculosis lesions in the badger **VLA**	01/11/98	39,002
R	Modelling badger populations, the epidemiology of natural infection with *M. bovis,* the risk of spread to cattle **CSL**	01/04/98	132,532
S	Perturbation study (culture and serology for *M. bovis* carried out at VLA) **VLA**	01/06/98	9,027
T	Developing innovative methods to estimate	01/04/99	882,089

	badger population density **CSL**		
U	An integrated study of perturbation, population estimation, modelling and risk **Oxford Univ**	01/04/99	1,252,592
V	Novel methods of estimating badger numbers in the wild **Bristol Univ**	01/07/99	230,426
W	The effect on viability of *M. bovis* of freezing samples prior to cultural testing **VLA**	01/09/98	30,646
X	The development of animal models to test candidate vaccines for *M. bovis* infection in badgers (continuation of earlier trial) **VLA**	01/04/98	202,445
Y	Blood tests to distinguish vaccinated from TB-infected cattle; IFN- assay to improve diagnosis in reactors **VLA**	01/04/98	374,924
Z	*TB Animal Vaccine Programme Advisor - Dr J Colston*	23/11/98	10,000 (annual cost)
AA	Development of vaccine candidates for protection of badgers against infection with *M. bovis* **VLA**	01/04/98	248,573
BB	Genome sequence analysis of *M. bovis* **VLA**	01/01/99	823,273
CC	Antigen presenting cells and T cell responses to Mycobacterium **IAH**	01/04/99	1,200,000
DD	Generation of vaccine candidates against *M. bovis* **VLA**	01/04/99	1,566,005
EE	Testing of vaccine candidates for bovine tuberculosis using a low dose aerosol challenge guinea pig model **VLA**	01/07/99	1,068,045

FF	Development of badger vaccines **VLA**	01/04/99	299,525
GG	Development of badger immunological reagents **VLA**	01/04/99	419,560
HH	Development of a turf model to assess the biological control of *M. bovis* using Mycobacteriophage **Centre for Applied Microbiology and Research**	01/07/99	80,000
II	Testing TB vaccines in cattle **VLA**	01/04/99	1,316,635
JJ	Badgers and bovine tuberculosis a proactive strategy for the control of bovine tuberculosis in badger populations **York Univ**	10/11/97	77,133
KK	An ecological and epidemiological study of a badger population naturally infected with *M. bovis* **CSL**	01/04/98	278,408
LL	Assessment of the potential impact of the large scale removal of badgers **CSL**	06/1998	4,912
MM	Ecological consequences of removing badgers from an ecosystem **CSL**	01/02/99	1,000,810
NN	Development of badger DNA fingerprinting - pilot study **CSL & Sheffield Univ**	02/1999	13,614
	Total research cost 1998 - 2004		17,435,615

November 1999

Key

VLA - Veterinary Laboratory Agency

CSL - Central Science Laboratory

APPENDIX G - Glossary of Key Terms

BCG

"Bacille Calmette Guerin" - a changed strain of *M. bovis* developed by the Drs Calmette and Guerin in the early part of this century, repeatedly sub-cultured until it became non-virulent. Used since the 1920s as a vaccine to protect against human tuberculosis.

Breakdown

MAFF define a breakdown (or a TB incident) as occurring when one or more reactors are revealed by the tuberculin skin test or when disease is suspected in either live cattle showing clinical disease or in carcases with lesions at post-mortem examination.

Buffer zone

an area separating different trial areas and different triplets. There are inner and outer buffer zones; these are explained in Section 4.5. Data on the incidence of TB in cattle in the buffer zones will not be included in the main analysis of the trial.

Confirmed breakdown

a herd breakdown where the disease has been confirmed in one or more animals, usually reactors, by detection of lesions at post~mortem and/or through culture of *M. bovis*.

Dirichlet tesselations

technique used in the delineation of likely badger territories, based on the location of main setts. Dirichlet tessellations are polygons, each centred on a main sett; every point within the polygon is closer to its own main sett than to neighbouring setts.

Epidemiology

the study of the distribution and dynamics of disease in a population. Its purpose is to identify factors which determine the occurrence of disease, and to provide a basis for intervention programmes. Epidemiological methods are also used to assess the variance, severity and magnitude of disease and related risks.

Gamma interferon

a product of white blood cells generated during an immune response.

Genome

the genetic composition of a cell or individual.

Genotype

the distinctive DNA fingerprint distinguishing one individual from another.

Incidence

the rate at which new cases of infection arise in a population.

Incident

(see breakdown)

Multivariate (or multifactorial)

a general term for the many methods of analysis important in investigating multiple variables.

Mycobacterium

a family of related bacteria characterised by a lipid~rich waxy coat that results in acid fast staining, which include species that cause TB.

Pathogenesis

the processes within an individual involved in the development of disease.

Perturbation

disruption of the social organisation or spatial structure of badger populations, such as that caused by culling.

Power (statistical)

the probability that a difference between treatments will be detected given a particular magnitude of underlying difference between them.

Prevalence

the proportion of a population infected at a particular time.

Randomised field trial

technique for comparing treatments in which specific treatments are allocated to trial areas by physical randomising device in order to avoid allocation biases and to ensure comparability.

Reactor

animal which gives a positive result (i.e. 'reacts') to the tuberculin skin test.

Sensitivity

the proportion of true positives detected by a diagnostic method.

Sett

burrow system which badgers use for shelter and breeding.

Social group

group of badgers (averaging six to eight in a group, although a maximum of 25 has been recorded) occupying one or more setts within a well defined territory from which badgers of other social groups would be excluded.

Specificity

the proportion of true negatives detected by a diagnostic method.

Spoligotyping

spacer~oligonucleotide typing (a molecular typing technique), used to distinguish different strains of the TB organism.

Strain

isolate of a bacterial species which is differentiated from other isolates of the same species by particular characteristics.

Treatment

term used to refer to the relevant action, i.e. proactive culling, reactive culling or survey only, which will be applied in the trial areas. Each triplet has three trial areas and each trial area will be subject to one of the three different treatments

Triplet

group of three trial areas, each subject to a different treatment. Within each triplet, one area will be allocated to proactive culling, one to reactive culling and one to survey only.

Tuberculin

a sterile protein extract derived from the tubercle bacterium and used to diagnose TB in cattle by skin testing (also known as Purified Protein Derivative or PPD).

APPENDIX H - Discussions with Interested Parties and Participation in Meetings and Conferences

1.Agriculture Select Committee oral evidence session - 16 February 1999

2.Organisations met:

British Veterinary Association
Countryside Council for Wales
Department of Agriculture Northern Ireland
English Nature
Micropathology Limited
National Assembly for Wales - Agriculture Department
National Farmers Union
National Federation of Badger Groups
National Trust
Royal Society for the Prevention of Cruelty to Animals
Royal Society for the Protection of Birds
Wildlife Trusts

3.Public Meetings and Conferences attended:

Triplet B - Open Meeting - Launceston - December 1998
Farmer-organised Open Meeting - Berkeley - February 1999
Triplet C - Open Meeting - Launceston - April 1999
Triplet D - Open Meeting - Leominster - June 1999
VLA Conference - Scarborough - September 1999
RASE Seminar - Shepton Mallett - October 1999
RASE Seminar - Warwickshire - October 1999
Triplet E - Open Meeting - Wootton Bassett - November 1999
Country Landowners' Association meeting - Seale-Hayne - November 1999
Mammal Society symposium - London - November 1999
London School of Hygiene and Tropical Medicine seminar - London - November 1999

APPENDIX I - Membership Of The Independent Scientific Group On Cattle TB

Professor John Bourne MRCVS CBE (Chairman) - former Professor of Veterinary Medicine at the University of Bristol (1980 - 1988), former Director of the Institute for Animal Health and Professor of Animal Health at the University of Reading (1988 - 1997), and Professor of Animal Health at Bristol since 1988.

Dr Christl Donnelly (Deputy Chairman) - Head of Statistics Unit at the Wellcome Trust Centre for the Epidemiology of Infectious Disease at the University of Oxford.

Sir David Cox FBA, FRS - Honorary Fellow of Nuffield College, University of Oxford since 1994.

Professor George Gettinby FRSE - Professor in the Department of Statistics and Modelling Science at the University of Strathclyde.

Professor John McInerney OBE, FRSA, FRASE - Glanely Professor of Agricultural Policy and Director of the Agricultural Economics Unit at the University of Exeter.

Professor Ivan Morrison FRSE - Head of the Division of Immunology and Pathology at the Compton Laboratory of the Institute for Animal Health.

Dr Rosie Woodroffe - Lecturer in Biological Sciences at the University of Warwick.