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**Bovine TB
Special Edition**

GVS
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Food and Rural Affairs

The Government Veterinary Journal (GVJ) is the official journal of the Government Veterinary Service and those who support its work or have an interest in state veterinary medicine. It is compiled and produced by the Department for Environment, Food and Rural Affairs (Defra) for, and on behalf of veterinary surgeons and those who support them across all parts of Government.

Its key aims are to enhance the contribution of veterinary expertise within and across government, promote the work of Government veterinary surgeons and provide a range of technical, factual and interesting articles in the fields of:

- Disease control
- Animal welfare
- Public health
- Consumer protection

It is intended that the GVJ will also highlight progress in relation to the Animal Health and Welfare Strategy and focus on issues identified by the Veterinary Head of Profession in Government.

Editorial Board

Editor:

Linda Smith
Defra
Block A Government
Offices
Coley Park
Reading
Berkshire
RG1 6DT

Board:

Jose Bis
Martyn Blissitt
Wyn Buick
Adrienne Conroy
Paul Gethings
Andrew Gresham
Angela Jones
Sivam Kumar

Alex Morrow
Richard Rowland
Linda Smith

Production Team:

Peter Green
Angela Greenaway
Emily Thorne

Correspondence, including distribution inquiries and requests for additional copies should be sent to:

GVJ Production Team
Internal Communications and
Stakeholder Management Team
Department for Environment
Food and Rural Affairs
Area 206
1a Page Street
London
SW1P 4PQ

Tel: 020 7904 6482
Email: GVJ@defra.gov.uk

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Front Cover: State Veterinary Service vet recording details about the tuberculosis inspection.

Contents

Foreword	4
1 TB policy developments	5
2 Some lessons from the history of the eradication of Bovine Tuberculosis in Great Britain	11
3 Bovine Tuberculosis in the European Union and other countries: current status, control programmes and constraints to eradication	19
4 Bovine TB: Modelling and predicting its distribution in GB using CTS data	46
5 The laboratory diagnosis of Bovine Tuberculosis	53
6 The introduction of pre and post-movement TB testing in Scotland for cattle from high incidence TB areas	58
7 Ante mortem diagnosis of Bovine Tuberculosis: the significance of unconfirmed test reactors	65
8 The BOVIGAM® assay as ancillary test to the Tuberculin skin test	72
9 Tuberculous pneumonia and BVD in housed calves	81
10 TB in domestic species other than cattle and badgers	87

Are you interested in writing for the GVJ?

Ideally, articles should be 1,000-3,000 words long, preferably with related illustrations. Full instructions for authors are available from any board member or the Production Team. Subject matter should be related to Government veterinary medicine in its broadest sense, and you do not have to work for Defra or the State Veterinary Service to be a contributor to the Journal. Articles for consideration can be submitted through any board member or the Editor (via the Production Team), a full list of which appears on the preceding page.

Foreword

I am proud to present to you the first Government Veterinary Journal bovine TB special edition. I am sure you will both enjoy it and learn from it; contributions have been made by many of the UK's leading authorities on bovine tuberculosis. The first line of the first article sums up the importance of this special edition; bovine tuberculosis is the most difficult animal health problem we face in Great Britain today. This opening article eloquently summarises policy in such a difficult area and includes reference to current disease trends, and policy changes during the last few years.

How can I summarise such a diversity of papers all on one subject? Andrew Proud presents a historical perspective on the virtual eradication of bovine tuberculosis in days gone by. Putting the experience of Great Britain in perspective, Ricardo de la Rúa-Domenech then describes the situation in other countries in a long but very useful article.

The following item, by Andy Mitchell, describes how Cattle Tracing Scheme (CTS) data can be used for disease modelling purposes, and the laboratory diagnosis perspective is represented by Keith Jahans' and Danny Worth's article.

Closely linked to the opening policy position paper, Martyn Blissitt discusses the introduction of pre- and post-movement testing in Scotland. The subsequent article helps explain the significance of unconfirmed TB test reactors and is of particular value to staff working in the field. Martin Vordermeier et al then explain the significance and importance of the BOVIGAM® test.

Finally, and on a more specific basis, Bob Monies describes an on-farm incident involving tuberculous pneumonia in calves, and Wyn Buick has adapted an existing document which originated from Ricardo de la Rúa-Domenech which outlines bovine tuberculosis in domestic species other than cattle.

Putting together a TB special has been a considerable challenge and we have attempted to provide a balance of articles ranging from the highly scientific to the historical. We would not, however, wish to mislead the reader. This is by no means the sum total of knowledge about bovine tuberculosis; it is, rather, a range of articles which address some important aspects of the disease.

I certainly believe that this edition provides significant Continuing Professional Development value for all readers throughout the Government Veterinary Surgeons network. We all benefit from a reminder of the science surrounding TB epidemiology and diagnosis and it should be noted that, where indicated, the authors hold an abundance of references or further reading material information which could not be included in the GJV.

My grateful thanks to the Editorial Board and Production Team, who have done a sterling job in pulling all of the articles into publishable format. Special thanks to those from the Production Team who have moved on.



Linda Smith, Editor

TB policy developments

Introduction

Bovine tuberculosis is the most difficult animal health problem we face in Great Britain (GB) today. The scale of the challenge facing both Government and industry in seeking to reverse the long-term upward trend in the disease, and preventing its spread into new areas, is significant. In leading the changes required to make this happen, the Government is committed to developing policies based on the full range of evidence available. We are committed to working ever more closely with our delivery partners and stakeholders throughout the policy development process and in formulating plans for the successful delivery of measures to reduce the level of the disease. These principles are enshrined in the 'Government strategic framework for the sustainable control of bovine tuberculosis in Great Britain'. This article sets out the key policy developments since publication of the strategic framework in March 2005, with particular emphasis on England.

Headline bovine TB statistics for GB in 2005

- 3.4% apparent prevalence at the end of the year (herds under restriction due to a TB breakdown, excluding herds with overdue test restrictions)
- 4.3% incidence of confirmed new herd breakdowns throughout the year (7.8% if all breakdowns are included)
- 93.6% of cattle herds were considered officially TB-free at the end of the year
- 4.8 million cattle were tuberculin tested in more than 43,000 herd tests

- 3,653 new TB incidents were recorded (up 9.1% on 2004). Infection was confirmed in 2,023 of those (up 14.7% on the previous year)
- 25,755 tuberculin test reactors were slaughtered (0.53% reactors per 100 animals tested)

The scale of the problem

Although bovine TB currently affects only a relatively small proportion of the national herd, the problem is severe in those areas where the disease is concentrated, such as the south west and west of England and south west Wales.

The costs of dealing with the disease have also escalated in recent years and in the financial year 2004-2005 we spent £90.5 million on the TB programme in

Author

Dr Debby Reynolds

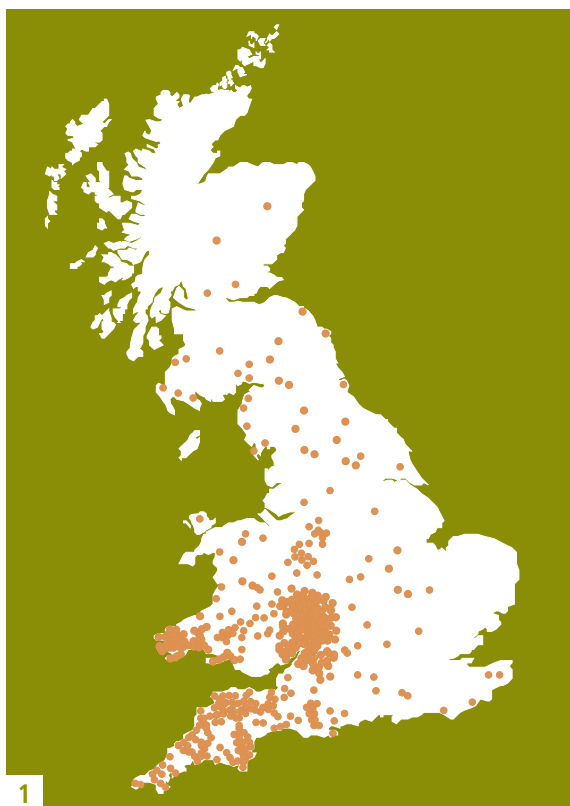


Figure 1
Geographical distribution of cattle herds with confirmed new TB incidents (red dots) in 2005

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GB. This covered expenditure on bovine TB testing and surveillance, compensation and research.

Very recently it has become apparent that there has been a substantial reduction, in the region of 20%, in the number of new TB incidents in the first few months of 2006, compared to the same period in 2005. However, the emerging figures should, for the time being, be treated with caution. The statistics on TB have historically gone up and down on a month by month basis due to changes in disease risk and the population of cattle under test. Real changes in disease incidence can only be established over a protracted time series by assessing long-term trends.

At this stage it is far too early to draw conclusions about whether the decrease is an exaggerated seasonal fall, or whether it indicates a more sustained reduction. The reasons for any

long-term fall, if maintained, are likely to be due to a complex combination of factors, including the introduction over recent years of a suite of enhanced cattle control measures along with a possible reduction in transmission risks. In addition, we are giving consideration to the potential effect that a switch in tuberculin supply towards the end of 2005 may have had on the number of new TB incidents disclosed. At the moment there is no evidence that the change in tuberculin supply could have caused this reduction, but further investigation is required before this can be confirmed.

Current policy position – the GB TB Strategic Framework

In March 2005 Defra, the Welsh Assembly Government and the Scottish Executive announced a joint ten-year

Government strategic framework for the control of bovine TB in cattle and farmed deer within GB. The framework was developed following extensive consultation with farmers, veterinary bodies and wildlife interests. It builds upon the Government's previous Five Point Plan (which focussed on public health protection measures; TB testing and control; development of a TB vaccine; research into transmission and spread of the disease and carrying out a badger culling trial), and applies to bovine TB the guiding principles set out in the overarching GB Animal Health and Welfare Strategy.

The strategic framework sets out a vision for a new partnership with

Government, working with its delivery partners and stakeholders, to reduce the economic impact of bovine TB, to maintain public health protection and ensure animal health and welfare.

The strategic framework sets out a vision for a new partnership with Government, working with its delivery partners and stakeholders, to reduce the economic impact of bovine TB, to maintain public health protection and ensure animal health and welfare.

Its aim is to slow down and stop the spread of disease into areas currently free of the disease, and achieve a sustained and steady reduction in TB incidence in high incidence areas. The framework recognises that TB control policies need to be tailored to reflect the regional variation in disease risk, and be adjusted to make best use of emerging scientific findings. The document also sets out a process for making decisions on whether badger culling may form part of future policy.

As part of the TB strategic framework, Government is committed to evidence-based policy development. There is an ongoing programme of research to improve our scientific understanding of bovine TB, for instance how it is spread and the role of badgers. The framework also formally recognises the importance

of other strands of evidence in relation to policy development – economics, environmental impact assessments, societal issues and the practicality of delivering policies.

Since the framework was published we have been working extensively with the State Veterinary Service, private vets, representatives of the farming industry, livestock auctioneers, Local Authorities and wildlife groups amongst others, to share ideas and to develop a greater understanding of the practical issues associated with the delivery of new bovine TB measures. For example, we convened a stakeholder sub-group, with an independent chair, to develop a detailed proposal for the introduction of statutory pre-movement testing. The eventual policy was based largely on the stakeholder group's proposals except where there were practical or legal constraints.

Recent developments in TB policy in England

The pre-movement testing policy was one of a series of measures announced by Ministers on 15 December 2005 to tackle bovine TB in England and help achieve the vision set out in the TB Strategic Framework. The key components of the announcement were:

- the introduction of a statutory requirement for pre-movement testing of cattle to help reduce the risk of spreading bovine TB through movement of cattle;
- the introduction of a new compensation scheme for farmers whose cattle are affected by bovine TB;
- a public consultation on both the principle and method of badger culling as a measure to control bovine TB in cattle;
- a renewed commitment to pursuing the development of a TB vaccine; and
- a commitment for extended use of a blood test based on a gamma-



2

interferon assay as an adjunct to the skin test to help improve diagnosis of the disease.

Figure 2
Local veterinary inspectors testing a cow

At the same time the Independent Scientific Group on Cattle TB (ISG) reported on the scientific findings of the proactive element of the Randomised Badger Culling Trial, or 'Krebs trial' (RBCT). The ISG reported that proactive culling of badgers in response to a herd TB breakdown, as conducted in the RBCT, while possibly benefiting farms where badgers were removed (20% reduced incidence) worsens the disease incidence in neighbouring farms (30% increased incidence). These figures have been updated since those published in *Nature*, 14 December 2005. The RBCT was designed to test the effect of two different approaches to badger culling, implemented under field conditions, and in a way that could be extended into a viable TB control policy option. These findings supported the ISG's earlier conclusions, arising from the reactive element of the RBCT, that localised culling of badgers is likely to be ineffective in controlling TB in cattle.

The RBCT is just one component of the TB research effort put in place by Defra, and which the ISG has directed since 1998. During the remainder of 2006 and the first quarter of 2007, the

→ CONTINUED ON p8

ISG will continue to undertake detailed evaluation of the results, submit further reports to Ministers and prepare papers for scientific journals. The ISG expects its final report – to include a full review of the RBCT and its findings, and other related research – to be ready in March or April 2007. That will conclude their work.

Pre-movement testing

A statutory requirement for pre-movement testing was introduced in England on 27 March 2006. The policy is being introduced in two phases to allow sufficient time for herd owners and the veterinary profession to adjust to the new requirements. The current arrangements, phase one of the policy, apply to cattle over 15 months of age moving from one and two year tested herds, unless the herd or movement meets one of a number of exemptions. All pre-movement tests must be arranged and paid for by the herd owner. Routine TB surveillance tests, paid for by the Government, qualify as pre-movement tests if animals are moved within 60 days of the test. Prior to the planned implementation date for phase one of the policy in February 2006, serious concerns were raised by farming organisations about the readiness of their members to comply with the policy and of the adequacy of veterinary resources to carry out the increased level of testing. We listened carefully to the concerns and as a result implementation was delayed by just over a month to give industry more time to prepare for the measure and allow a speedy independent review of veterinary capacity and preparedness to be carried out.

The review found no evidence to support an additional delay to the introduction of pre-movement testing.

To further ease the transition to the new arrangements, Government agreed to pay for one pre-movement test per herd owner in England between 20 February and 30 June.

The new arrangements are being closely monitored and early indications are that the new system is settling down well. The policy is being kept under review to enable modifications, if necessary, prior to the planned implementation of phase two of the policy, in March 2007, when pre-movement testing will be extended to movements of cattle over 42 days old.

Related measures have been introduced in Scotland and Wales. In Scotland, compulsory pre- and post-movement testing requirements were introduced from 23 September 2005, and in Wales pre-movement testing was introduced on the same basis as that in England on 2 May 2006.

Compensation

Following public consultation, a new compensation scheme for farmers whose cattle are affected by bovine TB, brucellosis and Enzootic Bovine Leukosis was introduced on 1 February 2006. The scheme was extended on 1 March to cover BSE. The new system was developed following the findings of a number of independent reports showing significant and widespread overpayments under the previous compensation system. It is more transparent than the old system and is designed to be fairer to both cattle owners and taxpayers.

Compensation in England is now determined primarily using table values, which reflect the average market price of bovine animals in 47 different categories. The categories are based on the animal's age, gender, type (dairy or beef) and status (i.e. pedigree or non-pedigree). As with pre-movement testing, Government has taken account wherever possible of the detailed concerns raised by stakeholders and, following consultation, made significant enhancements to the system. In particular, the proposed number of table valuation cattle categories was increased from 29 to 47, with separate tables for commercial and pedigree cattle.

We have also set up a Cattle Compensation Advisory Group, to help monitor the new compensation arrangements. The group includes representatives from NFU, valuer organisations, pedigree beef sector, pedigree dairy sector and the Meat and Livestock Commission.

Consultation on badger culling

Results from the RBCT carried out in England, combined with other scientific evidence, including that from the Republic of Ireland, led us to conclude that we needed to consult on whether or not badger controls should form part of our strategy to tackle bovine TB, and if so, what forms of control would be most appropriate. The resulting 12-week public consultation, which ran from 15 December 2005 to 10 March 2006, looked at the principle and method of a badger culling policy in areas of high TB incidence in cattle. The consultation document presented the scientific evidence, the balance of costs and benefits, and considered the implications of a badger cull for animal welfare and conservation.

We received over 47,000 responses to the consultation. A report summarising these showed that 95% of all respondents were opposed to a cull of badgers, but that opinion was much more evenly divided amongst organisations with a particular interest in TB. Of the interested organisations which responded, 50% were opposed to a cull whilst 41% supported culling badgers to control the disease. The remaining 9% of

responses were neutral. A further report summarising the outcomes of Citizen's Panels held to consider the issue, showed there was an even division of opinion amongst the individuals involved. However, in group discussions the view was marginally in favour of a cull as part of a multi-faceted strategy and with many conditions attached such as improved biosecurity and continued research into TB vaccines for cattle and badgers.

At the time of writing, no decision on badger culling has been made.

Research and TB Vaccines

The Government's research programme is focused on improving understanding of bovine TB in cattle and wildlife, trialing disease control options and developing new tools to fight the disease. The research priorities are improving diagnosis of the disease, developing vaccines both for badgers and cattle, improved understanding of the epidemiology (including modeling), more in depth understanding of wildlife behaviour and ecology and the impact of this, and cattle husbandry on TB, and modeling of the disease for use in cost benefit analyses of control policy options.

As well as funding a significant TB science programme, GB researchers are collaborating closely with researchers from overseas. It is critical that we work closely with other countries, to share experience and apply what has been learned elsewhere to the GB situation.

We are committed to developing a TB vaccine for badgers and cattle. Our announcement in December 2005 re-emphasised our commitment to this work. A substantial part of the Defra research programme focuses on this and over the past seven years we have invested more than £10.5 million in vaccine development and associated research. Although a vaccine is a long-term aim, and we do not anticipate that a new



3

Figure 3
Meles meles (badger)
Photographer:
Richard Yarnell
(2002)

→ CONTINUED ON p10

vaccine will be developed and licensed within the ten-year timeframe covered by the TB Strategic Framework, we are pressing ahead and making real progress.

In January this year the Veterinary Laboratories Agency (VLA) began further work looking at new vaccine candidates and delivery protocols in a natural transmission study in cattle. This will run in parallel with existing studies at the VLA and the Institute of Animal Health (and in collaboration with research in New Zealand and Ethiopia).

Research also continues on differential diagnostic tests, which are needed to distinguish vaccinated from infected cattle, since vaccines based on BCG make cattle react to the current tuberculin test as if they were infected with *Mycobacterium bovis*. Progress would need to be made on the legislative and licensing front before use in cattle would be possible.

The present programme of research into the use of a vaccine for badgers has been underway since 1999. A three year badger vaccine field study led by the VLA, in collaboration with the Central Science Laboratory, using parenteral BCG has recently started to obtain safety data together with some efficacy data for licensing purposes. Trapping and sampling badgers to obtain baseline data has started, and vaccination of wild badgers is likely to start in September.

Further work on oral vaccine and bait delivery systems for badgers has also started at the VLA in collaboration with colleagues at the Central Science Laboratory and New Zealand, and in close liaison with similar work in the Republic of Ireland.

Gamma interferon blood assay

Defra is already using the gamma interferon blood-based diagnostic test on an ad hoc basis in identified problem TB herds to improve test sensitivity and the removal of more infected cattle. About 14,000 tests were undertaken in 2005-2006. Preparations are now being made

for wider use of the gamma interferon test as an adjunct to the TB skin test in prescribed circumstances, and a Gamma Interferon Working Group has been established to prepare and deliver a policy for increased use of the test.

Engagement with interested organisations

The GB strategic framework set out a commitment for an annual conference for organisations with a particular interest in bovine TB, with the aim of reaching a wide audience and focussing on exchange of information. We held our first GB conference on 6 March 2006. The programme for the meeting was drawn up to provide a balanced coverage of bovine TB issues including cattle controls, wildlife issues and research. Nearly 60 organisations sent delegates, representing farming, veterinary, wildlife and other interests from across GB. The outcomes of the discussions on wildlife controls are being considered alongside the responses to the public consultation on badger culling and will be taken into account in the decision-making process.

We are making progress in establishing new bovine TB policy advisory arrangements. Any new group will be made up of individuals representing a broad range of stakeholder interests and will have close links with the Animal Health & Welfare Strategy's England Implementation Group.

Conclusion

The Government in GB is committed to working in partnership with others to achieve the vision set out in the GB TB Strategic Framework. The range of policy mechanisms available for controlling TB depends largely on achieving a better understanding of the disease, how it is spread, and the effectiveness and practicality of interventions and the outcomes of our research programme and other evidence will help with this.

Information

Dr Debby Reynolds is the CVO (UK), Director-General of Animal Health & Welfare and Veterinary Head of Profession.

Some lessons from the history of the eradication of Bovine Tuberculosis in Great Britain

The First Half-Century – Developing Understanding and Reluctance to Act

The Public Health Act of 1875 lit the fuse, which led, via a series of delayed explosions, to government action to control bovine tuberculosis. The Act empowered Medical Officers of Health and Inspectors of Nuisances (later known as Sanitary Inspectors, then Public Health Inspectors and finally (we hope!) Environmental Health Officers), to seize unfit meat. Eventually, some of them did. Spurred on by the report of a departmental committee in 1888, which, while conceding that ‘the bacilli may be found but rarely in the flesh’, considered the risk ‘too probable to ever allow of the flesh of a tubercular animal being used for food under any circumstances’, the more enthusiastic inspectors began to seize the whole carcase of any ox in which any tuberculous lesion was found.

And so it was that in 1889, the first explosion thrust bovine tuberculosis on the attention of an unwilling government, when a deputation of outraged butchers and cattlemen arrived at the Board of Trade to protest. The protest had the sympathy of thinking veterinary surgeons, including the Chief Inspector of the Veterinary Department of the Privy Council Office, Professor G T Brown, who wrote of ‘the frequent seizure by sanitary authorities of carcasses of... healthy cattle in fine condition... confiscated without any compensation to the purchaser, who had acted throughout in good faith.’ Brown revealed his sound scientific bent by referring to ‘the *presumption* that the use of meat from tuberculous animals is prejudicial to public health’ (author’s italics).

The Government responded in classic style by setting up a Royal Commission in 1890. For the next 21 years, a series of Royal Commissions deliberated, took evidence, and even did their own research, finally coming to firm conclusions in 1911, but apart from a wise response to the grievance of the initial protesters, their findings and recommendations, if not entirely ignored, were met with less than prompt and enthusiastic implementation. Critics of modern Government attitudes to food safety would do well to reflect that it took over a quarter of a century before an interim report of the Royal Commission, which concluded that tuberculous milk was the cause of a significant loss of human life, resulted in effective action to make milk safe. By contrast the speed of action and the extreme precautions taken in response to BSE are breathtaking. An Order was made in 1909 in response to the interim report of 1907 but was never put into operation. Orders made in 1913 and 1914 were revoked on the outbreak of the first World War, but these Orders went no further than requiring the notification of clinical cases, veterinary investigation and slaughter with compensation.

The 1925 Order resulted in the slaughter of an annual average of some 17,000 cows but, in the judgement of Gowland Hopkins, did ‘nothing to reduce the incidence of disease. Nor has it done much to protect the public from infected milk, as the majority of cows are not reported until towards the end of their lactation or when in an advanced state of the disease.’

Author
Andrew J Proud

→ CONTINUED ON p12

Although meat inspection remained a voluntary pastime for Local Authorities until wartime regulation of the meat industry in 1940, where they did carry it out, they adopted a less draconian judgement for localised tuberculous lesions. 'The French system' which M'Fadyean favoured, in his evidence to a Royal Commission in the 1890s, was essentially the same as that embodied in legislation in 1961 in Scotland and 1963 in England and Wales, later to be carried, virtually unchanged, into EEC Directives and present UK legislation; only generalised tuberculosis required total rejection, a fact of which some modern Meat Inspectors seem to be unaware.

'The French system'... was essentially the same as that embodied in legislation in 1961 in Scotland and 1963 in England and Wales, later to be carried, virtually unchanged, into EEC Directives and present UK legislation; only generalised tuberculosis required total rejection.

Were the Conclusions of the Royal Commissions Right?

At a time when voices are being raised in protest against TB reactors being salvaged for human consumption, and public money is being spent on testing animals which are all due for slaughter within a year or two, we might pause to ask whether the Royal Commissions were right to support the Smithfield protesters.

The controversy was informed by the views of two giants of medical science. The great Koch, whose best-known postulate is still a cornerstone of bacteriology, was also postulating, presciently that bovine tuberculosis was caused by a separate strain of the bacterium, but wrongly that bovine tuberculosis was insignificant in human disease. M'Fadyean, however, urged that the bovine strain was significant for human disease; nevertheless he had the wisdom to distinguish between the risks of contracting the disease from milk and from meat.

While he advocated the heat treatment of milk by 'steaming' as early as the 1890s, he supported the Smithfield protesters. He estimated that, while total rejection of the meat of all tuberculous animals would lead to the destruction of the carcasses of 15-20% of all adult cattle and probably 30% of dairy cows, the French system would result in the seizure of only 0.5%.

It would have been hard for the Royal Commissions to have missed the area of agreement between these opposing views. No doubt the French system of Meat Inspection owed something to Koch's views, but that it was supported by the leading advocate of the heat treatment of milk, must surely have weighted the scales of the argument. It is hard to believe that M'Fadyean had not noticed the obvious fact that is so commonly missed today, that if tuberculous milk is rendered safe by minimal heat treatment, beef will be rendered safe by cooking. The Gowland Hopkins report certainly grasped this point: "But as meat is generally well cooked, and as the tubercle bacillus is destroyed by exposure to comparatively low temperatures, this is unlikely to be a serious source of infection. *It is to infection from milk, the food of childhood, that most of the bovine tuberculosis in human beings is attributable*" (author's italics).

It is an irony that the French method of meat inspection arose and took root in continental Europe where, unlike, Britain, there is a tradition of eating uncooked meat.

M'Fadyean's estimate of prevalence may be open to question, as may the tentative estimate of the learned Fellows of the Royal Society who wrote the Gowland Hopkins Report of 1934. They accepted

that 40% of dairy cows were infected and referred to several witnesses who estimated that 40% of all cattle would react to the tuberculin test, but the conclusion is inescapable that between the 1880s and at least the mid 1930s the disease was endemic.

Fresh data available to Gowland Hopkins also endorsed M'Fadyean's view that only a minority of cattle developed generalised disease. In Edinburgh, 70% of carcasses from cattle with tuberculous lesions could be passed as fit for human consumption, 20% were subject to partial rejection while only 10% were totally rejected.

While the pasteurisation of all milk not arising from tuberculin tested cows has been enforced since 1935, the attempt to ensure inspection of all meat during the fourteen years of rationing, which began with the Second World War did not approach success until towards the end of that control in 1954. Not until the delayed implementation of the Meat Inspection Regulations of 1961 (Scotland) and 1963 (England and Wales) was all legally sold meat subject to compulsory inspection. According to 'Animal Health: A Centenary', compulsory meat inspection was introduced in Scotland some 30 years earlier than in England and Wales.

The author's view of the effectiveness of this inspection is prejudiced by the account of an Aberdonian Meat Inspector, the late Charles Knights, who started work at the age of 12 as a part time slaughterman, during wartime regulation. He recalled that one of his early duties was to remove the retropharyngeal lymph nodes and conceal them in the paunches. Clearly these nodes were commonly tuberculous but their total absence escaped the notice of the inspectors!

Meat inspection was not, therefore, universal until well after the whole country had been declared an attested area and the incidence of Bovine Tuberculosis had fallen to 0.06%. The dramatic decline of human tuberculosis attributed to *Mycobacterium bovis* accords much

better with the sudden removal of risk from milk after 1935 than with the slow implementation of national, compulsory meat inspection, which was finally overtaken by virtual eradication. Quite apart from the uninspected meat, which was reaching the consumer routinely, during and after the decade in which eradication took place, all reactors, which were not sold on the open market, were slaughtered and subjected to the 'French' method of inspection. It beggars belief that, if M'Fadyean was wrong, a clear occupational risk among slaughterman, butchers and beef-eaters would not have emerged. It also betrays a strange inconsistency of thought, if cooking is regarded as a satisfactory *primary* preventive measure for salmonellosis in poultry meat and not as an effective second line of defence for tuberculosis after the elimination of the vast bulk of infection by visual inspection and selective rejection.

Early Action

The Tuberculosis Order of 1925 and the Milk and Dairies Order of 1926, had the combined effect of making clinical tuberculosis notifiable and giving power to Local Authorities to undertake veterinary inspection of dairy herds. The good intention of this legislation was frustrated by the reluctance of farmers to report disease promptly and a marked, although not universal tardiness on the part of the Local Authorities, to appoint sufficient veterinary inspectors and to undertake regular herd inspections. Gowland Hopkins' summary of responses from Local Authorities as to the number of inspections undertaken indicated that many had no veterinary inspectors and over a third undertook no routine herd inspections!

Really effective inspection had to await the amalgamation of the veterinary services of local and central government in 1938. In Scotland, where annual veterinary inspection of herds was compulsory,

→ CONTINUED ON p14

Grade	Turberculin Test	Veterinary Inspection	Total Bacteria/cc	Coliforms	Other
Certified	6 Monthly	6 Monthly	<30 000 (see note below)	0 in 0.1 cc	
Grade A (TB tested)	6 Monthly	6 Monthly	<200 000	0 in 0.1 cc	May be bottled on farm
Grade A	Not required	Not required	<200 000	0 in 0.01 cc	May be bottled on farm
Pasteurised	Not required	Not required	<100 00	Not specified	

1

Table 1
Comparison of grades of milk at the time of the Gowland Hopkins report, 1934. Note that the Total Bacteria figure for Certified milk is NOT a misprint; it is the figure given in the Report, but may have been a misprint in that source

experience led to the opinion that annual inspection was insufficient.

Milk legislation provided for milk to be sold under one of four designations, but did not require all milk to be so designated. Table 1 sets out the requirements for the different grades.

The obtuseness of the categories may explain Gowland Hopkins' observation that although the effect was to provide 'milk of a quality maintained by a system of public inspection for those who are prepared to pay for it... sales of designated milk as such are far below the supply and show no signs of expansion.' Nevertheless, the grading system reflected and fuelled private sector activity in tuberculin testing so that Gowland Hopkins could also report that although 'many herds are free of tuberculosis but are not officially registered for the production of designated milk... under 1% of dairy cows are in herds officially recognised as being free of tuberculosis.' However, at that time 'free from tuberculosis' only meant that reactors at regular tests were immediately removed. A herd could be officially free even if it never had a clear test!

The Gowland Hopkins Report and a Quarter Century of Spectacular Action

Possibly in response to the last annual report of a retiring Chief Veterinary Officer, in 1932, the Prime Minister appointed the Economic Advisory Council Committee on Cattle Diseases, with the

president of the Royal Society, Sir Gowland Hopkins as chairman. It was the report of this committee two years later, which led to effective control and virtual eradication of bovine tuberculosis in the following 25 years despite interruption by the Second World War.

The Committee had the opportunity to take evidence from witnesses who had long experience of endemic bovine tuberculosis and who were informed by the experience of other countries. It realised that the prevalence of disease was so high that early compulsory eradication was not feasible but, recommended measures for reducing the risk to human health, cutting the load of bovine infection and paving the way for eventual eradication.

The members of the Committee grasped three important principles. That the pasteurisation of milk, properly regulated and enforced, would prevent most transmission to humans. That it was possible to split the national herd (and indeed individual herds) into clean and infected sections, since the disease needed close and prolonged contact of infected with uninfected cattle for transmission to take place. That the effective removal of clinical cases would reduce the prevalence of disease, since most infected animals were not significantly shedding the causal organism.

Although not formally recommending its adoption as a government measure,

they were clearly impressed with Bang's method of eradication as practised in Denmark. This depended on the fact that calves of infected animals were almost invariably free from infection at birth: calves were removed from their dams immediately and reared separately; when they calved they were milked and kept as an isolated group. As time went on, and clinical cases were removed, the clean herd eventually replaced the infected herd.

The immediate government response to Gowland Hopkins was threefold: rapid progress towards the effective pasteurisation of all milk not designated as 'tuberculin tested', regular veterinary inspections of all dairy herds to detect and slaughter clinical cases and a voluntary attestation scheme with bonus payments for attested herds.

During the following three years the average number of clinical cases jumped to 22,793, almost certainly as a result of increased veterinary inspection.

The subsequent decline was accelerated by wartime shortages which led to increased culling of unthrifty cows and in 1945 the annual figure fell below 10,000.

While pasteurisation made an immediate impact on new human infections (although that only became clear in hindsight when the mortality rate began to decline), the Attested Herds Scheme took much longer to make an impact. But a vocal section of the public did not appreciate their new safety, they complained that pasteurisation was unnatural, spoiled the taste, raised the cream line and reduced the nutritional value. Initial progress was brisk. By the

end of 1935 only 99 (out of at least a quarter of a million herds) were attested. During the next four years the total number at the end of each year increased between three- and fourfold, but the outbreak of war led to severe limits being placed on new applicants. To nearly 14,000 herds, which had gained attested status by the end of 1939, were added only 3,100 by the end of 1944. It is difficult to appreciate how a bonus of one penny a gallon for attested herds, to which was added a premium for 'TT' designated milk of 2.5 pence per gallon in 1938, translates into modern values. If one reflects that a new-graduate veterinary surgeon might have been pleased with a 'salary' of £3.50 a week and that as late as 1970 a penny a gallon was still considered a significant incentive

to accelerate brucellosis accreditation, one can only consider it as a very significant incentive indeed.

Modern critics of disease eradication programmes would be appalled by a scheme which allowed herdowners to gain attested status by selling their reactors on the open market. However,

the policy was totally defensible:

The unanswerable argument was that, with a suspected prevalence of 40%, purchasing on the open market was a certain way of adding more infected animals to a herd which was very likely already infected.

In addition, the Attested Herds Scheme, for the first time, was establishing a pool of animals known to be free of tuberculosis from which purchasers could buy with confidence. The effect was to redistribute infected animals rather than eliminate

The immediate government response to Gowland Hopkins was threefold: rapid progress towards the effective pasteurisation of all milk not designated as 'tuberculin tested', regular veterinary inspections of all dairy herds to detect the slaughter clinical cases and a voluntary attestation scheme with bonus payments for attested herds.

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	Total cattle	Total herds	Average herd size	% reactors	% herds
All herds	364,286	12,300	29.6	100	100
Herds with no reactors	127,141	6,971	18.2	34.9	57
Herds with < 10% reactors	114,701	2,897	39.6	31.5	23.6
Herds with < 10% reactors	122,444	2,432	50.3	33.6	19.8

2

Table 2
Summary of Results of
1937-1938 Survey

them; once a majority of animals were in herds known to be free of tuberculosis, national eradication could be considered.

It was fortunate that Gowland-Hopkins, did not recommend delaying action pending further surveys, trials or refinement of testing. Had the Attested Herds Scheme been delayed, pending the results of a trial undertaken during a 12 month period following August 1938, it would, almost certainly have been shelved for the duration of the war. Among 364,286 cattle in 12,300 self-contained herds, selected on the basis of a herd history of little or no clinical tuberculosis, the prevalence of reactors was 13%. More than half the herds had no reactors, but approximately one third of the cattle were in herds with a prevalence exceeding 10%. The results are summarised in Table 2. Clearly the estimates of M'Fadyean and Gowland Hopkins were not excessive.

The slow growth of the Attested Herds Scheme during wartime was not without its redeeming features. Research continued, particularly in the refinement of the tuberculin test. A variety of tuberculins of varying potency were eventually replaced by a standardised purified protein derivative (PPD), as recommended by Gowland-Hopkins. By the time the Scheme began, the subcutaneous test which depended on detecting rises in temperature in the 24 hour period

following injection, had been replaced by the double intradermal test which necessitated three visits to the farm. In 1940 the use of more potent tuberculins made it necessary to introduce a comparative test to reduce the number of false positives. In 1943 PPD tuberculins came into general use and were found to be of such high sensitivity and specificity that the double intradermal test conferred no advantage; it was abandoned in 1947. In the following 15 years this test proved itself to be dramatically effective. Critics of the present test would do well to realise that, following the replacement of 'mammalian' (i.e. human) tuberculin by the more specific 'bovine' in 1975, the present test is actually a refinement of that test.

The introduction of a bonus of 4d per gallon for milk from tuberculin tested herds in October 1943 and the reopening of the scheme to all applicants nine months later; set the scene for rapid acceleration after the war. Almost as many herds were added to the attested register in 1945 as in the whole of the previous five years. From then onwards progress was rapid so that by the end of 1950, the number of attested herds exceeded 55,000 and included 22% of the bovine population of around ten million. A year later another 19,000 herds including a million cattle had been added.

Since the geographical distribution of attested herds was far from uniform with

Scotland far in the lead and England lagging a long way behind Wales, it was now possible to consider eradicating the disease from designated areas. The plan was to select areas where a high proportion of herds were already attested and which were largely self-sufficient for herd replacements and marketing. The announcement that these areas were to be declared eradication areas preceded the declaration by two years which gave the minority of non-attested herds the opportunity, lubricated by further incentive payments, to gain attested status and avoid the movement restrictions and compulsory testing which would be imposed on the declaration date.

In October 1950 the first eradication areas (one each in Scotland and Wales) were announced and new incentive payments for both dairy and beef herds were introduced. However, before these areas were declared, voluntary attestation proceeded so well in the Scillies, the Shetlands and the islands of Arran and Cumbrae that these could be declared attested areas (without a compulsory eradication phase) in February 1951.

This policy worked well so that by the end of 1959 the whole of Scotland and Wales had been declared attested areas. The last attested areas (in the Midlands, North and East of England) were declared on 1 March 1960 and gained attested status only seven months later.

The response of farmers to the announcement of eradication areas fell into three categories. Many decided to take advantage of the incentives and sell their reactors on the open market; others,



either through inertia or belief that the compensation finally payable for reactors would be generous, waited for compulsory measures; a minority, especially in the later stages of eradication, deliberately bought cheap reactors expecting to make a large profit from the compensation which they would receive under compulsory measures. This latter category left a bitter memory in the collective consciousness of the farming community to such an extent that two decades later the NFU indicated their opposition to over-generous compensation during Brucellosis eradication. Farmers who had eradicated under the voluntary scheme, and received their incentive payments felt threatened when they found a close neighbour accumulating reactors. The author encountered one such case who believed that his herd had become heavily infected as a result. Consequently, having received incentive payments, he was not eligible for compensation and suffered heavy financial loss.

Despite the inevitable effect of the area eradication scheme in concentrating reactors in areas yet to be designated for eradication, some farmers must have been surprised to find herds which they had believed to be heavily infected were in fact clear. During the whole of compulsory eradication approximately half of all herds included in the first round of compulsory testing had no reactors. Perhaps this led to fraud. The author treasures a story told to him in 1967, about a veterinary surgeon in the highlands of Scotland who arrived at his most distant farm only to find that he had forgotten his tuberculin. He injected all the animals with air and was acutely embarrassed to find that they all showed large reactions at the mammalian site three days later. During the years when compensation was fixed at 75% of the value of the reactor up to a ceiling related to average values, there was little motive

Figure 1
Advanced case of
generalised TB

→ CONTINUED ON p18

for such fraud. Nevertheless the author encountered one such case where 18 reactors were faked. It is somewhat gratifying to report that the miscreant died in prison while serving a double life sentence – but for other offences. The overall prevalence of reactors was 17.5% and the herd range was 0.5-30.5%. It is hard to escape the conclusion that tuberculosis is not a disease, which spreads easily or rapidly between and within herds.

The progress of attestation was reflected in the decline of clinical cases. Having fallen below 10,000 during 1945, it had fallen below 400 before the first eradication areas were declared, and to only 28 in 1960. Not all of these suspect clinical cases, of course, were confirmed.

For an area to be declared attested, total disappearance of reactors was not required but only a fall to a very low prevalence. In 1960, when the whole country was declared attested, the prevalence was 0.19%. By 1964 it had fallen to 0.06% from which level it was not destined to rise significantly until the success of government policy to protect badgers showed its unfortunate by-product. The clear lesson from the history of the eradication programme is that bovine tuberculosis is readily susceptible to simple control measures where cattle are the sole reservoir population.

Space does not permit a consideration of the history subsequent to the declaration of national eradication. Although an interesting study, the need for it is less urgent as there are many people still living who remember most of it and a considerable literature is readily available.

Learning the Lessons of History

To the lessons explicit or implicit in the foregoing text should be added particular emphasis in three areas.

Those who claim that tuberculosis is now endemic in the national herd or in the South West, are clearly wrong. Currently prevalence is nowhere near

the 20-40% of the half century which preceded the area eradication scheme.

The methods of control developed and refined during the first half of the 20th century worked very well and allowed rapid reduction of prevalence to negligible levels. There is no reason to suppose that in the absence of a protected wildlife reservoir population they would no longer work today. With the same proviso, the control of the disease by isolation – within farms, of farms, and of designated areas – is as feasible today as it was in the 1940s and 50s. In those days it was understood that double fencing between farms was an adequate safeguard and that tying cows tail to tail, as opposed to nose to nose, in the shippon significantly reduced risk. While not able to quote a contemporary source, the author's clear memory is that the two yard space required between the fences during the Brucellosis Incentives Scheme provoked the comment from older farmers that only one yard had been required during tuberculosis voluntary attestation.

The lack of urgency attached to the slaughter of reactors until modern times did not appear to hinder the progress of eradication; considerable economies could be affected by learning from Professor Bang's methods. If an unseemly haste in removing reactors was not necessary when the only risk was cattle to cattle transmission, it is still less appropriate in dealing with disease acquired from another source, which cannot be removed.

Bibliographical Note

The Gowland Hopkins Report, HMSO 1934 and Animal Health: A Centenary, HMSO 1965, were the main sources of the information given above, but readers of the latter work should note that the history of tuberculosis eradication is to be found concentrated in two entirely separate parts of the book and scattered piecemeal throughout a third.

Information

Andrew Proud was formerly a Veterinary Officer (VO) at the Gloucester Animal Health Divisional Office, and retired in 2005.

Bovine Tuberculosis in the European Union and other countries: current status, control programmes and constraints to eradication

Introduction

Bovine tuberculosis (TB) caused by infection with *Mycobacterium bovis* (*M. bovis*) is a zoonotic disease that affects cattle worldwide. Although domestic cattle (*Bos taurus* and *B. indicus*) are the natural hosts of the organism, nearly all warm-blooded animals appear more or less susceptible to the infection. Given the right conditions, the infection can become self-sustaining in some of these species, which then constitute maintenance hosts to *M. bovis* in addition to domestic cattle. Public health concerns in the first half of the 20th century led to compulsory pasteurisation of milk and campaigns to eradicate *M. bovis* from the cattle population in developed countries. Whilst the zoonotic risk posed by *M. bovis* has been largely controlled in the developed World, the success of TB eradication campaigns in cattle has been mixed. Despite the limitations of existing screening tests, bovine TB has been effectively eliminated in many countries and regions. This has been achieved through the application of long-term systematic programmes of tuberculin skin testing and removal of reactors, coupled with repeat testing and culling of infected herds, slaughterhouse surveillance, cattle movement restrictions and occasional slaughter of entire herds with intractable breakdowns. More recently, deployment of ancillary *in vitro* diagnostic assays alongside the skin tests has enhanced the detection of infected cattle and helped resolve problems of non-specific reactivity to bovine tuberculin. In other developed countries

the traditional 'test and slaughter' approach has been instrumental in reducing the incidence of bovine TB, but complete control of the disease in domestic cattle and farmed deer has been hampered by wildlife reservoirs and other factors. The problem of bovine TB in wildlife is not unique to the British Isles. In a survey conducted in 2000 by the Office International des Épizooties (OIE), 22% of countries reported that TB had been identified in wildlife within the previous 10 years. In the majority of those countries the role of infected wildlife in the maintenance and spread of *M. bovis* is not well defined. However, the experience accumulated over the last 30 years has highlighted the difficulty and controversy in controlling TB in cattle once the disease becomes established in a wildlife reservoir. Additionally, endemic *M. bovis* infection in livestock and wildlife constitutes a serious threat to public health in many developing countries. This is exacerbated by the pandemic of human immunodeficiency virus (HIV) infection, socio-political unrest, inadequate veterinary, financial and diagnostic support to conduct test and slaughter campaigns, lack of access to pasteurised milk and close human-to-livestock interactions in pastoralist communities. This paper reviews the current global situation of bovine TB, focusing on the prospects for eradication of this disease in the European Union and other parts of the World.

Author

Ricardo de la Rúa-Domenech

→ CONTINUED ON p20

The European Union (EU)

Historical background and legislative framework

The first attempts to control TB in cattle in Europe and other industrialised countries began in the late 19th century, following Robert Koch's identification of the TB bacillus and the discovery of a link between certain cases of human TB and consumption of raw cows' milk. In the years that followed, control measures consisted of clinical examination of herds, bacteriological examination of milk and voluntary slaughter of tuberculous dairy cows. These measures were intended to protect public health, rather than a serious attempt at reducing the rather high prevalence of bovine TB in the national herd. Screening of cattle with tuberculin-based tests was not widely adopted in those early days. The control of TB in cattle intensified in the 1930s, but it was only after World War II that European governments began to phase in national or regional TB eradication campaigns based on regular tuberculin herd testing, slaughter of test reactors and restriction of infected herds. By 1960 participation in TB eradication programmes had become compulsory across Europe (e.g. GB in 1950, France in 1955, Ireland in 1957 and Northern Ireland, Poland and former Czechoslovakia in 1959) except in the Southern European countries, where it took a little longer to introduce similar schemes (e.g. Italy in 1977). As expected, each country tailored its programme to suit the local conditions. For instance, the cervical single intradermal test (SIT) was adopted as the primary screening test for TB in virtually all the continental European countries. By contrast, the single intradermal comparative tuberculin (SICCT) test performed with both bovine and avian tuberculin remains the primary test in Portugal, GB (since 1947), the Republic of Ireland (1954) and Northern Ireland (1958). Elsewhere in

Europe, the SICCT is only used for re-testing of SIT reactors and inconclusive reactors.

The first attempt to co-ordinate bovine TB control efforts across Europe was introduced on 26 June 1964, date on which Council Directive 64/432/EEC came into force setting out specific animal health requirements for the intra-Community trade of cattle. In relation to TB, the aim of this Directive was to facilitate trade of cattle between the EU Member States whilst minimising the risk of spreading the disease by movements of infected animals. This Directive laid down the criteria for herds, regions and countries to achieve (and maintain) 'officially tuberculosis-free' (OTF) status. Trade in bovine animals (including Asian water buffalo [*Bubalus bubalis*] and North American bison [*Bison bison*]) for breeding and production purposes can only take place out of OTF herds. In brief, Directive 64/432/EEC states that a bovine herd is OTF if all the animals over six months of age have passed two skin tests at six-month intervals. Although herds must be tested subsequently at yearly intervals to maintain OTF status, the interval between tests may be extended as the national or regional incidence of confirmed herd breakdowns declines. When the annual percentage of infected herds in an Member State (or a region of it) has not exceeded 0.1% for six consecutive years and all bovines slaughtered are subject to official veterinary meat inspection, that geographical unit can be declared OTF and dispense with routine skin testing of cattle. The technical annexes of this Directive also lay down the technique and standard interpretation of the two primary screening tests approved for use in bovine animals in the EU (the SIT and SICCT test). Directive 64/432/EEC has been amended on several occasions since its enactment. Most recently, Commission Regulation (EC)

	2004 cases/100,000 population	2004	2003	2002	2001	2000	1999
		No. of cases					
Austria (OTF)	<0.1	4	4	4	5	0	0
Belgium (OTF)	0.05	5	5	2	2	0	0
Cyprus	0.14	1 (1)	-	-	-	-	-
Denmark (OTF)	<0.1	2 (1)	1	2	4	12	2
Finland (OTF)	0	0	0	0	0	0	0
France (OTF)	-	-	-	-	-	-	22
Germany (OTF)	<0.1	51	-	-	-	-	64
Greece	0	0	0	0	0	93	-
Hungary	0	0	-	-	-	-	-
Ireland	<0.1	2	6	7	3	1	8
Italy	<0.1	5	1	4	0	0	-
Latvia	0	0	-	-	-	-	-
Lithuania	0	0	0	-	-	-	-
Portugal	-	-	-	-	0	-	-
Slovenia	0	0	-	-	-	-	-
Spain	<0.1	4	6	2	3	5	2
Sweden (OTF)	<0.1	4 (2)	5	7	5	5	2
Netherlands (OTF)	-	-	-	8	10	13	19
United Kingdom	<0.1	8	30	20	30	21	40
EU-Total		86	58	56	62	150	159

1 Current OTF status (as of 2005) is indicated. Imported cases are noted in parentheses and included in the total number of reported cases. No data provided by Czech Republic (OTF), Estonia, Luxembourg (OTF), Malta, Poland and Slovakia (OTF).

No 1226/2002 of 8 July 2002 re-defined the rules for standardisation and calibration of avian and bovine tuberculin and allowed, for the first time, the deployment of the gamma interferon (γ -IFN) blood assay (BOVIGAM®) as an ancillary parallel test for TB in cattle.

A second important legal initiative was the promulgation of Council Directive 77/391/EEC, which laid down EU-wide measures for the eradication of bovine TB, brucellosis and leucosis. Under this

Directive, non-OTF Member State are expected to draft programmes in order to accelerate the eradication of bovine TB. The European legislation also provides for financial support of national eradication programmes from the EU budget (commonly known as the 'EU Veterinary Fund'). In 2005, TB eradication programmes in Cyprus, Greece, Italy, Poland, Portugal and Spain were all co-financed under that scheme.

Table 1
Total number of cases human TB caused by *M. bovis* infection reported in the EU over the period 1999-2004 (source: data as reported by Member States to the European Food Safety Authority)

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Table 2

Descriptive TB statistics for cattle in Member States of the EU recognised as officially TB free (OTF) in 2005 by the European Commission. Data for 2004, except where otherwise indicated (adapted from Reviriego and Vermeersch 2005)

Human *M. bovis* infection in the EU

Despite the harmonisation of TB testing rules for intra-Community trade of cattle and TB controls in milk, dairy products and meat, there is still room for improvements in the reporting of human *M. bovis* infections in the EU. The notification systems for human TB are not standardised across the EU: many Member State do not distinguish between the different species of *Mycobacterium* that cause TB in humans and, even in those countries that undertake speciation of human mycobacterial isolates, this may not be standard practice. As a result, the incidence and overall trend of human *M. bovis* infections cannot be reliably estimated in the EU. In 2004 a total of 83 cases of human TB caused by *M. bovis* were recorded across the Member State that reported this data (Table 1), but the actual number of infected persons is

probably higher than that. Nevertheless, the risk of humans contracting TB from animals in the EU is considered very low, even in the non-OTF states. Most incidents of *M. bovis* infection in humans arise from reactivation of latent disease in elderly or immunocompromised people, or infections acquired outside the EU.

Eradication of bovine TB in the EU: progress and constraints

Tables 2 and 3 summarise the situation of bovine TB in the 25 Member State of the EU at the time of writing, according to the most recent information supplied by the national veterinary authorities to the European Commission and the OIE. Figure 1 shows the classification of EU Member State according to their official TB status as at 30 September 2005. The success of systematic testing and slaughter programmes has so far resulted in the recognition of 11 Member States

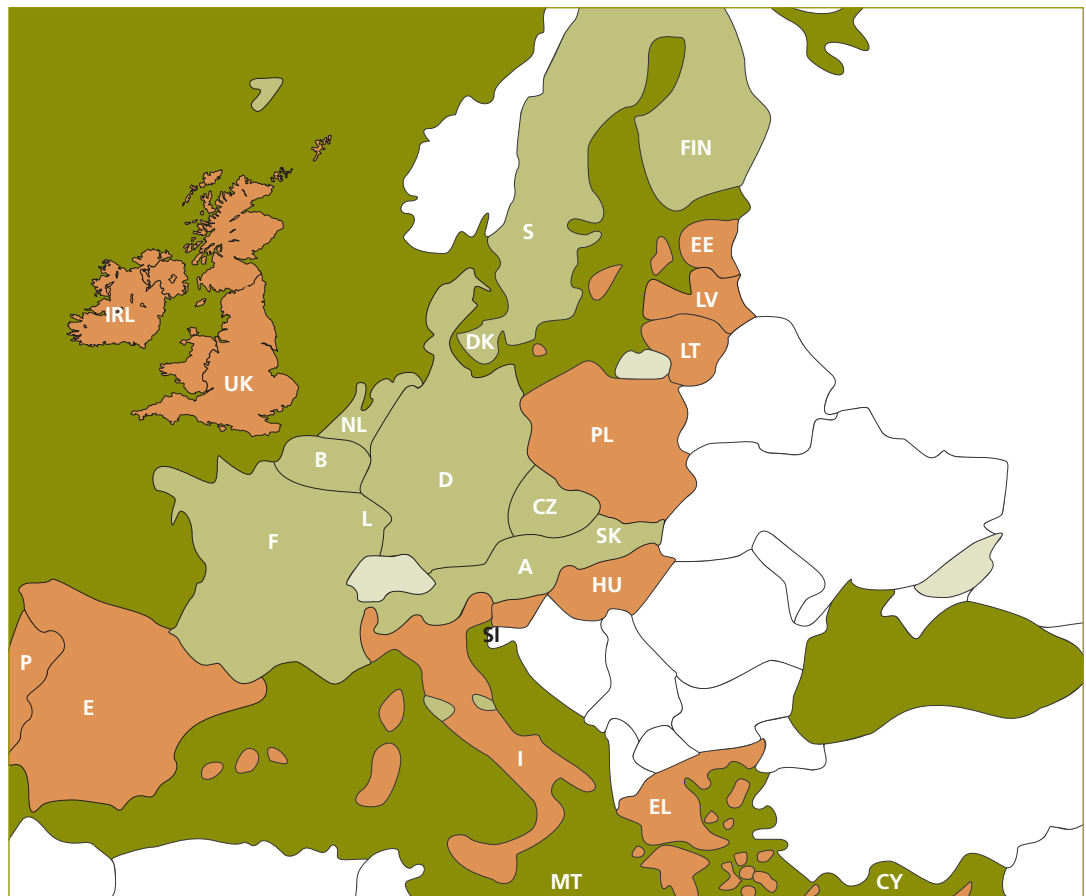
Figure 1

Map of the European Union showing the classification of Member States (or regions of them) according to their official TB status as at 30 September 2005

Light green: Officially TB free (OTF) Member States or regions (in Italy only)

Orange: Non officially TB free Member States

White: Non EU countries



Country	OTF since	Total number of existing bovine		Routine skin testing		Number of skin tests carried out before introduction of cattle into the herds ³	Number of cattle with suspect TB lesions submitted for histological and bacteriological examinations	Number of culture-positive animals	Officially TB free (OTF) herds		Infected herds	
		Herds	Animals	Interval between routine tests ²	Number of animals tested				Number of herds	%	Number of herds	%
Austria ¹	1999	95,401	2,088,273	N.A	803	N.A	51	0	95,401	100	0	0
Belgium	2003	42,553	2,807,915	(a)	0	450,000	134	46	42,549	99.99	8	0.01
Czech Republic	2004	27,806	1,428,329	(b) for bulls (c) for other cattle over 24 months	322,494	2,778	33	0	27,806	100	0	0
Denmark ¹	1980	28,270	1,759,192	(f) bulls and cattle intended for export	2,400	0	1	0	28,270	100	0	0
Finland	1995	22,882	969,140	(a)	814 ⁴	0	11	0	22,882	100	0	0
France ¹	2000	277,291	17,873,975	(a) (b) (c) (d) (e)	3.6 million (in 57,003 herds)	860,000	151	44	277,213	99.97	78	0.03
Germany	1997	235,897	14,028,087	(a)	6,784	175	86	44	235,886	99.99	0	0.01
Luxembourg ¹	1997	1,619	189,674	(a)	0	0	0	0	1,619	100	0	0
Netherlands ¹	1995	60,815	N.A	(a)	0	0	1	0	60,815	100	0	0
Slovakia	2005	11,352	563,771	(c)	321,127	0	28	0	11,352	100	0	0
Sweden	1995	29,456	1,666,164	(a)	445	1	0	0	29,456	100	0	0

N.A. data not available

1. Data for 2003

2. Explanation: a) No routine testing (slaughterhouse surveillance only), b) Tests once a year (excluding bovines under six weeks in most cases), c) Tests every two years, d) Tests every three years, e) Tests every four years, f) other (see details).

3. i.e. pre-movement testing

4. Bulls in artificial insemination stations

2

Country	Total number of existing bovine		Routine skin testing		Eradication programme	National animal TB incidence (reactors per 100 cattle tested in the year)		Officially TB Free (OTF) herds		National herd incidence (new reactor herds divided by herds tested during the year)	
	Herds	Animals	Interval between routine tests ³	Number of animals tested		%	Year of last TB incident	%	%	%	Trend over the period 1999-2004
Cyprus	363	60,000	(g) Last testing campaign in 2000-2001. Resumed annual testing in 2005	None in 2004, 7,000 in 2005	Yes	0	1928	100 (presumed)	0	No recent breakdowns Working towards OTF status	
Estonia ¹	9,180	248,160	(b)	149,813	No	0	1986	100 (presumed)	0	No recent breakdowns Working towards OTF status	
Greece	37,778	870,663	(b)	687,122	Yes	0.99	Ongoing	95.20	0.74	Stable	
Hungary	32,721	766,000	(c)	N.A.	No	<0.01	2004	99.99	<0.01	Declining. Working towards OTF status	
Ireland	124,414	6,992,264	(b)	9.5 million	No	0.33	Ongoing	96.90	5.72	Slowly decreasing	
Italy	187,098	6,456,993 (plus 240,000 buffalo)	(b)	3.8 million	Yes (for non-OTF regions)	0.21	Ongoing	90.90	0.62	Stable with wide regional variations in prevalence ⁵	
Latvia	71,799	376,540	(b)	166,767	No	<0.01	1989	100 (presumed)	0	No recent breakdowns Working towards OTF status	
Lithuania	195,226	916,715	(b)	512,863	No	0	2001	100	0	Declining. Working towards OTF status	
Malta ²	281	18,417	N.A.	N.A.	No	0	2001	100 (presumed)	0	No recent breakdowns Working towards OTF status	
Poland	882,761	5,649,362	(d)	1.7 million ¹	Yes	0.03	Ongoing	99.99	0.04	Stable	
Portugal	77,855	1,119,545	(b)	958,306 ¹	Yes	0.09	Ongoing	N.A.	0.17	Stable	

Slovenia	46,041	470,807	(g) Government prescribes ages and types of cattle eligible for testing each year	402,514	No	0	2002	100 (presumed)	0	Declining. Working towards OTF status
Spain	171,190	6,328,241	(b)	4.8 million	Yes	0.47	Ongoing	96.54	1.11	Declining
UK (GB)	93,165	8,924,000	(b) 25.6%, (c) 12.8%, (d) 0.7%, (e) 60.9% of herds	4.6 million	No	0.43	Ongoing	97.79 (at the end of 2004)	6.87 (3.6) ⁴	Increasing steadily Large regional variations in incidence
UK (NI)	28,568	1,677,583	(b)	1,865,671	No	0.81	Ongoing	91.87	9.17	Gradual increase accelerated in 2001 and 2002. Followed by a slow downturn accelerated in 2005

as OTF (Table 2). OTF status does not necessarily imply biological freedom from *M. bovis* and sporadic TB breakdowns continue to be reported in some OTF states. Of the ten Eastern and Southern European countries that joined the EU on 1st May 2004, eight had not achieved OTF status at the time of writing this article (Table 3 and Figure 1). Nevertheless, the outlook for these new Member State is quite favourable, as TB incidence has remained at very low levels for several years. By contrast, bovine TB remains a significant animal health problem in the UK, Ireland, Spain, Italy and (to a lesser degree) Greece and Portugal. The reasons for this vary from country to country, but are generally linked to the presence of reservoirs of infection in wild or domestic mammals, extensive use of outlying, rented or common grazing, high stocking densities, incomplete testing coverage, lack of compliance with scheme requirements or a combination of these factors.

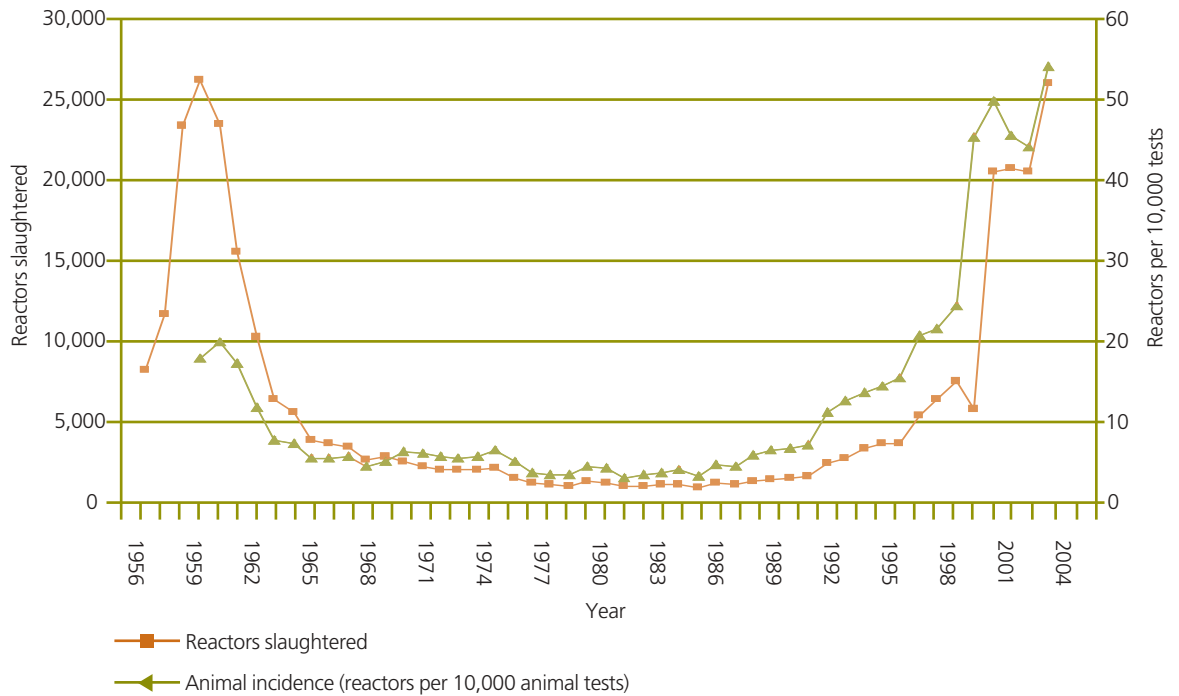
The comprehensive testing regimes that have eradicated TB in many European countries have failed to yield similar results in the British Isles. In GB, the highly successful herd attestation scheme launched in 1950 reduced the number and incidence of test reactor cattle from nearly 15,000 (16.2 reactors per 10,000 cattle tests) in 1961 to 569 (2.3 reactors per 10,000 tests) in 1982 (Figure 2). This remarkable progress came to a halt in the mid 1980s, when the situation began to gradually worsen to a point where GB now sustains one of the highest incidences of bovine TB in the EU (Table 3). In Northern Ireland the control of bovine TB has mirrored the experience in GB. A significant reduction in bovine TB incidence was observed after the introduction of a compulsory testing scheme in 1959. Disease incidence fluctuated in the 1970s and mid 1980s. This was then followed by a sustained

Table 3
Descriptive TB statistics for cattle in Member States of the EU not recognised as OTF by the European Commission in 2005. Data for 2004, except where otherwise indicated (based on data from Reviriego and Vermeersch 2005, Pavlik et al. 2005 and TB eradication programmes submitted by Member States to the European Commission)

→ CONTINUED ON p26

Figure 2

Total number (left y-axis) and incidence (right y-axis) of skin test reactor cattle slaughtered in GB every year between 1956 and 2005 (based on official data published in the CVO Annual Reports – Animal Health GB)



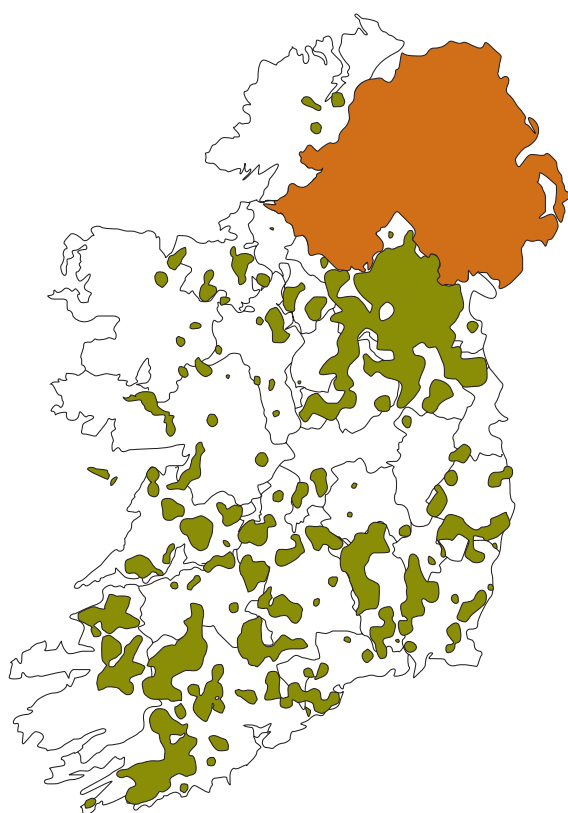
2

rise from 1987 to 2002 similar to the trend in GB, although both herd and animal-level incidence have fallen in Northern Ireland over the period 2003-2005. In the Republic of Ireland the bovine TB eradication programme made considerable progress in its early years. In the late 1950s and early 1960s the number of cattle culled as reactors exceeded 100,000 each year, but by 1965 this number had fallen to around 23,000. In contrast to the situation in the UK, the number of reactors identified in Ireland since the mid-60s has remained fairly static at between 20,000 and 40,000 reactors per year, despite annual testing of all herds.

M. bovis has been isolated from domestic animals other than cattle, wild deer, several species of carnivores and small mammals. However, the Eurasian badger (*Meles meles*) has been identified since the mid 1970s as a true maintenance host and the principal wildlife reservoir of *M. bovis* in the British Isles, where it remains a protected species. Nowadays, endemic *M. bovis* infection within badger populations in parts of the UK and Ireland is a major impediment to the eradication of bovine TB, although the

relative contribution of infected badgers to the incidence of TB in the national cattle herds is hard to quantify. A series of badger culling strategies have been in operation both in GB and Ireland since the mid 1970s and early 1980s respectively, but their success in helping control TB in cattle has been variable. Large-scale field trials have taken place in both GB and the Republic of Ireland to assess the impact of long-term badger removal operations on the incidence of TB in cattle. However, it is not yet clear how the lessons from the most recent trials will translate into cost-effective, socially acceptable and sustainable badger management policies. In the long term, both the UK and Ireland are committed to the development of an effective badger vaccine whilst retaining or enhancing the existing cattle controls. In the interim, Ireland continues to implement a national programme of reactive badger culling on affected farms where introduced cattle have been ruled out as the cause of a TB breakdown. This badger culling effort is more intensive in those areas of Ireland with persistent TB incidence in cattle (Figure 3).

M. bovis infection in wild deer was



3

first recorded in the UK in 1985 and the organism has since been isolated from five of the six species of free-living deer in the UK (Figure 4). Although TB is notifiable in deer, there is no evidence of widespread infection throughout the UK. Maintenance of infection and onward transmission to other species is most likely to occur when TB pathology is severe, the prevalence of infection is high, the species is abundant and infected individuals (or their excretions) encounter susceptible animals repeatedly or for prolonged periods. In general, wild deer in the British Isles do not fulfil these conditions, thus presenting a lower risk to cattle than badgers. Even in those areas of Britain where TB is endemic in badgers, prevalence estimates for deer are considerably lower. Furthermore, the relatively limited social interaction of deer and their free-living status act to limit transmission opportunities. However, the potential for wild deer to act as localised TB reservoirs for cattle exists where their populations are poorly

managed or artificially maintained above the natural carrying capacity. This could be happening in some populations of red (*Cervus elaphus*) and fallow (*Dama dama*) deer in South-West England. Based on anecdotal evidence, wild deer could also play a role in the maintenance of bovine TB in some regions of Ireland, but how (and how often) this may occur is still unclear.

Information about *M. bovis* infections in wild mammals in the rest of Europe is rather patchy. Surveys of TB in wildlife have been few and rather localised. With the exception of Finland and Sweden, TB in wildlife is not notifiable in EU Member State. Furthermore, in many Member State responsibility for bovine TB eradication programmes and wildlife conservation policies rests with different Government departments with competing agendas. All this makes it difficult to correlate any data on the presence of TB in wildlife with the incidence of the disease in cattle. In general, badger populations are estimated to be at much lower densities in continental Europe than in the British Isles. Furthermore, this species is huntable small game in several EU Member State such as Austria, Finland, France, Latvia and Sweden. Outside the British Isles sporadic *M. bovis* infection of badgers has only been documented in **Switzerland** in the 1950s. Rather than a maintenance host of *M. bovis*, badgers in that country were considered a spillover



4

Figure 3

Map of Ireland representing the 'chronic' TB areas (marked green). These areas comprise roughly 30% of the Irish agricultural land which yielded 70% of all standard interpretation reactor cattle during the period 1998-2000. Badger culling in the chronic TB areas does not take place over more than 60% of the agricultural land, whereas elsewhere in Ireland badger removal is limited to 20% of the land (source: James O'Keefe, Department of Agriculture and Food, Republic of Ireland)

Figure 4

Red deer (*Cervus elaphus*) stag in the Scottish highlands (photograph supplied by Charlie Wilson, RDS National Wildlife Management Team, Exeter). *M. bovis* infection in deer was first confirmed in Britain nearly 75 years ago. The bacterium is responsible for sporadic severe breakdowns of TB in farmed red and fallow deer. *M. bovis* has also been isolated from most species of wild deer in New Zealand and Europe. In continental Europe, TB appears to be an important re-emerging disease in wild deer and wild boar

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Figure 5

Wild boar (*Sus scrofa*) foraging in the Abruzzo region of Central Italy (photograph supplied by Charlie Wilson, RDS National Wildlife Management Team, Exeter). Wild boar populations in Europe are increasing in number and expanding in range. Tuberculosis caused by *M. bovis* was documented in free-ranging wild boar for the first time in Germany in the 1930s. The infection has been lately recorded across France, Northern Italy, Spain, Germany and other Central European countries, occasionally with a high prevalence of lesions in head and pulmonary lymph nodes. Although generally regarded as a spillover host, more studies are required to elucidate the role of wild boar in the epidemiology of bovine TB in continental Europe



host that became infected by scavenging carcasses of tuberculous roe deer (*Capreolus capreolus*). Switzerland has been officially free of bovine TB since 1959 and the disease has only been confirmed in five cattle herds between 1991 and 2003. Once eradicated from cattle, cases of TB in the Swiss wild deer and badger populations were no longer reported.

M. bovis has been isolated from carcasses of red and fallow deer, wild boar (*Sus scrofa*) (Figure 5), hare (*Lepus europaeus*), Iberian lynx (*Lynx pardinus*, an endangered carnivore) and fox (*Vulpes vulpes*) collected in surveys of wildlife killed on national parks and private estates in Central, Southern and Western **Spain**. In those regions it is quite common for herds of native Iberian pig and cattle breeds (including fighting bulls) to be reared in intensive free-range breeding systems. The incidence of TB in cattle has traditionally been high and difficult to control in those regions, where skin testing coverage is more patchy than in the predominantly dairy herds of Northern Spain. Furthermore, *M. bovis* infection is widespread among wild ungulates in Central, South and Western Spain, where red deer and wild boar populations have been artificially increased in recent decades to satisfy the demands of a thriving commercial game hunting industry. Geographical and temporal clustering of *M. bovis* strains isolated from cattle, domestic pigs, goats and wildlife is suggestive of interspecies

transmission within shared ecosystems. Based on the high prevalence of infection, repeated isolations over several hunting seasons, ecological interactions and pathological presentation, red deer and wild boar have been identified as potential maintenance hosts of TB in Spain. There are two additional challenges to the eradication of bovine TB from that country: a reportedly high prevalence of infection in goatherds and the seasonal movement of beef herds to mountain common grazing (transhumance). *M. bovis* infection of wild boar could also constitute a problem in some regions of **Portugal**, but the data currently available is scarce and does not allow a proper risk assessment of this species.

Wildlife does not appear to be a significant factor in the persistence of TB in cattle in **Italy**. *M. bovis* infection was identified in wild boar carcasses collected in Liguria and other regions in Northern Italy in the late 1980s and throughout the 1990s. The confirmation that most *M. bovis* isolates from wild boar were of the same genotype as those from cattle reactors in the same regions is suggestive of inter-species infection. Furthermore, a number of unusual TB recrudescences in cattle herds (on occasions after whole herd slaughter), also point towards a wildlife reservoir. However, in line with the experience of other countries, the incidence of bovine TB in wild boar has reduced as the prevalence of cattle infection continues to fall in Northern Italy.

France attained OTF status in December 2000, at a time when cattle were believed to be the sole reservoir of *M. bovis* in that country. The bacterium had never been isolated in French wildlife, with the exception of two wild boars. However, over the period 2001-2005 *M. bovis* infections were discovered by accident in wild boar and deer in four geographically distant regions of France. Epidemiological surveys have been conducted in some of these to establish

Region or Département	Species affected	Year of discovery	Apparent prevalence of infection	Self-sustaining infection in wildlife (maintenance hosts)?
Haute Normandie (Brotonne Forest)	Wild boar, Red deer and neighbouring cattle herds	2001-2002	28% and 14% of hunted boar and deer, respectively Animals of all age groups affected Respiratory and digestive lesions	Probable
Haute Corse	Wild boar	2003-2004	Low prevalence	Unlikely
Bourgogne	Wild boar and Red deer	2003-2004	Low prevalence	Unlikely
Pyrénées Atlantiques	Wild boar	2005	Very low prevalence	Unlikely

4

the extent of the wildlife reservoir (Table 4). It is still unclear why bovine TB is a re-emerging disease in French wildlife whilst almost eradicated in cattle. The discovery of these foci has coincided with alleged increases in wild ungulate populations in France over the last 25 years. Some have speculated that infection remained latent in some wildlife reservoirs throughout the cattle eradication campaign and that expanding wild deer and boar populations, coupled with more intensive wildlife disease surveillance, have led to the recent discovery of TB in those species.

Over the period 1999-2001, there have been reports of bovine TB confirmed by culture in free-ranging red deer from a natural park in the northern Alps straddling **Austria** and **Germany**. Molecular epidemiological data and the absence of TB in local cattle herds suggest that *M. bovis* had been circulating in the local wild deer population for some years. In the northern German region of Mecklenburg-Vorpommern tuberculous lesions were found in 102 (1.4%) of 7,419 carcasses of free-ranging wild boar killed during the period 1982 to 1988.

Of the carcasses with lesions, 55.6% yielded *M. bovis*. The alleged source of infection were TB breakdowns in neighbouring cattle herds. In other **Central European** Member States, where the TB eradication programmes in cattle have reduced the incidence of TB to very low levels, *M. bovis* has been isolated in several wildlife species. In the period 2000-2004, *M. bovis* infections were diagnosed in wild boar (n=14) and red deer (n=6) in Hungary, and in European bison (*Bison bonasus*) (n=14) and roe deer (n=2) in Poland. The true epidemiological significance, if any, of these sporadic cases of bovine TB in wild ungulates in Central Europe is unclear.

Finally, **Sweden** declared bovine TB freedom in 1958 under its own national eradication programme. However, *M. bovis* infection re-emerged in 1991 in the farmed deer population after the importation, four years earlier, of infected fallow deer from the UK. With at least 13 deer herds infected and faced with impossibility to trace all potentially infected deer due to lack of farm records,

Table 4
Location and epidemiological significance of recent *M. bovis* isolates from wild ungulates in France

There is no evidence at present of *M. bovis* infection in badgers or other susceptible wildlife species in France

→ CONTINUED ON p30

Figure 6

Tuberculin skin testing of cattle in Australia using the caudal fold test (source: Dr Brian Radunz, Department of Business, Industry and Resource Development, Northern Territory, Australia).



a voluntary scheme was put in place to allow deer farmers to regain OTF status through repeat tuberculin testing and long-term slaughterhouse surveillance. The outbreak was apparently contained with no evidence of spillover onto other species.

Russia and other countries of the former USSR

Apart from the three Baltic States that joined the EU in 2004 (Table 3), there is very little reliable data on the incidence and prevalence of bovine TB in the countries of the former Soviet Union, where 74 million head of cattle were kept in 2003. Systematic tuberculin herd testing and culling of reactors began in Russia in the late 1950s. This led to a gradual reduction in animal and herd incidence of bovine TB. Over the period 1996-2003, Russia reported a total of 2,012 TB breakdowns to the OIE, with the number of breakdowns falling from 712 in 1996 to 87 in 2003. All other countries in the former Soviet Union declared nil or very low incidences of TB herd breakdowns in 2003, except Armenia, Kyrgyzstan and Turkmenistan (no data available). Epidemiological investigations carried out in the 1960s and 1970s in various zones of Kazakhstan (Central Asia) revealed bovine tuberculin reactivity and *M. bovis*

infections in cats, dogs, sheep, goats and Bactrian camels.

Australia

Bovine TB was probably introduced in Australia early in the 19th century with the arrival of cattle from Britain. By the 20th century, the disease was well established in cattle herds throughout the country and posed a serious public health risk. The various States gradually introduced test and slaughter programmes from the early 1900s. Although these were initially voluntary, from the 1940s the testing of dairy herds supplying raw milk became compulsory. By the late 1960s TB in dairy herds was largely under control, but little progress had been achieved in beef herds outside the intensively farmed coastal strips of the southern parts of Australia. Faced with the prospect of losing vital export markets, on 1 July 1970 the State and Federal governments launched an eradication campaign with financial support of the beef and dairy industries. The Brucellosis and Tuberculosis Eradication Campaign (BTEC) relied on systematic and repeat skin testing of cattle herds using the caudal fold SIT (Figure 6), slaughter of reactors and movement controls. This was supplemented with slaughterhouse monitoring and traceback of carcasses with confirmed TB. In the latter stages of the campaign, entire groups or herds suspected of being infected were slaughtered out.

The *in vitro* γ -IFN test (BOVIGAM®) was developed in Australia in the late 1980s for the diagnosis of TB in cattle in combination with the caudal fold SIT. However, by the time the γ -IFN test had been optimised and validated for use in the field, bovine TB had already been brought under control by a conventional policy of skin testing and slaughter of reactors. So, this test played a minor role in the successful Australian BTEC

programme and was never routinely applied. It was, nevertheless, accredited as an official diagnostic test for bovine TB in Australia in 1991 and subsequently adopted as an ancillary test for TB diagnosis in other parts of the world.

One of the challenges the BTEC programme had to face was the large feral population of Asian water buffalo in the vast coastal floodplains of the Northern Territory. These animals had been introduced from south-east Asia in the 1800s and became well adapted to their new habitat. By the early 1960s the prevalence of bovine TB in feral buffalo had reached 16.4% and they were

regarded as a significant wildlife reservoir of *M. bovis* for domestic cattle.

Between 1984 and 1989 the feral population of 300,000 water buffalo in the Northern Territory

was extensively culled as part of the BTEC programme. 'Bush' areas across the country were also destocked of uncontrolled cattle and water buffalo living in a feral state, which could not be mustered for skin testing. By contrast, feral pigs (*Sus scrofa*) in Australia were considered an end host to *M. bovis* and not systematically culled. The high prevalence of TB recorded in feral pigs in the Northern Territory throughout the 1970s declined ostensibly after the eradication of the infection in cattle and the culling of feral water buffalo.

Australia finally became an OTF country according to OIE criteria on 31 December 1997. The following year, routine skin testing of cattle herds was replaced by intensive slaughterhouse-based surveillance (the National Granuloma Submission Programme). All meat plants are encouraged to submit for laboratory examination at least one suspicious TB lesion for every 1,000 cattle slaughtered

with two or more permanent incisor teeth. With *M. bovis* infection diagnosed in only four of 23,000 granulomas submitted in the period 2000-2004, Australia's objective to achieve biological TB freedom by December 2006 appears well within reach.

New Zealand

TB eradication programme in cattle and farmed deer: history, features and trends

Bovine TB was introduced in New Zealand in the early 19th century with the cattle of European settlers. A test and slaughter programme was rolled

out on a voluntary basis in 1945.

This became compulsory for all dairy herds in 1961 and for beef herds in 1968. The first case of *M. bovis* infection ever recorded in

farmed deer was diagnosed in 1978 in New Zealand. TB spread within the New Zealand farmed deer industry (the largest in the world) through movements of untested infected stock and captured infected feral deer. By the early 1980s it was recognised that farmed deer could act as maintenance hosts of *M. bovis*, resulting in severe and prolonged TB breakdowns and were a possible source of infection for other species. A voluntary test and slaughter scheme for farmed deer was thus introduced in 1983 and became compulsory in late 1989.

The New Zealand bovine TB eradication programme (known as Pest Management Strategy [PMS]), is administered by an incorporated society of stakeholders from the dairy, beef and deer farming interests, meat industry and local government, known as the Animal Health Board (AHB). Its activities are

Faced with the prospect of losing vital export markets, on 1 July 1970 the State and Federal governments launched an eradication campaign with financial support of the beef and dairy industries.

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funded by the Crown and Regional governments, a levy on the slaughter of cattle, grants from the agricultural industry and the salvage from the slaughter of reactors. The AHB's aim is the eradication of TB from New Zealand, but its medium-term operational objective by 2013 is to reduce the numbers of TB-infected cattle and deer herds to 0.2% annual period prevalence (APR: the number of herds classified as infected at the start of the financial year, together with herds found infected during the financial year, divided by total number of herds). From the mid 1990s, New Zealand has achieved a sustained downturn in numbers of infected cattle and deer herds (Table 5 and Figure 7) through a combination of control measures targeting reservoirs of

infection in both farmed and wild mammal populations:

- A test and slaughter programme for cattle and farmed deer, supplemented by routine slaughterhouse inspection;
- Herd- and area-based movement restrictions and controls, including pre-movement TB testing;
- Sustained TB vector control, designed to eliminate infection from wild animals in 'Vector Risk Areas' as well as preventing incursions of infected wildlife into 'Vector Free Areas' (see below);
- Buffer zones around populations of infected vectors and cattle and deer herds.

Table 5
Descriptive TB animal statistics for New Zealand cattle and deer in 1997-1998 and 2002-2003. The number of infected cattle and deer herds also fell in the years ending 30 June 2003 and 2004, continuing the downward trend in numbers of affected herds achieved over the previous nine years (source: Animal Health Board, New Zealand)

Measures used to evaluate progress	Cattle		Deer	
	1997-1998	2004-2005	1997-1998	2004-2005
Primary animal tests	4,775,672	5,307,138	552,129	756,782
Number (%) test positive	8,115 (0.17%)	7,200 (0.14%)	6,778 (1.23%)	9,717 (1.28%)
Number (%) serially re-tested	5,207 (64%)	6,779 (94%)	5,828 (86%)	8,744 (90%)
Percentage positive to serial re-test and slaughtered	16%	7.1%	3.3%	3.9%
Number of reactors slaughtered (% of all animals tested)	3,722 (0.08%)	1,073 (0.02%)	1,142 (0.2%)	1,318 (0.17%)
Number (%) of reactors with visible TB lesions	1,824 (49%)	307 (28.6%)	196 (17.2%)	125 (10%)
Number of tuberculous animals found during routine slaughter (% of the national kill)	722 (0.03%)	249 (0.01%)	156 (0.04%)	214 (0.05%)
Annual period prevalence of TB in animals*	0.03%	0.005%	0.03%	0.023%
Percent of TB animals from VRAs	83%	91%	74%	97%

* Based on the number of reactors slaughtered plus the number of lesioned non-reactors found at routine slaughter, divided by the population of the species.

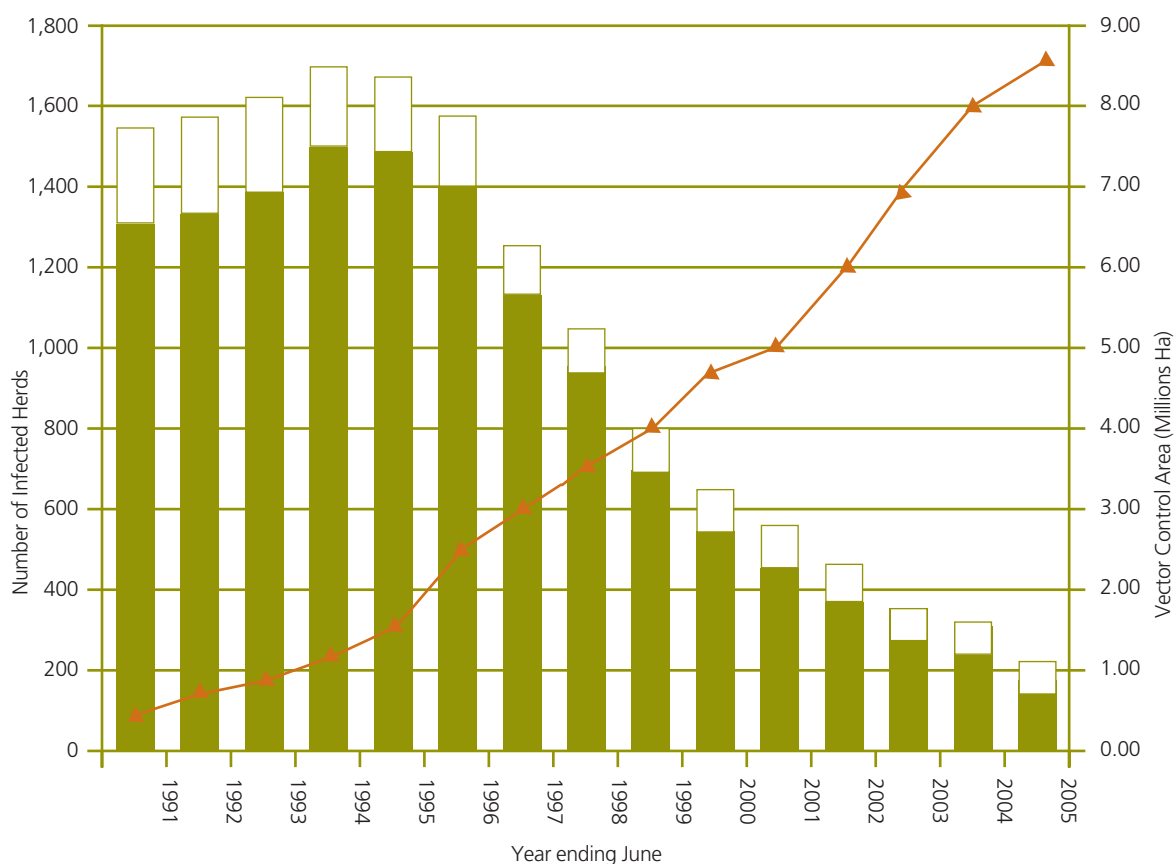


Figure 7
Evaluation of the numbers of infected cattle (green bars) and deer (white bars) herds in New Zealand relative to the cumulative area of the country under possum control operations (orange line), from June 1991 to April 2005 (source: Dr Paul Livingstone, Animal Health Board, New Zealand)

7

Primary TB screening of cattle herds in New Zealand is by the caudal fold SIT. Accredited lay technicians undertake the vast majority of routine skin tests in cattle and about 60% of deer tests. Cattle and farmed deer herds are categorised as either 'Infected' or 'Clear', together with an integer denoting the number of years that a herd has maintained its status (e.g. 'I5' or 'C8'). Herd testing takes place annually in declared movement control areas ('DMCAs', where herd period prevalence exceeds 1%) and triennially in clear ('C2' or better) herds elsewhere. All cattle and deer over three months of age moving out of or within a DMCA (except those going directly to slaughter) must be negative to a caudal fold SIT undertaken in the 60 days before movement. The γ -IFN blood test was adopted in New Zealand in the mid 1990s as an ancillary test for TB in cattle. As in other countries, this test is employed in very specific situations,

representing a small component of the overall testing effort (0.47% of the total of 5.33 million cattle tests administered in 2004-2005). In low TB incidence areas the γ -IFN test is an ancillary test of SIT-positive animals (serial testing) and has all but replaced the SICCT in this role. In explosive and chronic herd breakdowns ('problem' herds), the γ -IFN test is used as an ancillary test for skin test-negative animals to speed up the detection of infected animals (parallel testing).

Constraints to the eradication of TB from New Zealand

Wildlife reservoirs of *M. bovis* constitute a major impediment to the eradication of bovine TB in the New Zealand cattle herd. However, a fundamental difference with the situation in the UK and Ireland is that those reservoirs are non-native species and regarded as 'introduced pests'. Furthermore, agriculture is New Zealand's

→ CONTINUED ON p34

Figure 8

Map of New Zealand showing the Movement Control Area overlaid on Vector Free Area (Surveillance Area overlaid on Vector Free Area) and Vector Risk (possum control) and Vector Free Areas, as at January 2005 (source: Dr Paul Livingstone, Animal Health Board, New Zealand)



largest industry, representing 60% of the country's export earnings. All this makes the culling of wildlife (for livestock disease control purposes) a more socially acceptable proposition than it is in the UK. The brushtail possum (*Trichosurus vulpecula*) is a small, herbivorous marsupial imported from Australia in the 19th century, whose total population in New Zealand now exceeds 60 million. *M. bovis* infection was first described in possums in 1967 and today this species is considered a maintenance host of *M. bovis* and its main wildlife reservoir in New Zealand. The ferret (*Mustela putorius*) is also an important TB reservoir where its population density is high, although its status as a true maintenance or merely spillover-amplifier host is still debatable. Both possums and ferrets are

regarded as 'TB vectors' and the zones of where TB has been identified in these species are known as TB Vector Risk Areas (VRAs). As at June 2005, 39.5% of New Zealand's land area was classified as a VRA (Figure 8). More than 90% of infected cattle and deer herds are located in VRAs, even though such areas contain only 20% and 32% of New Zealand's cattle and deer herds, respectively. The role of possums, ferrets and other wildlife in the epidemiology of bovine TB in New Zealand is summarised in Table 6. All this philosophy is reflected in New Zealand's national TB vector control programme, which is systematically applied to possums in the VRAs and accounts for more than half of the AHB's expenditure. By contrast, other wild mammals (ferrets, feral pigs and wild deer, in decreasing

Table 6
Wild mammals of epidemiological significance in the transmission of *M. bovis* infection in New Zealand

	Brushtail possum	Feral ferret	Feral pig	Wild deer (mainly red, but also fallow and sika)
Year of first report of <i>M. bovis</i> infection	1967	1982	1990s	1970
Epidemiological status as hosts to <i>M. bovis</i>	<ul style="list-style-type: none"> • True maintenance host. • Highly susceptible to infection. • Major wildlife vector of TB for domestic livestock in New Zealand. • Main factor in the expansion of vector-risk (endemic) areas. 	<ul style="list-style-type: none"> • Certainly a spillover (amplifier) host and possibly a maintenance host. 	<ul style="list-style-type: none"> • Spillover (amplifier) host at worst. Generally considered a dead-end (not a vector). • Become infected through direct contact with (or by scavenging carcasses of) infected possums or ferrets. • Very sensitive sentinels of <i>M. bovis</i> in the environment. 	<ul style="list-style-type: none"> • Spillover (amplifier) host. • Could behave as maintenance host only in areas of very high deer density. • Wild deer dispersing over long distances seed infection in previously uninfected wildlife populations, when scavengers feed on infected carcasses. • Useful wildlife sentinels to survey for <i>M. bovis</i> infection in possums in large bush areas where there are no cattle or farmed deer.
Distribution and apparent prevalence of infection	<ul style="list-style-type: none"> • 60-70 million (up to 25/ha). • Average point prevalence of 5% (but as high as 60%). 	<ul style="list-style-type: none"> • Largest known feral ferret population in the world (3-8/km² in native grasslands, rarer in forests and wetter areas). • Prevalence as high as 32%. 	<ul style="list-style-type: none"> • 12-43/ha in un hunted populations. • 31% prevalence in some surveys, but always in association with infected possums. 	<ul style="list-style-type: none"> • As high as 37% in some areas, but always in association with foci of TB in possums. • Densities in NZ generally below the threshold required for self-sustaining infection.
Type of intervention	<ul style="list-style-type: none"> • Systematic culling in VRAs through a variety of methods. • Development of TB oral vaccine. 	<ul style="list-style-type: none"> • Culled only if epidemiological and survey information indicates that they could be acting as significant TB vectors. 	<ul style="list-style-type: none"> • Culled only if epidemiological and survey data indicate that they could be acting as significant TB vectors. 	<ul style="list-style-type: none"> • Culled if epidemiological and survey information indicates that they could be acting as significant TB vectors. • Targeted culling in some operations, to prevent establishment and spread of infected wild deer into VFAs.

order of probability) are targeted by ad-hoc control operations only if epidemiological and survey information indicates that they could be acting as significant vectors of TB. A variety of methods of vector control are used (e.g. aerial application of poisoned baits, ground application of toxins and trapping), depending on environmental considerations and cost-effectiveness. Vector control operations have reduced possum populations to very low densities within VRAs and this effort has been credited with the sustained decline in numbers of infected cattle and deer herds since 1996 (Table 5 and Figure 7).

Vaccination of cattle and farmed deer against TB in New Zealand would only be considered as a last resort in specific areas and would be based on a decision to

relinquish, at least temporarily, the eradication objective in those areas. By contrast, vaccination of possums has already been trialled in the field and would offer the potential to further reduce the rate of spread of infection in intractable wildlife TB hot spots where a low possum population density has already been achieved through poisoning. The development of an oral bait vaccine for possums containing a lipid formulation of BCG (an attenuated strain of *M. bovis*) is well advanced.

Canada

At the turn of the 20th century the prevalence of bovine TB in Canadian cattle was estimated at about 4%. An eradication programme was established in Canada in 1923. By 1961, animal prevalence of bovine TB had fallen to 0.11% through regular skin herd testing and slaughter of reactors.

M. bovis infection was first described in possums in 1967 and today this species is considered a maintenance host of *M. bovis* and its main wildlife reservoir in New Zealand.

From 1978 the thrust of the programme shifted from routine on-farm testing to routine slaughter inspection of cattle, with depopulation of infected premises. This includes slaughterhouse surveillance of Canadian cattle slaughtered in USA abattoirs (more than one million animals in 2002). In 1989 the TB eradication programme was extended to include farmed bison and cervids, resulting in the detection of 37 infected cervid herds through November 2003.

Currently, traceback investigations on all histologically suspect granulomas are supplemented by targeted on-farm testing of cattle herds in special eradication areas (see below). To compensate for the relatively small numbers of adult bison and deer consigned to slaughter, all herds of captive

bison and deer are routinely tested, usually at three-yearly intervals.

There are two known (but geographically and epidemiologically separate) reservoirs of TB in wild ungulates in Canada. *M. bovis* infection is endemic in the free-ranging wood bison (*Bison bison athabascae*) population in and around Wood Buffalo National Park (WBNP). This is Canada's largest national park, straddling northern Alberta and the Northwest Territories. Although WBNP was established in 1922 to protect the threatened wood bison population, the translocation between 1925 and 1928 of infected plains bison (*Bison bison bison*) from another national park in Alberta resulted in hybridisation of the wood bison and the introduction of bovine TB and brucellosis (eventually detected in 1937 and 1956 respectively). Because of its relative remoteness, the 4,700-strong wood

bison herd in WBNP poses a risk primarily to the healthy neighbouring wild bison populations. There has been no evidence of spillover of TB to other wildlife or domestic livestock. Plans were announced in 1990 to depopulate the WBNP, but were abandoned due to public opposition from the aboriginal and wildlife protection organisations. A series of multi-stakeholder and advisory committees and research programmes were set up in order to develop a management plan for the disease reservoir in the WBNP, but these were eventually dissolved due to lack of continuity in funding and stakeholder interest. An interim strategy for the containment of TB and brucellosis within the park was published in June 2004. This includes the creation of bison-free buffer zones around the park, an education programme and enhanced surveillance of commercial bison and cattle in the agricultural area west and south of the park.

Between 1997 and 2005 bovine TB was diagnosed in approximately 30 free-ranging elk, or wapiti (*Cervus elaphus manitobensis*) in and around the Riding Mountain National Park, situated in the middle of an agricultural area in south-western Manitoba. *M. bovis* infection is now considered endemic in the park's wild elk population, with some spillover detected in white-tailed deer (*Odocoileus virginianus*) and wolves (*Canis lupus*). Intensive skin testing of cattle herds within a special TB eradication area around the park has confirmed infection in six cattle herds in the same period. This has been attributed to direct and indirect contact of cattle with infected wild deer. Although both

the prevalence of infection and the deer density in the region appear to be low, infection is perpetuated by the congregation of wild deer in unnaturally high numbers at feed sites. In 2001, federal and provincial authorities instituted a TB management plan for this area, consisting of one to three-yearly testing of cattle herds around the park, improved TB surveillance in wild deer, erection of deer-proof fencing, legislation to deter deer baiting and management of the wild deer population by extended hunting seasons and wolf conservation.

Further challenges to the eradication of TB in Canada arise from sporadic TB incidents in exotic species at zoological collections and incomplete slaughterhouse surveillance of culled adult cattle, bison and deer.

Nevertheless, at the end of 2004 this country was nearing the complete eradication of bovine TB from cattle and farmed bison. According to domestic standards for cattle and farmed bison (harmonised with those of the USA since 1 January 2003) all ten provinces and territories of Canada, except Manitoba, are officially TB free. The TB incidence in cattle and farmed bison herds is very low. During the ten-year period from June 1993 through November 2003, *M. bovis* infection was confirmed in 12 herds of cattle and captive bison in eight separate outbreaks affecting five of Canada's provinces. This figure includes the cattle breakdowns around Riding Mountain National Park. A further breakdown was reported in 2004.

According to domestic standards for cattle and farmed bison all ten provinces and territories of Canada, except Manitoba, are officially TB free.

→ CONTINUED ON p38

United States of America

Brief history of the TB eradication programme in the USA

Bovine TB used to be the most prevalent infectious disease of livestock in the USA. In the early part of the 20th century *M. bovis* infection was also responsible for a significant proportion of TB cases in the human population. In 1917 the Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA) launched a state-federal TB eradication programme to eradicate bovine TB.

At that time, the national reactor rate in the USA was 5% of all cattle tested.

In 1940 this fell below 0.5% of all the cattle tested in every county.

There was some regression during World War II, but reactor rates had fallen to 0.06% by 1969. As TB

prevalence declined, the emphasis of the programme switched from area testing to slaughterhouse surveillance and infected herd depopulation, as in Canada and Australia. In 1987 regulations were tightened to reduce the risk of infection for cattle feedlots importing steers from Mexico. Most States and cattle herds in the USA are now officially TB free and the reactor rate in cattle is currently less than 0.02%. Endemic foci of TB in domestic cattle are confined to New Mexico, southern Texas along the Mexican border, northeast Michigan and, more recently, northwest Minnesota. These four areas comprise the vast majority of herds under restriction and have resisted TB eradication efforts for several years. The persistence of infection in at least one of these endemic foci is linked to the presence of a wildlife reservoir of *M. bovis* (see below). Meanwhile, herd breakdowns continue to occur sporadically

Endemic foci of TB in domestic cattle are confined to New Mexico, southern Texas along the Mexican border, northeast Michigan and, more recently, northwest Minnesota.

in accredited-TB free States at a rate of one to five per annum, with no obvious geographical pattern. The majority of these breakdowns are detected through slaughterhouse surveillance and herd trace-back.

Main features of the TB eradication programme

TB surveillance by skin testing, including periodic tests of cattle herds, plays a relatively minor role in the USA.

Fewer than one million skin tests per annum are conducted in a national cattle herd of approximately 99 million head (compare that with the 4.8 million

animal tests carried out in GB in 2005 in a population of nine million cattle).

In general, routine testing of cattle, farmed bison and captive cervid herds is limited to the few States or zones

where a TB eradication programme is underway to attain accredited TB free status. The remaining tests are conducted following trace-back of tuberculous cattle detected at slaughter and prior to interstate or international movement. Testing of captive cervids, bison and goats is also contemplated for herds wishing to attain or maintain TB accredited status.

The USDA's Uniform Methods and Rules (akin to the SVS TB Chapter), set out the minimum standards for the maintenance of TB-free accredited herds, the application and interpretation of official TB tests in cattle, farmed bison, goats and captive cervids and the requirements for interstate trade of cattle. Briefly, initial screening of cattle, bison or goats not known to be at risk is by the caudal fold SIT. The SICCT test is only applied for increased specificity on animals that react to the caudal fold SIT.

The cervical SIT with double strength tuberculin is the recommended test in exposed cattle and bison herds and the official test for routine use in captive cervids. Whilst skin tests are applied and interpreted in bison just as in cattle, special facilities are essential to safely handle bison and minimise the stress on the animals during testing. Although skin testing in the USA is normally undertaken by federal, State or accredited veterinarians, Federal and State technicians may perform routine screening tests under direct veterinary supervision. The γ -IFN test has been licensed in the USA for use in cattle and bison since December 2001.

This assay is now routinely used as a replacement for (or in parallel with) the SICCT when re-testing SIT reactors in herds presumed to be free from

In infected herds, the γ -IFN test is used in parallel with the caudal fold or cervical SIT as an alternative to depopulation.

infection. In infected herds, the γ -IFN test is used in parallel with the caudal fold or cervical SIT as an alternative to depopulation. Whole-herd slaughter is the option of choice if *M. bovis* infection is confirmed in a cattle or farmed bison herd. When this is not feasible, the herd is quarantined until it passes two tests at 60 days intervals, followed by one additional test 180 days later. Herds with unconfirmed TB breakdowns (non-visible lesion reactors only and no *M. bovis* isolated) can be released from quarantine after a single negative test of the entire herd 60 days after slaughter of the reactors. After release from quarantine, the herd is marked forward for five annual whole-herd tests.

Because of the very low incidence of the disease, bovine TB surveillance in the USA relies primarily on the examination of suspicious granulomas in slaughtered cattle, coupled with traceback investigations. Slaughterhouse surveillance for TB accounts for 95%

of all infected herds detected every year and is the responsibility of federal veterinarians and meat inspectors, supported by State meat inspectors in the smaller plants. In 2000, after a steady decline in the number of granuloma submissions, the USDA launched a plan for enhanced TB surveillance in the 40 large regional slaughterhouses processing 94% of all adult cattle. Meat inspectors are expected to submit at least five suspicious granulomas per 10,000 head of adult cattle killed in a plant. Inspectors receive cash awards for positive submissions and for each infected herd successfully back traced.

The sustained increase in the number of granulomas since 2001 has been attributed to this programme.

Despite these successes, however, herd traceback investigations only achieve a 50-70% success rate because of the lack of centralised cattle identification and tracing systems in the USA.

The USDA ranks states and zones into five possible stages as they make progress towards TB eradication. These stages are generally based upon the incidence of the disease in cattle herds. For a State or zone to be in the top category ('Accredited TB free' status) there must have been no confirmed TB breakdowns on holdings other than feedlots for at least five years. The State in question must also have a set of stringent regulations governing livestock dealers, maintain surveillance of cattle throughout the supply chain and require records to be kept to allow animal traceability. Accredited TB free State or zone status must be renewed annually. If *M. bovis* infection is confirmed in a herd within an accredited-free State or zone,

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this status can be retained as long as the herd in question is depopulated and an epidemiological investigation concludes that there was no evidence of TB spread. If two or more infected herds are detected within a 48-month period, the State or zone is downgraded one level to 'modified accredited advanced' status. As of January 2006, all States had 'accredited TB free' status for cattle and bison except Michigan, Minnesota, New Mexico and Texas. These four States (or parts of them) were classified by the USDA as 'Modified Accredited Advanced' due to endemic TB foci or (in the case of Minnesota) recent TB breakdowns of uncertain origin. Federal rules prescribe that all breeding cattle 18 months of age and older shipped out of those zones must be skin tested within 60 days of movement. This is without prejudice of any additional controls imposed by the importing States.

TB in wildlife in the USA

As described above, bovine TB has been nearly eradicated from cattle and captive bison in the USA. TB in farmed deer was identified as a potential problem in the USA in 1991, when an outbreak of TB in captive elk in Canada was back traced to stock imported from the USA. This incident led to the incorporation of captive cervids into the USDA TB eradication scheme in 1994. There are five possible State or zone designations for TB in farmed deer, which do not necessarily coincide with the status for cattle and bison. According to this system, all States in the USA are currently 'Modified Accredited' for captive cervids. Between 1991 and 2004, TB was disclosed in 41 captive cervid herds in 18 states

There is a strong association between the risk of TB breakdown on cattle farms and husbandry practices that favour deer access to cattle housing and feeding areas.

(30 of which were depopulated), but the incidence of TB in captive deer in the USA has been declining since 2000. In wild cervids, however, TB is an emerging disease that represents the greatest impediment to the complete eradication of TB from livestock in the USA.

Since 1994, an outbreak of bovine TB has affected cattle herds and free-ranging white-tailed deer in a 13-county area in the north-eastern lower peninsula of Michigan. This resulted in the loss of the State's accredited-free TB status in 2000. Between the spring of 1995 and 1 January 2005, more than 1,050,000 cattle in over 17,000 herds

have undergone skin testing in Michigan. Infection has been confirmed in 33 cattle herds and one captive deer herd, but no infected farms were identified in

the 2004-2005 testing season. Transmission from tuberculous free-ranging deer was the likely origin for a majority of these incidents. Between 1995 and 2005, 509 of 138,000 carcasses of free-ranging white-tailed deer examined by the State of Michigan tested positive for *M. bovis*. DNA typing of *M. bovis* isolates from deer, cattle and other mammals have showed that a unique strain is involved. This was the first self-sustaining outbreak of bovine TB described in free-ranging North American cervids and has become the most intensively examined example of TB in wild deer worldwide. *M. bovis* infection has also been reported in two deer hunters, four of 1,400 culled elk and in a variety of wild carnivores at a very low prevalence (except in the scavengers of deer carcasses such as coyotes *Canis latrans*). The low numbers of tuberculous non-cervid wildlife, the



Figure 9
Free-ranging white-tailed deer congregating at an artificial winter feed site in Michigan, USA (photograph reproduced from the Bovine Tuberculosis in Michigan Web site at: www.michigan.gov/bovinetb)

variety of species involved and the geographic spacing between cases are indicative of spillover from free-ranging deer, rather than endemic TB. Infection has not yet been disclosed in the North American badger (*Taxidea taxus*). American authors have estimated that *M. bovis* infection in cattle spilled over onto the white-tailed deer population in north-eastern Michigan in the first half of the 20th century, when TB in cattle was still quite prevalent. Although Michigan's cattle herds were declared accredited TB free in 1979, it is thought that the prevalence of *M. bovis* infection in wild deer was maintained at low values until it reached a detectable level in the mid-1990s, following a dramatic growth of the deer population. There is a strong association between the risk of TB breakdown on cattle farms and husbandry practices that favour deer access to cattle housing and feeding areas. It appears that environmental

(feedstuffs) contamination is a key mechanism of *M. bovis* transmission between white-tailed deer and cattle. White-tailed deer are a native wildlife species highly valued by the Michigan public and the hunting fraternity in particular. The practice of deer feeding and baiting by hunters, farmers and wildlife enthusiasts became popular in Michigan in the 1970s and 1980s to help deer survive during harsh winters. Deer gathering in unnaturally high numbers at feeding sites, increase the risk of deer-to-deer transmission of *M. bovis* (Figure 9). In 1995, the State Government passed legislation banning the feeding and baiting of deer across Michigan. Grants have been provided for farmers to erect deer-proof fencing around farms and feed storage areas. These measures, combined with extended deer hunting seasons and unlimited hunting quotas have reduced

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Table 7
Classification of Latin American and Caribbean countries according to the incidence of tuberculin test reactors (source: Pan-American Health Organisation, OIE and de Kantor and Ritacco 2006)

Incidence of reactors	Countries	Approximate total cattle population (2003)
Very low incidence (<0.1%) or virtually TB free countries	All the Caribbean countries except Haiti and the Dominican Republic Panama Honduras Belize Colombia Venezuela Surinam Uruguay	61.6 million
0.1 – 1%	Mexico Dominican Republic Nicaragua Costa Rica El Salvador Paraguay	47.4 million (of which 29.5 million head in Mexico)
>1% or unknown	Haiti Guatemala Argentina Bolivia Brazil Chile Ecuador Guyana Peru	265.1 million (of which 53.5 million head in Argentina and 182 million in Brazil)

7

the wild deer population by half since 1995. Considerable effort has also gone into educating cattle farmers and deer hunters and engaging the various stakeholder groups. The apparent prevalence of *M. bovis* infection in Michigan’s wild deer has fallen by 64% since 1997, but complete elimination of TB from cattle and wildlife may take another decade.

Latin America and the Caribbean

The prevalence of bovine TB and the measures applied to control it differ quite a lot across Latin American and Caribbean countries (Table 7). Argentina and Brazil (the two major cattle breeding and beef exporting countries in the region) launched national compulsory tuberculin test and slaughter programmes in 1999 and 2001, respectively. Despite these efforts, the TB situation in both countries is still far from being under control, as evidenced by a high incidence of reactor herds (and animals) and a rather worrying rate of cattle, water

buffalo and pig carcase condemnations resulting from TB at slaughterhouses. Furthermore, the occurrence of TB in large tracks of Argentina and Brazil is still largely unknown. Mexico has been ramping up its TB control programme over the last decade and made substantial progress in the beef herds of the northern states. However, significant pockets of infection remain in dairy herds and, as a result, the export of Holstein cattle to the USA is still banned. By contrast, Cuba, Jamaica, Panama, Uruguay and Venezuela have all but eradicated TB from their national herds after long-running campaigns. The main hurdles to the eradication of TB in Latin America appear to be an inadequate veterinary infrastructure, incomplete epidemiological data and a lack of standardised diagnostic methods and reagents across the region. On the positive side, no reservoirs of *M. bovis* infection have been reported in South American wildlife or in native species of domesticated mammals (e.g. New World camelids).

Africa

Bovine TB was considered a disease exotic to Africa, although it is nowadays present in cattle in almost all African countries. Information on the occurrence of bovine TB across this continent is very scarce, although there is sufficient evidence to indicate that *M. bovis* infection is widespread and found at high prevalences in some animal populations. It is believed that domestic cattle are the main host to *M. bovis* in Africa, but the organism has also been isolated from small ruminants, dromedaries and a large number of wild mammals. The public

health threat of *M. bovis* in Africa is also considerable, given that nearly 85% of its cattle and 82% of its human population are estimated to live in areas where the infection is prevalent or only partially controlled. However, it is difficult to assess the precise contribution of *M. bovis* infections from animal sources to the human TB epidemic in Africa. Limited laboratory resources preclude the differential diagnosis of human *M. bovis* and *M. tuberculosis* infections.

It is in South Africa where perhaps more information has been published about bovine TB in the continent. A national TB eradication scheme was introduced in that country in 1969. Although complete eradication of the disease has never been achieved, the overall incidence in commercial cattle remains relatively low. High morbidity

breakdowns in cattle herds have occurred sporadically since the first report of the disease in 1880. Those were believed to be the source of the first incident of TB in African wildlife, described in 1928 when *M. bovis* was isolated from lesions in free-ranging greater kudu (*Tragelaphus strepsiceros*) and lesser ungulates in the Eastern Cape province. There have been numerous reports of suspected and bacteriologically confirmed *M. bovis* infections in African wildlife since then (Table 8). Nowadays, the African (Cape) buffalo (*Syncerus caffer*) in Uganda and South Africa, the Kafue lechwe antelope (*Kobus leche kafuensis*) in Zambia and, possibly, the greater kudu in South Africa are all regarded as true maintenance hosts of *M. bovis* and a source of infection for cattle and other African wildlife. The best studied of these wildlife reservoirs (and probably the most

The public health threat of *M. bovis* in Africa is also considerable, given that nearly 85% of its cattle and 82% of its human population are estimated to live in areas where the infection is prevalent or only partially controlled.

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Country	Host species	Location	Year of report
Uganda	African buffalo (<i>Syncerus caffer</i>)* Warthog (<i>Phacochoerus aethiopicus</i>)	Ruwenzori Nat'l Park Ruwenzori Nat'l Park	1963, 1970s, 1997 1970s, 1997
Kenya	Olive baboon (<i>Papio cynocephalus</i>)	Maasai Mara Game Reserve	1980s
Zambia	Kafue lechwe (<i>Kobus leche kafuensis</i>)* Bushbuck (<i>Tragelaphus scriptus</i>)	Kafue river basin Private game ranch near Lusaka Private game ranch near Lusaka	1956, 1970s-90s 1998 1998
Tanzania	Eland (<i>Taurotragus oryx</i>) Wildebeest (<i>Connochaetes taurinus</i>) Topi (<i>Damaliscus lunatus</i>) Kudu (<i>Tragelaphus spp.</i>)	Northern Tanzania Northern Tanzania Northern Tanzania	1984 2005 2005 2005
South Africa	African buffalo* Greater kudu (<i>Tragelaphus strepsiceros</i>)* Duiker (<i>Sylvicapra grimmia</i>) Bushbuck Chacma baboon (<i>Papio ursinus</i>) Lion* (<i>Panthera leo</i>) Cheetah (<i>Acinonyx jubatus</i>) Leopard (<i>Panthera pardus</i>) Warthog Bushpig (<i>Potamochoerus porcus</i>)	Several private game reserves Hluhluwe-iMfolozi Park (HiP) Kruger National Park (KNP) Eastern Cape Province KNP and HiP Eastern Cape Province Eastern Cape Province KNP and HiP KNP and HiP KNP KNP KNP KNP HiP	1998 1986 Several reports from 1990 to date 1928, 1940, 1992 Several reports from 1996 to date 1928 1928 1995 1995 1995 1999 2005 After 1986

*presumed true maintenance hosts of *M. bovis* (in regions where infected individuals have been found). The role of the lion as a maintenance or spillover host in South Africa is debatable.

8

Table 8
Reports of *M. bovis* isolations from free-living African wildlife

important host for *M. bovis* in Africa) is the buffalo in the Kruger National Park, South Africa's largest wildlife reserve. There is strong circumstantial evidence that bovine TB was introduced between 1950 and 1960 by infected domestic cattle grazing in close proximity with buffalo on the southern border of the park. TB was eventually discovered in the Kruger's buffalo population in 1990 and has now reached epidemic proportions,

with prevalences of infection as high as 90% in some herds. Infection is maintained in buffalo herds mainly through aerosol transmission. Carcasses of tuberculous buffalo are a source of infection to predators and scavenging species via the oral route. Other wild animals contract bovine TB through environmental contamination. The expanding geographical range and prevalence of infected buffalo herds pose

a threat to rare wildlife species in the park and in neighbouring countries. This also represents a big challenge on long-term management strategies to prevent re-infection of domestic livestock. Where co-grazing of wild ungulates and domestic cattle takes place, recycling of infection is likely to continue with subsequent spillover to predators and scavengers at the top of the food chain. In the absence of an effective vaccine against TB and validated diagnostic tools for most species, *M. bovis* infection is likely to persist in free-ranging African wildlife for the foreseeable future.

Further reading

Available on request to the author.

Information

Ricardo de la Rúa Domenech is a Veterinary Advisor with Defra specialising in bovine TB

Bovine TB: Modelling and predicting its distribution in GB using CTS data

Author

Andrew Mitchell

Introduction

In April 2003 the Veterinary Laboratories Agency (VLA) received funding from Defra to carry out a nine month joint project with the Environmental Research Group Oxford (ERGO), to examine the Cattle Tracing System (CTS) data and assess the importance of cattle movement in the spread of bovine tuberculosis (BTB). The work followed on from an earlier VLA/ERGO collaboration assessing the environmental correlates of BTB distribution.

Geo-referencing and data transformations carried out on CTS data

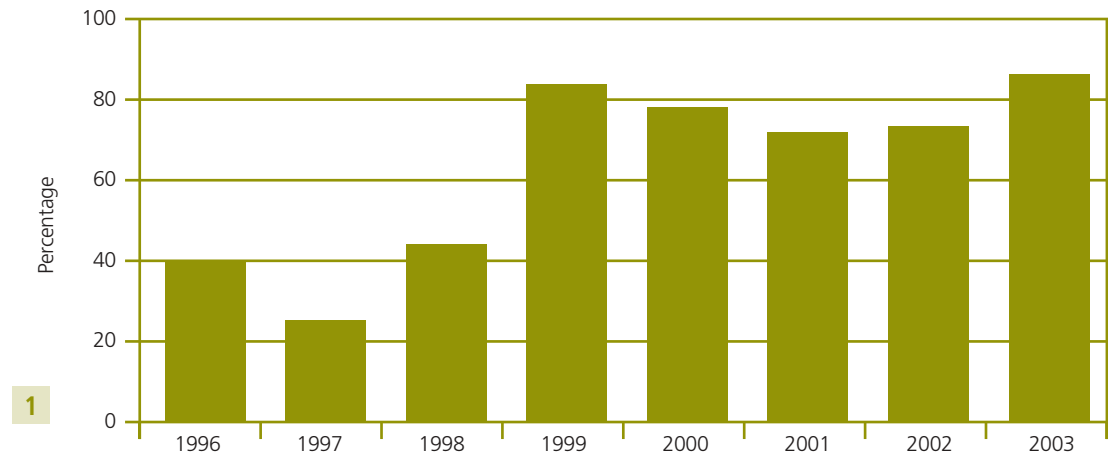
Before any modelling of CTS data could be undertaken, two main processing operations had to be carried out. These were firstly the geo-referencing of the CTS data – to enable its spatial representation in a Geographical Information System (GIS). The second stage required the ‘pairing’ of movement records. Because of the structure of movement records in CTS, where a single movement is stored in two elements – leaving a location (‘off’) and arriving at a destination (‘on’) – these two elements needed to be linked as a single paired record before further analysis could proceed.

Various methods were used to geo-

reference CTS locations. These methods either involved obtaining an Easting and Northing from CTS spatial data (the location address or its OS reference) or obtaining it by linking to other Defra data sets (e.g. VetNet or the Livestock Census). 98% of the locations associated with cattle movement were successfully geo-referenced.

The ‘pairing’ of movement records in CTS allows the creation of a logical movement history for a given animal. To enable the creation of logical movement histories the VLA developed a set of rules, with an underpinning rationale that if any part of an animal’s movement history could not be resolved, or was clearly incorrect (e.g. movements after death), then all movements associated with that animal were excluded from the paired movements dataset used in subsequent analysis. This approach resulted in 17.4 million animals being included in the analysis out of a total of 25.8 million, giving an animal rejection rate of 32.4%. Figure 1 shows the proportion of animals ‘included’ by birth cohort. This figure clearly shows the improvement over time of animals having a logical movement history which, since 1999 has stabilised at around 80%.

Figure 1
Proportion of Animals ‘included’ by Birth Cohort



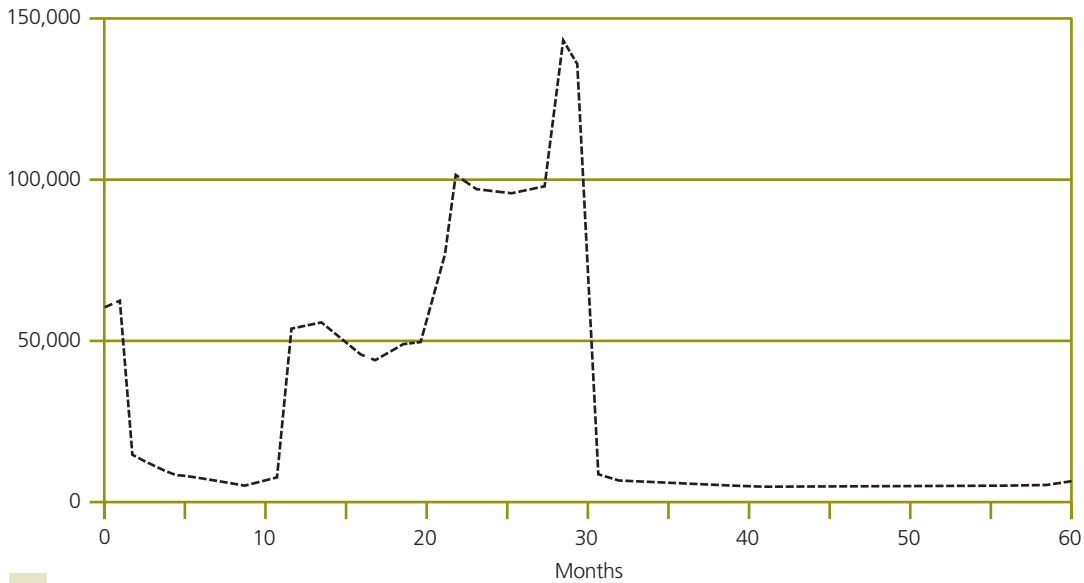


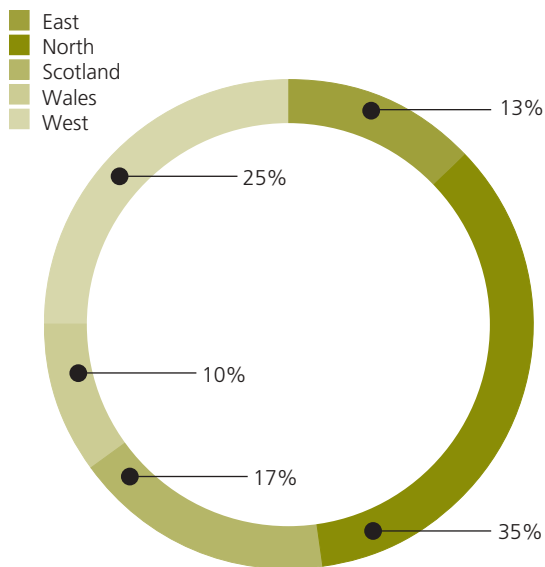
Figure 2
Age at death frequency distributions

2

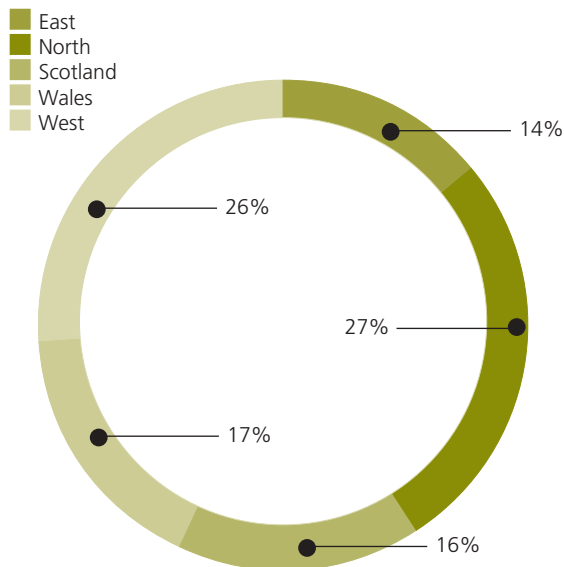
Characterisation of the national herd using CTS

Once the preparatory cleaning and data processing transformations had been carried out, it was possible to examine some of the basic characteristics of cattle movement and cattle populations in GB.

These characteristics included the age (at death) of the national herd (Figure 2); the movement of animals between Defra regions (Figure 3), and the details of movements out of particular regions (Figure 4).



3a



3b

Figure 3
The distribution of originating region of movement in 2002
(a) Originating region of movement
(b) Distribution of holdings on VetNet

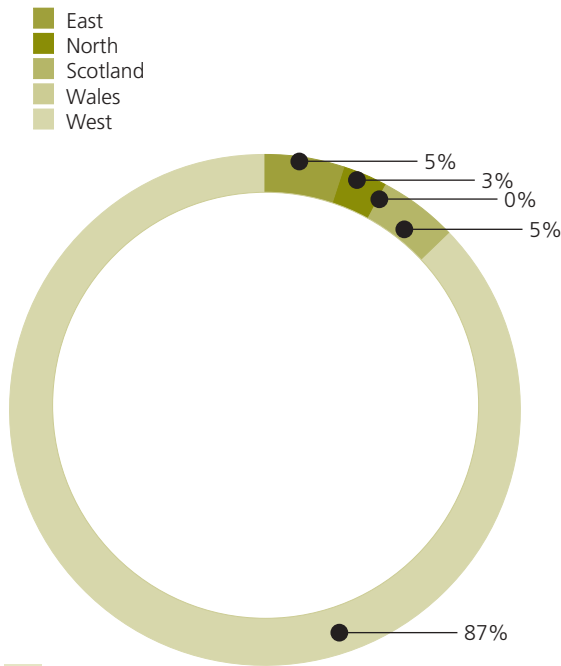
The regional origin of 1.84 million farm-to-farm cattle movements in 2002, shown in (a), was broadly similar to the distribution of cattle holdings in the VetNet database (b).

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Figure 4
Movements out of the Western region in 2002

(a) Receiving farm location

(b) Farm-to-farm cattle movements from the Western Region



4a

It was also possible, by combining CTS data with existing TB data sets, to investigate specific TB related questions such as the movement of cattle between the different parish testing interval regimes (Figure 5).

Extensive use has since been made of this type of data. The pattern of movements between different areas, holding types, herd types and parish testing regimes have been examined to attempt to gauge the effects of different pre-movement testing scenarios.



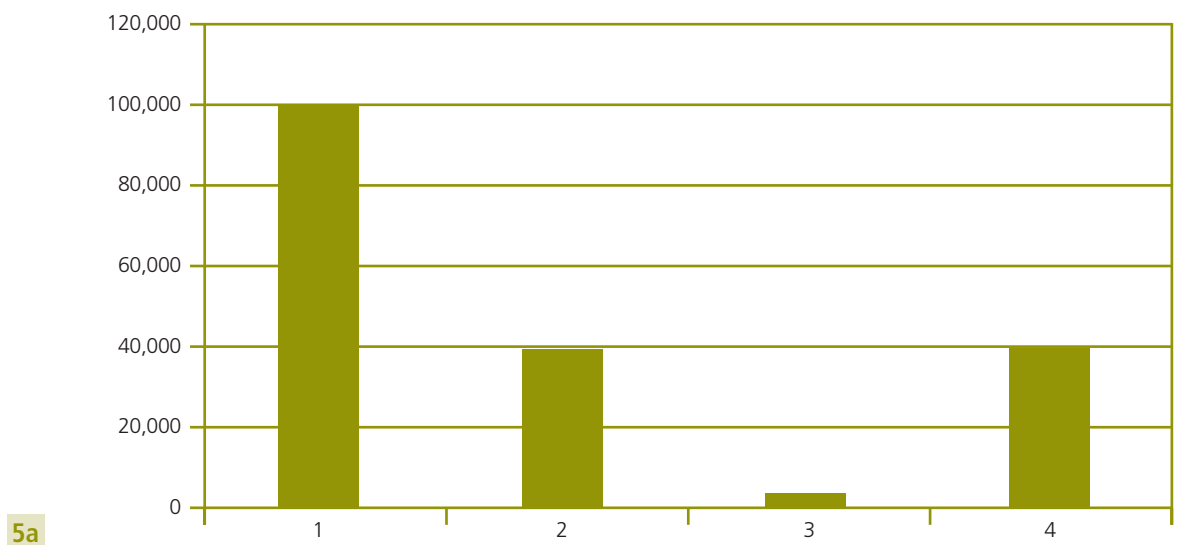
4b

In 2002, there were 453,505 farm-to-farm cattle movements originating from the Western Region.

Figure 5
Movements between parish testing regimes in 2002

Year 2002

(a) 1 Year



5a

5b

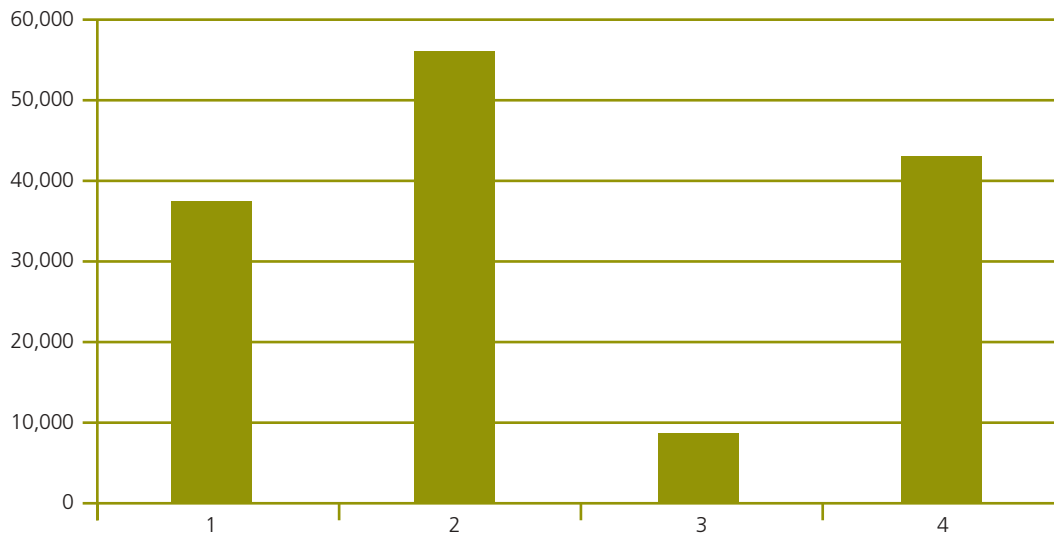


Figure 5
Movements between parish testing regimes in 2002

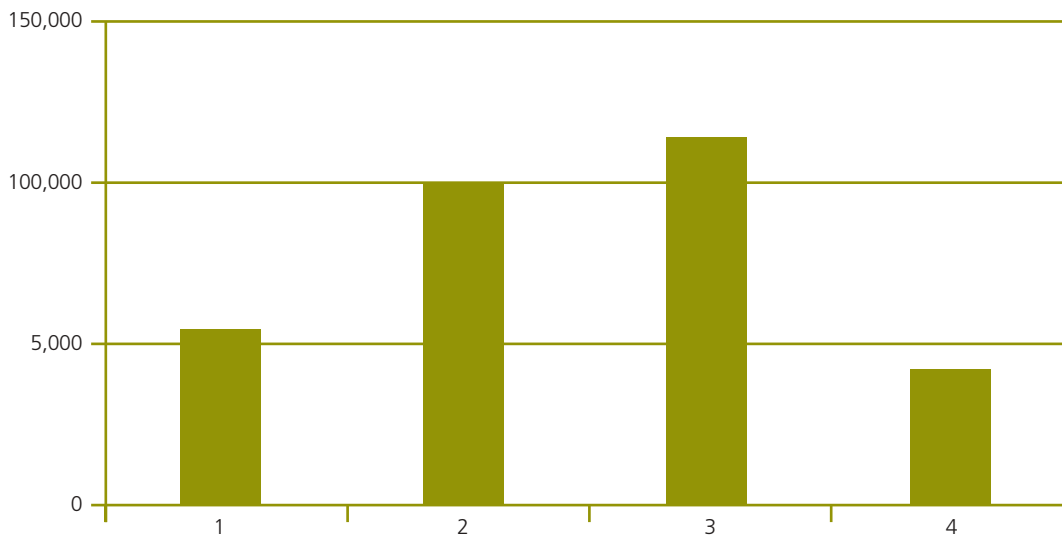
Year 2002

(b) 2 Year

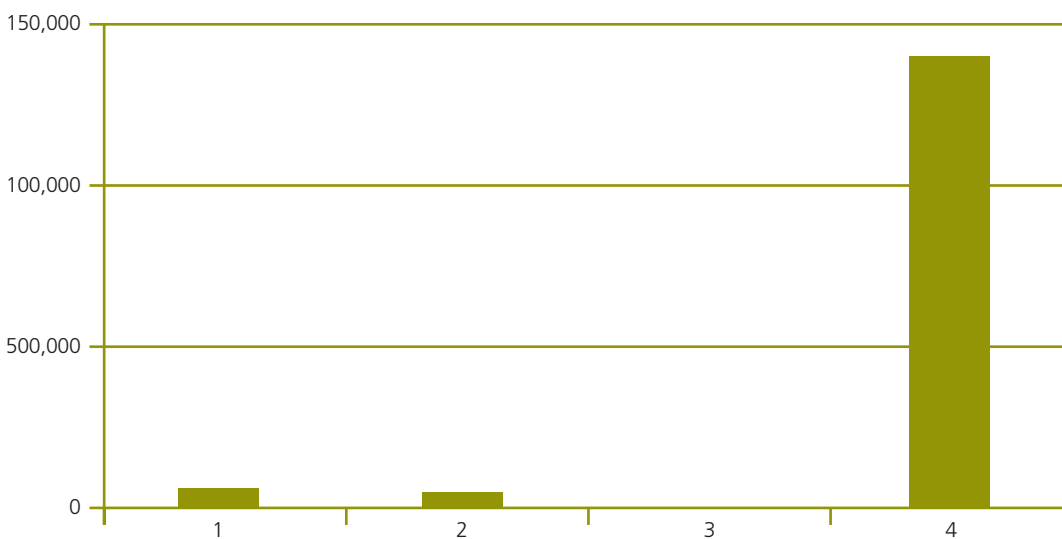
(c) 3 Year

(d) 4 Year

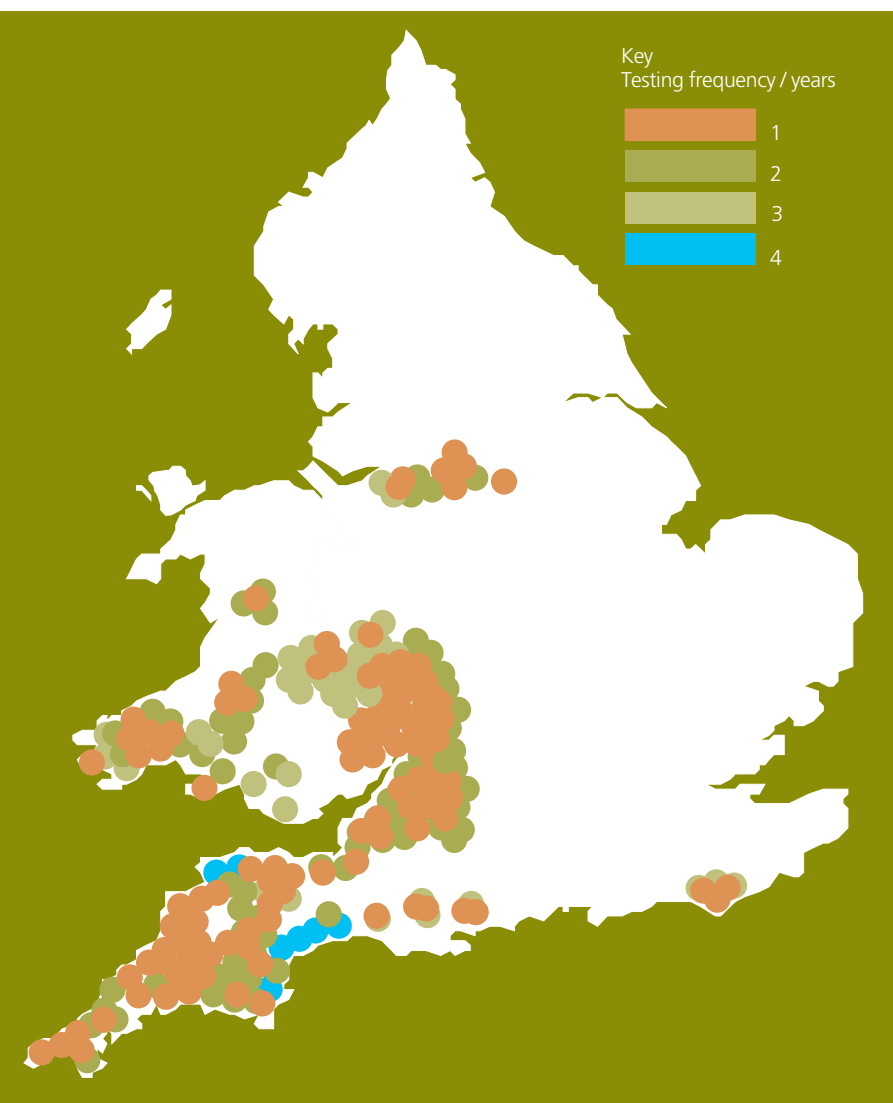
5c



5d



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5e

Figure 5
Movements between parish testing regimes in 2002

Year 2002

(e) Parish testing frequencies as recorded on VETNET @ 17/12/03

Results of modelling the spread of TB

The main purpose of the data cleaning and transforming of the CTS data was to allow the CTS data to be put into Grid Data format (Figure 6), so that it could be put into the GIS models and used as a potential predictor of BTB.

Two different modelling approaches were undertaken on the cleaned and transformed data. The first being distribution modelling, which seeks to establish what parameters are important in the spread of BTB and which can then be used for short-term BTB predictions. The second method used was stochastic simulation (a simulation governed by the laws of probability); this provides a dynamic disease spread model, which can be used for giving medium term predictions.

Distribution modelling uses logistic regression to establish a relationship between the presence of BTB and a series of significant predictor variables. The final equation is then applied to the complete predictor grid coverages to generate a predicted BTB distribution.

The predictor variables used are shown in Table 1. These included those found to be significant from the previous modelling work (including indicators of seasonality, climatic variation, vegetation, land use, demography, cattle density and an index of badger presence) plus new predictors obtained from the CTS. All the CTS variables were found to be significant predictors of disease presence, with the proportion of movements into an area from infected areas consistently outperforming all other variables in the model.

Stochastic simulation modelling again uses logistic regression to model the spread in BTB distribution. The model is applied to a known starting distribution and produces predictions over a number of years that can be projected into the medium-term future.

For the simulation method, predictors were identified separately for 'core' and 'remote' TB areas. Core areas were defined as those cells in which disease had been found in at least two of the previous three years, with remote areas being all other areas. For core areas only two variables were significant (these were the BTB status of both the cell and the surrounding area in previous years) whilst for remote areas the proportion of movements from infected areas was again the best predictor of BTB. This suggests that the variation in the levels of movement of infected animals has a greater impact on disease levels in remote areas where the disease has yet to become established.

Figure 7 shows an example of the predictions given by the simulation modelling. These show good agreement with those obtained by distribution

Table 1
Predictor data used
in the analysis of
BTB occurrence

Type	Variable
Anthropogenic	Distance to roads (DROADS) and city lights (LIGHT)
Demographic	Human population density (HPOP)
Land cover	<i>Percentage cultivation and managed grassland (PCU); Proportion urban and suburban land cover (PUR); Woodland (PWO), Open Woodland (POW); Grassland (PGR); Water (PWA); Normalised Deviation Vegetation Index (NDVI^a); Length of Growing Period (LGP).</i>
Geographic	Longitude (LONG), Latitude (LAT)
Topographic	Elevation (ALT)
Temperature	Air Temperature (AT ^a); Land Surface Temperature (LST ^a); Middle Infrared Reflectance (MIR ^a);
Water and moisture	Vapour Pressure Deficit (VPD ^a); Distance to Rivers (DRIV); Potential Evapo-transpiration (PEV)
Zoological	<i>Cattle density (CAD); Holding density (HOD); Proportion of dairy cattle (PDC); Herd size (HES); Badger record density (BAD); Distance to nearest recorded badger presence (DBR).</i>
Cattle movement	Total movements in (TM); Total movements in from infected areas (TMI); Proportion of movements from infected areas (PMI).
BTB persistence and local spread	Number of years of past BTB infection in the 5km cell (NYBTB); number of infected cells in the previous year in a 5km radius doughnut window (DNT); Distance to the nearest cell with BTB present (DBTB)

1

modelling and they highlight the potential for the local spread of the disease in Cumbria and the Scottish borders.

The future

The work carried out and the experience gained in using the CTS data will enable its future use for the epidemiological analysis of cattle movements and disease spread.

The modelling work undertaken will have three main uses for future TB control and policy. It will allow the identification of new TB risk areas and high-risk movement types. Also, by adjusting movement data within the model, the effect of future movement restrictions can be modelled.

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Figure 6
Example of CTS in
Grid data format
(grid cell resolution –
5km)

Figure 7
The probability of
BTB presence in
2005 as predicted by
simulation modelling



Distribution of cattle 'on' movements that came from grid cells classified as infected in 2000.

Further reading

Available on request to the author.

Acknowledgements

I would like to acknowledge the fellow contributors at the VLA to this project – Dr Richard Clifton-Hadley and Joshua Mawdsley. I also wish to acknowledge the large contribution made to this project by Dr David Bourn, Dr Willy Wint from the Environmental Research Group Oxford (ERGO) and Dr Marius Gilbert from the Free University of Brussels. The time given by Mel Reader (CTS consultant working for Defra) in explaining the complexities of the CTS data was very much appreciated.

Information

Andrew Mitchell is the TB database manager, in the Centre for Epidemiology and Risk Analysis at the Veterinary Laboratories Agency in Weybridge.

The laboratory diagnosis of Bovine Tuberculosis

Bovine tuberculosis is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*. This bacterium is an obligate pathogen, but it can survive for several months in the environment in moist soil and in darkness. It is highly susceptible to sunlight (UV radiation) and remains viable for long periods (>6 months) in frozen tissues.

Cattle are major hosts but some wildlife populations act as reservoirs for the agent. *M. bovis* has been isolated from nonhuman primates and also in buffalo, bison, zebu cattle, sheep, goat, equine, camel, pig, wild boar, cervidae, antelope, dog, cat, fox, mink, possum, ferret, rat, llama, kudu, eland, tapir, elk, elephant, situtunga, oryx, addax, rhinoceros, feline (exotic and domestic), squirrel, otter, seal, hare, racoon and other mammals. There is evidence to suggest that *M. bovis* has become established in the environment in Great Britain where badgers are

considered to be an important reservoir of infection.

Infection in cattle is usually detected in the live animal on the basis of the tuberculin skin test. After death, it is diagnosed by post-mortem examination and then confirmed in the laboratory by histopathological and bacteriological techniques.

The Foot and Mouth Disease outbreak in 2001 brought a temporary halt to the cattle skin testing programme and when this resumed there proved to be a

dramatic increase in the number of tissue samples submitted for laboratory examination.

Laboratory tests are

lengthy and expensive and consequently, the drive for improvement has intensified.

Infection in cattle is usually detected in the live animal on the basis of the tuberculin skin test.

Bacteriology

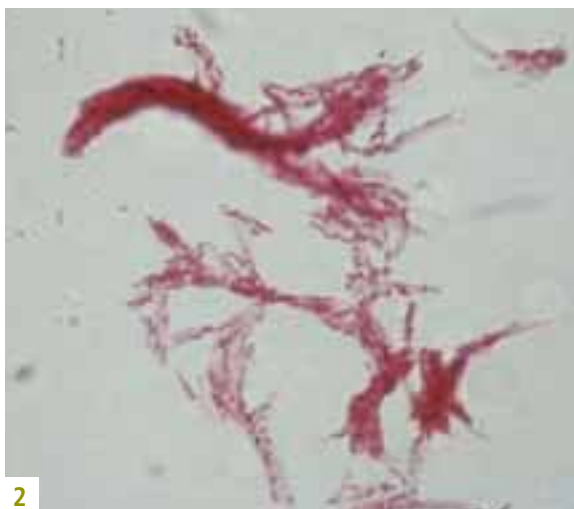
Due to its hazardous nature, all work is conducted in a containment level three laboratory. Field samples and cultures are

Authors

Keith Jahans and
Danny Worth



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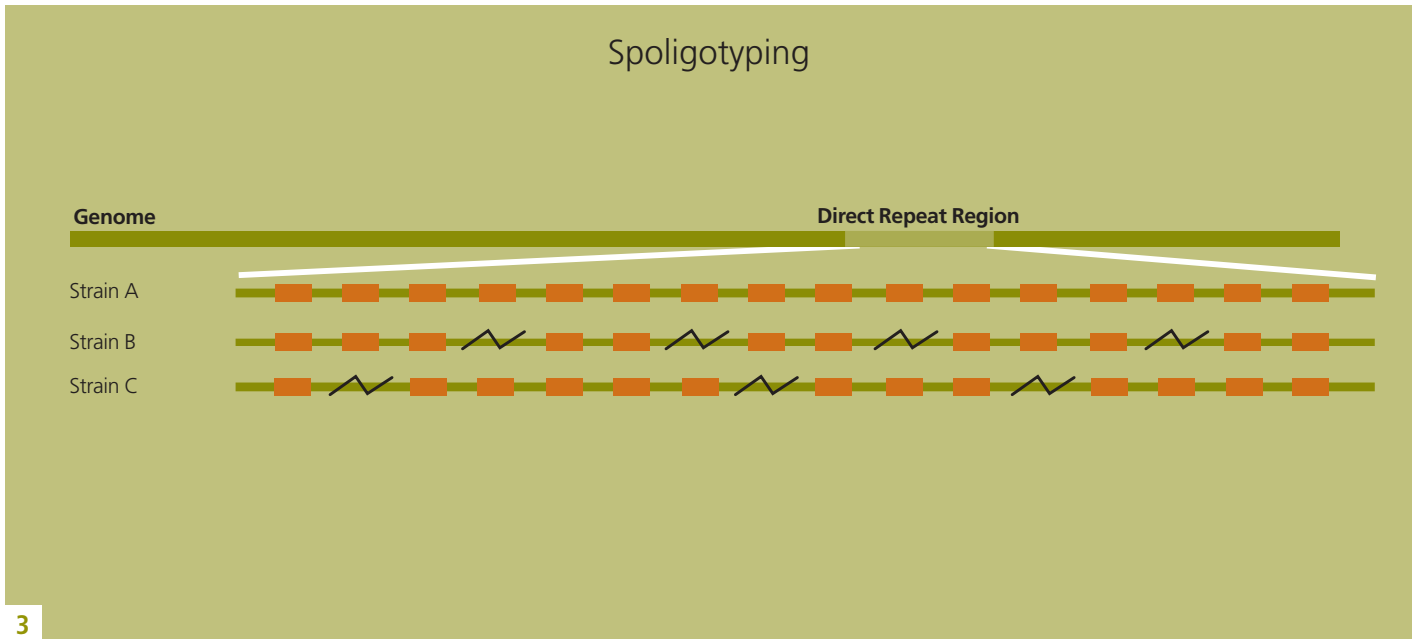


2

Figure 1
7H11, Stonebrinks and Lowenstein-Jensen media showing colonies of *M. bovis*

Figure 2
Ziehl-Neelsen stained slide of *M. bovis* showing 'chording'

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3

Figure 3
Representation illustrating differences in spacer oligonucleotides

handled in a Class one microbiological safety cabinet (MSC) with a Defra approved disinfectant to hand. Staff wear protective clothing and surgical gloves.

Tissues for cultural examination are homogenized with a solution of 5% oxalic acid, in order to destroy any contaminating organisms, centrifuged at 1100g for ten minutes and the resulting deposit washed and re-suspended in 10ml of 0.85% sterile saline. The suspension are sown on to the following media slopes: three Stonebrink's, two Lowenstein Jensen base (LJ), two Lowenstein Jensen + Glycerol (LJG), two Lowenstein Jensen + Pyruvate (LJP) and three Middlebrook 7H11. The slopes are incubated at 37°C for up to six weeks and then examined for growth.

M. bovis will grow on all these media but most strains are inhibited by glycerol and enhanced by pyruvate. The organism has a distinctive morphology on the media (Figure 1). On 7H11 in particular, it produces flat, matt colonies that float off the media when the condensation liquid is run up the slope. The media will

also support the growth of a wide range of other mycobacteria. Ziehl-Neelsen (ZN) staining may also prove useful in diagnosis. Mycobacteria are acid-fast and stain red. *M. bovis* sometimes produces a typical 'chording' effect (Figure 2). Diagnosis is based on these cultural observations with the help of histology.

Molecular typing

Individual strains of *M. bovis* can be distinguished according to their genetic finger-print. The principal typing method

is known as spoligotyping. It is based on DNA polymorphism present at one particular chromosomal locus,

the 'Direct Repeat' (DR) region, which is unique to the *Mycobacterium tuberculosis* complex bacteria of which *M. bovis* is a member. This method distinguishes between strains by identifying the presence or absence of known spacer oligonucleotides (Figure 3) in the DR region, hence the term spoligotyping.

Strains are grown, heat killed and subjected to Polymerase Chain Reaction

Diagnosis is based on these cultural observations with the help of histology.

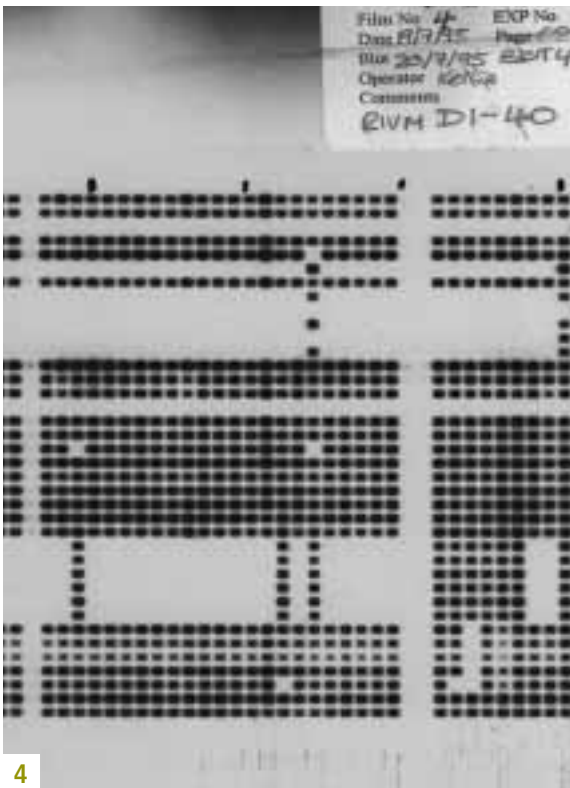


Figure 4
Autorad showing
different 'bar code'
spoligotype patterns

(PCR) using primers based on the DR region allowing amplification of the spacers in between. Forty-three unique spacer oligonucleotides are hybridised permanently to a nylon membrane. The PCR products from the killed strains are hybridised to the membrane at right angles to the spacers. After Hybridisation the membrane is developed by an enhanced chemiluminescence system. An image showing the presence or absence of spacers, giving the impression of individual bar codes representing each sample, is developed by autoradiography (Figures 4 and 5).

The different spoligotypes, combined with spatial clustering, has provided useful information on the spread of the disease (Figures 6 and 7). Data from spoligotyping is now available to the SVS to help trace the source of disease breakdowns. Types nine and 17 have been found to comprise approximately 70% of *M. bovis* positive cultures. Because of this, an alternative typing method is being introduced to provide further differentiation. Genetic loci containing variable

numbers of tandem repeats (VNTR) form the basis for human gene mapping and identification, forensic analysis and paternity testing. The genome sequence of *M. tuberculosis* revealed a large number of targets of tandem repeats and so this was the method chosen. Since applying this technique, VLA has shown that the same spoligotype may be split into a number of VNTR types. In addition, VNTR has been shown to discriminate between *M. bovis* isolates with the same spoligotype but from different geographical areas of Great Britain. Used in conjunction with spoligotyping it has provided valuable information about the evolution of the *M. tuberculosis* complex strains.

Acid-fast organisms which are not recognised as *M. bovis* by spoligotyping or do not show growth characteristics typical of *M. bovis* are tested by multiplex PCR. The amplification process is moderated by several oligonucleotide primers that, typically, are 20-30 nucleotides long. This is where the multiplex PCR differs from a conventional PCR which generally only uses one set of primers. The primers are single-stranded DNA that have sequences complementary to the flanking regions of the target DNA. Following PCR, the amplification product can be detected using gel electrophoresis. Visible bands are measured against reference DNA bands added at either end of the gel pre electrophoresis. When the gel is run the reference DNA produces a 'ladder' of bands at intervals of 100bp. All members of the Mycobacterium genus produce a band at 1030bp. However, the TB Complex (which includes – *M. bovis*, *M. microti*, *M. tuberculosis* and *M. africanum*) show a second band at 372bp, *M. avium* a second band at 180bp and *M. Intracellulare* a second band at 850bp.

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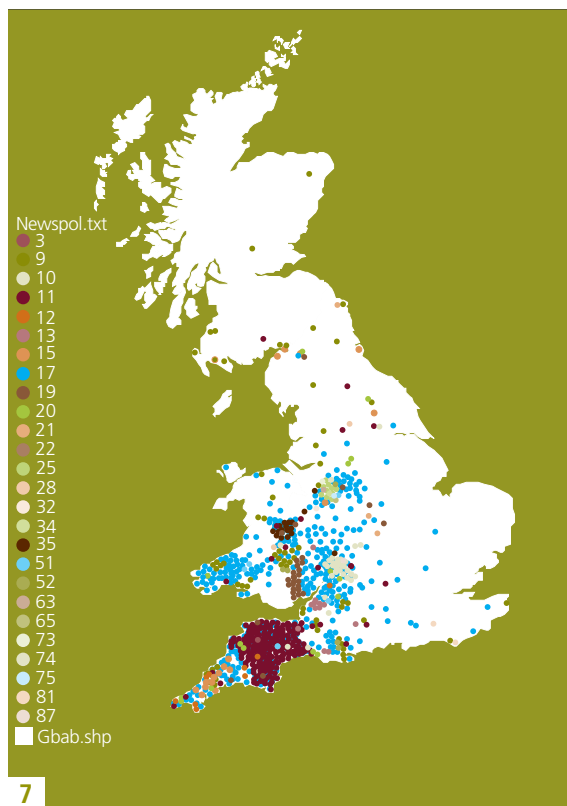
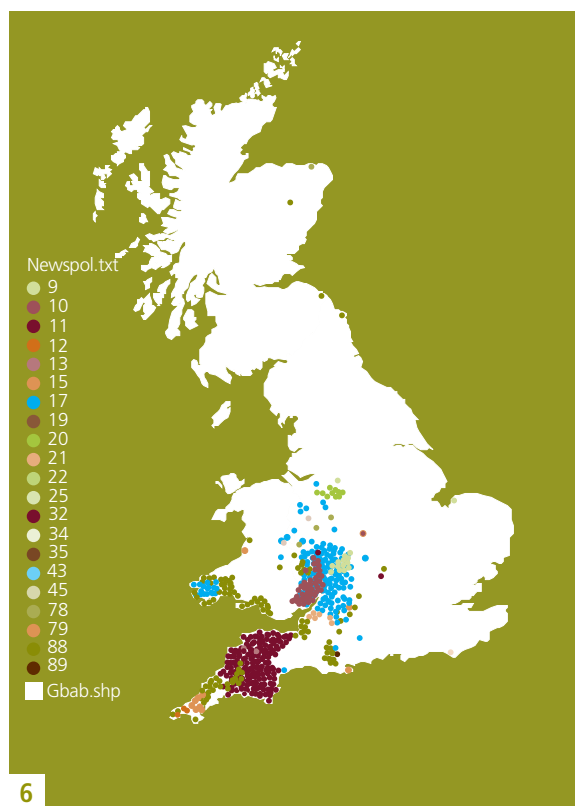


Figure 6
Cattle Spoligotypes
2000

Figure 7
Cattle Spoligotypes
2005

samples and are being used by some veterinary laboratories for isolating *M. bovis*. These systems are able to identify *M. tuberculosis* after only 15 days. Work at Weybridge by the TB research group has shown that *M. bovis* differs from *M. tuberculosis* in the way it metabolises certain key substrates. Recent studies at VLA Starcross and VLA Weybridge have shown that the Becton Dickenson MGIT 960 system could be used for *M. bovis* isolation and further work is being carried out to evaluate its use with farm animal and wild-life samples.

The sequencing of the *M. tuberculosis* and *M. bovis* genomes has meant that we are beginning to understand more about the biology of the organisms and the nature of the disease. This has already provided us with valuable information to fine tune and further develop our laboratory techniques. As our understanding increases so will the efficiency of diagnostic tests.

Thus the quality of the information that the VLA provides to the SVS can only be improved.

Information

Keith Jahans is the TB and Anthrax Diagnosis Workgroup Leader in the Statutory and Exotic Bacteria Unit at the Veterinary Laboratories Agency.

Danny Worth manages the TB Diagnostic Laboratory within the Statutory and Exotic Bacteria Unit at the Veterinary Laboratories Agency.

The introduction of pre and post-movement TB testing in Scotland for cattle from high incidence TB areas

Author

Dr Martyn
J Blissitt

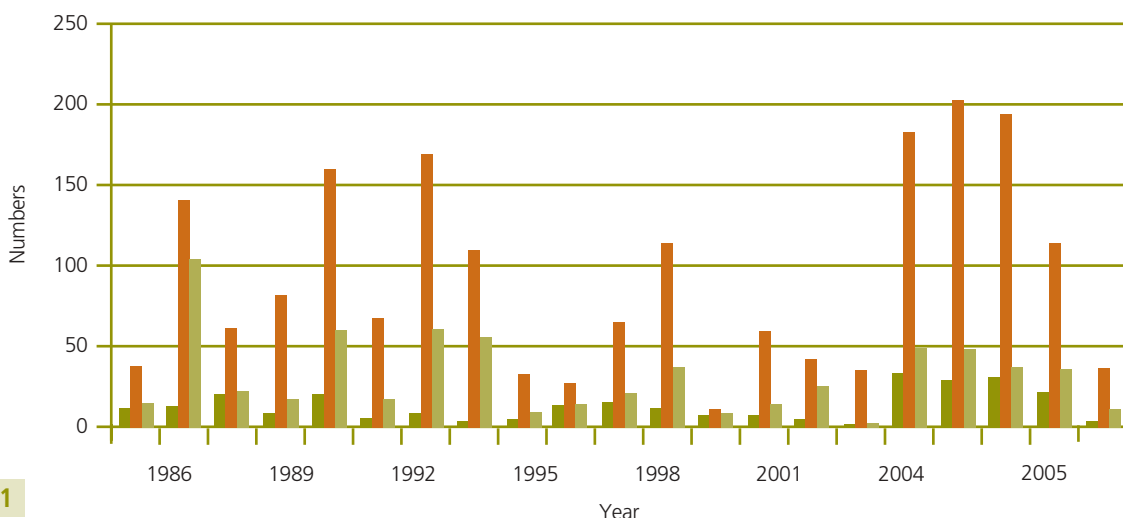
The incidence of bovine tuberculosis in Scotland

Scotland has, for many years, enjoyed a low and relatively stable incidence of bovine tuberculosis (TB) in its cattle herds (Figure 1). From 1986-2000, the number of new confirmed incidents each year was generally in single figures. The three exceptions were in 1988 and 1990 when 12 new confirmed incidents were recorded and in 1996 when ten new confirmed incidents were disclosed. The annual number of reactors over this period ranged from nine (1998) to 167 (1992) and the larger numbers of reactors reflect the years when a whole herd slaughter was required. Whole herd slaughters are occasionally required in four yearly testing areas (all parishes in Scotland are on four-yearly testing) because the disease has longer to establish itself between routine periodic tests.

In 2001, routine TB testing was suspended as all available veterinary resources were redirected to the eradication of the national foot and mouth disease outbreak. The two TB cases detected in Scotland in 2001 were both slaughterhouse cases. When testing resumed in 2002, 28 new confirmed cases were disclosed and it has taken until 2005 to see the numbers falling back to 13 new confirmed cases, with a reasonable prospect, in most areas, of now returning to pre-FMD levels of TB. The increase in the number of TB cases in Scotland post-FMD can be put down to a combination of:

- (a) The suspension of TB testing in 2001 leading to the detection of 'two years worth' of cases in 2002;
- (b) A reflection of the worsening TB situation in parts of England and Wales where Scottish farmers could trade cattle freely and;

Figure 1
Incidence of Bovine
Tuberculosis



1

Figure 2
TB in Scottish
Wild Animals



2

(c) Post-FMD restocking movements. In fact, only six new confirmed incidents of TB in Scotland were on farms restocking post-FMD (three in 2002 and three more in 2003). In each case disease was quickly eradicated under the existing policies of test and slaughter.

There remain two essential differences between the patterns of TB outbreaks seen in Scotland and those in the higher incidence areas of England and Wales. Firstly, there is no evidence of recurrence

of disease on farms in Scotland and secondly, there is as yet no evidence of 'hot spots' of disease involving similar spoligo/VNTR types on neighbouring farms. Both factors are taken as evidence that there are no significant wildlife reservoirs of infection for cattle in Scotland at the moment. This should also be true in the lower incidence areas of England and Wales. Indeed, there are

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Table 1
Origins of
Scottish cases

Origin of Scottish Cases				
	2002	2003	2004	2005
Breakdowns	28	22	23	13
Breakdowns where Origin identified	19	17	11	5
Origin associated with movements from EW&I	19	13	11	5

1

only five recent isolations of *Mycobacterium bovis* in Scottish wildlife – in four deer and a badger from widely different locations (Figure 2). None of these cases had any association with a cattle herd breakdown. Official surveillance programmes have, however, been limited to the 48 badgers submitted by the public under the Road Traffic Accident Surveillance (1972-1991 where one was positive) and the routine surveillance of deer at post-mortem examination. Without more significant evidence of wildlife reservoirs in Scotland it has not been considered a priority to conduct further surveillance more widely. In any case, there are limitations with the interpretation of data from surveys such as those reliant on road traffic accident data.

It can be reasonably concluded that traditional TB control policies still work quite efficiently in Scotland and other low incidence areas. The new confirmed annual incidence figures that have been achieved in Scotland are probably the closest that current policies can get to eradication – given the free trading position with high incidence TB areas in GB and Ireland.

Origins of infection

Table 1 shows the number of new confirmed breakdowns where the State Veterinary Service is satisfied that the origin of a new Scottish breakdown has been established. It is not possible to

definitely identify origins in all cases, however, where cattle have moved from known or previously unidentified infected farms and where spoligo/VNTR strain types match, then the origin is considered to have been established for tracing purposes. The data shows that the proportion of origins identified varies from year to year, however, where origins are identified, almost all cases are a result of ‘importing’ cattle from high incidence areas of England, Wales and Ireland. These account for 48 out of the 52 cases where an origin has been identified during this period.

Policy development

In 2003 the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the State Veterinary Service in Scotland, began to assess the possibility of introducing a statutory requirement for pre and post-movement TB testing of cattle coming to Scotland from high incidence TB areas.

Relative effectiveness of pre and post-movement testing

A model for pre and post-movement testing already existed for cattle coming to Scotland from Ireland, where cattle are moving from a high to low incidence area and are TB tested before and after the movement. Pre-export TB testing data from 1997-2002 was helpfully provided by veterinary colleagues in Belfast and Dublin and an analysis was undertaken



Figure 3
Herd of
highland cows

to determine the pre-movement test failure rate. However, it was apparent that pre export tests may have been coded as 'private tests' or 'part herd' tests and it was difficult to establish a failure rate specifically for the population of cattle tested prior to movement to Scotland. However, from the available data, and acknowledging the constraints on the pre-export testing analysis, the exercise indicated an approximate pre-movement failure rate (in terms of reactors) of between one in 1,000 animals (ROI data 1999-2002) and one in 250 animals (NI data for 2002).

A more specific post-import analysis for Irish cattle (1992-2002) revealed that, at 60 day post import testing, a further one in 847 were subsequently disclosed as reactors in Scotland. For Scotland this has meant revealing between one and ten reactors per year in an imported population averaging 3,380 cattle/per year. These failure rates are therefore similar to pre-movement failure rates.

The previously accepted view was that pre-movement testing would disclose the majority of reactors and that post-movement testing would disclose a much

smaller number of reactors that had been incubating disease at the first test. However, it appeared from the analysis above that the numbers of reactors detected at pre and post-movement tests, when cattle went from high to low incidence areas, are not dissimilar.

Cattle movements from high incidence areas of England and Wales to Scotland

Work was undertaken with British Cattle Movement Service (BCMS) and IBM colleagues to quantify the number of cattle in the 'at risk' population, coming to Scotland from high incidence areas of England and Wales. Preliminary analysis of Cattle Tracing Scheme data suggests that between 15,000 and 20,000 cattle make up the population that would be eligible for pre/post-movement testing each year.

Consultation

In February 2004, GB Agriculture Departments jointly published a consultation document 'Preparing for a New GB Strategy on Bovine Tuberculosis'. The consultation emphasised the need to reduce the risk of spreading TB from high to low

incidence areas and several options were considered including:

- (a) Zoning – banning all cattle movements from high incidence areas;
- (b) Pre-movement testing;
- (c) Post-movement testing;
- (d) Pre and post-movement testing.

Veterinary advisers in Scotland indicated that only a complete ban on movements would eliminate the risk of disease spread from cattle movements.

Preliminary analysis of CTS data suggests that between 15,000 and 20,000 cattle make up the population that would be eligible for pre/post movement testing each year.

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4
Figure 4
Highland cow being prepared for testing

However, the risk could be reduced, and pre and post-movement testing of all cattle moving from high incidence TB areas to low incidence areas (except calves under 42 days and those going direct to slaughter) was likely to yield the greatest risk reduction. Furthermore, cattle should be permitted to move freely for a period of 60 days after the pre-movement test. This was described as SEERAD's preferred option in the consultation document.

A further advantage of employing pre and post-movement TB testing is that the 80% sensitivity of the TB skin test (a generally accepted figure) can be increased to 95% by testing twice, i.e. also employing post-movement testing. In other words, instead of identifying an average of 16 out of 20 previously undetected infected cattle, testing twice provides the potential to identify 19 out of 20 previously undetected infected cattle before they enter the Scottish herd.

A further advantage of employing pre and post-movement TB testing is that the 80% sensitivity of the TB skin test (a generally accepted figure) can be increased to 95% by testing twice.

Stakeholders

A 'pre-movement testing stakeholder group' was set up on a GB basis towards the end of 2004 and chaired by Bill Madders. In addition, a Scottish post-movement testing stakeholder group was established in Edinburgh and included representatives of the National Farmers Union of Scotland, The Institute of Auctioneers and Appraisers Scotland, Local Authority representatives, National Beef Association and Scottish Association of Meat Wholesalers. Stakeholders were unanimously supportive of a pre and post-movement testing policy and SEERAD were able to refine the proposed policy to address the remaining concerns that stakeholders had. One such concern was the need to ensure that Scottish farmers could identify eligible cattle, i.e. those that should have been pre-movement tested before coming to Scotland and that would need post-movement testing after arrival.

The 'TB Strategic Framework for the sustainable control of bovine tuberculosis in GB'

In February 2005, the GB Agriculture Departments published the 'TB Strategic Framework' document and recognised that a regional approach was required to 'slow down and stop the geographic spread of the disease' and to 'keep clean areas clean'.

The Tuberculosis (Scotland) Order 2005

The new legislation came into force in Scotland on 23 September 2005. It requires Scottish cattle keepers to:

- (a) Ensure that cattle from high-risk parishes are TB tested no more than 60 days before the date of movement from the holding of origin;

Destination Country	Destination AHDO	Destination County Name	Section 1	Section 2	Section 3
Scotland	Ayr AHDO	Ayr	4	5	0
Scotland		Dumfries	37	162	5
Scotland		Kirkcudbright	34	42	0
Scotland		Renfrew	0	84	0
Scotland		Wigtown	1	20	0
	Sub-Total		76	313	5
Scotland	Galashiels AHDO	Berwick	70	117	2
Scotland		East Lothian	0	1	0
Scotland		Lanark	3	8	0
Scotland		Mid Lothian	21	6	0
Scotland		Roxburgh	45	39	18
	Sub-Total		139	171	20
Scotland	Inverness AHDO	Nairn	40	0	0
	Sub-Total		40	0	0
Scotland	Inverurie AHDO	Aberdeen	149	333	20
Scotland		Banff	57	13	19
Scotland		Kincardine	7	0	1
Scotland		Moray	2	8	0
	Sub-Total		215	354	40
Scotland	Perth AHDO	Angus	0	22	0
Scotland		Argyll	0	6	0
Scotland		Fife	2	8	0
Scotland		Perth	50	0	0
Scotland		Stirling	2	1	0
	Sub-Total		54	37	0
Total			524	875	65

Table 2
Eligible movements to Scotland

2

(b) Undertake a post-movement test of eligible cattle, 60 to 120 days after arrival.

The only exceptions are for calves under 42 days old and cattle moving direct to slaughter. TB tests are conducted at the

owner's expense. Government provides the tuberculin and administrative assistance required to support the new policy.

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Monthly Cattle Movement Report

BCMS and IBM colleagues have collaborated closely to produce a new monthly report of cattle movements to Scotland from high incidence parishes in England and Wales. These monthly reports now distinguish between cattle moved directly from holdings in England and Wales to a holding in Scotland, as well as movements from high incidence parishes to Scotland via a market in England and Wales, or via a market in Scotland. Table 2 is the summary sheet describing eligible movements to Scotland in January 2006. Section 1 describes the direct movements, Section 2 describes movements via English and Welsh markets and Section 3 describes movements via Scottish markets. The movement report is used in two ways.

Firstly, a monthly 'mailmerge' exercise sends advisory letters to recipient farmers to confirm that they have acquired eligible cattle that should have been pre-movement tested before arrival. The letter also provides advice about what to do if the cattle have not been tested and addresses the concern of stakeholders that farmers might not know the provenance of the cattle they had purchased.

Secondly, the report is sent to Animal Health Offices in Scotland. The State Veterinary Service then conduct 10% checks on consignments to assess compliance with pre-movement testing requirements. The second workstream within Animal Health Offices involves entering cattle data on to the VETNET IT system to 'mark forward' post-movement TB tests for 60 to 120 days after the arrival date on the movement report. Where post-movement tests have not been conducted by 120 days (or where pre-movement testing has not been done as required) whole herd movement restrictions are imposed until the overdue testing has been completed with negative results.

Initial compliance

From 23 September 2005 to 31 January 2006, 1,410 cattle in 326 consignments came to Scotland from high incidence parishes in England and Wales. 58 consignments (31%) of 387 cattle were checked by SVS staff and around 80% were fully compliant with pre-movement testing requirements. Most of the other consignments had only minor non-compliances – for example, pre movement testing outside of the 60-day window. At the time of writing, 'overdue pre-movement test codes' have been recorded as being required in only 15 consignments of cattle.

The compliance rates for pre and post-movement testing are, so far, very encouraging and the next milestone will be the disclosure of the first reactor. The post-movement test failure rate for Irish cattle between 1997 and 2002 suggested that, on average, one in 847 cattle was subsequently disclosed as a reactor in Scotland.

With 1,466 cattle having been post-movement tested (to 19 June 2006) we might have expected to find reactors in this population already – if test failure rates are similar for cattle from high incidence areas of England, Wales and Ireland. It remains to be seen whether this is the case. The overwhelming support for these measures at grass-roots level in Scotland is extremely encouraging and the extent to which farmers and other stakeholders in Scotland have adopted the new policy should make a significant contribution to an important objective of the TB Strategic Framework, namely the objective of keeping a 'clean area clean'.

Information

Dr Martyn Blissitt is a Veterinary Advisor for Notifiable Diseases at the State Veterinary Service (SVS), Scotland.

Ante mortem diagnosis of Bovine Tuberculosis: the significance of unconfirmed test reactors

"...the substance [tuberculin] will become an indispensable diagnostic measure in the future. It will enable us to diagnose questionable cases of early phthisis [tuberculosis] even when we fail to detect bacilli..."

Robert Koch (1891)

"These results and those of others show that the tuberculin test, like many other things in this world, is not perfect... [However, it would be a] great mistake to question this method because it does not do everything we want."

B. Bang (1892)

Authors

Ricardo de la Rúa-Domenech,
Tony Goodchild,
Martin Vordermeier,
Richard Clifton-Hadley

Introduction

'Tuberculosis' (TB) is a clinical or pathological diagnosis that, by convention, refers to the clinical signs (or lesions) arising from infections by organisms of the *Mycobacterium tuberculosis* (MTB) complex, a closely related group of bacteria that includes *M. bovis*, the causative agent of bovine TB. 'Skin' tests measuring a delayed-type hypersensitivity response to the intradermal injection of purified mycobacterial proteins (tuberculin) are used throughout the world, in both human and veterinary medicine, for the diagnosis of the pre-clinical stages of TB. In Great Britain (GB), the majority of new TB breakdowns (incidents) in cattle are disclosed by routine screening or targeted testing of herds with the single intradermal comparative cervical tuberculin (SICCT) test. This and other types of skin tests are the internationally accepted standard for the detection of *M. bovis*-infected cattle and are considered the best tests currently available for diagnosing TB in live animals. Many developed countries have eradicated bovine TB through systematic application of the skin test alone, followed by slaughter of all test reactors.

A smaller percentage of TB incidents in GB (10% to 40%, depending on the local frequency of skin testing) are initiated by suspect lesions of TB found by meat inspectors during normal meat production ('slaughterhouse cases'). Additionally, the gamma-interferon (γ -IFN) blood test (BOVIGAM®) has been used in GB since 2002 to maximise the detection of infected animals in herds with acute or persistent infection that cannot be cleared by short-interval skin testing alone.

Somewhat arbitrarily, a TB incident is not considered 'confirmed' for statistical and other purposes until at least one of the slaughtered animals presents itself with characteristic TB lesions on post mortem examination or, in the absence of lesions, *M. bovis* can be cultured from a pool of selected lymph nodes. According to Annex B of European Directive 64/432/EEC (as amended), all skin test reactors are considered to be affected with TB and must be slaughtered. Under EU legislation there is also a requirement to conduct post mortem and bacteriological examinations on those animals. The reason for this, however, is not so much the validation of skin test results, but

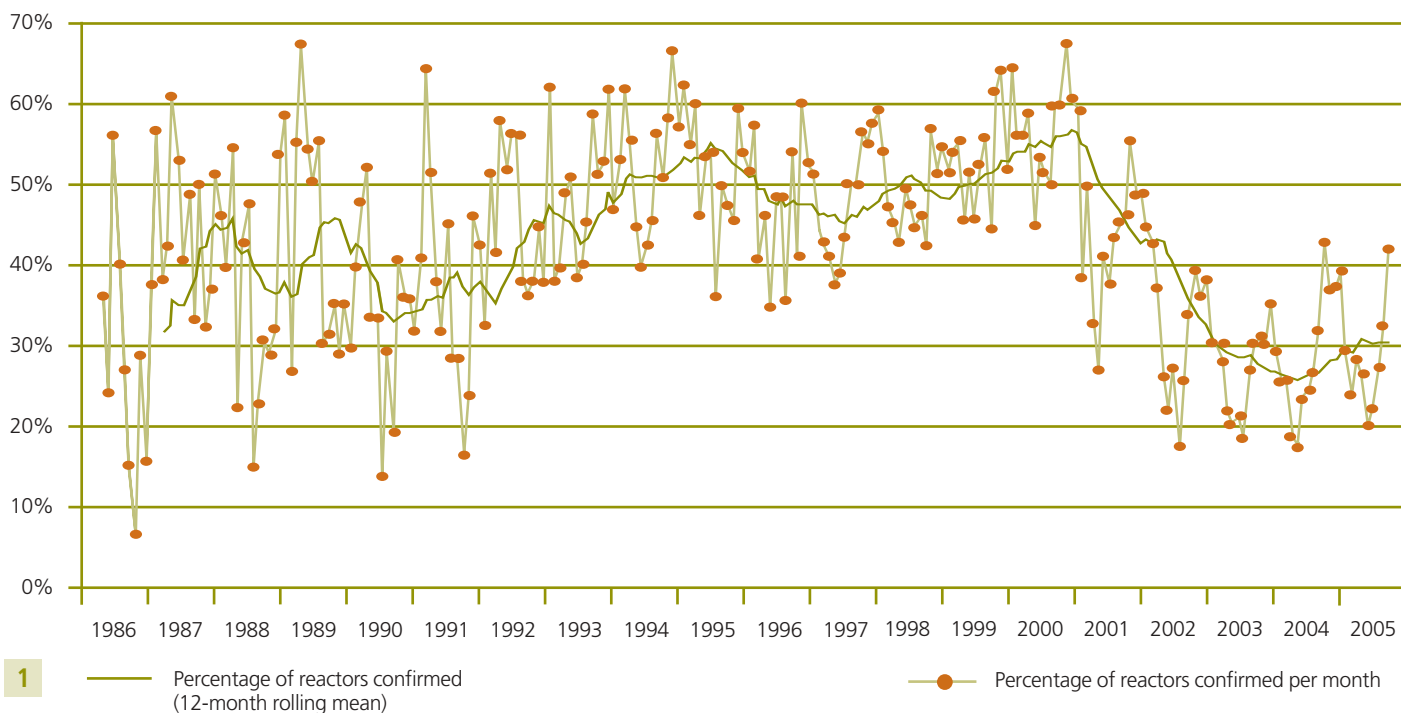
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rather to determine (1), the severity of disease (and thus the infectiousness of reactors to other cattle and human in-contacts) and (2) the number and interpretation of subsequent skin tests required to restore the official TB-free status of the affected herd. Moreover, the SVS uses the results of these post-mortem examinations to inform decisions on the reporting of bovine TB incidents to the medical public health authorities, and for the instigation of tracings and testing of contiguous herds. Finally, tissue samples from reactors are cultured to provide sufficient material for DNA extraction and molecular typing (spoligotyping) of *M. bovis* isolates. This information is important for epidemiological and geographical monitoring of the spread of *M. bovis* in GB and to support breakdown investigations by the SVS.

Throughout GB, the overall proportion of individual test reactors that are confirmed by *post mortem* and/or bacteriological examination is relatively low: approximately 40% of all skin test reactors (Figure 1) and 18% of cattle slaughtered due to a positive γ -IFN test result. Similarly low confirmation percentages have been observed for skin test reactors in the Republic of Ireland, Northern Ireland, New Zealand and other

countries. This may give the impression of a test accuracy far lower than that reported in the scientific literature and a wasteful policy that leads to the unnecessary destruction of 60% of all cattle reacting to the skin test. The presence of these so-called 'false positives' undermines the confidence of many veterinarians and farmers in the skin and γ -IFN tests. In this paper we will attempt to explain that this is a simplistic view and there are several reasons for the failure to 'confirm' the presence of TB in tuberculin reactors (or γ -IFN positive animals) by post mortem examination and bacterial culture. We will also show that the perception that too many false positive reactors are being slaughtered arises from two misunderstandings, namely (1), that post mortem examination and culture of the causative organism of TB are the 'gold standards' against which one should assess the validity of an *ante mortem* test result, and, (2) a failure to appreciate the difference between the false positive rate ('given that the animal is not infected, what is the probability that the test will be positive?') and the predictive value of a positive test result ('given that the animal has tested positive, what is the probability that the animal is infected?').

Figure 1
Overall monthly 'confirmation rate' for all tuberculin test reactors disclosed in the period January 1986 to December 2005 (Courtesy of Raquel Gopal, VLA Weybridge)



Epidemiological and Immunological principles relevant to TB screening tests

Predictive value of a positive test

The diagnostic accuracy of a screening test is primarily defined in terms of its sensitivity and specificity, which are calculated respectively, from the proportions of infected and uninfected animals that are correctly diagnosed. Test sensitivity is the proportion of diseased (infected) animals detected as positive in the diagnostic assay. Diseased animals that test negative are thus false negatives. Test specificity is the proportion of disease-free (uninfected) animals that are correctly identified as negative by a diagnostic test. Disease-free animals that test positive are then, by definition, false positives. No biological test for any disease is perfect (i.e. both 100% sensitive and 100% specific) and the tuberculin and γ -IFN tests are no exception. Furthermore, in all quantitative diagnostic tests a cut-off point for a positive result has to be defined on the continuous scale of possible test results. As a consequence, there is an inverse relationship between test sensitivity and specificity and a compromise between the two must be selected. Field studies evaluating the sensitivity of the SICCT test in various countries suggest that its sensitivity lies between 52.0% and 100%, with median values of 80.0% and 93.5% for standard and severe interpretations, respectively. Similarly, other studies conducted in TB-free cattle populations have found the specificity of the SICCT test to be between 88.8% and 100% (median of 99.9%). Looking at the prevalence of field reactors in locations of very low TB prevalence (as judged by post mortem examination), the specificity of the SICCT test used in GB can be estimated at 99.99%. This means that fewer than one

in 1,000 non-infected animals tested will be wrongly classified as skin test reactors. Imperfect test specificity leads to false positives, also known within the context of TB testing programmes as 'non-specific reactors' (NSRs). The causes of cross-reactions to the bovine tuberculin used in the skin and γ -IFN tests are complex and fall outside the scope of this paper, but they are summarised in the box below. The relevance of this to our discussion is that only a very small proportion of all Non Visible Lesion (NVL), culture-negative reactors disclosed in Great Britain should be attributed to non-specific (false positive) reactions.

Potential causes of false positive results to the tuberculin and other diagnostic tests for TB in cattle

- Infection with *M. tuberculosis* (human TB bacillus) or mycobacteria of the *M. tuberculosis* complex other than *M. bovis*
- Infection with *M. avium subsp. avium* (avian TB)
- Infection with *M. avium subsp. paratuberculosis* (Johne's disease)
- Immunisation of calves with Johne's disease vaccine
- Unidentified acid-fast bacilli associated with the presence of subcutaneous granulomas commonly known as 'skin TB'
- Experimental vaccination with *M. bovis* BCG strain
- Sensitisation to tuberculin by ingestion of various non-pathogenic environmental mycobacteria found in soil, stagnant water and vegetation. Bryophytes (mosses), in particular, appear to be a rich source of these organisms
- 'Atypical' mycobacteria recovered from mammals
- Non-mycobacterial organisms such as *Nocardia* spp.

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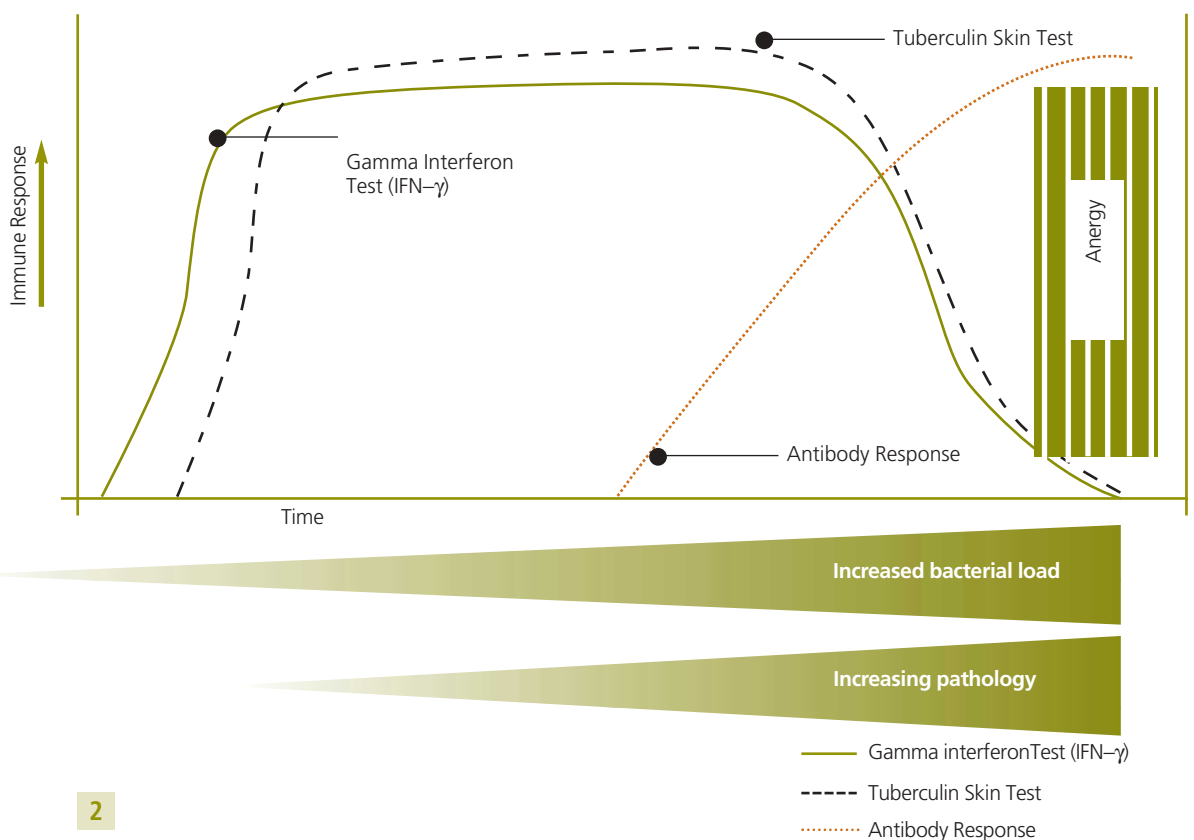
A key point to remember is that the ability of a diagnostic test to predict accurately the true status of a positive animal is not constant. Rather, this depends on the sensitivity and specificity of the test, as well as the prevalence of the disease in the population tested. The relationship between these three parameters is given by a probability formula, which defines what epidemiologists call the 'predictive value of a positive test result'.

For any test with less than 100% sensitivity and 100% specificity (i.e. virtually every diagnostic test), the higher the disease prevalence, the test sensitivity and test specificity, the more likely it is that a positive test result will be truly predictive of the disease (or infection). To illustrate this point, let us consider two herds. In herd A, the proportion of truly infected animals is 20%, while in herd B, the same proportion is 1%. The same test, with sensitivity and specificity both of 90%, would have a positive predictive value of 69% in herd A, but only 8% in

herd B. It is, therefore, misleading to use the proportions of NVL reactors (or herds) to judge the merits of the skin and γ -IFN tests. This is because the herd or reactor NVL proportion will vary according to the prevalence of *M. bovis* infection in the screened population, regardless of test accuracy. It also follows, therefore, that in an area of endemic high incidence of TB (e.g. annual and, to a degree, biennial testing parishes) or in a herd breakdown in which Visible Lesion (VL) cattle have been previously disclosed, one can assume with a high degree of confidence that the majority of cattle that fail the skin test (which is highly sensitive and extremely specific) are infected with *M. bovis* or have been exposed to the bacterium and developed an immune response against it.

Gold standards for the diagnosis of TB
Culture of *M. bovis* provides the definitive proof of infection. Disclosure of typical tuberculous lesions in itself does not prove infection, as such lesions can

Figure 2
Schematic representation of the spectrum of pathological and immune responses in cattle infected with *Mycobacterium bovis*



occasionally be caused by other mycobacteria. However, in combination with a positive skin or gamma-interferon test result, the presence of TB lesions is considered sufficient to confirm infection with *M. bovis*. In other words, bacteriology and *post mortem* examination of suspect animals are extremely specific methods for the diagnosis of *M. bovis* infections (the infection is almost always present in the animal if those tests yield positive results). The downside is that *post mortem* examination (particularly when conducted in a commercial slaughterhouse) is a less sensitive technique than the immunodiagnostic tests (e.g. skin and γ -IFN tests) conducted in live animals. These tests detect a cell-mediated immune response of the host against infection with *M. bovis*, which is often subclinical and may or may not have progressed to the disease stage (TB) when the infected animal is identified. Depending on animal, test and (possibly) *M. bovis* strain characteristics, it usually takes between one and nine weeks for cattle to become reactors to the skin test following infection with *M. bovis*. Most animals will be identified as skin test reactors from three to six weeks post-infection (this could be slightly less for the γ -IFN test). This lag period between infection and reactivity to tuberculin (pre-allergic phase) appears to be largely independent of the *M. bovis* infective dose. By contrast, it may take several months to reach the visible lesion (VL) stage in a majority of cattle infected under natural conditions. Due to this delay from infection to the development of detectable signs of bovine TB, cellular immune responses against the invading organism will be evident at an earlier

The diagnostic accuracy of a screening test is primarily defined in terms of its sensitivity and specificity, which are calculated respectively, from the proportions of infected and uninfected animals that are correctly diagnosed.

stage than the pathological changes caused by the infection, and probably well before bacterial loads are sufficiently large to be found by standard culture methods (Figure 2). This is one of the reasons why the VL percentage in reactors taken in tests that disclose a new breakdown is significantly greater than in reactors taken in short-interval tests. Furthermore, *M. bovis* is an intracellular pathogen with a slow generation rate and is not particularly easy to culture in the laboratory. Culture of the organism from a pooled sample of NVL lymph nodes becomes even more complicated than isolation from a VL animal, which is generally attempted from the lesion itself. Even when VLs are present, these do not always contain a sufficient number of viable bacteria to grow *in vitro*. For these reasons, the culture of *M. bovis* in the laboratory and the examinations for TB lesions at slaughter should not be considered the gold standards for the infection status of cattle. At post mortem examinations, a proportion of these test positive animals (reactors) will not have VLs, nor will *M. bovis* be subsequently cultured from tissue samples. To imply that such animals were 'false positives' is inappropriate, as they may have been at an early stage of disease when the bacillus could not yet be detected. Such animals could have been harbouring the bacilli, and when the disease had progressed further, became infectious to other cattle. From a TB control perspective, the early removal of skin test reactors and gamma-interferon test positive animals is a desirable outcome of testing, particularly in areas of endemic TB.

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The Empirical Evidence

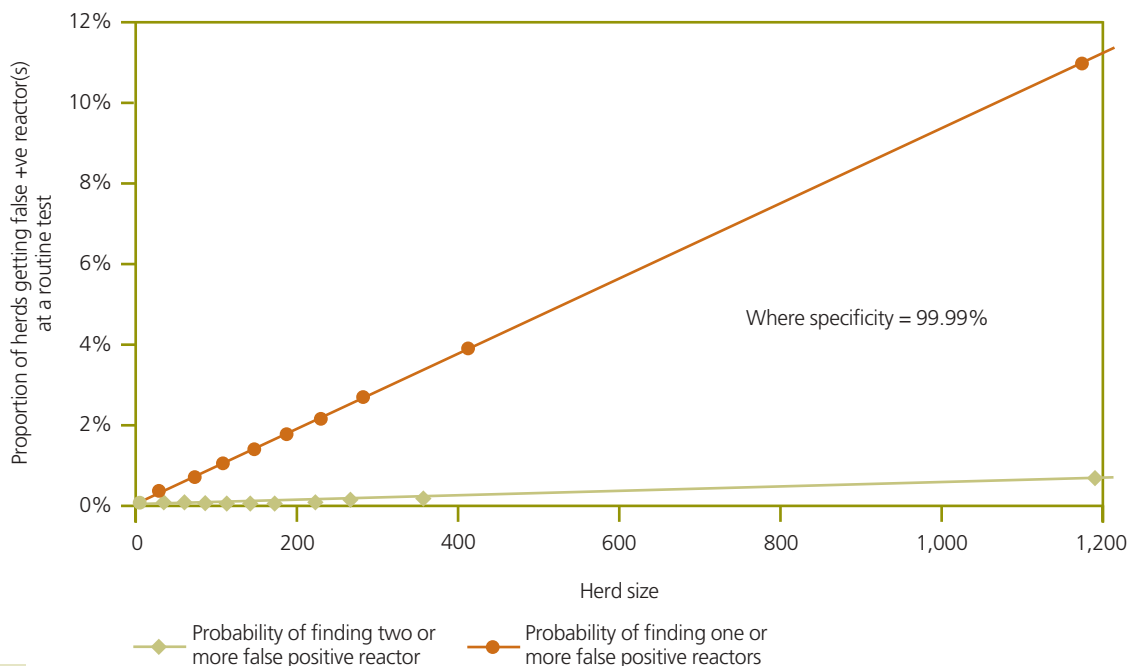
Assuming 99.99% specificity for the single intradermal comparative tuberculin test, we have estimated that the probability that any animal gives a false positive test result tends towards 0.00016 (i.e. 0.016% or 1.6 in 10,000 animals tested) in counties with low prevalence of TB. For herds in which 50 cattle were tested, one in 200 herds would have one false positive reactor. For a herd in which 200 cattle were tested, approximately one in 50 herds would have one false positive reactor. In other words, the probability of a false positive result increases with herd size,

and five unconfirmed incidents in those areas were due to non-specific reactors. The excess was the fraction of unconfirmed TB breakdowns attributable to *M. bovis* infections.

In areas where TB is endemic, unconfirmed breakdowns tend to cluster around foci of confirmed TB. The historical TB data for Great Britain show that, following an unconfirmed TB breakdown, the risk that a confirmed breakdown will occur in the next 12 months is five to ten times greater than for herds in the same county without a history of breakdowns (data for 1996-1999). In other words, disclosure of

Figure 3

The percentage of herds with false positives (skin test reactors) increases with herd size. It is, however, very rare to find more than one false positive in a herd



3

although it is extremely rare to find more than one false positive in a herd (Figure 3). However, in areas of Great Britain with a high incidence of confirmed TB breakdowns, there are far more 'unconfirmed' breakdowns than could be accounted for simply by the estimated rate of false tuberculin reactors. Based on data from the period 1996-1999 only one in between two

unconfirmed reactors is often the first indication of a developing TB problem in a herd, which will manifest itself in the presence of VL animals in future herd tests. In relation to the γ -IFN test, studies in Northern Ireland have demonstrated that gamma-IFN test positive, skin test negative cattle that are not immediately slaughtered are far more likely to react to subsequent skin tests.

Conclusion

In summary, saying that a TB breakdown or a reactor is 'not confirmed' means different things in different circumstances, depending on local TB prevalence, thoroughness of post-mortem examination, number of reactors detected and herd size. The chronic and latent nature of TB means that NVL and culture-negative reactors in herds with confirmed TB or herds in endemic TB areas should not be automatically regarded as false positives. Such animals may well have been exposed to *M. bovis* and infected even if the presence of the disease cannot be established. This is because the 'gold standard' tests (*post mortem* examination and culture) are significantly less sensitive than the *ante mortem* diagnostic tests for TB (the skin test and gamma-interferon tests) that measure indicators of infection rather than disease. Animals that are positive to a skin or gamma-interferon test, but NVL and culture negative can, therefore, be indicative of:

- non-specific cross reactions to the antigens used in the ante mortem tests (true false positives);
- early detection of *M. bovis* infection, when tuberculous granulomas are still too small to be detected by routine *post mortem* examination and there are very low numbers of bacilli in the lymph node pool collected for culture in the absence of lesions;
- infection with lesions in a location that is not normally checked at routine *post mortem* examination;
- animals with arrested infection, i.e. temporarily able to contain the bacterium in a condition of latency, a phenomenon well characterised in human TB.

In regions where the prevalence of TB is low or the disease is believed to be absent, the predictive value of a positive skin test result will be less than in the endemic TB areas. In other words, NVL, culture-negative reactors in low-TB prevalence regions are more likely to arise from non-specific sensitisation to tuberculin. For that reason, where NVL, culture-negative reactors are persistently found in herds in 4-, 3- or two-yearly testing areas and non-specific sensitization is suspected, it is possible to use the γ -IFN test for sequential testing of those animals prior to slaughter. The γ -IFN test results, the evidence from a detailed necropsy, laboratory culture, the herd's testing history and the TB history of the area, are all taken into account when assessing the likely infection status of those animals and before considering if such herds should have their TB restrictions lifted under the so-called 'non specific reactor' procedures (described in VIPER 23). For the reasons explained above, such protocols are never applied on reactors in annually tested herds or herds in which infection with *M. bovis* has already been confirmed.

In the end, from a disease control perspective, test performance comes down, not to arguments about specificity and gold standards, but whether a given test is useful in the field. This was the pragmatic approach that underpinned the γ -IFN field trial of 2002-2005 and the SVS instructions for ad hoc application of the γ -IFN test. Namely, that the γ -IFN test is most likely to be useful to the SVS as a supplement to the skin test in identifying early infected animals in severe or chronic TB breakdowns, where removal of small numbers of false positives is only a minor concern.

Information

Ricardo de la Rúa-Domenech is a Veterinary Advisor, TB Division, Defra.

Tony Goodchild is a bovine TB epidemiologist, Veterinary Laboratories Agency Centre for Epidemiology and Risk Analysis, Weybridge.

Martin Vordermeier is Leader of the Immunology Group, TB Research Unit, Veterinary Laboratories Agency, Weybridge.

Richard Clifton-Hadley is Head of Statutory and Exotic Bacteria Unit, Veterinary Laboratories Agency, Weybridge.

The BOVIGAM® assay as ancillary test to the Tuberculin skin test

Authors

Martin Vordermeier, Adam Whelan, Katie Ewer, Tony Goodchild, Richard Clifton-Hadley, John Williams, R. Glyn Hewinson
Veterinary Laboratories Agency (VLA) – Weybridge, TB Research Group

Abstract

The BOVIGAM® IFN- γ test constitutes an additional ante-mortem test to identify cattle infected with *Mycobacterium bovis*, the causative agent of bovine tuberculosis. We will explain why we think that at present its most advantageous use will be as an ancillary test to the tuberculin skin test with a view to maximising the numbers of infected animals detected. This paper will also describe the principle of the test and interpretation of the results in the context of the patho-biology of tuberculous infections. Furthermore, the results of studies carried out to determine the sensitivity and specificity of this test will be addressed, including the presentation of unpublished results from recent GB trials. In addition, we will discuss the potential use of defined antigens to differentiate *M. bovis* infected cattle from those vaccinated with future vaccines currently under development.

1. Infection biology of bovine tuberculosis in cattle in relation to diagnostic tests

It is generally accepted that cell-mediated immune (CMI) responses are the principal and earliest immune responses to develop after infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis (Figure 1, (1-3)). Antibody responses develop considerably later than CMI responses (Figure 1) and it is therefore not surprising that assays based on measuring CMI, like the tuberculin skin test and the BOVIGAM® IFN- γ assay, have been used extensively for the diagnosis of bovine tuberculosis (BTB) in cattle. Currently there is no recognised serological test available for TB diagnosis.

To better understand the underlying principles of the tuberculin skin and the BOVIGAM® IFN- γ tests, it is important to understand the kinetics of the immune response. BTB in cattle is presented primarily as visible lesions in the lungs and associated lymph nodes and/or in the lymph nodes of the head. *M. bovis* can also be cultured from tissues with or without visible lesions. As *M. bovis* is closely related to the pathogen causing human tuberculosis, (*M. tuberculosis*) it has been proposed that the principles of *M. bovis* pathogenesis in cattle follows closely that of *M. tuberculosis* in humans, which is described below.

Humans in contact with TB patients will be exposed to *M. tuberculosis* (as will be cattle in a herd with confirmed bovine TB). A certain proportion (10-30% in the case of humans) of exposed individuals will actually become infected (as defined by an acquired delayed type hypersensitivity and/or IFN- γ response). About 5% of infected humans develop disease within one year of infection (*primary tuberculosis*), whilst 95% of infected individuals do not. Such individuals – i.e. those that are tuberculin skin test positive or show IFN- γ responses *in vitro* to antigens such as ESAT-6 but do not present with clinical or radiological signs of disease – are classified as latently infected. However, 5-10% of latently infected humans develop clinical tuberculosis during their lifetime through re-activation of the latent infection (*re-activation tuberculosis*). Thus, the population of latently infected humans presents a huge reservoir of *M. tuberculosis*. Although exposed, latent and diseased states are likely to be equally applicable to bovine tuberculosis

in cattle (Figure 2), the proportions that these disease states occur in cattle are not known. It is likely that the proportion of latent infection will be considerably lower in bovine tuberculosis than in human tuberculosis. However, the argument that latently infected individuals (culture-negative NVL, skin test reactors for example) constitute a continuous and unpredictable source of re-infection, is equally valid for cattle as it is for human TB.

It is generally accepted that cell-mediated immune (CMI) responses are the principal and earliest immune responses to develop after infection with *Mycobacterium bovis*, the causative agent of bovine tuberculosis.

The concept of infection without signs of disease (latency) as defined in the previous paragraph, as opposed to detectable disease, has important implications for the interpretation of tests like the IFN- γ test or the skin test (see also Figures 1 and 2). At the early stages of infection, or in latently infected cattle, a period will occur when *M. bovis* appears to be absent because the bacillary load is not

large enough to be detected by culture. In addition, the pathological changes caused by the bacilli are not yet profound enough to be detected during routine abattoir inspection. However, cellular immune responses will be detectable in these animals at an earlier stage of

infection than the pathological changes caused by the disease (e.g. visible lesions), or before the bacterial loads exceed the numbers necessary to be able to culture *M. bovis* from tissue samples (Figure 1).

This has two consequences: firstly, immuno-diagnostic assays of cellular immunity (which includes the IFN- γ test as well as the tuberculin skin test), because they detect infection rather than disease, can be very sensitive at identifying *M. bovis* infected cattle. Secondly, at abattoir inspections, a proportion of these identified animals will not have visible lesions, nor can *M. bovis*

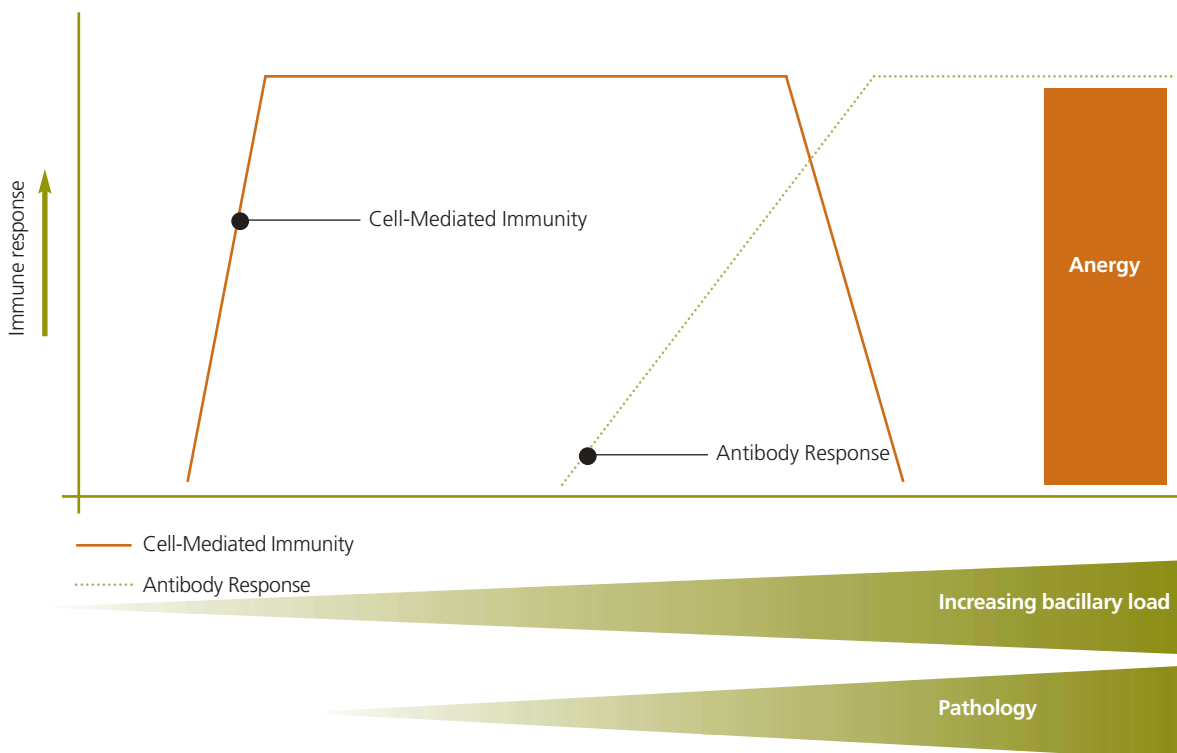


Figure 1
Development of immune responses in cattle following *M. bovis* infection: Bovine tuberculosis as spectral disease

be subsequently cultured from tissue samples. To designate these animals *a priori* as 'false-positive' is inappropriate as they can harbour bacilli, and become infectious to other cattle, specifically when the disease has progressed further.

2. Tuberculin skin tests

The most commonly applied diagnostic tests for BTB in cattle are tuberculin skin tests. Two types of tuberculin skin tests are in use, namely, the single intradermal test (SIT), and the single intradermal comparative cervical tuberculin test (SICCT). The SIT, using bovine tuberculin purified protein derivative (PPD) alone, can be carried out as the caudal fold test, (CFT) (which is used in countries such as USA, Australia, New Zealand) or as an injection into the skin of the neck (cervical SIT). The SICCT, used in the UK and Ireland, compares responses to avian and bovine tuberculin PPD. The following sensitivity and specificity ranges have been cited in recent reviews. SIT: sensitivity, 68-96.8% (CFT), 80-91% (cervical SIT); and specificity, 96-98.8 (CFT), 75.5-96.8 (cervical SIT). For the SICCT the following

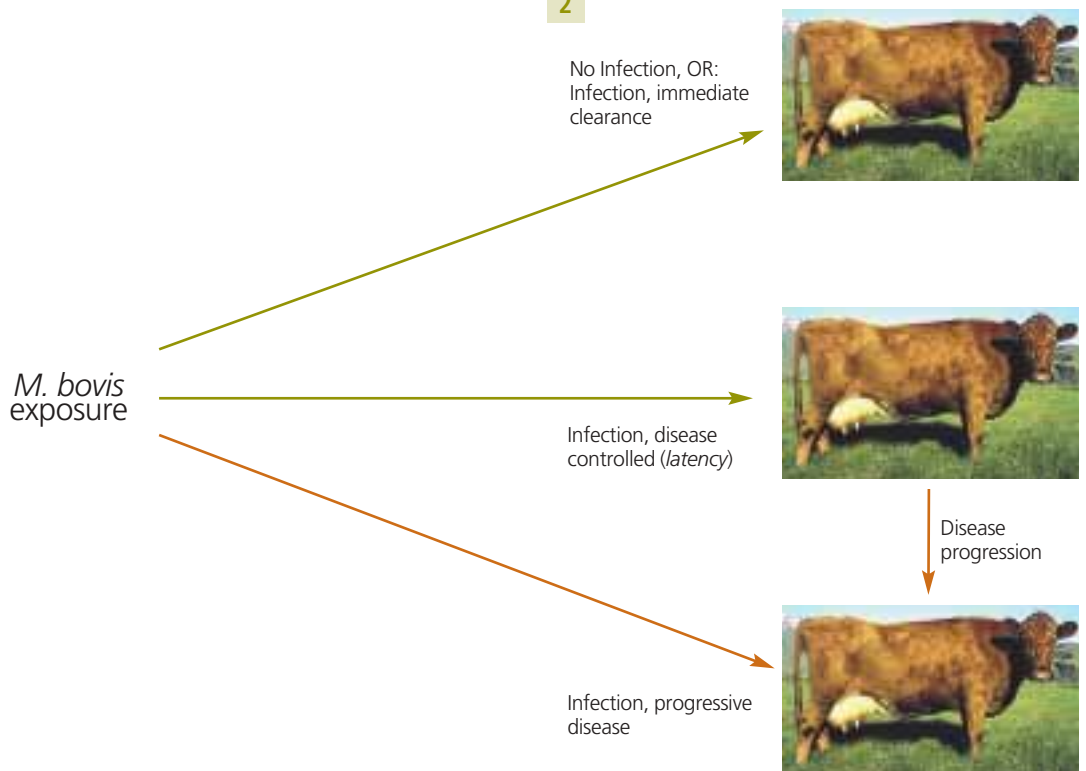
values have been reported for sensitivity and specificity, respectively (standard interpretation): 55.1-93.5% and approximately 88.8-100%.

Importantly, BCG vaccination will result in positive tuberculin responses to the skin test (and the BOVIGAM® assay, see below) in a large proportion of vaccinated animals, thus compromising the use of tuberculin as a diagnostic antigen in conjunction with vaccination. As the principle of the tuberculin skin test has been amply described previously, we will

CMI diagnosis (TT or IFN- γ)	Visible lesions / <i>M. bovis</i> +ve
Negative	Negative
Positive	Negative or Positive
Positive (or negative, e.g. anergic cattle)	Positive

2

Figure 2
Biology of bovine tuberculosis and the consequences on the outcome of diagnostic tests. Please note that the degree of latency in cattle is unknown



2

not expand on it further in this review but will concentrate on discussing the principle of the IFN- γ test (BOVIGAM® assay).

within 24 hours of sampling, and cultured at 37°C in the presence of bovine or avian tuberculin.

3. BOVIGAM® IFN- γ blood test

The BOVIGAM® IFN- γ test is an *in vitro* laboratory-based immuno-diagnostic test which detects gamma interferon (IFN- γ) that is produced after the stimulation of blood cells with antigens such as bovine tuberculin. The test is performed in two stages; firstly, blood samples are transported to the laboratory

Practical advantages of the test include:

- (i) flexibility in its interpretation by setting appropriate cut-offs;
- (ii) only one farm visit is required;
- (iii) the test does not interfere with the host's immune status, so that assays can be repeated more frequently than tuberculin skin tests;

Country	IFN- γ Sensitivity (%)	Specificity (%)
Australia	84.4	98
Australia	81.8	99.1
USA	80.9	ND
Brazil	100	ND
Spain	84.9	ND
Spain	87.6	ND
Northern Ireland	89.3	99.2
New Zealand	85	93
New Zealand	94.6	(73.6 ^a)
Italy	ND	88.4
Italy	96.6	98
Italy	NT	97.3-98.6
Ethiopia	95.5	87.7
Romania	92.5	ND
Eire	84.2	96.2
Eire	88.5	NT
GB	88.2	92
GB	89.7	96.6
Median	88.4	96.6
Range	80.9-100	87.7-99.2

Table 1
Previous studies of sensitivity and specificity of the BOVIGAM® assay

A number of different cut-off values and interpretation protocols were applied in the different trials. However, results in all studies are based on the comparison of responses to avian and bovine PPD. A study concentrated on false-positive skin test positive animals, i.e. specificity of skin test is 0% in this cohort, data not used to determine median specificity. ND, not determined.

(iv) it is highly amenable to the inclusion of defined antigens that allow the differentiation between infection and vaccination (*differential diagnosis*, see below).

After 24 hours incubation, plasma supernatants are collected, and the amount of IFN- γ produced quantified by enzyme-immuno-assay (EIA) using the commercially available BOVIGAM® kit. A test result is interpreted as positive for bovine tuberculosis when the blood cells from an infected cow produce more IFN- γ after stimulation with bovine tuberculin than after stimulation with avian tuberculin. It is also accepted that the BOVIGAM® test detects animals that escape skin testing, probably because it detects animals earlier after infection than tuberculin skin testing.

The BOVIGAM® test is generally considered to be more sensitive than tuberculin skin testing. Whilst specificities have been reported that, although not widely dissimilar to those seen with tuberculin skin testing, were lower in most studies than those reported for the SICCT. However, direct comparisons of the two tests in the same study have rarely been made.

A number of trials assessing the BOVIGAM® assay have been conducted since 1991, and Table 1 summarises 18 of these trials conducted in 11 countries with respect to sensitivity and specificity. The assay was applied in these trials in its basic form using bovine and avian tuberculin. However, a range of cut-offs were used, and in some studies cattle were also tested before skin test application, whereas in others they

Table 2
Increase in overall sensitivity by using SICCT and BOVIGAM® test in parallel

Study	IFN- γ ⁺ (%)	ST ⁺ (%)	ST ⁺ /IFN- γ ⁺ (%)
A. Sensitivity increase			
Wood, 1991	94	66	95
Whipple, 1995	73	80	91
Gonzalez, 1999	85	80	93
Collins, 2002	88	74 ^a	93 ^a
Goodchild, 2004	70 ^b	65 ^b	88 ^b

B. Maximising detection of infected animals (project SB4008) ^c			
Numbers of VL and /or Cu+ cattle detected			
	IFN- γ ⁺ ST ⁻	ST ⁺ ^d	IFN- γ ⁺ and ST ⁺
Goodchild, 2006	142	387	529

a) Severe interpretation of skin test. b) Whole herd removal operation. c) Data based on results from 78 herds. d) Skin test reactors were not tested with the BOVIGAM® IFN- γ assay. NA, not applicable. ST, Skin test, IFN- γ , BOVIGAM® test.

were tested after skin test application. Interestingly, despite the application of different cut-offs across different study populations, the results are often very similar, which also highlights the ease with which it is possible to adjust BOVIGAM® cut-offs to local conditions without overtly affecting test performance. As Table 1 indicates, median sensitivity and specificity levels across these trials were 88.4% and 96.6%, respectively. During 2005, a Defra-funded study was conducted to determine the specificity of the test in GB with tight confidence intervals by using a larger number of animals from TB free herds than had been tested previously in GB. Results based on 874 cattle determined test specificity to be 96.7% (95% CI: 95.6-97.8, unpublished results). This underscores the relatively high specificity of this test and is in line with previous published results. Furthermore, it confirms the specificity values determined previously (96.6%, Table 1).

Although the majority of infected animals will be positive in both tuberculin skin test and BOVIGAM® assay, the populations of animals reacting to either of the assays are not fully overlapping and a proportion of animals will be IFN- γ -positive/skin test negative, IFN- γ -negative/skin test positive or negative in both tests (Figure 3). Therefore, the most beneficial application of this test is alongside the tuberculin skin test as an ancillary test in herds with high disease prevalence or with persistent infection that cannot be cleared with the tuberculin skin test (also called parallel testing). This increases the overall diagnostic sensitivity and will thus have a major impact on disease control.

The BOVIGAM® test is generally considered to be more sensitive than tuberculin skin testing, whilst specificities have been reported that, although not widely dissimilar to those seen with tuberculin skin testing, were lower in most studies than those reported for the SICCT.

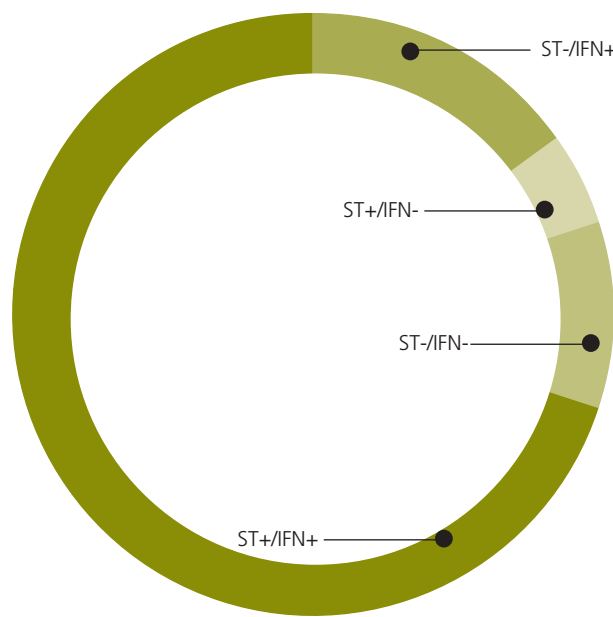


Figure 3 Outcome of parallel testing using tuberculin skin testing and IFN- γ assay in combination. Pie chart represents VL and/or *M. bovis* culture positive animals. ST, tuberculin skin test; IFN, BOVIGAM® IFN- γ test results

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The advantage of applying the two tests together has also been acknowledged by the European Union who allow the use of the test to 'maximise the detection of infected animals'. Table 2 presents published and unpublished data

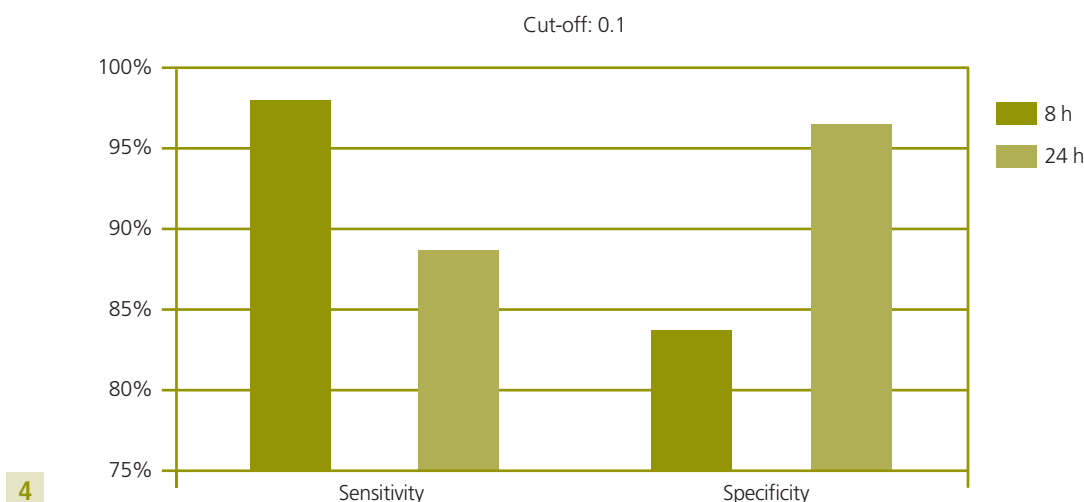
illustrating this point. The recently completed 'National IFN- γ Trial' (project SB4008) has underscored the fact that the BOVIGAM® test will identify a significant portion of skin test-negative animals with visible lesions (VL) and/or

which are culture positive for *M. bovis*. In the national trial, 27% more of such animals were identified by the IFN- γ test than had been found at the disclosing skin test (Table 2).

What would be the possible consequence of leaving IFN- γ +/ST- animals in affected herds? It was recently reported that animals that were IFN- γ positive/skin test negative animals were seven to nine times more likely to become

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Figure 4
Overnight storage improved specificity of BOVIGAM® assay. Antigens: PPD-B, PPD-A responses, cut-off: OD450 PPD-B minus PPD-A > 0.1



subsequently skin test positive than IFN- γ negative animals. The authors of this study therefore concluded that not removing IFN- γ + animals could leave a significant source of re-infection in these herds.

4. Influence of blood storage on BOVIGAM® test performance

In practical terms this can be condensed to the question as to whether blood should be tested on the day of sampling (within eight hours), or could be left overnight (within 24 hours) without loss of sensitivity. Studies in New Zealand and the USA have concluded that about equal proportions of animals could be defined as positive when the BOVIGAM® test was performed on the day of sampling or within 24 hours of sampling, despite the observation that the OD values after overnight storage of the blood samples were substantially lower than when the blood culture was started on the day of sampling. These results formed the basis of New Zealand and USA policy of starting the assay on the day after the sample was taken but within 24 hours.

Our own results confirmed these findings. Despite decreases in signal strength (OD450), we did not observe a reduction in the numbers of experimentally infected cattle detected. In field reactors we detected a modest drop in relative sensitivity from 96% to about 90%, when we assessed SICCT

(comparative tuberculin skin test) field reactors with confirmed TB (Figure 4) using a cut-off value (PPD-B minus PPD-A > 0.1 OD450). More dramatically however, we observed an increase in specificity when the blood was kept overnight rather than cultured freshly from about 85-97% (Figure 4). An increase in specificity following overnight storage has also been observed by others.

In contrast to these findings that suggest that overnight storage of blood has no significant impact on sensitivity, a recent paper suggested that the delay in blood culture reduced test sensitivity significantly. Applying the BOVIGAM® test to visibly lesioned animals within eight or 24 hours did not decrease sensitivity significantly in line with what the other studies described above had shown. However, they observed a decrease in the percentage of animals testing positive in the BOVIGAM® test after 24 hour storage compared to eight hour for SICCT-negative animals. However, it is impossible to assess the disease status of this skin-test negative group as no post-mortems were conducted. It is therefore not possible to assess if this decrease in the number of animals testing BOVIGAM®-positive is caused by a decrease in sensitivity due to overnight storage, or indeed an increase in specificity due to the detection of less false-positive animals.

5. Blood sampling in relation to skin test application

Early data suggested that IFN- γ responses were affected by the application of tuberculin skin tests. In these studies, increases in the OD450 values after *in vitro* stimulation with avian and bovine PPD were observed between seven and 59 days post-skin test. However, the test applied in these studies was the caudal fold test (CFT).

Since then numerous studies have looked at the development of IFN- γ responses post-skin test, and reached conflicting conclusions.

However, by comparing these studies, a general conclusion can be drawn that CFT can boost IFN- γ responses whilst the application of the SICCT does not. Furthermore, our data, as well as that of others, demonstrated that blood could be taken three days post-SICCT application without affecting test sensitivity.

Although the majority of infected animals will be positive in both tuberculin skin test and BOVIGAM® assay, the populations of animals are not fully overlapping.

6. Specific antigens and differential diagnosis of vaccinated and infected animals

One of the main advantages of the BOVIGAM® assay is the ease with which defined antigens can be added. The defined antigens used commonly are antigens like ESAT-6, CFP-10, whose genes are absent from the genome of the vaccine strain *M. bovis* BCG or proteins that are not highly expressed by some strains of BCG like MPB70. Such defined antigens may also increase test specificity compared to the use of avian and bovine

tuberculin in animals sensitised by environmental mycobacteria. This is in contrast to the use of defined proteins for skin testing in cattle, where positive responses could only be elicited by applying large protein doses, or when the antigens were applied with immunomodulating agents like lipo-peptides.

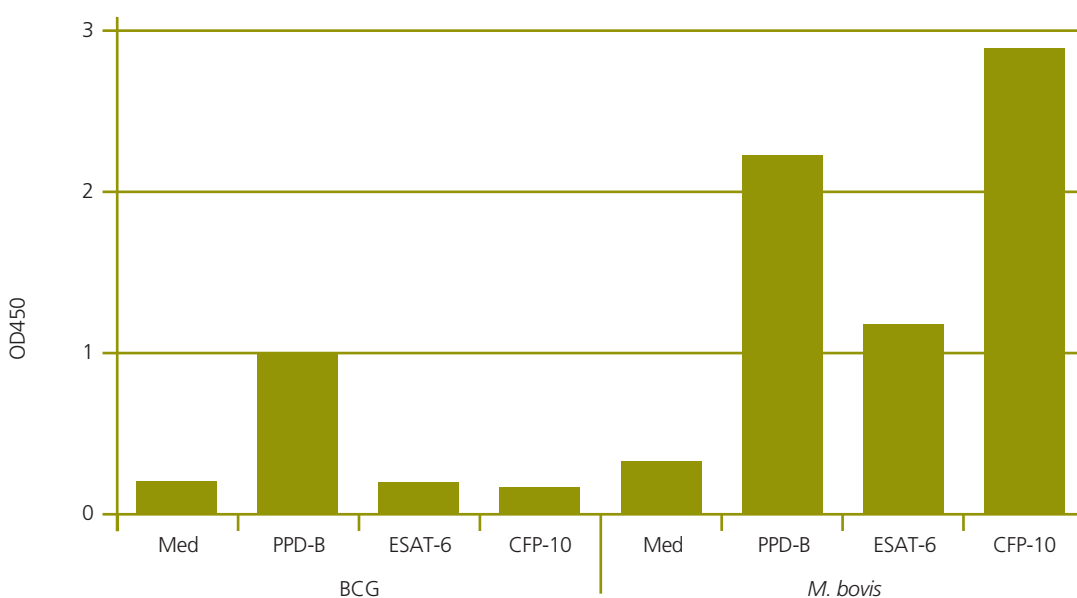


Figure 5 Differential diagnosis of *M. bovis* infected from BCG vaccinated cattle by the use of ESAT-6 and CFP-10 in the BOVIGAM® IFN- γ test. Heparinized blood was collected from BCG vaccinated cattle (n=5) and from cattle experimentally infected with *M. bovis* (n=5) and incubated with bovine tuberculin (PPD-B), or recombinant ESAT-6 or CFP-10 protein. IFN- γ production was determined by ELISA and data is expressed as mean OD450 values \pm SE

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Information

Martin Vordermeier is the Immunology Team Leader at the VLA.

Tony Goodchild is a bovine TB epidemiologist at the VLA.

Adam Whelan is a research scientist specialising in bovine tuberculosis.

Katie Ewer is an immunologist studying bovine immunity to tuberculosis at the VLA.

Tony Goodchild is a bovine TB epidemiologist at the VLA Centre for Epidemiology and Risk Analysis.

Richard Clifton-Hadley is Head of Statutory and Exotic Bacteria Unit at the Veterinary Laboratory Agency.

John Williams is Deputy Work Group leader within the Laboratory Testing Unit at the Veterinary Laboratories Agency.

Professor Glyn Hewinson is Head of the TB Research Group within the Statutory and Exotic Bacteria Unit at the Veterinary Laboratories Agency.

Interestingly, in the recent GB specificity study, the use of a peptide cocktail of ESAT-6 and CFP-10 increased the specificity compared to PPD only marginally (from 96.7-97%, unpublished data), whereas the combined use of both PPD and the peptide cocktail (i.e. only animals giving a positive result to both antigen preparations were deemed test-positive) lead to a specificity of around 99% (unpublished data). However, these latter results should be viewed with caution as this approach would inevitably also result in reduced sensitivity.

The selection of antigens absent from the BCG genome highlights the interest in antigens applicable for the differentiation of (BCG) vaccinated from *M. bovis* infected animals (*differential diagnosis*), which may constitute the major application for such defined antigens. BCG vaccination will result in positive tuberculin responses both in the skin test and the BOVIGAM® assay, thus compromising the use of tuberculin as a diagnostic antigen for vaccinated animals. However, several studies have shown that by using ESAT-6 and CFP-10, which are absent in BCG, as diagnostic antigens in the BOVIGAM® assay, *M. bovis* infected animals could be distinguished from BCG vaccinated cattle (Figure 5). For example, in one study approximately 70% of BCG vaccinated animals tested positive after stimulation with tuberculin, whereas none responded to stimulation with a cocktail of ESAT-6 and CFP-10. However, the test sensitivity compared to tuberculin was still lower, and studies are currently underway to identify further antigens that, when used in combination with ESAT-6 and CFP-10, will increase test sensitivity to levels akin to tuberculin. This endeavour has been greatly facilitated by the elucidation of the genome sequences of *M. tuberculosis*, *M. bovis*, and BCG. This has allowed the application of *in silico* methods of comparative genomics to predict 'specific antigens', which can

then be tested in cattle, either in the form of synthetic peptides, or recombinant antigens, for their suitability for *differential diagnosis*. Several antigens have been identified in this way that are able to increase test sensitivity when used in combination with ESAT-6 and CFP-10. This approach of identifying specific antigens is an on-going and successful research effort and steady progress is being made to close the sensitivity gap between these specific antigens and tuberculin. However, this gap, whilst narrowing, still exists, and further specific antigens will have to be identified to close it completely.

7. Concluding remarks

Both IFN- γ and tuberculin skin tests are based on the detection of cellular immune responses and therefore detect infection rather than disease. A corollary of this statement is that animals will be identified by these tests that are in early or latent disease states where it may be impossible to detect visible pathology or to culture *M. bovis* from tissues. Such animals should not be dismissed as false-positives; by allowing the disease to progress they could become critical sources of re-infection. As the populations of animals detected by the BOVIGAM® test and tuberculin skin test do not completely overlap, their combined application in parallel, with the BOVIGAM® test used as a strategic ancillary test, will maximise the numbers of infected cattle that can be detected. The BOVIGAM® test is a flexible test format that can readily be tailor-made to particular applications by refining cut-offs for positivity or by the inclusion of more defined antigens than tuberculin. The most promising application of such defined and specific antigens could be in the differentiation of vaccinated from infected cattle, and genome-based approaches have been successful at identifying additional specific antigens.

Tuberculous pneumonia and BVD in housed calves

Veterinary Laboratories Agency (VLA) – Truro

A three-month-old calf showing signs of pneumonia died within 48 hours of the first clinical signs becoming apparent despite veterinary treatment. The calf was submitted to VLA Truro for post mortem examination which revealed extensive lesions of tuberculous pneumonia from which *Mycobacterium bovis* (*M. bovis*) was subsequently isolated. This case report describes the clinical, post mortem and laboratory findings in a group of 22 'in-contact' calves.

History

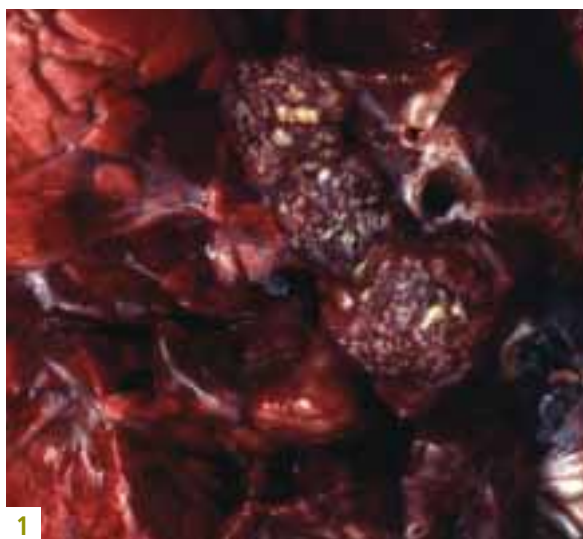
The farm carried 100 Holstein milking cows, together with 150 dairy and beef followers. Cows and youngstock were fed on silage, straw and bought in concentrates alone. Calves were fed on hay, straw and bought in concentrate together with raw milk, in buckets, including mastitic milk withheld from the bulk tank.

In a five month period between November and the following March eight young cows had aborted two to three

months before full term. In June and July two calves had been born apparently blind and one of these was ataxic. The area surrounding the farm had a history of TB in cattle and badgers but this farm had no experience of TB during the last 20 years. In July of the same year, however, the eventual slaughter of an inconclusive reactor milking cow revealed tuberculous lesions in the retropharyngeal lymph nodes from which *M. bovis* was isolated. A herd TB test followed on 24 August and this identified 11 reactors, eight of which showed visible lesions of TB. Two of these were adults in which the lesions were confined to the lymphatic glands of the head and chest. The other six were calves, between six and two months of age, and in four of these the tuberculous lesions were again confined to the lymphatic glands of the head and chest. However, two also showed miliary caseous lesions in the apical lobes of the lungs. *M. bovis* was isolated from the tuberculous lesions of the two cows and five of the six calves. *M. bovis* was also isolated from the lungs of one calf which was considered to have shown lesions

Author

R J Monies,
BVMS, MRCVS,
Veterinary
Investigation
Officer



1



2

Figure 1
Lung of calf no. 400
– granulomatous
mycobacterial
pneumonia

Figure 2
Bronchial lymph
nodes of calf
no. 400 caseous
lymphadenitis

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typical of acute pneumonia rather than tuberculosis.

Three weeks after the herd TB test, a three-month-old calf developed pneumonia and died three days later despite veterinary treatment. Unfortunately, post-mortem examination was not carried out and the carcass was removed to the local hunt kennels. The following week a three month old calf (No. 400) developed hyperpnoea, dyspnoea and a temperature of 103°F. It showed a mucopurulent nasal discharge, developed a cough and again, despite veterinary treatment, it died 24 hours later. The calf was submitted to VLA Truro for post-mortem examination on the 21 September, and this revealed caseous foci in sub-mandibular, retropharyngeal, bronchial and mediastinal lymph nodes as well as extensive pneumonia. Acidfast bacilli were present in smears prepared from the lesions and *M. bovis* was subsequently isolated from these. Histological examination of the lungs showed a granulomatous and necrotising mycobacterial pneumonia and lymphadenitis. Because of the apparent rapid onset of disease, the age of the calves and the severity of the changes seen, a visit was made to examine the remainder of the calves on the farm.

Clinical Examination of the In-Contact Calves

Twenty two calves were present on the farm and these were kept in two houses, both of which were open at one end to the cows covered yard and separated only by a gate. The calves, therefore, shared air space and had nose to nose contact with the cows. Fourteen, one-three month-old calves were kept in one house and eight, three-four month-old calves were in the other. There had been

frequent movements of calves between the houses in both directions.

A number of the calves showed respiratory signs including hyperpnoea, coughing and nasal discharge. In addition five calves showed either developmental abnormalities or central nervous signs which were suggestive of in-utero Bovine Viral Diarrhoea Virus infection (BVDV) (Animal Numbers 396, 397, 398, 411 and 420). Two had short tails, one calf had bilateral cataracts and two calves showed ataxia and circling. The Animal Health Office decided to slaughter all 22 calves as TB

contacts. Heparin and plain blood samples were collected from all the calves prior to their slaughter on the 30 September.

Laboratory Investigations

Postmortem examinations of all 22 calves were carried out.

Formalised tissue samples of brains from the five calves showing clinical signs suggestive of in utero BVD virus infection were collected. Sections of these tissues were examined histologically and immune staining for pestivirus antigen was carried out on sections from all five calves.

Samples of affected lungs from all calves showing lung lesions were taken aseptically and inoculated onto 5% sheep blood agar and MacConkey agar. In addition, the samples were inoculated onto modified Middlebrook 7H11 agar semi-solid slopes and any suspect colonies were inoculated onto specialised Lowenstein Jensen and Stonebrinks media at different temperatures (Gallagher and Horwill, 1977).

Serum samples were examined by the complement fixation test for antibody to *Haemophilus somnus* and by the ELISA test for antibody to *Mycoplasma bovis*.

Three weeks after the herd TB test a three-month old calf developed pneumonia and died three days later despite veterinary treatment.



Figure 3
Calf no. 397
cerebellar hypoplasia

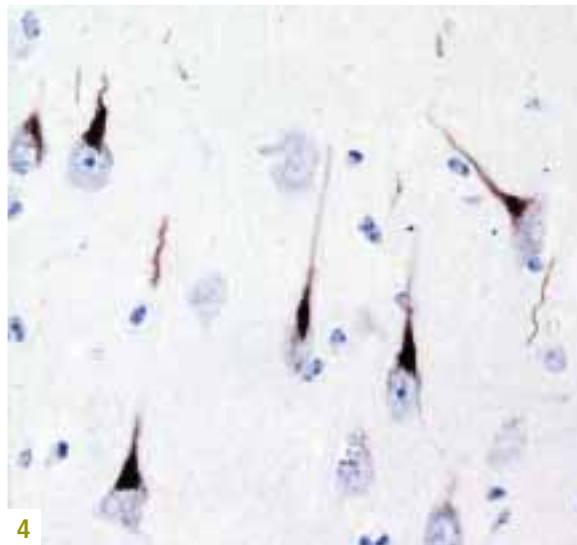


Figure 4
Distribution of
antigen (brown)
indicates persistent
infection in Cerebral
neurones

Serum samples were screened by the ELISA tests for antibodies to Respiratory Syncytial Virus (RSV), Infectious Bovine Rhinotracheitis (IBR), Parainfluenza 3 (PI3) and BVD.

Heparin blood samples were screened for BVD antigen and clots from all the blood samples were screened for BVD virus by virus isolation. Pooled samples of thymus and spleen tissue from the five calves showing clinical signs suggestive of in utero BVD virus infection were also screened for BVD virus by virus isolation.

Laboratory Findings

Of the five calves showing central nervous signs or developmental abnormalities two showed cerebellar abnormalities – hypoplasia in one (397) and aplasia in the other (398). Histopathology confirmed granulo-prival-type cerebellar dysgenesis in these and also revealed similar histopathology in a further one (396). Immune-staining failed to identify pestivirus in these but did identify pestivirus in the cerebral neurones of the remaining two (411 and 422), ie those showing no gross or histopathological evidence of changes to the central nervous system. This finding was considered to be indicative of persistent pestivirus infection.

Lesions suggestive of tuberculosis were identified in 14 of the 22 calves, 13 of these showed lung lesions ranging from

miliary granulomatous abscesses to caseous abscesses and generalised bronchopneumonia. In the majority of these caseous nodules were present in enlarged bronchial, mediastinal and retropharyngeal lymph nodes. The remaining calf showed caseous nodules in the bronchial and mediastinal lymph nodes only.

Pasteurella multocida was isolated from the lung lesions of one of the calves only – 398.

Mycobacterium bovis was isolated from the lesions in nine of the calves – 386, 394, 396, 398, 402, 404, 405, 411 and 422.

BVD antigen was detected in a heparin sample taken from one calf (385). BVD virus (type I) was isolated from whole blood samples taken from two of the calves (385 and 411). In addition BVD virus (type I) was isolated from a pooled sample of thymus and spleen tissues from two calves (411 and 420).

Serum samples showed no evidence of antibody to either *Haemophilus somnus* or *Mycoplasma bovis*. ELISA examination for antibody to RSV, IBR and PI3 viruses showed titres to all three viruses, a decline in antibody titres to all three viruses with the increasing age of the calves was apparent. ELISA examination for antibody to BVD virus showed that all

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except three had titres greater than 0.4 OD units. One (385) had no titre and calves 411 and 390 had titres between 0.2 and 0.3 OD units. Interestingly, calf 420 had a titre of 0.75 OD units.

Further Investigations

Whilst awaiting the 60 day tuberculin test the farmer culled a cow because of persistent mastitis. The cell count had risen from 500 (x 1,000/ml) to 2,558 (x 1,000/ml) over the previous four months. The affected quarter was swollen and firm and during several periods of antibiotic treatment the milk had been withheld from the bulk tank and fed to the calves. At post mortem several small caseous abscesses were present in the liver and a mesenteric lymph node, showing caseation and calcification, was enlarged to 5cms in diameter. Multiple tuberculous miliary abscesses were present throughout one quarter of the udder. Examination of Ziehl-Neelsen stained smears from the udder lesions showed very large numbers of acidfast bacilli. Histological examination of mammary tissue showed sheets of epitheloid macrophages together with extensive areas of necrosis and central

Lesions suggestive of tuberculosis were identified in 14 of the 22 calves, 13 of these showed lung lesions ranging from military granulomatous abscesses to caseous abscesses and generalised bronchopneumonia.

mineralisation. A Ziehl-Neelsen stained section showed numerous acidfast bacilli within the lesions and also occasional bacilli within the ducts. *M. bovis* was subsequently isolated from the lesions.

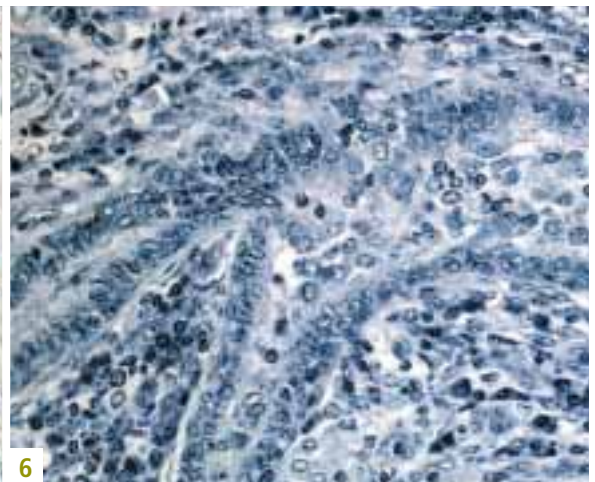
Examination of the udders of all the cows in the herd and scrutiny of their individual cell count records was then carried out. Milk samples were collected from any cows showing abnormal udders or persistently high cell counts. The samples were centrifuged and microscopic and cultural examinations of the sediments for acidfast bacilli were carried out. All of these proved negative.

A total of eight cows were culled before the next 60 day herd test and each time the carcasses of these were examined. Two showed lesions of TB which were confined to the retropharyngeal lymph nodes and liver of one cow, caseous abscesses were present in the lungs and liver of one more cow. The 60 day herd test identified a further five reactor cattle. Three of these were older cattle showing tuberculosis lesions in the lymphatic glands of the head or liver. Two, however, were calves aged four and six-weeks-old both of which showed extensive pulmonary tuberculosis.

Figure 5
Mycobacterial mastitis, large numbers of acidfast bacilli within necrotic areas of mammary gland tissue



Figure 6
Two acidfast bacilli within lumen of ducts of mammary gland



The two calves were part of a group of four which were the last animals to receive milk from the cow with tuberculous mastitis before its eventual slaughter. The remaining two calves of the four were slaughtered but showed no lesions of TB.

Blood samples were also collected from all the cattle on the farm at the same time as the TB herd test was carried out. Samples were screened for both BVD antigen and BVD antibody. Antigen was detected in only one animal – an 18-month-old steer which was then removed from the herd. Surprisingly, 15% of the herd was still sero-negative to BVD virus. The subsequent 60 day tuberculin tests did not reveal any more tuberculous animals and has since remained clear. Since the episode the herd embarked on a trial to eradicate BVD. This has been based upon a programme of vaccination but also involves testing calves at three months of age and the removal of persistently infected animals. The preliminary findings of this suggest a correlation between improved calf health and freedom from BVD virus. However the results of the trial will be the subject of a future report.

Discussion

The significant feature of this case is the high morbidity and severity of the tuberculous disease in the calves. Advanced and disseminated lesions were found in calves which had passed the TB test less than one month previously. The youngest calf, showing lung lesions from which *M. bovis* was isolated, was only 25 days old. This would suggest that bovine TB is not always a slow and chronic disease, rapid progression resulting in clinical disease can occur, especially in calves. It is also of note that, despite good evidence that infection was introduced orally to one calf at least, none of the calves showed alimentary tract lesions. However, feeding milk in

buckets to calves may well create aerosols resulting in respiratory infection. It cannot be assumed that all of the calves were infected from the milk. It is likely that airborne spread also occurred between the calves, the lung lesions in some of the calves would have been an abundant source of bacilli for respiratory infection of other contact calves. The position of the calf houses meant that the spread of infection from the calves back to the cows was indeed possible.

Involvement of respiratory viruses in the syndrome was not demonstrated. It is unfortunate that it was not possible to collect paired serum samples from the calves. The decline in antibody titres to RSV, PI3 and RSV viruses was seen with the increasing age of the calves and this would suggest that the titres were most likely the result of maternally derived antibody.

A combination of antigen detection and virus isolation techniques on blood samples together with virus isolation and immune staining procedures on tissue samples identified three calves which were persistently infected with BVD virus. A further three calves showed post mortem changes resulting from in-utero BVD infection. The presence of an 18 month old viraemic animal suggests that BVD virus had been circulating in the herd for approximately two years at least. The titres to BVD virus seen in these group of calves relate well to the accepted understanding of BVD in cattle. Calves born with cerebellar anomalies would be expected to have antibody to BVD virus but not to be viraemic (396, 397 and 398). Calves infected at an earlier gestation age would be expected to be viraemic but to have no antibody to BVD virus (385). There are also those calves which fall in between the two groups. In this case these are represented by the two animals from which virus was recovered and in which virus was demonstrated by immune staining.

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These calves (411, 420) showed varying levels of antibody to BVDV. The importance of BVD virus and its role in immunosuppression and intercurrent disease has been recognised for some time. It is known that acute BVD virus infection in calves can influence the onset of bovine respiratory tract disease and this is most probably a sequel to the period of leucopaenia which accompanies the onset of acute BVD infection. This poses some interesting questions.

Could a calf which is either persistently or acutely infected with BVD virus develop extensive tuberculous lesions when challenged with *M. bovis* more quickly than a calf not infected with BVD virus? Also, would such a calf once infected with *M. bovis* present an unusually high level of challenge to in-contact calves which may themselves be transiently infected with BVD virus? Could such calves, as a result of their suppressed immune response produce a lowered response to the intradermal skin test for bovine tuberculosis?

Calves 385, 390 and 420 were demonstrated to be persistently infected with BVD virus but cultural and histological examination failed to confirm the lung changes were caused by *M. bovis*. Calf 411 was also demonstrated to be persistently infected and in this instance *M. bovis* was isolated from the lung lesions. However, as one of the younger animals the opportunity of calf 411 to influence the older calves in the group would have been limited. Before the group of 22 calves were investigated in detail, six calves with tuberculous lesions had been removed from the farm. A further two calves, one a confirmed case of tuberculous pneumonia, had died. Unfortunately, at that stage, the involvement of BVD virus had not been suspected and the BVD

status of those calves remains unknown. It is likely to have been similar to the BVD status of the 22 calves described here.

Whether or not persistent and acute BVD infection influenced the course of the mycobacterial infection in this herd is open to speculation. What is important to recognise is that *M. bovis* is a cause of clinical disease in cattle and that whether or not *M. bovis* bacilli are excreted in the milk of a dairy cow or in aerosols from the respiratory tract of a calf there is an opportunity for zoonotic transmission particularly if the immune system of the in contact individual is suppressed.

Acknowledgements

The assistance of Roger Sainsbury (Animal Health Office, Defra), John Head and Stephen Trethewey (Head and Head, Helston) for bringing the case to my attention and assisting in examining and collecting blood samples from calves is gratefully acknowledged.

The author would also like to thank Dr Sandra Scholes, VLA Lasswade, for carrying out histological examinations of

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The significant feature of this case is the high morbidity and severity of the tuberculous disease in the calves.

Information

Robert Monies is a Veterinary Investigation Officer VLA, Truro.

TB in domestic species other than cattle and badgers

Background

The spectrum of hosts susceptible to *M. bovis* infection is very broad.

Although cattle are the natural hosts of the bacterium, all terrestrial mammals are susceptible to infection to a variable degree, as determined by the exposure level, innate resistance, predominant immunological pathways and type of husbandry. However, their opportunity to transmit the disease to other animals (particularly cattle) varies according to their ability to maintain TB within their own populations. Coleman and Cooke have classified host species of *M. bovis* as follows:

- **maintenance hosts** (e.g. bovines, badgers and occasionally farmed and wild deer), where the infection persists by vertical, pseudo-vertical or horizontal transmission within the species, without the need for input from other species; and
- **spillover hosts**, where TB occurs within the species only as long as there is input from an external source. Spillover hosts may in turn be either 'dead-end' hosts (if the incidence and pathology of the disease indicates they play no significant role in its onward transmission) or 'amplifier' hosts if they appear capable of increasing the prevalence of TB in livestock or other species.

1. Sheep

TB is rare in sheep, despite the fact that many flocks in TB hotspots must be exposed to *M. bovis*. The first reported case of TB in sheep in Britain occurred in

2003 involving a flock in close association with infected cattle. Six sheep reacted to the tuberculin test, four of which had tuberculous lesions at post mortem.

2. Goats

Bovine TB does occur in goats. Milk from infected goats is a potential human health risk. Goats may be considered spillover (amplifier) or maintenance hosts, depending on the type and location of the herd. In Mediterranean countries, for example, goats are known to maintain *M. bovis* infection and there is some evidence of transmission of goat-adapted clones of *M. bovis* (*M. bovis* subsp. *caprae*) to humans and cattle.

SVS standing instructions state that 'Goats should be placed under movement restrictions and officially tuberculin tested at the Departments' expense if located on premises where TB has been confirmed in cattle, or if *M. bovis* infection has been confirmed in the goat herd itself. Consideration should also be given to testing other goat herds on contiguous premises. This is strongly recommended if any dairy goat herds supplying/retailing unpasteurised milk are at risk of infection'.

3. Horses

Horses are reported to be resistant to human, bovine and avian TB. Isolated cases do however occur. Thus horses are true dead end hosts.

4. Pigs

The oral route is considered the most important route of infection in pigs,

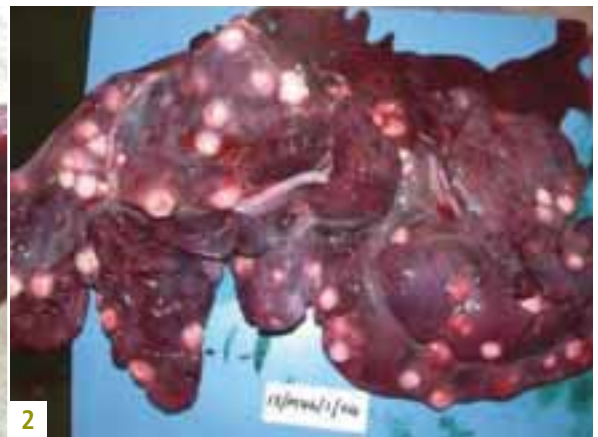
Author

Wyn Buick,
State Veterinary
Service (SVS),
Carmarthen

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Figure 1
Tuberculous Liver – Pig

Figure 2
M. Microti Liver – Llama



most frequently caused by feeding milk or milk products from infected cows (domesticated pigs) or scavenging carcasses of tuberculous animals (feral pigs). The status of the pig as a true dead-end or amplifier host of TB is still being debated. TB is not considered to be particularly contagious amongst pigs or to spread easily from pigs to other animals. In most cases, the disease is self-limiting and no control measures are required. However, evidence from New Zealand and Spain suggests that cross-infection between feral pigs/wild boar and extensively reared domestic pig populations is possible in the absence of cattle.

M. bovis was a common cause of TB in domesticated pigs in the early to mid 1900s. In recent years, the vast majority of suspect TB lesions detected by the Meat Hygiene Service during slaughterhouse inspection of pig carcasses have proven positive for *M. avium*. Still, in the period 1999-2003 four incidents of *M. bovis* infection were confirmed in pigs. The first one took place in 2000 on a wild boar farm in Cornwall. The second one was disclosed in 2002 in two fattened pigs in an outdoor unit in Wiltshire. Both pig premises were located in areas with a well established and

documented history of bovine TB in cattle and badgers. The other two incidents were in annual cattle testing areas.

5. South America Camelids (llamas, alpacas, vicuñas, guanacos)

TB is not a major health problem with camelids, but these species do occasionally develop the disease. Although reports of infection in their natural habitat in South America are few, cases of bovine TB have been diagnosed

in llamas and alpacas in New Zealand, the USA and in Great Britain. *M. avium* and *M. microti* infections have also been documented. Some authors have concluded that TB should be considered

Although cattle are the natural hosts of the bacterium, all terrestrial mammals are susceptible to infection to a variable degree, as determined by the exposure level, innate resistance, predominant immunological pathways and type of husbandry.

in the differential diagnosis in all cases of ill-thrift in these species, with or without obvious respiratory signs. As with other non-bovine species, there is little legislation underpinning the management of TB incidents in camelids in Great Britain. There is no requirement to identify camelids or record their movements. Divisional Veterinary Managers (DVMs) or Local Authorities have no legal powers to enforce TB testing in camelids and slaughter any reactors. Similarly, there are no provisions to compensate owners

for the loss of such animals.

TB testing of camelids should be by the single intradermal comparative cervical test (SICCT), as in cattle. Although not perfect, the SICCT is a suitable test for assessing the status of individual animals in a camelid flock with confirmed TB. This test provides reasonable sensitivity and specificity and it is the official testing procedure for camelids imported into the UK. The γ -IFN test (BOVIGAM®) is not a valid test for the diagnosis of TB in camelids.

6. Dogs

Although TB in dogs was a relatively common infection in the late 19th century and first half of the 20th century, more recent case reports of canine TB in the veterinary literature are scarce. The lower frequency with which TB is currently diagnosed in dogs probably reflects the reduced prevalence of TB in human beings and cattle in the developed countries.

Epidemiology

Dogs, like any other terrestrial mammals, can be naturally infected with *M. bovis*, *M. tuberculosis*, *M. avium* complex and other mycobacteria. However, the consensus of opinion is that dogs do not represent an epidemiologically significant source of infection for cattle, other dogs or other animals.

At present the disease appears to be extremely rare in dogs in GB. Typically less than five canine samples are processed at VLA annually for mycobacterial culture. Only one submission has proven positive to *M. bovis* since 1999.

Pathology

In the dog the primary complex occurs most frequently in the lungs and associated lymph nodes. Pleuritis (pleurisy) is a common complication. Lesions in the liver and mesenteric lymph nodes are also common. TB lesions in the skin and superficial nodes are more commonly observed in cats than in dogs.

Treatment

Because of the risk of transmission to other animals and human contacts, Defra's official line is to discourage the treatment of companion animals infected with *M. bovis* or *M. tuberculosis*. Furthermore, there are significant difficulties and financial costs associated with any anti-tuberculosis treatment, which involves a prolonged course of antibiotics for at least six months. The prognosis depends on the extent and severity of infection, but it is generally guarded.

In general, the antibiotics used to treat mycobacterial infections in dogs and other companion animals are the same as those used in humans. Some of these are inherently toxic.

There has been only one confirmed case of TB caused by *M. bovis* in dogs in the last five years. This was in a dog from Gloucestershire. *M. bovis* spoligotype ten (the most prevalent type of *M. bovis* in cattle in the eastern half of the county) was isolated from pulmonary lesions submitted to VLA for culture in February 2002.

7. Ferrets

In New Zealand, high rates of infection have been found in feral ferrets (up to 66% on infected properties). In the UK



Figure 3
Mesenteric lymph node – Ferret

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infection has been found in domestic ferrets and in one feral ferret. Wales has a significant population of polecats (closely related to feral ferrets) which are well known to regularly frequent farmyards.

8. Cats

Three infectious diseases caused by bacteria of the genus *Mycobacterium* have been documented in cats. These include classical tuberculosis (TB), feline leprosy (a cutaneous disease) and opportunistic mycobacterial

infections (again, typically, cutaneous conditions). Only classical TB in cats is of relevance to State veterinarians in the course of their duties and, as such, it is discussed in detail in this paper.

Classical TB in cats is quite a rare disease, traditionally caused by two members of the *Mycobacterium tuberculosis* complex: *Mycobacterium bovis* and *M. microti* (the vole tubercle bacillus). Infection of cats with *M. tuberculosis* (the human tubercle bacillus) appears to be even more rare, probably because cats are naturally more resistant to the human than to the bovine bacillus. Sporadic infections of cats with *M. avium* have also been described in the veterinary literature.

Cats are considered spill-over hosts of *M. bovis*, *M. microti* and *M. tuberculosis* because removal of the source of infection in cattle, wildlife or people results in a reduction in the incidence of TB in cats. However, cats are not true end hosts as the disease presentation makes them (at least theoretically) capable of transmitting these infections to other cats and other mammals, including humans. Cats are thus better regarded as 'spill-over amplifier' hosts of *M. bovis*.

Because of the risk of transmission to other animals and human contacts, Defra's official line is to discourage the treatment of companion animals infected with *M. bovis* or *M. tuberculosis*.

Cats become infected by exposure to infectious material from tuberculous cattle (or other maintenance hosts of *M. bovis*), tuberculous wildlife or through close contact with infected people. Therefore, the incidence of TB in the cat population is often a reflection of the local prevalence of bovine TB in

cattle/badgers/wild rodents and, to a lesser degree, *M. tuberculosis* infection in people.

Bovine TB in cats used to be quite common in GB. Most cases of feline TB were believed to have resulted from

the ingestion of contaminated unpasteurised milk from tuberculous cattle. More recently, after the introduction of regular TB testing of cattle and milk pasteurisation, other sources of infection for cats have been postulated:

- wounding by tuberculous possums in New Zealand
- hunting prey (voles, mice) infected with *M. microti*
- direct or indirect contact with tuberculous badgers in GB
- scavenging of tuberculous wild deer carcasses or offal in Michigan, USA.

Predisposition

Most cases of feline TB are probably subclinical. Infection usually occurs after protracted exposure, e.g. repeated exposure to infectious small mammals, living on a farm housing tuberculous cattle or living for prolonged periods with infected humans. TB is therefore seen mainly in adult cats and, interestingly, is seen most commonly in males. Infection with feline immunodeficiency virus (FIV) could predispose cats to infection with



Figure 4
Popliteal Lymph
Node – Cat

TB is particularly rare (but not unheard of) in horses and sheep, which should be considered true dead-end hosts.

Pigs, farmed wild boar, dogs, cats and camelids should be treated as potential amplifier hosts of TB.

Acknowledgements

Much of this article has been edited from the SVS standing instructions on Bovine tuberculosis – Viper Ch 23 section Y prepared by R. de la Rua-Domenech.

mycobacteria. However, very few cats with mycobacterial infections have actually tested positive for FIV or feline leukaemia virus (FeLV).

Incidence

Between 1980 and 2003 *M. bovis* was isolated from cats on 41 occasions at VLA. The incidence of tuberculous disease in cats caused by *M. bovis* has been sporadic in GB. However in 2005 the VLA received 90 suspect feline samples, 13 of which were positive for *M. bovis* and 23 for *M. microti*-like or *M. avium*-complex organisms. This is more than double the recorded incidence in previous years.

Conclusion

Among domestic animals in Great Britain, pigs, horses, sheep, cats, dogs and camelids are all considered spillover hosts as is perhaps the case with all other mammalian species (badgers, cattle and deer excluded). In other words, these species become infected only when the challenge level is relatively high, and they cannot sustain the infection within their own populations in the absence of infected cattle or a wildlife reservoir. This does not mean that, if infected, these species cannot occasionally transmit the disease to other animals and humans, i.e. some of them may act as amplifier hosts.

Information

Wyn Buick is a
Veterinary Officer
with the SVS,
Carmarthen.

Contents

Foreword	4
1 TB policy developments	5
2 Some lessons from the history of the eradication of Bovine Tuberculosis in Great Britain	11
3 Bovine Tuberculosis in the European Union and other countries: current status, control programmes and constraints to eradication	19
4 Bovine TB: Modelling and predicting its distribution in GB using CTS data	46
5 The laboratory diagnosis of Bovine Tuberculosis	53
6 The introduction of pre and post-movement TB testing in Scotland for cattle from high incidence TB areas	58
7 Ante mortem diagnosis of Bovine Tuberculosis: the significance of unconfirmed test reactors	65
8 The BOVIGAM® assay as ancillary test to the Tuberculin skin test	72
9 Tuberculous pneumonia and BVD in housed calves	81
10 TB in domestic species other than cattle and badgers	87

PB 12125
Nobel House
17 Smith Sqaure
London
SW1P 3JR

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