

BOVINE TUBERCULOSIS CASE RESPONSE MANUAL

MANAGING AN INCIDENT OF BOVINE TUBERCULOSIS

Edition 2, 2009

This manual is a technical response plan that describes the proposed Australian approach to a bovine tuberculosis incident. The document provides guidance based on sound analysis, linking policy, strategies, implementation, coordination and eradication plans.

Primary Industries Ministerial Council

This disease response manual is a stand-alone document

This response manual will be reviewed regularly. Suggestions and recommendations for amendments should be forwarded to:

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Subsequent changes will be endorsed by the Animal Health Committee

Approved citation:

Animal Health Australia. Bovine Tuberculosis Case Response Manual, Edition 2, 2009. Primary Industries Ministerial Council, Canberra, ACT.

Publication record:

First Published: 2007

Edition 2 published: 2009

The manual is available on the internet at <http://www.animalhealthaustralia.com.au>

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ISBN 978-1-876714-71-0 (printed version)

ISBN 978-1-876714-72-7 (electronic version)

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IMPORTANT NOTE: Important regulatory information is contained in the OIE Terrestrial Animal Health Code for bovine tuberculosis, which is updated annually and is available on the internet at the OIE website: http://www.oie.int/eng/normes/en_mcode.htm¹

DISEASE WATCH HOTLINE

1800 675 888

The Disease Watch Hotline is a toll-free telephone number that connects callers to the relevant state or territory officer to report concerns about any potential emergency disease situation. Anyone suspecting an emergency disease outbreak should use this number to get immediate advice and assistance.

¹ All URLs in this document were accessed on November 2009

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Abbreviations

AHC	Animal Health Committee
APP	Approved Property Program
AQIS	Australian Quarantine and Inspection Service
ARLBTB	Australian Reference Laboratory for Bovine Tuberculosis
BCG	Bacillus Calmette Guerin
BTEC	Brucellosis and Tuberculosis Eradication Campaign
CCA	Cattle Council of Australia
CVO	chief veterinary officer
HIV	human immunodeficiency virus
IFN- γ	interferon gamma
NCI	national cattle industry
NGSP	National Granuloma Submission Program
NLIS	National Livestock Identification Scheme
NPL	no palpable lesion
NVL	no visible lesion
OIE	World Organisation for Animal Health (formerly Office Internationale des Epizooties)
PCR	polymerase chain reaction
PIC	property identification code
PPD	purified protein derivative
PPG	Property Program Group
RFLP	restriction fragment length polymorphism
SDR	Standard Definitions and Rules
TB	tuberculosis
TFAP	Tuberculosis Freedom Assurance Program
TFAP2	Tuberculosis Freedom Assurance Program, stage 2
VNTR	variable number of tandem repeats

1 Introduction

Moves to eradicate bovine tuberculosis (TB) from Australian cattle began in the nineteenth century. A formal national approach to eradicating bovine TB began in 1968, when the Australian, state and territory governments agreed on a formal program, the national Brucellosis and Tuberculosis Eradication Campaign (BTEC). Internationally acceptable freedom from TB was achieved in 1997, but concern over the potential for resurgence of the disease led to targeted surveillance and response programs — the Tuberculosis Freedom Assurance Program (TFAP) in 1998 and an extension (TFAP2) in 2002. At the time of preparation of this manual (2006), there have been no cases of TB in cattle since December 2000 and no cases in buffalo since January 2002. Because of the success of risk reduction strategies under TFAP and the accumulated negative monitoring data from abattoirs, including no detections of TB cases for the past four years, further outbreaks are considered unlikely. Animal Health Australia will continue to manage bovine TB surveillance from 2007 to 2010 under the direction of the Animal Health Committee. It is intended that bovine TB will be included in the Emergency Animal Disease Response Agreement² from 2010.

Purpose of this manual

This manual provides a legacy of information, for future veterinary managers and field operators, to assist rapid and effective eradication of any future outbreak of bovine TB. It is based on a wide range of publications and advice from those who were closely involved in the latter stages of eradication and surveillance initiatives. This information is specifically oriented to the Australian environment and Australia's disease control practices.

The manual describes nationally accepted practices for eradication of bovine TB that have been endorsed by the Animal Health Committee and approved by the Primary Industries Standing Committee as the standard for any action to eradicate a resurgence of bovine TB. The primary consultants for the development of this manual have been Dr Geoff Neumann and Dr Jim Tolson. The process has been managed by Animal Health Australia, with input from TFAP2 Coordination Committee members.

Because this document is an amalgamation of several internal sets of instructions to staff, pragmatic decisions have been made to ensure that protocols and procedures are described in a reasonable and effective way, based on best practice at the time of publication. As this document is intended for use in operational situations, few formal references to the activities and procedures described are provided.

² <http://www.animalhealthaustralia.com.au/programs/eadp/eadra.cfm>

Overall policy for bovine tuberculosis

Bovine TB is an OIE³ listed disease that is significant in the international trade of livestock and livestock products. TB is also a zoonosis and a safety hazard in human food.

The disease response policy is to find all cases, to determine the extent and origin of the disease in the livestock population, and then to eradicate the disease using a ‘whole herd depopulation’ approach. A number of strategies to achieve and then verify eradication will be used, including the following:

- **Initial quarantine will occur for all cattle and any other at-risk susceptible livestock on affected and suspect premises. Since TB is spread through close contact, only in exceptional circumstances would it be necessary to establish a restricted or control area.**
- **Epidemiological investigations will be undertaken to identify the source of infection. Cattle and other at-risk livestock that might have been infected by exposure will be permanently identified. Full laboratory characterisation of any isolate is recommended for comparison with the TB reference collection. Trace-back may need to consider all movements of animals over the preceding 10–15 years.**
- **The confirmed case or cases, and part or all of their herds, will be destroyed and disposed of, depending on the findings of veterinary investigations. The preferred approach is removal of all at-risk populations with possible exposure. Destruction and disposal plans will be documented in an Approved Property Program.**
- **Surveillance and monitoring will be undertaken using the tuberculin test and carcase inspection in the field and at abattoirs, in order to determine the limits of the outbreak and provide evidence of eradication and ongoing freedom from disease following the response. The extent of surveillance and monitoring will be determined by veterinary investigations.**
- **Any remaining at-risk animals will be permanently identified and subjected to a program of early slaughter.**

Managing bovine tuberculosis in the Australian environment

Achieving national consistency in disease control procedures and supporting measures was challenging. The Australian environment has large variations in climate and topography, and cattle are often managed under conditions that are not conducive to standard disease control procedures. Nationally consistent control is further complicated by the state-based animal health system, which has significant implications for policy development, legislation, resources and implementation.

³ World Organisation for Animal Health (formerly Office Internationale des Epizooties)

Successful Australian disease control programs have often involved overcoming obstacles, adopting innovative approaches and achieving consensus for complex and often costly decisions. During the bovine TB eradication programs, important aspects included:

- The nature and frequency of consultation and communication between governments and with the national cattle industry, which ensured consistent application and real progress
- Sound policy, good record keeping, regular analysis and reporting and performance review by high-level government and industry committees, which led to progressive refinement of bovine TB eradication policy, procedures and funding
- Continually improving knowledge of the disease and its behaviour under the wide range of conditions faced, which enabled the development of specific support for the involvement of affected producers — in particular, the compensation and additional assistance required for owners of large pastoral holdings in central and northern Australia
- Regular analysis of performance and expenditure and the commissioning of specific reports at critical points in the program.

One commissioned report (Tolson and Jervois 1990), examined records of the National Tuberculosis Case Register and the National Granuloma Submission Program (NGSP) to identify where risks from future bovine TB might occur. The outcome confirmed what many feared — that the disease could reappear up to 15 years after apparent eradication.

An appreciation of this critical epidemiological feature of the disease was the major driver of TFAP and TFAP2, which followed BTEC. It has also stimulated the preparation of this manual to provide a lasting legacy of the combined experience of many individuals and help to prevent the disease regaining a foothold in Australia.

Historical background

The following information is provided to place any future disease control activity into the context of previous activities. This historical context and the lessons learnt about bovine TB eradication are also addressed in Appendix 1 — Legal capacities critical to success of bovine tuberculosis control and eradication.

Activities before 1970

Bovine TB control began in the 1870s after it had been demonstrated that people could die from TB contracted via milk. Use of the tuberculin test (see Section 6) began in Australia in the 1890s. Over the following decades, state and territory control programs in dairying areas, based on voluntary tuberculin tests and slaughter of infected cattle, significantly reduced transmission of TB to humans. Other sanitary methods that contributed to control of the disease included slaughter of clinical cases and test reactors, not feeding infected offal to pigs, separation of grazing cattle from grazing pigs, and disease tracing to infected properties.

The widespread adoption of pasteurisation in the 1940s finally ensured the safety of milk. Mandatory controls, such as herd testing of whole milk suppliers, were introduced. At the same time, synthetic medium tuberculin became available for testing in cattle.

Compensation for loss of infected cattle, mostly from state industry funds, was adopted in most areas as a means of encouraging farm control of bovine TB. In the 1950s, all states and territories invoked various formal control or eradication measures. The introduction of compensation for infected animals and tuberculin test reactors meant that, by the start of the national eradication campaign in 1970, bovine TB prevalence was very low in all dairying regions and in most beef herds in the closely settled areas. However, in the pastoral areas of central and northern Australia, the environment and cattle management practices made bovine TB eradication difficult, and many properties had a high prevalence of bovine TB.

In 1967, after a protracted campaign, Australia was declared free from bovine pleuropneumonia. In the same year, the Joint FAO–WHO Expert Committee on Zoonoses recommended the adoption of eradication of bovine TB by test and slaughter (FAO/WHO 1967). Concerns that disease in Australian cattle might affect buoyant beef exports spurred Australia to consider a national eradication campaign.

Before 1970, the policy and activities in each state and territory largely originated in that jurisdiction; there was no obligation on the states and territories to adopt consistent approaches, although state and territory chief veterinary officers (CVOs) conferred regularly and no doubt influenced each other's approaches. Finance for bovine TB control was provided by each government, and producers contributed to the all-important compensation schemes (except in Tasmania, where control was funded solely by the state government).

The Brucellosis and Tuberculosis Eradication Campaign

A national Brucellosis and Tuberculosis Eradication Campaign (BTEC), based on test and slaughter and abattoir monitoring, began in Australia in 1970. Its objective was to protect international markets for beef and dairy products, following apparent rapid progress in disease eradication in the United States. Under BTEC, a national slaughter levy on cattle was established to provide funds, disease targets were set, and the beef and dairy cattle industries adopted common goals for state and territory governments.

BTEC was originally based on a formal agreement between the Australian Government and the states and territories to provide funding for specified activities and conduct eradication according to agreed protocols. As problems emerged in northern Australia, especially concern over financial hardship for some producers, a new 'BTEC Committee' was formed in 1984 to take over the management and financial coordination of the eradication campaign. This committee — which included representatives of the Australian, state and territory governments, the cattle industry and specialist financial and economic advisers — provided an important national cohesion to activities.

The agreed protocols and national technical rules for the conduct of eradication activities were prepared by a national technical committee and termed the national Standard Definitions and Rules (SDRs; Australian Animal Health Council Ltd 2003). These were first published in 1975 and continued (with minor changes to reflect progress and new challenges) to provide the technical framework throughout TFAP and TFAP2; the final version was published in 2003. There were also standardised laboratory techniques that detailed minimum diagnostic standards for laboratory tests to confirm a diagnosis of TB.⁴

⁴ <http://www.scahls.org.au/asdts/asdt.htm>

This case response manual for bovine TB, which contains the policies for the period after TFAP2 (beginning in 2007), constitutes the current version of the SDRs.

Funding for BTEC was a collaborative effort, with each party funding a specified proportion of key activities. From 1973, the Australian cattle industry contributed through levies, initially on exports and later on cattle transactions.

Throughout the campaign, technical workshops and reviews were regularly held to monitor progress and transfer technical information between jurisdictions. National progress reports documented activities throughout Australia and provided the basis for continuous reassessment of the procedures adopted in each state and territory.

In 1992, the National Granuloma Submission Program (NGSP) was implemented to address concerns about efficient detection of bovine TB at abattoirs as the prevalence in the cattle population declined. NGSP emphasised inspection procedures, the need for well trained inspectors, the examination of sample handling and submission and the importance of laboratory confirmation for diagnosis of bovine TB.

BTEC was successful for many reasons. These are summarised below because they provide a framework for future eradication activity:

- A simple and clear campaign goal — the elimination of *Mycobacterium bovis* from all cattle and buffalo herds
- A national approach driven by both a committed cattle industry and supportive governments
- The establishment and mutual recognition of standardised procedures and quality control over all aspects of management, field testing and laboratory procedures
- Adequate funding provided by the industries and by state, territory and Australian governments
- Financial support mechanisms to encourage the adoption of sound eradication programs
- Effective legislative support
- Adequate compensation to owners of suspect and diseased animals that had to be slaughtered
- A high level of competence in farmers, stock inspectors, veterinarians and campaign managers
- A high-quality veterinary laboratory diagnostic service
- An active research program funded by governments and the cattle industry.

Australia adopted a status of ‘Impending Free’ for bovine TB in 1992 and was declared a ‘Tuberculosis Free’ area at the end of 1997, using a definition that exceeded the internationally recognised tuberculosis-free status defined by the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE, formerly Office International des Epizooties).⁵

⁵http://www.oie.int/hs2/sit_mald_cont.asp?c_mald=35&c_cont=5#e

The extended period before eradication was formally recognised in Australia results from the knowledge that this insidious chronic infection has the potential for resurgence many years after detection of the last active case.

The Tuberculosis Freedom Assurance Program

TFAP followed BTEC from 1998 and ended in December 2002. Unlike previous phases of TB eradication in Australia, this phase was established under the auspices of Animal Health Australia within a formally established deed between the participating state, territory and Australian governments, the Cattle Council of Australia (CCA) and Animal Health Australia.

The deed defined the essence of the program as follows:

- Ensure the maintenance of Australia as a TB-free area, and eventual eradication of bovine TB
- Maintain surveillance that efficiently detects any remaining bovine TB
- Provide financial and human resources to complete eradication within the proposed timeframe
- Adopt risk management techniques and provide assistance to owners to lessen the impact of eradication
- Collect adequate granulomas for laboratory exclusion of bovine TB
- Conduct targeted testing of herds that had inadequate monitoring
- Provide financial assistance to accelerate the removal from properties of older cattle
- Implement Approved Property Programs that would ensure that a property affected by bovine TB returned to a disease free herd status within two years.

The original TFAP program had seven administrative components: field operations, NGSP, the TB case register, the Tuberculosis Reference Laboratory, the NGSP database, assistance measures and corporate activities.

Funding for TFAP was aligned with the responsibilities of each party. The state or territory concerned funded field operations; the Australian Government funded NGSP, the TB case register and the national reference laboratory; and the national cattle industry funded assistance measures. A TFAP Coordinator was responsible for technical, administrative and financial aspects of the program as a key corporate activity funded by all parties. A representative group, the TFAP Coordination Committee, provided program oversight, and a TFAP Property Program Group reviewed and approved proposed property programs.

As with BTEC, ongoing review was important, and a midterm review was a provision of the deed. This review examined the effect of operations, provided recommendations on key issues and proposed changes to arrangements for the remainder of the program. It also investigated and reported on the need for a further period of surveillance for bovine TB after the program ended in December 2002.

The Tuberculosis Freedom Assurance Program 2

TFAP2, which began in 2003, was the final stage of formal national processes to confirm eradication of TB from Australia. As with TFAP, it involved the Australian Government,

all states and territories and the national cattle industry in a formal deed of agreement that described the agreed policies and processes to be employed. These were based on knowledge of the chronic and insidious nature of the disease and the significant management complexities arising from cattle management in the north of Australia. A further period of surveillance was thought necessary to:

- Continue to reduce the risk of TB incidents
- Deliver a managed and phased end to formal national management of eradication
- Transfer responsibility for the various components to the appropriate parties.

Surveillance was by agreed field operations and abattoir inspection, with an agreed transition by all jurisdictions to standard meat inspection during the life of the program. Suspicious samples continued to be sent to veterinary laboratories and examined for evidence of bovine TB according to a national protocol described in the SDRs. Other significant features were maintenance of the Australian Reference Laboratory for Bovine Tuberculosis (ARLBTB) and arrangements to provide assistance measures to support field surveillance and management of TB incidents and cases.

Throughout TFAP2, the states and the Northern Territory continued to provide funding and to be responsible for all activities necessary to effectively monitor for, diagnose and manage the eradication of bovine TB. The Australian Government and CCA provided component funding, and Animal Health Australia coordinated management and funding for the program.

From January 2005, the submission of granulomas continued at meat inspectors' discretion in accordance with the Australian standard meat inspection procedures and instructions from the Australian Quarantine and Inspection Service (AQIS).

Government and cattle industry cooperation in TB eradication

The successful eradication of bovine TB largely resulted from the ongoing support for BTEC and the TFAPs provided by governments and the cattle industry. The combination of government and national cattle industry funding, accountability and management is a successful model that should be emulated. State and territory managers should continue, wherever possible, to involve the local and national cattle industries at the earliest opportunity following detection of a suspect case of bovine TB.

The major area in which local and national cattle industry representatives will be able to assist is in development of an appropriate property program. Those knowledgeable about bovine TB programs should routinely be included in expert consultations for resolving a bovine TB detection.

Terminology

The terminology used in this manual follows conventions used during BTEC and the TFAPs. For those unfamiliar with herd classifications used during these programs, the SDRs prepared for TFAP should be consulted. Any difference in common usage of a term that has been advised by a state or territory is pointed out at the time of first use.

The approach in the current document is to use general terms that are likely to be readily recognised in the future. Descriptions of procedures that will no longer be used (e.g.

comparative tuberculin test) are not included, and terms describing herds and area status are not used. Terms in common usage are not defined in the manual. Other terms are explained in the Glossary or defined on first usage.

2 Nature of the disease

Aetiology and taxonomy

Tuberculosis (TB), caused by *Mycobacterium bovis*, is an infectious, chronic respiratory disease that affects cattle, water buffalo, deer, goats and a wide range of other animal species. The disease is usually characterised by the formation of nodular granulomas or tubercles within the respiratory system, and possibly in other parts of the body.

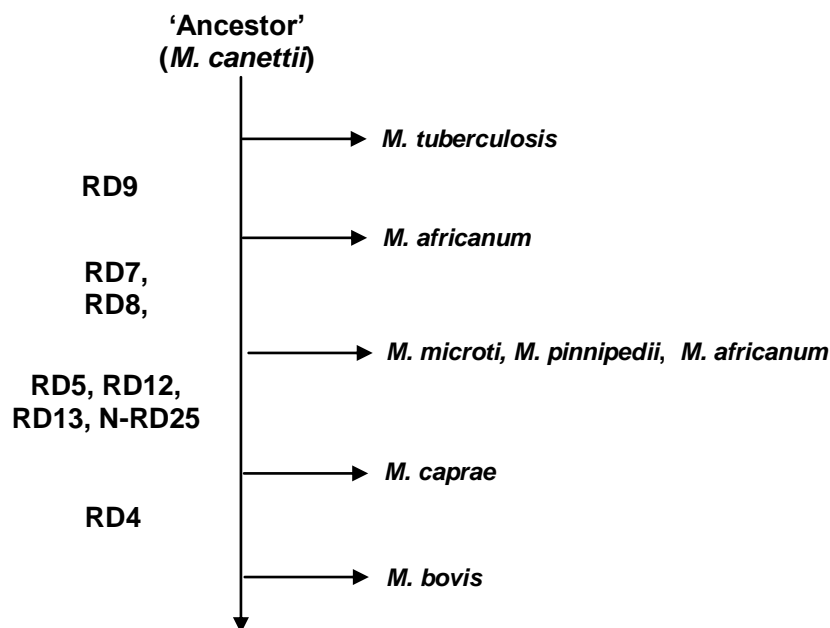
M. bovis belongs to the *M. tuberculosis* complex (closely related group) of organisms; members of this complex are the primary cause of TB in a number of species. All members of the *M. tuberculosis* complex have been reported to cause infection in animals.

The other members of the complex have traditionally included the classical human pathogen (*M. tuberculosis*), an African variant of the human tubercle bacillus (*M. africanum*) and *M. microti*, which infects rodents, particularly voles. *M. bovis* Bacillus Calmette Guérin (BCG), an attenuated form of *M. bovis* produced by a series of subcultures, is also commonly included in the *M. tuberculosis* complex.

Recently, the advent of molecular techniques has led to recognition of further species within the *M. tuberculosis* complex. One of these is *M. caprae*, primarily a pathogen of goats (Aranaz et al 2003), but also reported to cause TB in cattle indistinguishable from disease caused by *M. bovis*. Another, *M. pinnipedii*, is a pathogen of seals now known to be responsible for endemic TB in at least 7 seal species in the southern hemisphere (Cousins et al 2003). *M. bovis*, *M. pinnipedii* and *M. caprae* are all zoonotic organisms.

Other variants belonging to the *M. tuberculosis* complex that have recently been characterised include the 'oryx bacillus' and the 'dassie bacillus'. The oryx bacillus, which appears to be a subtype of *M. bovis*, infects oryx; the dassie bacillus, apparently a variant of *M. microti*, causes TB in rock hyrax or dassie (*Procavia capensis*) (Cousins et al 1994) and surikat (*Surikat surikat*).

Historically, taxonomic segregation of the *M. tuberculosis* complex has been based on each species' unique combination of host preference, and its characteristic growth, morphology, physiology and biochemistry (Vestal 1975). In the past decade, molecular techniques have allowed a better understanding of the taxonomy and evolution of the *M. tuberculosis* complex. *M. bovis* was previously considered to be the precursor of *M. tuberculosis*: it was believed that TB spread to humans after the domestication of infected animals. Recent molecular work has shown that *M. tuberculosis* evolved from *M. canettii* (which infects humans) and that *M. bovis* has evolved relatively recently from *M. tuberculosis* (Brosch et al 2002, Mostowy et al 2002). *M. africanum*, *M. microti*, *M. caprae* and *M. pinnipedii* are intermediate (between *M. tuberculosis* and *M. bovis*) in terms of evolution. These relationships are shown in Figure 1.



Note: Suggested phylogeny is derived from analysis of genomic deletions (RD [region deleted] and N-RD [new region deleted]). The vertical axis represents the loss of genomic regions within isolates of the *M. tuberculosis* complex. Each horizontal axis clusters isolates on the presence or absence of genomic regions. Isolates labelled *M. africanum* could not be assigned to a single deletion type.

Source: Mostowy et al (2002)

Figure 1 Suggested evolutionary relationships within *Mycobacterium*

Other mycobacteria of significance to bovine TB

M. avium, *M. intracellulare* and other unidentified *Mycobacterium* species are known to cause granulomas in cattle that may be indistinguishable from those caused by *M. bovis* on necropsy examination and histopathology (D Cousins, Principal Microbiologist and Manager, Animal Health Laboratories, Department of Agriculture and Food, Western Australia, pers. comm., September 2006). In most cases, these granulomas are limited in distribution. Infections with these species are not considered to be infectious between animals. In countries or regions where Johne's disease (paratuberculosis) is common, granulomas caused by *M. paratuberculosis* may be confused with TB, especially in deer species. In such cases, the affected lymph nodes are often found in the alimentary tract, particularly the mesenteric lymph nodes. In this manual, *M. avium* refers to *M. avium* subspecies *avium* and *M. paratuberculosis* refers to *M. avium* subspecies *paratuberculosis*.

In water buffalo, there is limited evidence that mycobacteria other than *M. bovis* cause tuberculosis-like granulomas (K de Witte, Northern Territory Department of Primary Industry, Fisheries and Mines, pers. comm., 2005).

A range of mycobacteria may be isolated from lymph nodes taken from tuberculin reactors (that is, animals that react to the tuberculin test; see Section 6). Care must be taken in the interpretation of results because of the potential for environmental mycobacteria to contaminate cultures and overgrow the target mycobacteria if insufficient care is taken in the collection of specimens at necropsy.

Susceptible species

M. bovis has the widest host range of any of the *M. tuberculosis* complex and can infect a wide variety of domestic animals and wildlife. Several good reviews are available on this subject (O'Reilly and Daborn 1995, de Lisle et al 2001). Species in which *M. bovis* infection has been reported are listed in Table 1.

World distribution and occurrence in Australia

Bovine TB has a worldwide distribution. Because of its ability to cause zoonotic infection and productivity losses and to impede trade, many developed nations have embarked on eradication or control programs. A list of current country status situations can be found on the website of the World Organisation for Animal Health (OIE, formerly Office International des Epizooties)⁶ or in OIE reports.⁷

In Australia, bovine TB was widespread in cattle and water buffalo before early attempts at eradication. *M. bovis* had rarely been recorded in other species in Australia before 1970. It had been reported in a sheep, several horses, and a dog (Seddon and Albiston 1965).

In the early part of the twentieth century, the prevalence of TB in domestic pigs was very high in some Australian piggeries (Cousins et al 1998b). Domestic pigs were infected primarily as a result of ingestion of infected offal or milk.

Feral pigs in northern Australia were infected following ingestion of infected carcasses. In line with research findings in other countries, domestic and feral pigs are dead-end hosts in Australia. Research in northern Australia demonstrated a consistent decrease in occurrence of *M. bovis* in feral pigs when eradication efforts reduced the prevalence of TB in cattle and water buffalo (Corner et al 1981, McInerney et al 1995).

Sheep are reported to be less susceptible to *M. bovis* infection than bovines. However, TB has been found in sheep grazing on heavily contaminated material in New Zealand (Cordes et al 1981, Davidson et al 1981). *M. bovis* infection of sheep has not been confirmed in Australia.

The only report of TB in goats was in 1989, when a single goat was found to be infected with *M. bovis*. The goat was in a herd in the southwest of Western Australia that was co-grazing with cattle with a high prevalence of *M. bovis* infection. The affected property was severely overstocked and the animals were in generally poor condition (Cousins et al 1993a).

Infection in farmed deer has been reported: *M. bovis* established in three deer herds in a serious outbreak in South Australia in 1986 (Robinson et al 1989). In March 1990, TB was confirmed in an aged, male fallow deer in a Victorian herd. This animal had originated from the South Australian herds, and there was no further evidence of TB infection (J Harkin, Principal Veterinary Officer, Victorian Department of Primary Industries, pers. comm., March 2007).

⁶ http://www.oie.int/eng/info/en_infoan.htm

Table 1 Examples of free-living or captive wildlife reported as infected with *M. bovis*⁸

Free-living wildlife hosts	Captive wildlife hosts
Antelope, marsh (<i>Kobus leche</i>)	Baboon (<i>Papio hamadryas</i>)
Baboon, olive (<i>Papio cynocephalus anubis</i>)	Baboon (<i>Papio papio</i>)
Baboon, chacma (<i>Papio ursinus</i>)	Camel, bactrian (<i>Camelus bactrianus</i>)
Badger (<i>Meles meles</i>)	Chimpanzee (<i>Pan troglodytes</i>)
Bear, black (<i>Ursus americanus</i>)	Deer, axis (<i>Axis axis</i>)
Bison (<i>Bison bison</i>)	Deer, fallow (<i>Dama dama</i>)
Bobcat (<i>Lynx rufus</i>)	Deer, red (<i>Cervus elaphus</i>)
Buffalo, African (<i>Syncerus caffer</i>)	Deer, roe (<i>Capreolus capreolus</i>)
Buffalo, water (<i>Bubalus bubalis</i>)	Deer, sika (<i>Cervus nippon</i>)
Cat, feral (<i>Felis catus</i>)	Dusky langur (<i>Presbytis obscurus</i>)
Cheetah (<i>Acinonyx jubatus</i>)	Fox, fennec (<i>Vulpes zerda</i>)
Coyote (<i>Canis latrans</i>)	Gibbon, siamang (<i>Symphalangus syndactylus</i>)
Deer, axis (<i>Axis axis</i>)	Kudu, greater (<i>Tragelaphus strepsiceros</i>)
Deer, fallow (<i>Dama dama</i>)	Lemur, Mayotte (<i>Lemur mayottensis</i>)
Deer, mule (<i>Odocoileus hemionus</i>)	Leopard (<i>Panthera pardus</i>)
Deer, red (<i>Cervus elaphus</i>)	Leopard, snow (<i>Uncia uncia</i>)
Deer, roe (<i>Capreolus capreolus</i>)	Macaque, lion-tailed (<i>Macaca silenus</i>)
Deer, sika (<i>Cervus nippon</i>)	Macaque, stump-tailed (<i>Macaca arctoides</i>)
Deer, white-tailed (<i>Odocoileus virginianus</i>)	Monkey, colobus (<i>Colobus guereza caudatus</i>)
Duiker, common (<i>Sylvicapra grimmia</i>)	Monkey, rhesus (<i>Macaca mulatta</i>)
Ferret (<i>Mustela putorius furo</i>)	Oryx, Arabian (<i>Oryx leucoryx</i>)
Fox, red (<i>Vulpes vulpes</i>)	Rhinoceros, black (<i>Diceros bicornis</i>)
Goat, feral (<i>Capra hircus</i>)	Rhinoceros, white (<i>Ceratotherium simum</i>)
Hare, European (<i>Lepus europaeus occidentalis</i>)	Sea lion, Australian (<i>Neophoca cinerea</i>)
Hedgehog (<i>Erinaceus europaeus</i>)	Sea lion, South American (<i>Otaria byronia</i>)
Kudu, greater (<i>Tragelaphus strepsiceros</i>)	Sea lion (<i>Otaria flavesceus</i>)
Leopard (<i>Panthera pardus</i>)	Seal, New Zealand fur (<i>Arctocephalus forsteri</i>)
Lion (<i>Panthera leo</i>)	Tiger (<i>Panthera tigris</i>)
Lynx, Siberian (<i>Lynx pardinus</i>)	
Mink, American (<i>Mustela vison</i>)	
Mole, European (<i>Talpa europaea</i>)	
Pig, feral (<i>Sus scrofa</i>)	
Possum, brushtail (<i>Trichosurus vulpecula</i>)	
Rabbit, European (<i>Talpa europaea</i>)	
Raccoon (<i>Procyon lotor</i>)	
Rat (<i>Rattus norvegicus</i>)	
Seal, Australian fur (<i>Arctocephalus pusillus doriferus</i>)	
Seal, New Zealand fur (<i>Arctocephalus forsteri</i>)	
Seal, subantarctic fur (<i>Arctocephalus tropicalis</i>)	
Sea lion South American (<i>Otaria flavesceus</i>)	
Stoat (<i>Mustela erminea</i>)	

⁷ http://www.oie.int/eng/en_index.htm

⁸ Includes examples of species from which *M. bovis* or a closely related variant has been isolated. Sources: de Lisle et al (2001); Cousins (2004).

Warthog (*Phacochoerus aethiopicus*)

Between 1986 and 1988, *M. bovis* infection was recorded in a colony of sea lions in a marine park near Perth, Western Australia (Forshaw and Phelps 1991, Cousins et al 1993b). A few years later, a case of pulmonary TB occurred in a seal trainer who had worked at the affected marine park. DNA fingerprinting techniques were able to confirm that the seal trainer had been infected by the seals (Thompson et al 1993). The strain of mycobacteria was determined to be a new species within the *M. tuberculosis* complex and was named *M. pinnipedii* (Cousins et al 2003).

TB has not been recorded in the Australian population of feral camels. Nor has it been found in the native marsupial possum, despite possums being a serious reservoir of the disease in New Zealand after being introduced in the early part of the twentieth century.

Bovine TB is a well-known zoonosis; the fact that *M. bovis* can cause disease in humans is one of the reasons so many developed countries have embarked on control and eradication programs. A number of risk factors for infection in humans were identified in a longitudinal study of human cases of *M. bovis* infection from 1970 to 1994 in Australia (Cousins and Dawson 1999). These risk factors included working with stock and in meat processing plants.

Wild or feral animals infected with *M. bovis* can hamper eradication and control efforts (Davidson 1976, Tweddle and Livingstone 1994). Australia is fortunate not to have a wildlife host that could not be controlled during the eradication program; infected water buffalo in Australia were treated in the same way as cattle.

Historical occurrence in Australia

1847–1970

Bovine TB was probably introduced into Australia with the early importations of cattle as Australia was settled. The disease was recorded in 1859 in cattle that had been killed in an effort to control pleuropneumonia. By 1880, TB was common in coastal dairy populations. It was widespread in cattle and water buffalo before early control efforts, and later records show individual herds of dairy cattle with up to 90% of animals infected. Less disease was present in the beef cattle introduced to the vast plains of northern and central Australia.

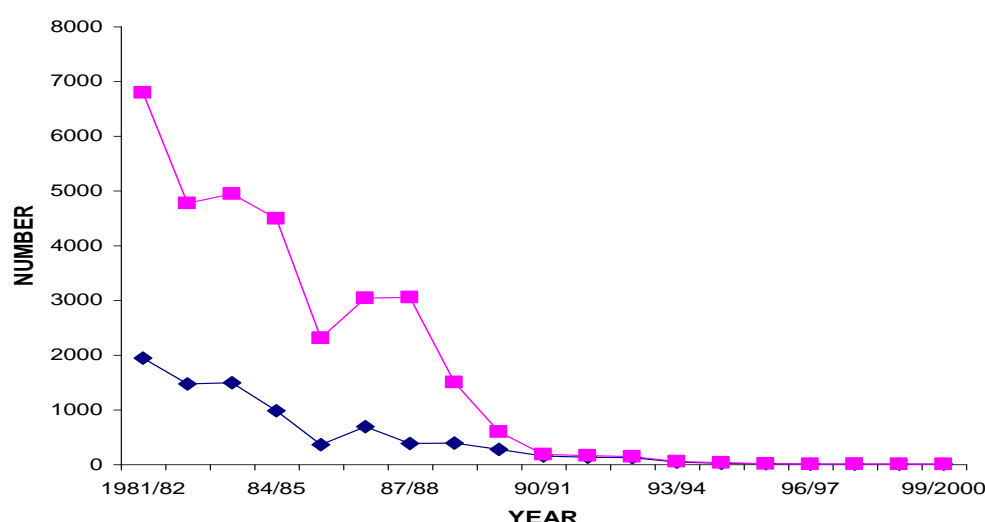
Control measures introduced for bovine TB during the period to 1970 are described in Section 1.

1970–1997

Activities under the national Brucellosis and Tuberculosis Eradication Campaign (BTEC), which began in 1970, are described in Section 1. Over the decade from 1970, cattle tail tags were introduced to improve disease tracing from abattoirs to property of origin. This measure, together with existing cattle brands and movement permits, and computerisation of disease records, meant that Australia had a disease eradication program that was envied by many around the world (Cousins et al 1998b). As a result of achievements under BTEC, the OIE recognised Australia as free from bovine brucellosis in 1989. Disease monitoring for brucellosis in abattoirs ceased in 1993. The eradication of brucellosis was assisted by the failure of the disease to establish in the vast herds of north-western Australia.

TB eradication proved far more difficult. In central and northern Australia, large free-roaming herds presented many challenges. Innovative measures were adopted to provide for testing of all beef cattle and water buffalo, either by repeat testing or consignment to slaughter (Tolson and Jervois 1990, Glanville and Roberts 1992, Lehane 1996). Cattle and water buffalo that were running wild were rounded up, and many were shot on the spot, sometimes from helicopters. Radio-tracking devices found many straggler cattle and water buffalo. The water buffalo population of the Northern Territory was reduced by 400 000 to assist with eradication of bovine TB (BL Radunz, Chief Veterinary Officer, Northern Territory, pers. comm., June 2006).

Official disease programs for the large individual properties saw removal of many infected cattle resulting in the rise in the number of cases detected around 1987/88 in Figure 2. Significant gains were made in the decade leading up to 1992; disease incidence decreased 100-fold from the late 1980s to the early 1990s (Roberts et al 1998). Figure 2 shows the number of TB-infected cattle in Australia from 1981 to 2000.



Source: Roberts et al (1998)

Figure 2 TB-infected cattle in Australia (1981–2000), as detected by abattoir monitoring (■) and field testing (◆)

As with farmed pigs many years earlier, eradication of bovine TB in the feral pig population was closely monitored. Disease control was achieved by destroying infected animals or by eradicating infection from nearby cattle or water buffalo (Corner et al 1981, McInerney et al 1995).

Australia was declared a 'Free Area in respect to Bovine Tuberculosis' on 31 December 1997.

Recent history and current situation (1998–2006)

Disease monitoring activities under the Tuberculosis Freedom Assurance Program (TFAP and TFAP2) are described in Section 1.

Eight new primary cases of bovine TB occurred during TFAP, compared with 36 cases in the last 5 years of BTEC. However, in 2001 and 2002, 9 secondarily infected herds (14 cases) were found after the herd owners purchased cattle from earlier herd dispersal. No new primary cases were recorded during this time (Turner 2003). In 2002, 2 adjacent small herds of water buffalo in the Northern Territory were the last recorded cases of bovine TB in Australia.⁹

From 1998 to 2002, a phase of more sensitive abattoir monitoring took place. During this period, the National Granuloma Submission Program (NGSP) resulted in more than 21 000 laboratory submissions (a submission rate of 1 in 1945 of the 41 million cattle slaughtered). Only 10 tuberculous animals were detected in abattoirs from 1998 to 2002, compared with 57 during the previous 5-year period.

During this period, the live cattle export trade of aged cows to slaughter continued to improve, providing an outlet to reduce the number of cattle that might previously have been exposed to infection. The program also called for a further test of herds that retained cattle if they had been infected after 1988. Targeted culling of aged cows was assisted with financial incentives to get them to slaughter.

Dr Brian Radunz, the Chief Veterinary Officer in the Northern Territory, reported to the 4th International Conference on *Mycobacterium bovis* in 2005 that TFAP2, the continuation of TFAP, maintains Australia's internationally recognised status as a bovine tuberculosis Free Area (Moore et al 2006).

All targeted herds had their final evaluation by field testing during TFAP2 (2003–2006). Risk assessment strategies are used to streamline abattoir monitoring (Moore and Roe 2002).

Diagnostic criteria

In the early stages of TB control, great reliance was placed on detecting macroscopic lesions during gross post-mortem examination. If there was doubt about the cause of the lesion, histopathology was employed. Culture for *M. bovis* was rarely performed (media for culture of mycobacteria were only developed in the 1950s). When Australia's BTEC commenced, the need for a definitive diagnosis became more important and routine culturing of all suspected lesions became commonplace. In the later stages of the campaign, exhaustive cultural examination of tissues from suspects became essential.

Clinical signs

When present, clinical signs can include variable pyrexia, weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and coughing, particularly with advanced TB. These signs are not unique to bovine TB. Although normally a chronic debilitating disease, bovine TB can assume a more acute, rapidly progressive course.

⁹ <http://www.animalhealthaustralia.com.au/aahc/index.cfm?7339E022-B68F-AB04-264E-AAA1B958ED0D>

Gross pathology

TB causes abscess-like lesions commonly referred to as granulomas or tubercles. The area of the body affected is usually related to the route of entry. Because of the frequency of respiratory transfer, lesions are often seen in the lungs and associated lymph nodes. However, the lymph nodes of the head are often the only ones affected. Macroscopic lung lesions are not essential for the spread of TB by the respiratory route. Small and even microscopic lung lesions, which often occur concurrently with thoracic lymph node lesions, are often not detected by normal abattoir or field autopsy techniques. Once the organism has entered the bloodstream, lesions may be found in any part of the body and may result in animals with ‘generalised TB’.

In the late stages of eradication, infected animals usually only had head lesions. Animals with generalised TB, ‘spreader’ animals (animals that discharge mycobacteria, usually by the respiratory or salivary routes) and animals with thoracic lesions were rare. This may be because such animals had died by this stage, and testing only detected more recently infected secondary cases.

The detection of macroscopic lesions at necropsy is an important aspect of the diagnosis of bovine TB. A presumptive diagnosis of bovine TB is often made on the basis of gross pathology and examination of smears or histological sections made from lesions. However, a definitive diagnosis can only be made by isolating *M. bovis* from animal specimens.

Lesions in cattle are most frequently seen at necropsy in the retropharyngeal, bronchial, and mediastinal lymph nodes, which may be the only affected tissue. The lung, liver, spleen and the surface of body cavities may also be affected. Lesions in other species can differ from the classical picture seen in cattle. In farmed deer, lymph node lesions may be liquifactive rather than having a caseous appearance. Liquifactive lesions are the most common presentation in possums with bovine TB.

Lesions in cattle may vary in size from 1 mm to more than 10 cm in diameter. There may be single lesions in lymph nodes or a primary complex — that is, lesions in a parenchymatous organ and a lymph node draining the organ. Most lesions appear as firm or hard, white, grey or yellow nodules. The cut surface usually shows a yellowish, caseous centre, which is dry and firm. Calcification is common, particularly in lymph nodes, and on sectioning the lesion, a gritty sensation and grating sound indicate its occurrence. Conglomerate tubercles, formed by the growth and coalescence of one or more adjacent tubercles, may occur over the pleural or peritoneal surfaces. Metastases give rise to myriad tubercles of the same size, usually 2–3 mm in diameter. Old lesions may be encapsulated by connective tissue, heavily calcified and inspissated (very dense).

Collection of specimens

Because *M. bovis* is a known zoonosis, care must be taken to protect the operator performing a necropsy. Specimens must be collected using strict aseptic technique that avoids slicing at the time of collection. If lesions are found, at least three are collected for culture.

M. bovis may also be isolated from a small proportion of no visible lesion (NVL) reactors. Because *M. bovis* numbers are low in infected tissues in which no lesions have developed, it is important to minimise any environmental bacterial contamination that could reduce

the sensitivity of culture techniques by overgrowth. If no obvious lesions are detected in a reactor at the time of necropsy, the following tissues must be collected for culture:

- Left and right medial retropharyngeal lymph nodes
- Left and right tracheobronchial (bronchial) lymph nodes
- Interior and posterior mediastinal lymph nodes.

Left and right, and anterior and posterior, lymph nodes may be collected into the same jar. These tissues are then examined for lesions in a laboratory or in clean surroundings to prevent contamination. Even if no lesions are found, the tissues must be cultured to determine if *M. bovis* is present.

For histopathology, thick (5 mm) sections of lesions are placed in 10% buffered formalin. The section should include both normal tissue and lesion and should not be more than 2 cm² in area. The volume of formalin should be 10 times the volume of the specimen.

For bacteriology, sections of lesions must be collected using aseptic technique. The sections should be placed in sterile, leak proof containers and refrigerated before submission to the laboratory. If the specimen will not reach the laboratory within 24 hours of collection, it should be frozen. Alternatively, it can be placed in either sterile saturated borate solution or coated with sodium tetraborate (borax, Na₂B₄O₇) powder and refrigerated (about 4°C). If specimens will not reach the laboratory within 48 hours, they should preferably be frozen (–10°C or less) and should remain frozen, including during transport, until cultured. All specimens must be packaged and transported according to International Air Transport Association (IATA) regulations for diagnostic specimens.

Further details on sample selection and submission are in Appendix 2.

Laboratory tests

Occupational health and safety

M. bovis is classified as a risk group 2 organism in the current Australian/New Zealand Standard, *Safety in laboratories — microbiological aspects and containment facilities* (AS/NZS 2243.3:2002), and as a designated risk group 3 organism in Europe and the United States. Because of its public health risk, it is recommended that *M. bovis* be treated as Risk group 3 organisms in Australian laboratories, using appropriate precautions to minimise the risk of human infection.

Examination of fresh fixed smears made from lesion material

Detection of acid-fast bacteria resembling *M. bovis* may give an early presumptive diagnosis of bovine TB. Smears are prepared from the caseous material lining the inner wall of the lesion and stained for acid-fast bacteria using the Ziehl–Neelsen stain. Mycobacteria appear as red, medium-length, acid-fast rods, singly or in clumps.

Histopathology

Histopathology, together with clinical signs, gross pathology, and/or tuberculin test results, can be used for a presumptive diagnosis of bovine TB. However, this technique is not specific for *M. bovis*, and diagnosis of bovine TB can only be confirmed by culture.

Sections for histological examination are prepared using routine procedures. Sections are stained for normal tissue elements using haematoxylin and eosin or azure–eosin, and also for acid-fast bacteria using a recommended Ziehl–Neelsen method.

In cattle, *M. bovis* infection evokes a characteristic granulomatous reaction, the tubercle, with the following characteristics:

- A central area of necrosis
- Some degree of calcification that usually occurs in the centre of the caseated area
- A zone of epithelioid cells surrounding the caseated area.
- Usually, Langhan's giant cells on the margin of the zone of epithelioid cells and elsewhere
- A zone of lymphocytes, macrophages and plasma cells towards the periphery of the tubercle
- Encapsulation of the tubercle by fibrous tissue
- *M. bovis* (which appears in Ziehl–Neelsen stained sections as red, medium-length rods, singly or in clumps) in the cytoplasm of the macrophages and giant cells on the periphery of the lesion, and scattered through the necrotic debris in the centre of the lesion; the *M. bovis* cells are usually only present in low numbers in most natural cases of bovine TB.

In other species, the histological picture of bovine TB will differ from that in cattle. Differences may be observed in the extent and type of necrosis, and the presence or absence of mineralisation, Langhan's giant cells, and fibrosis.

Bacteriology

Laboratory procedures for the isolation of M. bovis

Handling and manipulation of infectious material, both tissue specimens and cultures, should be done in a Class I or Class II biosafety cabinet to protect the operator from infection. *M. bovis* infection has been recorded in laboratory workers in Australia (Cousins and Dawson 1999).

Mycobacteria are slow-growing compared with other bacteria. Because samples collected in the field or abattoir may be contaminated with other bacterial or fungal species, it is necessary to treat the samples with a decontaminating agent, such as a weak alkali or acid or a detergent, to destroy any contaminating bacteria. This provides the mycobacteria with an optimum chance to grow without being overgrown by other organisms. The decontamination reagent chosen should have minimal effect on the viability of *M. bovis* in the specimen, while rendering contaminating organisms nonviable.

Very few *M. bovis* organisms may be present in tissue, and the bacteria in small lesions (such as those present in tissues of NVL reactors) may be diluted by the surrounding tissue when processed. The chances of isolating *M. bovis* are improved by increasing the amount of material cultured. This is most easily achieved by increasing the number of slopes used. The decontaminant must be neutralised and the suspension centrifuged to deposit the tissue and any mycobacteria present. The deposit is then inoculated onto a series of media. Australian standards¹⁰ require samples to be inoculated onto at least two slopes of agar-

¹⁰ <http://www.scahls.org.au/asdts/asdt.htm>

based media and two slopes of egg-based solid media. Some laboratories may also use the liquid medium, 12B (available from Difco) designed for the BACTEC system (BD Biosciences); this is the base liquid medium used for Johne's disease culture. The BACTEC liquid culture system may provide a faster result. BACTEC cultures are held for 8 weeks after inoculation before being discarded as negative.

Incubation

The inoculated media are incubated at 37°C in air, with or without the addition of carbon dioxide (maximum 5%), and examined for evidence of growth at weekly intervals. They should be held for a minimum of 10–12 weeks before being discarded as negative. Because *M. bovis* is slow growing, evidence of growth is commonly seen between 3 and 5 weeks after inoculation. In ideal circumstances, colony growth may be detected after 2 weeks' incubation. Suspect colonies are stained and examined using the Ziehl–Neelsen stain.

Identification of *M. bovis* and other mycobacteria

Isolated acid-fast organisms can be identified using a variety of biochemical tests (Vestal 1975). Immunological techniques were used from the mid-1980s (Corner et al 1988), and DNA techniques have been used since the early 1990s. The immunological technique is no longer used in Australia because of difficulties in obtaining monoclonal antibody. DNA techniques are very sensitive and specific and have largely replaced both biochemical and immunological techniques for identification of mycobacteria.

DNA techniques for identification and direct detection

The most commonly used DNA technique for identification and direct detection of mycobacteria is the polymerase chain reaction (PCR). In Australia, a number of PCR tests are accepted for identification of *M. bovis* and for differentiation of *M. tuberculosis* complex from other commonly isolated mycobacteria species, such as *M. avium* and *M. intracellulare* (Cousins et al 1991, 1992, 1996; de los Monteros et al 1998). These techniques can also differentiate *M. paratuberculosis* from *M. tuberculosis*-complex organisms. PCR techniques are also sometimes used for direct detection of *M. bovis* in tissue samples. Although direct detection may offer considerable advantages in terms of speed, PCR applied directly to tissue is not as sensitive as culture, and false negative results are possible.

PCR can provide a result within a day of isolating colonies on culture media or within 2 days using fresh or formalin-fixed tissue. To maximise the chance of success for direct PCR, tissue should remain in fixative for no longer than 24 hours before being embedded in paraffin wax.

DNA fingerprinting techniques to study origin of isolates

DNA fingerprinting techniques can be very useful in determining the origin of infection of *M. bovis* and other *M. tuberculosis*-complex strains. These techniques can be used to study transmission between different species and within and between properties; to provide evidence of residual infection; and to examine population dynamics, evolution and clonal expansion. Differences between strains of *M. bovis* can be detected by a number of techniques, some more effective than others.

Spoligotyping (**spacer oligonucleotide typing**) is a PCR-based method that is very effective for typing *M. tuberculosis* strains and is a rapid and useful screening test to identify major differences between strains of *M. bovis* (Aranaz et al 1996, Kamerbeek et al

1997). The technique can also be used to identify *M. bovis*, which lacks 5 spacers at one end of the standard hybridisation pattern compared with other species from the *M. tuberculosis* complex. Spoligotyping can provide a result within 1–2 days of isolating bacterial colonies or from liquid media with evidence of growth. It can also be attempted directly from DNA extracted from tissues. However, the results using bacterial colonies are more reliable and more likely to be unambiguous. Results of spoligotyping can be compared relatively easily between laboratories, and there is a globally accepted nomenclature for recording spoligotype results. *M. bovis* and *M. tuberculosis* databases are available for recording and comparing results.

Restriction endonuclease analysis (Collins and de Lisle 1985) and restriction fragment length polymorphism (RFLP) analysis (Cousins 1996; Cousins et al 1998a, 1998c) are the best techniques for discriminating between strains of *M. bovis* (and other *M. tuberculosis* organisms) in New Zealand and Australia, respectively. RFLP is a time-consuming and logistically complicated technique and results may take up to 2–3 months. Four different gene probes have been used for RFLP in Australia: the insertion sequences 6110 and 1081, the polymorphic GC-rich sequence, PGRS, and the direct repeat pUCD (a repetitive element isolated at the University College, Dublin). Each of these techniques has advantages and disadvantages for typing *M. bovis* strains; these include differences in turnaround time, cost, level of technical ability required to perform the test, and ease of comparison. PGRS RFLP provides the best differentiation between Australian strains of *M. bovis*, but is also the most difficult to analyse.

Since 2005, the Australian Reference Laboratory for Bovine Tuberculosis (ARLBTB) has been trialling a PCR-based typing technique that identifies a variable number of tandem repeats in various alleles (VNTR typing). This is a relatively rapid technique that takes approximately 2–4 days and is performed on bacterial colonies. It provides a reasonable degree of differentiation between *M. bovis* isolates and, with additional alleles, may approach the sensitivity of PGRS RFLP. It is also very easy to perform comparisons between isolates on a global basis.

A culture collection containing approximately 1900 strains of *Mycobacterium* spp, including many reference strains, is held at ARLBTB. Results of DNA fingerprinting for approximately 600 *M. tuberculosis* complex strains (including *M. bovis* and reference strains of the *M. tuberculosis* complex) are also kept at ARLBTB. DNA fingerprinting results are analysed using a commercially available computer program (GelCompar/Bionumerics — Applied Maths, Belgium).

Differential diagnosis

Other conditions may be confused, both macroscopically and microscopically, with TB. The most important of these are non-*M. bovis* mycobacterial granulomas. A number of mycobacterial species can infect animals and cause lesions often identical to those caused by *M. bovis*. The most common of these are members of the *M. avium* complex, which are found in cattle, farmed deer, pigs and some wildlife species. In farmed deer, *M. paratuberculosis* can cause lesions whose appearance is virtually identical to those caused by *M. bovis*.

In the final stages of a bovine TB eradication program, it is important to use culture techniques to confirm cases that have been diagnosed by histology because a significant proportion of tubercle lesions are likely to be caused by mycobacterial species other than *M. bovis*.

Other conditions that may be confused with bovine TB include granulomas due to: *Rhodococcus equi*, *Nocardia–Streptomyces*, fungi, club-forming organisms, oil, neoplasms, foreign body abscesses, hydatid cysts and other parasites. These may cause lesions that are macroscopically similar to TB, but most are readily distinguishable histologically.

Detailed information on histological differential diagnosis can be found in the Australian and New Zealand Standard Diagnostic Procedures.¹¹

The diagnosing veterinarian may consider a number of tests to rule out other mycobacteria and to rule in *M. bovis*. These are summarised in a cascade of options, as follows:

- histopathology diagnosis
- direct detection of *M. bovis* and other mycobacteria in tissue specimens (application of PCR to tissue sections is under development)
- conventional mycobacterial culture using solid media
- BACTEC liquid radiometric culture for mycobacteria
- identification of mycobacterial isolates using conventional procedures (biochemical tests)
- rapid identification of mycobacteria using
 - multiplex PCR to differentiate *M. tuberculosis* complex, *M. avium*/*M. paratuberculosis* and *M. intracellulare* species from other mycobacteria
 - *M. tuberculosis* complex multiplex PCR to differentiate *M. bovis* from other *M. tuberculosis* complex organisms
- DNA fingerprinting of confirmed *M. tuberculosis* complex isolates using spoligotyping, RFLP and/or VNTR typing
- 16S rRNA sequence identification of *Mycobacterium* spp other than *M. tuberculosis* complex.

In summary, all isolates of *M. tuberculosis* complex identified in Australia should be confirmed using an internationally recognised bovine TB reference laboratory, and their DNA profiles should be established. ARLBTB stores and maintains the Australian National Culture Collection of *M. bovis* isolates that are used in DNA fingerprinting studies, and records isolate types in an electronic database. The laboratory can perform individual fingerprinting tests, and provide a computer-assisted analysis of results to determine matches of any new isolates with previously identified types.

Resistance and immunity

Innate and passive immunity

As a result of natural or innate immunity, many cattle that are exposed to *M. bovis* will not develop disease.

¹¹<http://www.scahls.org.au/asdts/asdt.htm>

Active immunity

Most cattle infected with *M. bovis* produce a cell-mediated immune response and contain the infection within localised foci for long periods (Thorns and Morris 1983). If an antibody response occurs, it is normally associated with the development of more progressive disease. There appears to be a reciprocal relationship between T-cell reactivity and localised infection, on the one hand, and antibody levels and progressive disease, on the other.

Delayed-type hypersensitivity is the basis for the single intradermal caudal fold tuberculin skin test (tuberculin test) in cattle. Gamma delta T-cells, particularly those bearing the WC1 molecule, are present at an early stage of development of a response. However, their role remains unclear (Kennedy et al 2003).

Vaccination

TB vaccines have never been used in cattle in Australia, although BCG has been used to control human TB. Internationally, a number of research groups are working to develop and evaluate vaccines that may be used in cattle and wildlife (e.g. possums and badgers), as well as humans. In most cases, the aim is to develop better alternatives to BCG. Vaccines would only be useful in countries in which wildlife reservoirs are persistently infected with *M. bovis*. Since animals vaccinated with BCG respond to the bovine purified protein derivative (PPD) in the tuberculin test, research is also under way on diagnostic tests that can differentiate vaccinated from infected animals.

Epidemiology

Source of infection and modes of transmission

M. bovis infection is spread to cattle primarily through the inhalation of infectious aerosols, but has also been reported to be spread by ingestion of infectious material from drinking infected milk or ingesting contaminated pasture or feed. Cutaneous, congenital, and genital infections have been recorded but are considered rare. Carrier animals are significant in spreading and perpetuating the infection, but transmission is intermittent and mimics a point source epidemic.

Aerosol transmission occurs in all environments and the infective dose by inhalation can be very low. However, transmission is only effective over short distances, of 1–2 m, and cattle density is therefore a significant factor in the rate of transmission. Infection is spread more rapidly in intensive animal husbandry situations than in extensive or rangeland conditions, such as those existing in many parts of northern Australia. Disease also appears to spread more rapidly where animals are kept in crowded conditions, such as zoos; under such conditions, stress may adversely affect the ability of the animal to withstand infection.

Under Australian conditions, most TB infections are considered to be acquired by inhalation because 70–90% of lesions are found in either the lymph nodes of the head or in the thoracic cavity. Cattle have also been infected with *M. bovis* by ingestion of hay contaminated by humans with urinary TB.

Ingestion of contaminated carcasses or offal was the most common mechanism of infection for feral pigs. The same mechanism, as well as ingestion of infected cow's milk, was incriminated as the source of infection for domestic pigs.

Before the advent of pasteurisation, the most common source of infection for humans (and particularly children) was through ingestion of infected cow's milk. Such cases would often result in infected lymph nodes known as scrofula. Ingestion of unpasteurised infected milk remains a problem in developing countries or in cultures in which milk is not heated before drinking. Even in developed countries, clusters of cases of *M. bovis* TB have been associated with eating under processed or soft cheeses produced in countries with high rates of bovine TB.

Humans can also become infected through inhalation of infectious aerosols and through direct exposure of cuts and abrasions (known as 'butcher's wart').

Laboratory-derived cases of *M. bovis* TB have been recorded, and infection has been reported in patients with human immunodeficiency virus (HIV; Dankner et al 1993). Reports of person-to-person spread of *M. bovis* infection are rare, strengthening the belief that *M. bovis* is not as infective for humans as *M. tuberculosis*.

Incubation period

Bovine TB can progress within weeks or years, depending on the immunity of the host, the size and frequency of the infectious dose and host genetics. In many cases, infection will be localised and cleared by the immune system, such that disease never develops. In humans, only 10% of people infected with *M. tuberculosis* will develop TB disease in their lifetimes.

Persistence of agent

M. bovis is an obligate intracellular parasite and has a limited survival period outside the host (depending on the environmental conditions). It is susceptible to drying and ultraviolet light, but is relatively resistant to detergents and moderate changes in pH. In one study, under the high temperature conditions experienced in northern Australia (average maximum over 20 weeks was 32–43°C), *M. bovis* survived for 4 weeks in soil in $\geq 80\%$ shade, but no isolation was made after 4 weeks from dry or moist soils exposed to sunlight, or from faeces held under any conditions (Duffield and Young 1985).

Epidemiological factors influencing transmission

Consistent with the mostly aerosol spread of *M. bovis*, disease prevalence is higher under intensive farming practices, such as on dairy farms or where animals are housed indoors. In beef herds, prevalence will generally be lower, but high prevalence (around 35%) has been observed where cattle are overstocked and/or in poor condition. In very extensive farming systems, such as in pastoral cattle management, herd prevalence will generally be lower than in intensive systems, but small family groups can have high prevalence. Under pastoral conditions in northern Australia, opportunities for transmission were provided by cattle congregating around waterholes during the dry season, or on reduced amounts of dry land during the wet season.

Typically, the disease does not spread uniformly within a herd. In an extensive management situation, animals tend to stay together in discrete social groups that run in their own area of the property, and TB infection is likely to spread only within the group. This stable situation remains undisturbed until fire, flood, drought or human interference drives animals together or away from their home range. Infection may then spread between social groups. To a large extent, TB eradication involves detecting and removing such infected social groups.

Manner and risk of introduction to Australia

A recrudescence of bovine TB in Australia is possible. Although measures to ensure removal of higher-risk animals and improved targeting of monitoring during the latter phases of eradication were implemented, it is possible that the disease could reappear from a previously undetected infected animal. In Tasmania, which has a relatively isolated cattle population, 17 years elapsed between the last two cases of the disease. In the final stages of eradication in New South Wales and South Australia, two infected herds were recorded 15 and 16 years, respectively, after previous known exposure to the disease.

Humans remain as a source of introduction of *M. bovis* into Australia, but the risk of transmission to cattle is low. Throughout the world, some surprising foci of infection remain (Grange 2001). A study in San Diego in the United States found that one-third of all paediatric TB infections were due to *M. bovis*, and the source was rarely determined (Besser et al 2001). Humans with HIV infection are more susceptible to concomitant TB infection, and travellers with both diseases present a risk for re-entry of *M. bovis* (Pasquali 2004). There is also a risk of introduction in exotic animals (such as South American camelids), zoo animals (Cousins et al 1994), dogs, domestic farm animals (such as deer, sheep and goats) and purebred cattle, even though quarantine measures are well established.

If an outbreak occurs, the national scheme for identification of all sale and slaughter cattle will be of tremendous benefit in tracing potential sources of disease introduction into a herd and all spread from that herd. Meat and Livestock Australia maintains the National Livestock Identification Scheme (NLIS), which is compulsory in all states and territories.¹²

¹² <https://www.nlis.mla.com.au/index.aspx>

3 Detecting and confirming bovine tuberculosis

Identifying tuberculosis at abattoirs

Detection of granulomas at meat inspection is the usual way in which tuberculosis (TB) is found. The National Granuloma Submission Program (NGSP) and a later, more risk-based version of this program (NGSP2) used during the second phase of the Tuberculosis Freedom Assurance Program (TFAP2), aimed to maximise the number of granulomas from cattle detected at post-mortem inspection and submitted for laboratory examination. This was achieved through improved inspector training and awareness, and facilitation of the submission process.

Meat inspection is now based on ‘inspector discretion’, and requires the submission of granulomas where an inspector is unsure of the cause of a lesion or suspects TB.

Upon finding a suspect lesion, the inspector or the officer-in-charge of the abattoir is required to ensure that a record is kept of the tag number(s) of the animal(s) involved and the size, location and description of the lesion(s). This description is submitted with the specimen(s) to an approved laboratory.

Other information that may assist with trace-back includes sex, age, breed, coat colour and any brand, ear tag, tail tag and health certification that accompanied the animal. Identification of other animals in the group is expected, especially if the suspect animal is not fully identified.

Where animals are slaughtered as part of an approved property program (APP), they will be identified with a National Livestock Identification Scheme (NLIS) device. The NLIS will be advised to identify the property identification code (PIC) for an increased level of surveillance.

Further details on the granuloma submission program are available from the Animal Health Australia website.¹³

Other means of detecting tuberculosis

Field testing

During TFAP and TFAP2, abattoir surveillance for TB was supplemented by targeted field testing of herds that were considered to have had insufficient surveillance via meat inspection. The two diagnostic tests used for field testing cattle are the tuberculin test and the interferon gamma test. These are described in detail in Sections 6 and 7. Field testing is unlikely to be a part of TB detection in future.

¹³ [http://www.animalhealthaustralia.com.au/programs/adsp/tfap2\\$/tfap2_home.cfm](http://www.animalhealthaustralia.com.au/programs/adsp/tfap2$/tfap2_home.cfm)

However, export testing may be required by some cattle-importing countries, and reactors to the tuberculin test (almost certainly ‘false positives’) will continue to be found. If such reactors are found to have TB, the procedures in this manual should be adopted for investigation and subsequent action.

Laboratory identification

Laboratories that receive samples that are suspect for TB submitted from a post-mortem examination (and the submitter) are required to advise state and territory authorities of such instances.

Laboratory confirmation of tuberculosis

When TB eradication commenced, the disease was often only diagnosed by detecting typical macroscopic lesions at post-mortem examination. Laboratory confirmation at that time was by histological examination. Positive histopathology requires the identification of characteristic cellular pathology (using haematoxylin and eosin stain) and acid-fast bacilli by the Ziehl–Neelsen stain.¹⁴

In the latter stages of the Brucellosis and Tuberculosis Eradication Campaign (BTEC), it became increasingly important to confirm the cause of lesions found in tuberculin test reactors or in granulomas on abattoir examination.

Bacteriology became an essential tool and was used to confirm the diagnosis of *M. bovis*, to investigate no visible lesion (NVL) reactors and to study the cause of any apparent false positive reactors from field testing. However, culture methods were slow. In the latter stages of eradication, several developments improved the diagnosis and understanding of the epidemiology of the disease. In particular, improved culture methods enabled more rapid and more sensitive results. Several new methods for the rapid identification of *M. bovis* were also developed and introduced for routine use. These included the immunoperoxidase test and polymerase chain reaction (PCR) techniques on tissue and cultures. DNA fingerprinting techniques provide an understanding of the transmission of disease and will have application in tracing outbreaks in Australia.

State and territory laboratories continue to process abattoir samples and some have considerable experience in diagnostic histopathology and culture. Laboratories performing histopathology and culture of suspected TB lesions must be approved as part of the Australian National Quality Assurance Program.¹⁵

The final confirmation of diagnosis of TB is routinely performed at the Australian Reference Laboratory for Bovine Tuberculosis (ARLBTB), which is designated as a centre of excellence for the diagnosis of *M. bovis*. All isolates identified as *M. bovis* in approved laboratories must be submitted to the ARLBTB for:

- confirmation of identification
- long-term storage of isolates in the National Culture Collection

¹⁴ <http://www.scahls.org.au/ASDTs/19-BovineTuberculosis.pdf>

¹⁵ <http://www.anqap.com/>

- DNA fingerprinting.

All Australian laboratories approved to carry out examinations for TB use methods described in the Australian Standard Diagnostic Techniques for Animal Diseases or the more recent Australian and New Zealand Standard Diagnostic Procedures.¹⁶

‘TB incidents’ and ‘TB cases’

This terminology was introduced during TFAP, as protocols for managing herds tightened. The terms ‘TB incidents’ and ‘TB cases’ provide a ready way to clearly define the status of a herd implicated in a detection of TB.

TB incidents

The detection of any evidence of TB is referred to as a ‘TB incident’. The procedures outlined here ensure that all important factors are considered in the investigation that takes place. Such an event could arise when a laboratory that has received abattoir samples is unable to confirm TB by histopathology or culture, but is unable to establish an alternative diagnosis.

The term ‘TB incident herd’ is used to describe a herd in which a suspect lesion has been traced to an animal and the chief veterinary officer (CVO) has approved procedures to assist in excluding TB as a diagnosis and establishing the cause. Such further action would include:

- Provision of part of each suspect sample to the ARLBTB
- An epidemiological investigation and risk analysis of the herd and its neighbours.

If appropriate, increased turn-off of associated cattle and intensive monitoring through abattoirs or field testing may be approved by the CVO (see Section 5). As a guide, surveillance measures should seek to achieve 99% confidence of detecting 1% disease in the TB incident herd if these measures are to be used to indicate TB freedom.

The term ‘TB incident’ is important in event management and public relations. It should be recognised and used consistently given the important implications for Australia’s reputation as a country that has eradicated the disease.

TB cases

A ‘TB case’ refers to an animal from which TB has been confirmed because:

- *M. bovis* has been cultured from a lesion; or
- Despite negative culture, there has been positive histopathology, with a professional decision based on evidence such as gross pathology, laboratory findings and epidemiological investigation.

¹⁶ <http://www.scahls.org.au/asdts/asdt.htm>

Where TB has been unequivocally traced to a herd, the herd is referred to as a 'TB case herd'. If there is some doubt, or there are multiple associated traces, then a herd is termed a 'suspect TB herd' until the disease situation is clarified.

Secondary TB cases may be found by tracing from a TB case herd. Recipient herds must be further investigated, with tracing to determine whether infection has been transferred into such herds. If evidence of spread is found, these herds also become TB case herds.

Details of TB incidents and TB cases must be reported to the Animal Health Australia TB case database.

4 Action on advice of a potential case of bovine tuberculosis

Initial action

When advice is received of a bovine tuberculosis (TB) incident, the local veterinary manager should:

- Promptly contact the owner
- Confirm ownership of the suspect animal, if possible
- Put a hold on any cattle movements other than for slaughter until a property visit is carried out by the local veterinary manager to obtain more details.

A formal movement control or quarantine should be issued until investigations are completed and the nature of the problem is understood.

The state or territory veterinarian responsible for TB (see Section 5) should advise the Animal Health Committee (AHC) through the chief veterinary officer (CVO).

Where there is concern that the event may receive undue attention from the media, the procedures outlined in Section 11 should be followed.

Documents required for initial property visits

The following types of documents may be required during an initial property investigation:

- Quarantine order
- Cattle compensation guidelines
- Claim for compensation
- Order for destruction or removal of diseased cattle
- Permit to move.

Each state and territory will have its own requirements and these should be referred to.

Quarantine/initial movement control

Prompt control over movements of cattle from a potentially infected herd is important to ensure that the infection is limited to that property. It also signals to the owner, the cattle industry and the public the importance placed on continuing control of TB.

The specific protocol for placing a property ‘under quarantine’ depends on the legislation in each state or territory. In essence, quarantine is an official order restricting cattle to a specified location and requiring authorisation for cattle movements to and from that location. It may also impose treatment or control requirements.

Investigation of a tuberculosis suspect herd

The initial investigation of a herd associated with a suspect TB case aims to confirm whether or not TB exists on the property. This initial investigation may take the form of:

- An epidemiological investigation, including collection of a herd TB history
- Testing of the herd or source group
- Destocking the affected group, combined with testing of at-risk cattle
- Destocking defined age groups and testing remaining cattle.

Where there is no further evidence of tuberculosis

If no additional evidence of *M. bovis* is found in the initial investigation, further action depends on the nature of the detection. If the herd of origin is not in doubt, the actions required will be included in the approved property program (APP). If the herd of origin has not been confirmed, herds under investigation may be required to undertake one or more tuberculin tests.

The number of tests required will depend on the nature of the group — that is, whether animals are related to the infected group (that is, have had contact with it) or have been segregated from the infected group for a significant period (at least 60 days). If TB is not confirmed, any further action will be at the discretion of the state or territory CVO.

Where further infection is detected

Where the investigation confirms that further TB is present in the herd, destocking of the source group or herd will be the preferred action. Animals may be retained for testing, subject to approval of the TB Property Program Group and AHC, if:

- There is no evidence of transmission to younger age groups
- The specified groups of animals have been continuously segregated from the infected group
- Destocking of all animals is not economically feasible.

The number of repeat tests required will depend on the circumstances.

Tracing cattle movements

Where TB is confirmed, trace-back and trace-forward to all contact herds is necessary. If tracing to a herd and further investigation reveals that the infected or suspect animal was not born on that property, further tracing is required to determine what other properties are involved. Appropriate action is then taken on herds on these other properties.

To identify the direct contacts of all infected animals, tracing may be required for the lifetime of the infected animal(s). In the absence of knowledge of the source, tracing should go back at least 5 years. All herds that have received animals from a case herd or secondary case herd must be investigated. The action required on such herds may be subsequently modified or limited to the groups containing, or that have contained, the cattle identified as contacts.

5 Property and program management

The following description of procedures, which is an amalgam of procedures provided in the Standard Definitions and Rules (SDRs) and various state and territory instructions, is a basis for developing a suitable program for managing an incident or case of bovine tuberculosis (TB).

In addition to this information, the responsible officers should also be familiar with or consult relevant legislation and any additional local information that explains the special features of TB control in their state or territory.

Approved property programs

The development of a property or herd TB eradication program agreed by the owner and the relevant state or territory department was fundamental to implementing appropriate control procedures in the extensive areas of Australia. An approved property program (APP) is a formal document that details the nature of the property and cattle, the proposed program operations and the responsibilities of the relevant parties in implementing the program.

APPs were used throughout the Brucellosis and Tuberculosis Eradication Campaign (BTEC) and the Tuberculosis Freedom Assurance Programs (TFAPs) to provide a basis for developing nationally consistent programs that would provide a common understanding of the actions proposed and reliably lead to eradication. They also provided a basis for assessing various forms of assistance to producers. Although additional assistance is no longer available, the principles for establishing a formal documented plan remain.

Traditionally, APPs could be of different types depending on the activity to be undertaken. It is now more appropriate to recognise that an APP is an essential prerequisite to any activity on a property related to investigation or eradication of TB.

An APP is a documented agreement between an owner and the state or territory government on the implementation of a program that can be physically completed within two years. It provides details of:

- The nature of the program
- The obligations and responsibilities of each party
- The consequences of noncompliance
- The consequences of detecting further TB as a result of the program
- The requirements for annual review
- The availability and nature of compensation.

An APP may also be a useful management tool for neighbouring herds, herds implicated by trace-back or trace-forward, other herds containing at-risk, in-contact cattle, or any other herd subject to testing.

A model for the process, content and structure for APPs is provided in the following sections. It is also valuable to examine previous TB cases to better understand local requirements and gain an appreciation of the finished document.

Responsibilities of key personnel

The government personnel involved in developing and implementing an APP will differ between jurisdictions. The following provides a well-tested framework that ensures inclusion in the APP of all the detail and process necessary for a successful eradication program.

Chief veterinary officer and Animal Health Committee

The chief veterinary officer (CVO) has primary responsibility for developing and implementing animal health programs in the jurisdiction, including seeking endorsement of APPs through the Animal Health Committee (AHC). AHC is responsible for endorsing and monitoring APPs.

State or territory tuberculosis case manager

A senior veterinarian (preferably with previous experience of TB eradication) should have overall responsibility for managing a TB incident or case on behalf of the state or territory. This person also has ongoing responsibility for ensuring that staff from state or territory agencies:

- Are aware of and are trained to follow the procedures described
- Are able to access instructions that clarify local requirements.

The state or territory TB case manager is also involved in reviewing and approving draft APPs.

Manager of an approved property program

The APP manager will usually be a district veterinarian who will, either personally or via delegated veterinarians and inspectors, ensure that the activity on a property follows the guidance provided in this manual and leads to successful eradication. The APP manager is also responsible for keeping records on actions taken on a property under the APP.

The APP manager normally negotiates the APP with the owner of the cattle. The draft program will be reviewed by the state or territory TB case manager and the CVO. It will then be analysed by a TB Property Program Group set up by AHC to provide expert assistance to design and management of the program.

Supervisors and managers of programs must be knowledgeable about:

- Pastoral cattle mustering, handling and management
- The purpose, nature and content of an APP
- The technical aspects of tuberculin testing
- Post-mortem technique and sample collection
- The documentation associated with testing and management of reactors.

Departmental officers

A departmental officer should be present at all eradication procedures involving extensive herds to:

- Check the identity of cattle
- Check the accuracy of identification of samples
- Supervise any age culling
- Check the effectiveness of mustering
- Check the effectiveness of destocking
- Supervise ear tagging
- Manage stock movements, including maintaining group security and keeping records of all movements
- Report to the CVO on the effectiveness of tuberculin testing and compliance with the APP.

Owners

In addition to requirements imposed on a property owner resulting from the disease control legislation in a state or territory, owners are expected to cooperate with the state or territory government agency to:

- Develop a suitable APP
- Provide facilities and staff to assist
- Present cattle for testing as agreed
- Destock agreed groups of cattle in a timely manner.

Providing advice to property owners and managers

The process of developing a suitable APP with a property owner or manager is complex, with possible ramifications at the state/territory and national levels. To reduce potential problems during this process, an officer with previous TB eradication experience should be involved and, if necessary, specifically engaged to assist in developing the APP.

Owners need to be fully informed of the implications for their operation should TB be confirmed and/or extensive eradication activity become necessary. Information on TB and any instructions should be in writing so that misunderstandings are minimised.

Given the period of time since TB eradication was common, owners and managers may have little understanding of the importance of a case, or the need for prompt eradication and management under an APP.

Any suspicion of TB must be treated as a serious animal health incident. As a minimum, eradication is likely to involve an order to slaughter all in-contact cattle. Officers with responsibility for investigating a TB trace should be aware that the investigation and associated quarantine, testing and destocking will be sensitive issues for owners. Working cooperatively with the owner to develop an agreed program will be more successful than dictating requirements.

The nature of TB is such that it is unwise to make predictions about the outcome of control and eradication procedures. Owners should not be given any advice about expected progress or quarantine release that cannot be reasonably assured.

Once a suitable APP is negotiated, owners must be advised that final approval is subject to approval by the state or territory CVO, scrutiny by the TB Property Program Group and endorsement by AHC.

Preparing for program development

Before officers visit a property to discuss the trace-back with the owner or manager, they should prepare for the visit. This includes internal discussion within the state or territory government agency to familiarise key personnel with the property and any previous TB history and surveillance, and conducting a preliminary costing of the activity and potential financial impacts of an APP.

It is useful for an APP to be prepared in draft form before a face-to-face meeting with an owner so that there is a clear picture of the issues to be discussed and some feel for the impact the program may have on the enterprise.

Before discussing an APP with an owner, the APP manager should brief the TFAP Manager about the intended activity and its estimated costs.

The initial property visit

At the initial property visit, the cattle movements on and off the property for at least the past 2 years are ascertained and a chronological record prepared using the owner's waybill book and/or records of stock sales and purchases. The record must include accurate numbers, age, breed and type of cattle that could have been in contact with the infected animal. The owner must be made aware that a formal movement permit is required before any quarantined stock can move on or off the property.

The owner should be asked about neighbouring properties and herds. A sketch map of the property, including boundaries and neighbouring property positions and owner details, should be drawn up. The owner should be advised that the neighbours will be notified of the nature of the quarantine order placed on the property.

Capacity of property management to implement an approved property program

Experience has shown that the property owner/manager can have a critical effect on the performance of a proposed APP. Key issues that may influence the outcome include the owner's:

- Ability to plan a realistic and detailed management routine that will integrate with the eradication program
- Reliability in implementing routine or extra management activities
- Ability to fund the proposed program
- Willingness to provide competent staff to support the testing program
- Ability to undertake paddock inspections at the required frequency and thoroughness.

Assessing the impacts of an approved property program

An APP could have significant effects on the daily operations of the property, as well as placing a financial impost on the owner. Careful attention to detail and documentation is essential to manage these effects.

In addition to receiving advice and assistance to determine the financial implications of eradication activity on their operation, owners must be well briefed on:

- The availability of cattle compensation
- Other government assistance programs that may apply
- Potential impact on their ability to sell cattle.

Developing a program

An APP should be designed to eradicate the infection within two years of detection. It must consider the total cattle enterprise and identify all key elements that may impact on its success. Before the APP is finalised, it is therefore essential to ensure that sufficient time is available to accumulate relevant information and appropriately assess all factors.

The model provided here for developing suitable APPs is a summary of documents originally prepared by state and territory agencies. It should not constrain officers from providing additional detail where it supports the outcome of eradication.

The property eradication strategy

The development of an appropriate APP depends on a careful analysis of the disease situation, the nature of the property, the cattle herd and its management, the time period during which eradication must be achieved and the resources that can be reasonably applied.

Wherever possible, the person(s) responsible for developing the APP should consult with someone experienced in developing and implementing similar programs.

Overall philosophy

When national eradication commenced in the 1970s, it relied heavily on the tuberculin test (see Section 6); when reactors were identified, they were treated as infected. This was satisfactory while the prevalence of TB was high. However, as prevalence declined in the later stages of eradication, the proportion of false positive reactions predictably increased. This created problems with owners because of a perception that the tests were ineffective.

This led to a change from relying on the tuberculin test to using epidemiological investigations of newly detected or problem herds as a key to successful eradication. Mycobacteriological isolation was used as the definitive diagnosis.

The following summarises the major considerations in developing a suitable strategy for achieving eradication in a timely and cost-effective manner.

Diagnostic testing

Tuberculin testing (see Section 6) was previously the mainstay of detecting and eliminating animals that have TB. In the later stage of formal eradication programs, the use of the interferon gamma (IFN- γ) test (see Section 7) also had some impact. However, drawbacks to testing programs include the relatively poor sensitivity of tuberculin testing (especially in extensive areas) and the need to regularly retest groups of cattle until a series of tests without detecting disease (accepted as a minimum of 4 tests over 3 years) has been completed. The IFN- γ test presents logistical problems because of its requirement for collection of whole blood samples and their delivery to an approved laboratory within 24 hours of collection. All testing programs are resource intensive and demand a high level of control over the cattle involved.

Whole herd test and slaughter was the classic eradication method; it involved testing all animals presented and slaughter of reactors. The technique was enhanced in 1982 to require paddock checks for unmustered cattle and removal or destruction of these animals.

If further TB cases were detected, it would be likely to precipitate further serial, targeted destocking of the herd. Test programs may be of benefit in large herd situations where the level of TB risk is considered acceptable for eradication (that is, around 1 or 2 cases from a herd of 10 000 breeder cows). A typical status progression would be as modified from the TFAP2 SDRs below:

(3.4.2) Testing

- (a) An Infected (IN) Herd requires one Negative Test without evidence of infection not less than 60 days after the previous test, to attain RD status.
- (b) A Restricted (RD) Herd requires one further Negative Test without evidence of infection not less than 6 months after the test by which it attained RD status to attain PC status.
- (c) A Provisionally Clear (PC) Herd requires one further Negative Test without evidence of infection at an interval of not less than 6 months after the test by which it attained PC status to attain CF1 status.
- (d) A Confirmed Free One (CF1) Herd requires one Negative Test not less than 12 months after attaining CF1 status to attain CF2 status.
- (e) A Confirmed Free Two (CF2) Herd requires a review 8 years after the last known Tuberculosis and all Cattle previously exposed to Tuberculosis require one Negative Test. Such a test is a Surveillance Test and the Herd achieves CF3 status. Where exposed cattle remain in the herd, additional surveillance testing may be required as agreed in the APP.

Age culling

Experience has shown that the cumulative risk of TB increases with age. Given the increased risk with age of diseased animals not reacting to the tuberculin test (because of declining immune status with advancing age and decrepitude), testing programs that concentrate on younger animals are very successful. Other epidemiological factors that support this approach include the chronic nature of TB infection and the increased risk of transmission as the period of infection increases.

Age culling involves the forced sale of all cattle over a specified age, commonly 5 years, before tuberculin testing.

The greater the severity of age culling, the more likely it is that diseased animals will be removed from a group. Unfortunately, this also means that fewer productive breeders are left, and this has a greater impact on the financial viability of the property. Thus, age culling should be part of a long-term strategy and only used when the size and nature of the cattle operation require this approach.

Destocking

Destocking or depopulation means the removal of all animals from a property, area or premises to slaughter. This has proved a cost-effective means of eradication, especially in herds with chronic TB or where there are substantial areas containing infected cattle or buffalo. Destocking was also favored on properties with limited resources or expertise to conduct test-and-slaughter programs. Producers often preferred this option, despite the medium-term financial disadvantages, because it ensured eradication and allowed new stock to gain early access to markets.

Factors supporting a decision to destock a herd or group include:

- A previous history of TB
- Concern over the effectiveness of a testing program because of factors such as age or poor nutrition of the cattle
- Management that is incapable of implementing a testing program
- Holding and testing facilities that preclude effective control
- Inadequate stock control, or geographical/environmental conditions preventing adequate musters
- A financial and economic advantage to destocking.

The National Livestock Identification Scheme (NLIS) should be informed when cattle with a TB risk go to slaughter, to facilitate a more intense inspection procedure.

Travelling and communications

Eradication procedures often require long-distance travel, sometimes in difficult terrain and road conditions. Inspectors should ensure that they are equipped to deal safely with these conditions. They should ensure that:

- Vehicles are appropriate, in sound condition and equipped for the task
- Sufficient food and water are carried to deal with a vehicle breakdown or delay
- Accurate directions and/or maps are obtained from the owner or manager
- Advice is received as to whether the meeting is at the homestead or cattle yards
- The owner/manager is advised of the approximate time of arrival
- A satellite phone or equivalent communication capability is carried.

Punctuality and a willingness to assist the process and accommodate changes in routine are essential. Supervisors of programs must maintain effective liaison with the veterinary officer in charge of the APP, the owner/manager or head stockman, and any testing veterinarian.

Managing extensive and difficult-to-muster areas

The variable climatic and environment conditions in central and northern Australia mean that cattle are grazed over very large areas. These areas are often heavily vegetated, flood prone or mountainous.

Actions that assist integrity of cattle groups include fencing and other barriers, individual identification, paddock inspections for stray stock, repeat musters and destruction of unmusterable stock.

Area or premises management

In some parts of Australia, there are neither boundaries nor internal fences. In such areas, the 'premises' or home area concept has been used to segregate cattle for a testing program.

Each premise must include a self-contained group of cattle that is managed separately from other premises. In particular, there must be secure and defined physical boundaries, such as fencing or impassable topography.

Premises should have an individual property identification code (PIC) number. Cattle identification and movement conditions should be similar to those applying to movements between properties.

The criteria for successful premises management can be summarised as follows:

- Each premises must be self-contained.
- Each premises must have secure and readily defined boundaries.
- Each premises must be issued with its own PIC number.
- Movement conditions should apply that are similar to those for movements between properties.

Unmusterable areas

It is important to repeatedly test the same group of cattle. However, many properties have areas that cannot be effectively mustered. Such areas should be secured from any retained cattle and destocked over a period to be determined by the CVO.

Proving areas free of cattle

In all testing and destocking programs, paddocks and premises must be thoroughly inspected for cattle by ground and/or air to ensure that all are mustered, tested or destocked. Cattle that cannot be mustered must be destroyed. In some cases, post-mortem examination of such cattle for TB may be warranted to support the disease status of the whole group.

Information from mustering crews and helicopter pilots is often very useful in gaining an accurate picture of the efficiency of mustering. Any conclusion should be confirmed by the owner/manager.

Before conducting an aerial inspection of a premises or paddock and destroying any unmustered cattle, those responsible must obtain signed permission from the owner/manager. Any unmusterable cattle that are destroyed must be described and recorded so that appropriate compensation can be determined.

Helicopter shooting

Shooting cattle from a helicopter is a specialised task that requires specific training and should only be undertaken by experienced marksmen.

Pilots involved must also be trained so that the flight characteristics provide a suitable platform from which cattle can be humanely and efficiently destroyed.

Further information and detailed advice are contained in Brumm (1992), AUSVETPLAN¹⁷ and animal welfare standards.¹⁸

Judas cow procedure

Radio tracking of ‘Judas cows’ is an effective method of detecting residual feral cattle and buffalo. The technique capitalises on the social nature of cattle and buffalo, which results in their seeking out other animals in an area.

Radio transmitter collars are placed on suitable cattle, usually cows or young bulls, after they are immobilised by an anaesthetic dart fired from a helicopter.

Pre-collared steers can also be released into new areas, where they tend to travel along watercourses and locate local cattle. Stock with radio collars are located monthly by helicopter and the accompanying stock are destroyed. The collared animals are then released to locate further unmusterables.

Further information and detailed advice are contained in Caple (1992) and Carrick (1992).

Test groups

Effective testing programs rely on managing test groups so that all eligible cattle are tested repeatedly. Key factors to consider include:

- Group size — one that can be safely handled and securely held in the available facilities and that does not impair the testing veterinarian’s ability to carry out the test as specified
- Integrity — strict isolation of groups from each other is the most efficient way of reducing the potential for spread within a property
- Identification — to record and monitor the composition of a group and enhance traceability of strays or any animals moved from the property.

Further detail is provided in Section 6.

¹⁷ http://www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm

¹⁸ <http://www.publish.csiro.au/nid/22/sid/11.htm>

Managing cattle movements

The importance of controlling cattle movements associated with a potentially infected property cannot be overemphasised. A government officer, usually a stock inspector, will have local authority to manage all stock movements and will be responsible for maintaining group security and keeping records of all movements, whether controlled or inadvertent.

Controlling necessary movements

Properties affected by TB are placed under a formal notice of quarantine as quickly as practicable. Cattle movements from the property and between groups are only permitted with the approval of the CVO.

Movements to slaughter are routinely permitted, but records of the animals moved must be maintained so that their absence from a test group is recognised.

Cattle straying from an infected property

In addition to managed movements, straying of cattle from potentially infected properties also has significant implications. In particular, straying cattle may cause concern in adjacent properties if they are found mixed with disease-free cattle. Assessing disease status in such circumstances is difficult, given the uncertainties in identifying infected animals and the long incubation period of TB.

Straying animals may prolong testing programs and result in additional costs to the owner. A process for managing them should be written into the APP. It should, as a minimum, require the owner of the stray stock to advise the supervising inspector of dates and numbers of stock involved. It is usually preferable for such animals to be routinely consigned for supervised slaughter. Post-mortem collection of suitable lymph nodes from the straying cattle may be warranted to exclude TB.

Cattle straying onto an infected property

Ingress of cattle from adjacent groups may result in considerable additional work to remove them. Because their status will be uncertain, the situation may best be resolved by consigning them for slaughter.

Collection of lymph nodes and laboratory examination for TB are not necessary.

Contingency plan

APPs should be reviewed in the event of drought, flood, and fire or market depression. Contingency plans developed in these situations should attempt to maintain the integrity of test groups.

Administration of an approved property program

Documentation

Carefully prepared and accurate documentation of an APP is essential, because the information in the APP provides the basis for all parties to have a common understanding of actions, responsibilities and support for the agreed activities. In some jurisdictions, APPs will have a legal basis, requiring careful consideration of their structure, content and ramifications.

APPs should include, as a minimum:

- The start, finish and any interim target dates
- A timeline outlining activity over the full two years of the program
- The number, sex, age and disease status of all cattle on the property
- The ear tag identification system to be used
- The measures to prevent spread to neighbouring groups or herds
- A map of the property, indicating the area occupied by the cattle and surrounding herds
- A description of the holding paddocks and testing yards
- A description of any testing program
- A description of any destocking.

Detailed documentation is expected for the first year of the program, and an outline should be provided for the following year(s).

APPs involve significant financial commitments and implications for both agencies and owners. The written agreement should clearly describe these.

Once the owner or manager and state or territory TB case manager have approved the draft program, two copies should be prepared for signing. The owner/manager retains one copy and the other copy is usually held by the TB case manager. A photocopy is commonly retained for local records.

When an APP is completed, the owner/manager should be notified by letter, which should thank them for their efforts and cooperation and summarise key outcomes of the program, the final status of the herd and any final conditions or requirements.

Approval

Final approval of an APP lies with the state or territory CVO, who is expected to follow the guidance provided in this manual and to obtain the support of the TB Property Program Group (PPG) and AHC.

Endorsement of the APP by the PPG, which includes cattle industry representatives, is central to the success of the APP process. Industry expertise is important for the success of APPs on large properties. Industry is also a key stakeholder and will contribute funding for compensation for slaughtered stock from the Cattle Diseases Compensation Fund on the basis of an APP. Further details on the operation of the PPG are in Appendix 3.

Maintaining records

Keeping records of what occurs on a property during the conduct of an APP is important. These records provide:

- A basis for preparing annual reports
- Information relevant to an epidemiological analysis of the factors that may impact on the efficiency of eradication
- Assurance to all concerned that the disease is being managed as agreed
- A basis for defending the actions taken and the progress achieved, recognising that such action may occur many years after the APP ends.

Responsibility for keeping records is generally assigned to the APP manager (district veterinary officer).

Review

The APP should be reviewed at the end of each cattle season, taking into account any additional information and experience obtained while carrying out disease eradication. Review of an APP at the end of each cattle season provides an opportunity for veterinary officers, stock inspectors and property owners/managers to examine progress and assess the effectiveness of the actions taken to control the spread of TB on the property. This in turn assists all parties to make sound decisions on future program direction and activity.

A review of an APP should be carefully planned to ensure that all key issues are appropriately and efficiently explored. Issues that are routinely examined include:

- Prevailing conditions on the property, including weather and feed availability
- An assessment of any destocking
- An account of progress with testing, including its effectiveness, and an estimate of future testing required
- An assessment of management issues, such as mustering, maintaining test group integrity, provision of resources and general stock management.

The documented review of the program should be discussed with the owner before being forwarded to the CVO. A summary report should be provided to AHC.

6 Tuberculin testing

The *single intradermal caudal fold tuberculin test* (tuberculin test) has been the main technique for the diagnosis of tuberculosis (TB) in cattle. It is a cell-mediated assay in which the immune response (an inflammatory reaction) at the site of inoculation of antigen results in a swelling of variable size after 72–96 hours. The test is official when performed by an Approved Person.

The antigen used is a derivative of the mycobacterial tuberculin protein known as purified protein derivative (PPD) tuberculin (CSL Tuberculin PPD [bovine] 3 mg/mL).

Reactors to the test are removed and a postmortem examination is conducted to look for evidence of infection. Animals may be sensitised to tuberculin for several reasons and thus not all reactors are infected. Conversely, animals that have a negative reaction to the test may be infected. An understanding of how to use the test effectively is therefore important. The reasons for the test not being 100% efficient in detecting tuberculous animals are described in the following sections.

Characteristics of successful test programs

Because of the limitations of the tuberculin test and especially its poor sensitivity (about 70%), tight quality control is necessary to achieve consistent results. The major quality controls include:

- restricting use of the test to younger animals, preferably less than 5 years of age
- maintaining stock under test in good condition
- reducing stress during mustering, yarding and testing
- repeating the test at least twice per year until no evidence of disease is found
- achieving consistency in groups presented for testing by
 - maintaining strict group segregation
 - Identifying and recording the animals in each group at both test and reading.

In many situations, meeting all of these conditions is difficult. It is essential that such situations are recognised because of the impact they may have on achieving eradication within a reasonable timeframe, effective management of testing programs, and advice provided to owners and managers.

All cattle over 6 months of age should be included in a testing program. Testing of animals younger than 6 months may be authorised by the chief veterinary officer (CVO).

Test groups

Size

Test groups should be restricted to a size that can be safely handled and securely held in the available facilities and that does not impair the testing veterinarian's ability to carry out

the test as specified. The size of groups that can be successfully handled also depends on whether ear tagging is to be carried out at a test and the number of people available to tag and record/read ear tags.

Management factors (such as paddock and yard size, available labour, age of stock) may have a major influence on group size. Officers should ensure that owners do not have unreasonable expectations of the number of cattle that can be effectively tested in a day.

Maintaining integrity of test groups

Because of the limitations of the tuberculin test, repeated testing of groups is required to build confidence that TB is not present. Strict isolation of groups from each other also provides the most efficient way of reducing the potential for spread of infection from one group to another within a property.

Separation of potentially exposed cattle from other groups must be maintained until eradication is achieved or the cattle are disposed of. It is important that property managers are aware of the importance of this and understand the implications for management of the property.

Most commonly, fencing is used to provide the required degree of separation, but natural barriers may also be used if they will allow separation to be maintained throughout the approved property program (APP).

Knowledge of the disease status of cattle in the surrounding areas is important in maintaining test group security and must be assessed. This would usually be achieved by a tuberculin test of such cattle.

In all cases, unidentified or stray cattle must be assessed to ensure that their role or potential role in managing a case is understood.

Identifying cattle under test

All cattle tested as part of an APP must be individually identified. Accurate identification also provides enhanced traceability of any animals that are moved from the property or that stray to another group.

The method of identification will be specified in the APP and approved by the CVO. It will be compliant with the National Livestock Identification Scheme (NLIS), and the NLIS database will be informed of the herd identity for future tracing and surveillance. Identification by numbered ear tags (radio frequency identification) is the preferred method for recording and monitoring the composition of a test group.

Understanding the tuberculin test

Despite widespread use and examination of many variables involved in its use, the tuberculin test has a number of well-documented problems, including a large reduction in sensitivity under some circumstances. Various studies report the sensitivity of the tuberculin test as ranging from 48% to 95%, depending on the physiological state of infected animals, the selection process for test animals, and operator variation. In northern

Australia, local consensus during the 1980s and 1990s (the peak of test program activity) was that the test had a sensitivity of about 70%.

Although the specificity can be apparently high in some herds, false positive reactions from antigenic cross reactivity with atypical mycobacteria may be significant. The reasons for variation in test performance are not understood.

Animals that react but show no lesions

These were commonly referred to as no visible lesion (NVL) reactors. In the latter stages of the TB eradication programs, when the protocol for examining lymph nodes was changed to eliminate slicing that can contaminate specimens, they were referred to as no palpable lesion (NPL) reactors.

Two major reasons for the occurrence of NVL reactors are recognised:

- Nonspecific reactions to other microorganisms can occur — in particular, to other mycobacteria that the animal may have come into contact with. While sensitising the animal to tuberculin, they rarely result in observable lesions.
- Recently infected cattle may not yet have developed lesions.

Non-reacting infected animals

There are many reasons that infected animals may be anergic or non-reacting to tuberculin, including the following:

- Injection of tuberculin decreases the antigenic response to subsequent testing for a period of 60 days (desensitisation), and cattle cannot be reliably retested during this period.
- The presence of other diseases may affect the animal's immune system.
- Cattle with 'generalised TB' (the infection has spread widely within the body) may not react, usually because of the impact of the disease on the animal's immune system.
- Incorrect injection of tuberculin can result in subcutaneous deposition.
- Recently infected animals may not react.
- Malnutrition can reduce the effectiveness of the animal's immune system. This may be significant in central and northern Australia where seasonal conditions may leave cattle in poor condition.
- Other stress factors, including pregnancy, recent calving, mustering, transport and age, may lead to non-reaction.

Repeat testing

The true sensitivity of the tuberculin test is not known. The accepted sensitivity of about 70% in northern Australia may be further reduced by factors such as herd nutritional and lactation status, tractability of the cattle, local weather effects and operator effects. This means that, on average, the test will only identify 70% or fewer of infected animals. Thus, if used as a single test, it would be unreliable and eradication based on testing would be unlikely.

Repeat testing of stable groups of animals, with removal of any reactors, is essential to improve the performance of the test. The aim should be to test all cattle in a group at least twice per year. More frequent testing can be carried out if environmental conditions are conducive, but there are practical limitations to such programs on most northern properties.

Testing once per year rather than twice per year may be approved by a CVO if there are special features of the APP, the behaviour of the disease or other epidemiological features. However, it must be accepted that progress towards eradication will be significantly reduced with this frequency of testing, making it impossible for a program operating on this basis to be completed within 2 years.

Because the animal becomes desensitised to tuberculin, a minimum of 60 days must elapse before the test can be repeated. During the Brucellosis and Tuberculosis Eradication Campaign (BTEC) and the Tuberculosis Freedom Assurance Programs (TFAPs), a formal program of tests and herd statuses was used to ensure that all herds subjected to testing programs fulfilled minimum requirements for the number of tests without the detection of TB. These requirements recognised the need to progressively increase the period between tests (from 6 months to 12 months) once several 'clean' tests had been achieved. The increased period between tests provided an opportunity for animals in the early stages of infection to progress to a sensitive state and for any undetected anergic animals to regain sensitivity.

Storage and care of tuberculin

Tuberculin must be treated with care to ensure that no loss in potency occurs as a result of environmental conditions. It should be protected from light and stored at 2–8°C until used. A portable refrigerator or icebox containing sufficient frozen blocks to maintain this temperature is required. Tuberculin must not be frozen because this will seriously reduce its potency.

Tuberculin must only be used if it is within the expiry period printed on the bottle.

Used and partly used containers must be disposed of at the completion of a test.

Equipment

A single or multidose syringe that can accurately dispense 0.1 mL of tuberculin is required. The preferred syringe for accuracy and speed is the McIntock pre-set syringe.¹⁹ Needles should be 22 gauge or finer, with the unsheathed portion of the needle no less than 2 mm and no more than 5 mm in length. Spare needles are essential.

Equipment should be checked at least a week before a test. Syringe washers dry out over time and it may be necessary to dismantle the syringe and soak washers in water for a day or two.

Syringes must be kept free of dirt and faecal contamination by routinely washing with water and must be cleaned before refilling.

¹⁹ <http://www.bkmcintock.com/index.htm>

Checking the efficiency of mustering and yard work

Departmental officers attending eradication procedures are expected to supervise all aspects of a tuberculin test on behalf of the local veterinary officer in charge.

Attending officers must count and identify the cattle when injected and then supervise the reading of the test to ensure that the same cattle tested are present at reading and that no stranger cattle have intruded. To this end, cattle being tested can be paint branded at testing, with a symbol and colour that distinguishes them from other recently tested cattle in other mobs or premises.

The details of animals tested are recorded on a 'TB test report sheet'. Details recorded should include ear tag and button colours, the serial numbers of ear tags applied to any untagged eligible cattle, and the destination of any subgroups formed following the test.

Observations of the mustering process, such as the number of station staff in attendance and the number and type of aircraft and vehicles used, should also be recorded. Additional information that may be relevant to the effectiveness of the test includes the number of station staff in attendance at the yard, the method of holding the cattle and the amount and type of feed provided for the cattle between the needle and the read. Such records are submitted to the veterinary officer in charge and should be kept on the station file.

Whether any cattle remain unmustered in the paddock or premises must be determined. The objective is to obtain a 100% muster.

Testing facilities

For the safety and comfort of personnel, animals must be appropriately restrained during the tuberculin test. Without appropriate facilities, operator error may impact on the effectiveness of the test.

The crush should be narrow enough to restrain the majority of cattle tested. It should be of a height that obviates the need to climb over the crush or reach through it, since both actions are potentially dangerous. Crushes should be loaded with sufficient cattle to inhibit their forward and backward movement

Conduct of the test

The same caudal fold must be used for all animals in a test group.

The fold should be examined for any abnormality. If any abnormality is detected, this should be recorded as it may disguise a reaction. If necessary, the opposite fold can be used and the animal specially identified to ensure that the same fold is examined at reading.

Injecting tuberculin

The injection site is cleaned if obscured by mud or faeces. The test is performed using two hands and visual appraisal of the injection site. The fold to be injected is located and immobilised at the level of the third or fourth coccygeal vertebrae using the fingers, or

fingers and thumb of the hand not holding the syringe. The forefinger of the controlling hand can be used to turn the caudal fold out to expose the bare portion.

An intradermal injection is made by inserting the needle at 45° to the skin surface.

Intradermal deposition of tuberculin is indicated by the immediate formation of a small palpable bleb at the site of injection.

Reading and interpretation

The test is read by lifting the tail with one hand so that the injection site is visible, cleaning the area if it is obscured by mud or faeces and palpating the injected caudal fold with the free hand.

Palpation is done by running the entire length of the injected caudal fold between the finger and thumb. The operator must also visibly check for a blanched area surrounding the injection site, which can indicate hypersensitivity.

A positive reaction is any swelling other than a small hard scab caused by the needle wound.

A negative test is no perceptible swelling at the site of injection.

The caudal folds may be compared to help determine if there is any swelling, thickening or oedema at the injected site.

If there is any doubt, it is best to identify the animal as a reactor for a detailed, standard, post-mortem inspection and collection of essential lymph nodes for laboratory examination and culture.

The role of veterinary practitioners

Veterinary practitioners were used extensively during BTEC to perform tuberculin testing. While it is unlikely that they will be required to assist in future, the following general conditions are provided as a guide should this be necessary. Local requirements and specific protocols will be determined by the respective state or territory.

A veterinary practitioner would normally be subject to a contract that will specify the following requirements:

- To give 72 hours notice to an inspector before performing a test
- Not to test any cattle in which the veterinary practitioner has any financial interest except with the approval of the state or territory CVO
- To arrange with the owner or manager of the stock to carry out a test
- To store and carry the tuberculin under specified conditions
- To perform the test correctly
- To conduct testing and reading only in daylight
- Only to test groups of cattle that are of such a size they can be tested comfortably in one day, allowing for fatigue and the nature of the facilities

- To maintain and keep in good working order all necessary equipment
- To keep on hand adequate supplies of tuberculin, and containers and preservatives for the collection of samples
- To personally perform all aspects of the test.

7 The interferon gamma test

The interferon gamma (IFN- γ) test is an additional tool for elimination of tuberculosis (TB) in cattle. It may help to minimise destocking or add confidence in the effectiveness of a testing program as an alternative to destocking. When used in combination with the tuberculin test, the increased sensitivity of the IFN- γ test may enable the number of repeat tests to be reduced.

The test was introduced in the closing stages of the Brucellosis and Tuberculosis Eradication Program (BTEC) as a diagnostic assay. It is based on the in vitro measurement of cell-mediated immunity.

The sensitivity of the IFN- γ assay (93.6%; Rothel et al 1990) is significantly greater than that of the tuberculin test (70%; Cousins et al 1998b). The use of the two tests in parallel has given an overall sensitivity of 95.2%, with a specificity of 96.3%. Although the IFN- γ assay detects many infected cattle missed by the tuberculin test, some infected animals may test positive using the tuberculin test and negative using the IFN- γ test.

How the interferon gamma test works

Tuberculin is processed by antigen-presenting cells and recognised by sensitised T-cells in infected animals. The IFN- γ test duplicates in a test tube the cell-mediated immune response to tuberculin. It measures the release of IFN- γ by white blood cells in a simple whole blood culture.

The assay is available commercially (Bovigram, CSL Limited, Melbourne). It requires overnight culture of whole blood with phosphate-buffered saline (nil-antigen control), bovine purified protein derivative (PPD) tuberculin or avian PPD tuberculin. If white blood cells have been sensitised by previous exposure to TB, IFN- γ will be released. After 16 to 24 hours' incubation, the plasma is separated by centrifugation and assayed for IFN- γ in an enzyme immunoassay using monoclonal antibodies raised against recombinant bovine IFN- γ .

Procedure

A blood sample is collected into a vacuum tube (vacutainer) that contains heparin to prevent clotting. Within 24 hours (necessary for viable white blood cells), the sample is divided into three parts as described above.

Interpretation

M. bovis infection is indicated when bovine PPD stimulates more IFN- γ than does avian PPD or the nil-antigen control. A false positive reaction is indicated when the avian PPD stimulates a higher level of IFN- γ than bovine PPD and nil antigen.

Avian tuberculin is used because *M. avium* and related organisms are a common cause of nonspecific reactions. Only those samples where a higher reading is obtained in the sample incubated with bovine PPD tuberculin are considered positive.

Facilities

Facilities adequate to allow safe collection of blood using vacutainers must be available.

Application in Australia

The assay can be completed within 24 hours but is limited to testing up to 400 cattle per day, even with experienced staff in the field and laboratory. It must not be used until 60 days after the last intradermal tuberculin test, to avoid possible desensitisation by antigen overload (Radunz and Lepper 1985).

The logistical difficulties involved in testing cattle in remote areas meant that IFN- γ assay was not used routinely in eradication programs. Where it was used, it was used in combination with a tuberculin test to help identify residual infection in problem herds.

If use of this assay is being considered, there are several practical matters to take into account:

- The test requires the collection of heparinised blood samples and thus use of vacutainers. This may be slow if operators are inexperienced.
- Laboratory processing must be carried out on the day of collection (preferably within 6–8 hours).
- The laboratory procedure is labour intensive and requires specialised training.
- About 400 samples per day are the most that can usually be processed at a time. This leads to logistical problems if testing of large groups is necessary.
- The test costs about \$15 per animal (in 2005), making routine use potentially expensive compared with the benefits of the test.

A benefit of the assay is that the cut-off value used can be varied to suit the requirements of a testing program. In an eradication situation, the most stringent cut-off would be used to maximise sensitivity.

The high sensitivity of the IFN- γ assay means that it is ideally suited to situations in which the prevalence of TB is high or the disease is spreading rapidly and it is desirable to detect as many diseased animals as possible. If the IFN- γ and tuberculin tests are conducted in parallel, overall test sensitivity can be enhanced.

The IFN- γ test has some advantages over the tuberculin test:

- It tests for both bovine and avian tuberculin sensitivity at the same time.
- It is a laboratory assay and does not require a second visit to obtain a result.
- There is no post-injection period during which the condition of the animals (e.g. stress or nutrition deprivation) may interfere with the response.

Although the unit cost of testing an animal will be higher than for the tuberculin test, the assay's higher sensitivity means that fewer rounds of testing would be required to clean up a herd and thus the total cost of eradication from a herd may be lower.

8 Handling of test-positive cattle

Reactors and any cattle deemed suspect on clinical grounds, must be identified and placed in isolation from all other cattle pending their disposal (by either on-property slaughter or consignment to an abattoir) OR retested, this time with a comparative cervical skin test (CCT) as described in Appendix 5. Reactors to the second test must be destroyed of by either on-property slaughter or consignment to an abattoir.

The CCT has a lower sensitivity than the single intradermal caudal fold test however retesting with the CCT increases the overall specificity of testing. The test should only be used if there is a suspicion that a tuberculin test reaction is due to previous exposure to *Mycobacterium avium* complex or if destruction is not acceptable because the animal has a special value.

Identifying and recording test reactors

An inspector supervises the identification, description, holding and destruction of any reactors found by a testing veterinarian. The decision by the testing veterinarian that an animal is a reactor is final and must be unequivocally supported.

Inspectors will be aware that the nature of the tuberculin test is such that some non-infected cattle will react. Experience in pastoral areas indicated that one reactor can be expected for every 400–500 head tested.

Owners and/or managers must be made aware that any cattle with a reaction must be destroyed and samples collected for laboratory examination or retested, not before 60 days, with the CCT.

Unless a retest will be done, reactor cattle must be disposed of within 21 days of a test, either by consignment to an abattoir for immediate slaughter or by slaughter on the property. Slaughter on the property is common, especially when there are only small numbers. The other major factor that will assist in making a decision on disposal is the ability to collect sterile samples during a post-mortem in the prevailing local environment and weather.

On-property slaughter and examination

If an officer is asked to humanely destroy reactors, he/she must use an approved method and take appropriate safety precautions. The use of high-powered firearms should be avoided in yards because of concern for the safety of others and the potential for destruction of lymph nodes required as samples. Officers should ensure that a suitable rifle is available. Appendix 2 describes the sample selection and submission requirements for laboratory diagnosis of bovine TB.

The area where a post-mortem is conducted must be protected from contamination, especially wind and dust, as these may render samples unfit for laboratory culture.

The attending departmental officer is expected to:

- Assist the testing veterinarian to conduct a post-mortem on any reactors
- Supervise the collection and packaging of samples
- Transport the samples and ensure that they reach the laboratory in good condition.

Where a post-mortem is carried out on the property of origin, the carcass must be disposed of in a manner approved by the state or territory chief veterinary officer (CVO).

Abattoir procedure

If there are several reactors and their meat value is such that it justifies their transport to an approved abattoir, each is identified by placing a reactor ear tag in the left ear. The veterinarian or departmental officer records the numbers of all reactor ear tags on the appropriate form and witnesses the removal of the animals from the herd and their isolation in a secure area before transport. Recording and reporting of National Livestock Identification Scheme (NLIS) data are also essential.

Reactors are transported under a permit issued by an inspector.

The officer-in-charge of inspection at the abattoir must be advised before the dispatch of the reactors. Following meat inspection, he/she will report to the state or territory CVO on the:

- Identification of all reactors examined
- Description of any suspect lesions
- Specimens submitted for laboratory examination.

As set out in AQIS Export Control Orders, lesions are divided into two equally representative portions and submitted — one refrigerated in a sterile leak proof container, and the other in 10% buffered formalin — within 48 hours of collection. If a lesion is too small to be divided, a fresh refrigerated sample for bacteriology must be submitted as soon as possible.

Retesting with a comparative cervical skin test

Appendix 5 describes the technique and the interpretation of the CCT. An animal with a positive, or a suspected positive CCT result, must be destroyed and sampled as described in Appendix 2.

9 Post-mortem examination for TB

The postmortem examination of cattle is a critical part of diagnosing bovine tuberculosis (TB).

Stringent procedures have been developed for collecting samples from reactor animals, especially where lesions are not evident on post-mortem. This includes use of aseptic techniques for collecting samples in the field and the use of sterilised instruments and containers for specimens to maximise the likelihood of identifying *M. bovis*.

Any deterioration in conditions (such as rain or excessive wind and dust) requires increased care and attention to sterility. This may mean a more frequent change of instruments or postponing the post-mortem until environmental conditions improve.

Instruments and other materials

The following are widely accepted as necessary for conducting a field post-mortem for TB:

- skinning knife
- boning knife
- rib cutters; long-handled pruning shears are ideal
- toothed forceps
- curved scissors
- straight scissors
- scalpel handle and blades; tin with lid for used blades
- ample supply of clean drapes
- paper towels
- buckets
- scrub-brush (nail/kitchen)
- clean, and preferably boiled, water
- quality disinfectant (Savlon or Inhibac)
- 10% buffered formal saline (10% formalin in buffered saline) and 100 mL (minimum) leak proof containers
- plastic bags
- insulated carriers and ice or freezer blocks
- gloves — post-mortem gloves must be used to protect both operator and samples.

A good supply of sets of sterilised instruments is essential. Operators must be prepared to clean and sterilise instruments during or between post-mortems if necessary.

Preparation of instruments

Following a post-mortem, all instruments should be prepared as follows for subsequent use:

- Scrub until free of blood and tissue using Savlon or Inhibac (preferably warm) and then fully immerse in fresh disinfectant for at least 15 minutes.
- Rinse each instrument with lots of clean water to remove the disinfectant. Bore or tank water used for rinsing should be boiled.
- Dry all instruments.
- Wrap knives separately in paper towels or drapes.
- Prepare and roll up in clean drapes at least six sets of instruments (1 × forceps, 1 × curved scissors and 1 × straight scissors) for collection of lymph nodes.

Conduct of the post-mortem examination for tuberculosis

- With the skinning knife, retract the skin from the underlying tissues extending from the point of the mandible down the neck and over the thorax and abdomen.
- Make two deep longitudinal incisions over the ribs, one just above the brisket and the second next to the backbone.
- Cut the ribs along both incisions and remove the freed rib cage.
- Free the tongue, larynx and pharynx from the hyoid bones by cutting through the cartilaginous joint, and separate from other tissues for about 15 cm down the neck. Take care to avoid cutting the oesophagus as the contents may contaminate lymph nodes.
- Discard the skinning knife and immerse it in disinfectant solution.
- Using the boning knife, trim through tissues to locate retropharyngeal, bronchial and mediastinal lymph nodes. Again, take care not to cut or remove lymph nodes with the knife or touch them with hands.
- Discard the boning knife and immerse it in disinfectant solution.
- Wash gloved hands in diluted disinfectant and rinse with clean water.
- Remove lymph nodes, as described in Appendix 2.

Carcase disposal

Following a field post-mortem, the carcase(s) must be disposed of in such a way that the area is left free of contamination and access to feral or native animals and birds is restricted. Deep burial or burning is best but if this is not practicable then the area should be fenced off to prevent access by cattle, feral or native animals and birds.

Decontamination

Equipment, personal clothing and footwear should be decontaminated in a visible and effective manner so that there is no opportunity for contaminated material to be transferred to another person or property.

10 Destocking

Destocking is expected to take one of two forms:

- group destocking, to remove a discrete high-risk group of animals from a herd
- age destocking, to provide a suitable group for tuberculin testing.

Group destocking involves strategic removal of cattle from specific areas because of high disease risk or difficulties in achieving full musters. This is likely to be the favoured option because experience shows that such a strategy is likely to return the property to normal operations in the shortest period of time.

Age destocking creates young age groups for tuberculin testing and may have use for in-contact groups where the likelihood of infection is low. Repeat tests will still be required to provide confidence that bovine tuberculosis (TB) is not present.

Despite these potential applications, destocking can be very expensive, causing loss of production and cash flow. Consequently, the implications for the business must be carefully considered. Owners must be advised to consult their financial advisers and to take advantage of any economic advisory service offered. Compensation payments may also be very high.

In any situation, destocking should be carried out as expeditiously as possible. It must be completed within one season to avoid an increase in the number of animals to be removed as a result of cows calving or ingress of cattle from surrounding areas. Early completion will increase the efficiency and cost effectiveness of the task and provide the best option for the owner in terms of early access to destocked areas and restocking.

Legal basis for destocking

Each state and territory will have its own requirements for enforcing destocking for disease control purposes. This will involve some form of legal order requiring an owner to take actions to achieve the agreed outcomes. Such orders will usually be issued by the Chief Inspector of Stock because of the serious implications of disease. They can usually be served on either the owner or occupier of the land or the owner of the cattle, whichever is appropriate.

In practice, such orders are implemented through negotiations on destocking as a part of an approved property program (APP).

Details that may be included in an order include:

- the area of land that the order applies to
- the number and description of the cattle
- the destination abattoir or other point of delivery
- the number and class of cattle that may be destroyed as unmusterable if necessary
- the process (including supervision) for destruction of unmusterables
- limits on compensation that will be paid

- the period of time that the area of land must remain vacant before restocking
- the completion date.

Once issued, orders can be cancelled, suspended or amended according to circumstances.

Destocking precautions

Destocked areas must remain free of cattle for a minimum of 60 days to provide confidence that no viable *M. bovis* remains. This implies secure boundary fencing or other effective barrier to control cattle movements from adjoining areas.

All cattle turned off under an order are consigned for immediate slaughter or as otherwise approved by the chief veterinary officer (CVO).

Care is needed to minimise the potential for cattle to escape during yarding, transport and unloading. If cattle escape, the owner, agent or carrier must promptly notify the nearest inspector so that immediate action can be taken to minimise mixing with other stock.

An aerial and/or ground survey of destocked areas will be required 30 days after the removal of the last animal. It may also be necessary to perform one or more checks within this period if there are concerns about unmusterable cattle or strays. If these are found in the destocked area, they must be removed for slaughter or destroyed on site. If slaughter on the property is necessary, then post-mortem examination for TB may be required.

Supervision

Supervising inspectors or veterinary officers must supervise the whole process of removal of cattle to an approved abattoir for slaughter. This will require considerable liaison with the owner, agents, trucking contractors, yard operators, meatworks management and meat inspectors.

The supervising officer must understand the implications of quarantine and ensure that records are kept of all aspects of destocking. Records should be sufficiently detailed to provide accountability for adherence to agreed timelines, compensation and movements, so that groups and classes of cattle can be traced from the property of origin through to abattoir slaughter. For example, cattle may need to be staged via a dip or other yard on one truck and leave on another, and this may necessitate keeping various groups/truckloads of destock cattle separate from one another in dip-yards or other staging yards.

The supervising officer must also ensure that the welfare of the cattle is appropriately managed throughout the process of destocking.

Attendance at drafting and trucking

Punctuality and a willingness to accommodate the process of drafting and trucking of destocking cattle and likely changes to routine are essential. Owners, property managers and supervising inspectors must accept that problems will occur and must have a means of effective communication with the veterinary officer in charge and others involved in the destocking.

Agents will usually conduct the actual drafting, and inspectors should record the number of each class of cattle and provide assistance as appropriate.

There may be situations in which the inspector will need to decide whether any cattle are untruckable. He/she must be prepared, with the agreement of the owner, to humanely destroy such animals. The description and identification of the animals must be noted so that the owner can be compensated. The owner is responsible for disposal of the carcasses.

Cattle ordered to slaughter are identified by special tail tags and usually a yellow or light blue paint stripe applied to the midline of their backs.

Travel must be accompanied by a permit authorising movement of the animals from property to abattoir. This must include accurate numbers, identification (brands/earmarks) and descriptions. It is also useful for the permit to have an attached diagram denoting the decking configuration of the truck and the number and class of stock as loaded. Figure 3 shows a stylised diagram of trucks, indicating prime mover and specific deck layout.

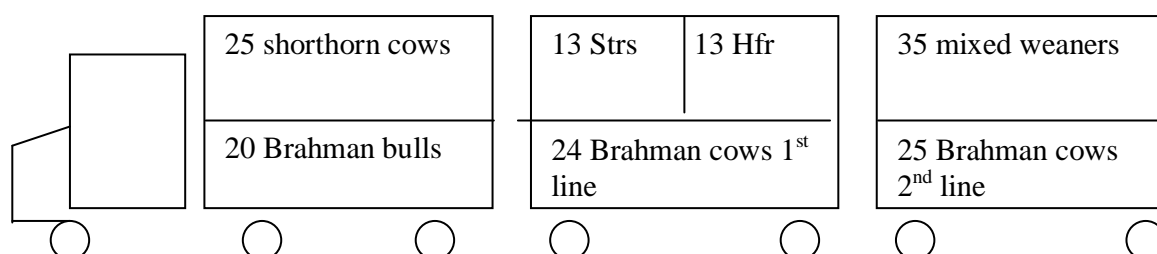


Figure 3 Sample diagram of decking configuration for truck used in destocking

Interstate movements

Prior approval is required for destock cattle to travel to an approved abattoir in another state or the Northern Territory. This usually involves the veterinary officer in charge advising the inspector or veterinary officer in the district of destination of the details of each consignment before they are dispatched. The supervising officer should carry appropriate telephone contact numbers for the officers in the district of destination.

Managing long distance movements and spelling

Where destock cattle are consigned to abattoirs distant from the property, they may need to be rested, dipped and prepared for movement at facilities other than those at the property of origin.

Offloading at dip yards and other suitable facilities must be supervised by the nearest inspector or veterinary officer, who must be forewarned of the movement by the veterinary officer in charge. The supervising officer must ensure that a record is kept of the number and type of cattle and any deaths that occur during the journey or following unloading. The cause of death should always be investigated, and a post-mortem examination may be warranted to exclude the presence of TB.

The owner or manager of dip yards/spelling facilities must also be forewarned of any proposed movement of destock cattle, and must agree that the destock cattle will be able to be held in isolation, watered and fed. The facilities and availability of feed should be checked by the inspector to ensure that they will satisfy all requirements.

The supervising officer should forewarn the yard owner or manager that the destock cattle may need to be held under quarantine conditions for longer than expected if the condition/health of the cattle or prevailing weather conditions deteriorate.

The owner or manager of the dip yard must also be advised of any decontamination measures required (e.g. cleaning troughs and removing remnant feed) before healthy cattle can use the yards that held the destock cattle.

Managing truck rollovers and escape of destock cattle

Destock cattle that escape from a truck rollover must be recaptured or destroyed as quickly as possible. To facilitate this, truck drivers must be made aware of the nature of the cattle being trucked and must be given phone numbers for the supervising officer and the veterinary officer in charge.

On being advised of any rollover, the veterinary officer in charge will liaise with the police and available inspectors to ensure that all cattle are accounted for by yarding or destruction.

The implications of the escape on a property will depend on the individual circumstances. Prompt removal of the animals will obviate the need for any further action on the cattle they may mix with.

Abattoir issues

The veterinary officer in charge or supervising inspector will maintain daily contact with the abattoir to check on the safe arrival of all destock cattle.

He/she must also ensure that abattoir management and meat inspectors are aware of the destock cattle and the need for careful inspection. Any granulomas detected, along with the description of the source animals and any identifying marks or tags, should be dispatched to the appropriate laboratory within 24 hours of collection.

The fate of carcasses with TB suspect lesions will depend on the extent and location of the lesions, according to AQIS Export Control Orders. Carcasses with no lesions detected are processed normally. Export and domestic abattoirs have a legal obligation to keep records of carcasses from destock animals and carcasses that are condemned.

11 Media response in the event of a new case

A detection of bovine tuberculosis (TB) may elicit media attention due to its unusual and potentially zoonotic nature. TB will be viewed as unusual or as an 'exotic disease' and reports of an 'outbreak' could have a negative impact on cattle and beef sales and export markets. It is thus important for accurate, factual information to be used consistently throughout Australia.

The strategy described is intended primarily for use in situations in which media attention on a TB case has potential to affect the national interest.

Spokespeople must be credible and respected by the media and public. In most cases, the relevant chief veterinary officer (CVO) will handle the media because he/she will be in the best position to provide the community with clear and accurate information. The information in Appendix 4 provides a basis for this role.

As TB is a zoonosis, enquiries about the implications for human health should be referred to a health spokesperson.

The key messages are:

- Australia has remained vigilant for TB in cattle since the last case in 2000.
- This case was detected because of the close and continuous monitoring of the health of Australian cattle.
- When a case of bovine TB is found, the following steps are taken:
 - The property that the cattle came from is quarantined.
 - All in-contact and suspect cattle are slaughtered.
 - The surrounding areas and properties are tested and monitored.
 - A careful watch over the area is maintained until national authorities are satisfied that TB has been eradicated.
- Meat is safe. Affected animals will not enter the food chain. The current risk to field staff and others is low. Direct contact with infected animals is required for exposure and infection.

Information on TB in cattle, Australia's programs that led to its eradication, and on *Mycobacterium bovis*, the causative organism, is provided in Appendix 4.

Management of the media should be coordinated by Animal Health Australia's communication section.

Appendix 1 Legal capacities critical to success of bovine TB control and eradication

Despite the fact that the vast majority of approved property programs (APPs) were concluded successfully because of the cooperation of the affected livestock owners, at times it might be necessary to use regulatory powers to enable the necessary actions for an APP to proceed. During the Brucellosis and Tuberculosis Eradication Campaign (BTEC) and the Tuberculosis Freedom Assurance Programs (TFAPs), a list of legal powers was developed for future guidance of APP managers. The following consolidated list of legal powers was agreed by campaign managers as being necessary for jurisdictions to deal with the full range of program circumstances that had been experienced.

- To enter and search any land, vehicle or place or premises for the purpose of determining the suspected presence of bovine TB.
- To require a person to take reasonable steps to provide information.
- To muster, inspect, confine, count, examine, mark for identification, or test, any livestock.
- To require the permanent identification of cattle and buffalo and the recording of movements to ensure whole-of-life traceability.
- To take and remove for analysis or examination samples from or specimens of any livestock that the inspector reasonably believes is infected with bovine TB and to conduct any analysis or examination of any livestock or sample or specimen of the livestock.
- To require an owner (including an owner's agent or person in charge of the stock) to muster, yard or secure the owner's livestock or to provide adequate facilities and assistance to allow the safe and efficient handling of livestock during inspection and during the taking of samples and specimens.
- To impose quarantine to control the movement of animals, into and out of suspect or infected premises or areas.
- To require destruction of livestock, including livestock showing no signs of disease, and to restrict the purposes for which they may be used.
- To control the keeping, transport or management of livestock, livestock products, fittings and fodder, in the quarantine area.
- To require identification of livestock and livestock products, and to trace the movement of animals, animal products and vehicles.
- To control and monitor feral animals and wildlife as required to effect control.
- To provide compensation for animals destroyed for the purpose of controlling or eradicating bovine TB.
- To enlist the assistance of other emergency management agencies if necessary for the purposes of controlling and eradicating a bovine TB outbreak.

Appendix 2 Sample selection and submission for laboratory diagnosis of bovine TB

The following describes the minimum requirements for samples suitable for laboratory examination for tuberculosis (TB). Any reduction in the standard of collection, preservation or transport of samples may affect the quality of the samples, resulting in the laboratory results being questioned.

Collection of samples for laboratory examination

Reactors to the tuberculin test are classified as either having lesions visible and/or palpable or no palpable lesion (NPL). A range of lymph nodes is routinely submitted in addition to any granuloma or other suspicious lesion to determine whether the histologic features are consistent with TB and to detect the presence of *M. bovis*. To obtain consistent and reliable results, it is essential that:

- rigorous precautions are taken to prevent contamination of samples with mycobacteria, either saprophytes from the environment or pathogens transferred on instruments
- any tissues intended for histological examination are fixed immediately to preserve cellular detail and bacteria.

Sample selection

Samples from NPL animals

The lymph nodes listed in Table A2.1 are required to exclude the presence of TB and must always be submitted.

Table A2.1 Lymph nodes required for exclusion of TB

Priority	Tissue	Location of tissue
Essential	Medial retropharyngeal lymph node	Left and right
	Tracheobronchial (bronchial) lymph node	Left and right
	Mediastinal lymph node	Anterior and posterior
Highly desirable	Tracheobronchial (bronchial) lymph node	Cranial and medial
	Other thoracic lymph nodes	
Desirable	Mandibular lymph node	Left and right
	Parotid lymph node	Left and right
	Lateral retropharyngeal lymph node	Left and right
	Medial iliac lymph node	Left and right
	Mesenteric lymph nodes from the region of the duodenum, jejunum and ileum	
	Superficial inguinal (mammary or scrotal) lymph node	Left and right

Procedure

- Using sterilised forceps and scissors, remove lymph nodes from surrounding tissues and inspect for lesions.
- If no lesions are palpated, using sterile forceps place the intact node into a plastic bag.
- Place each plastic bag with node onto ice in an insulated container.

Samples from lesions

- If a lesion is seen or palpated, it must be divided, using a sterile scalpel blade (part for culture and part for histology examination).
(Note: Cutting boards should be scrubbed with disinfectant and rinsed with clean water. The surface of cutting boards can be covered with a new plastic bag opened by splitting along one side and the bottom and using the inside surface for cutting. Use each bag only once to avoid cross-contamination).
- The portion for histological examination should not exceed 1 cm in thickness and must include part of the lesion. Place in a leak proof container containing 10% formal saline so that the volume is at least ten times (10 ×) that of the specimen.
- Place the remaining portion of the lymph node (for culture) into a sterile container that is in turn placed immediately onto ice in an insulated container. Avoid formalin contamination of fresh samples for culture.
- If the lesion is very small, submit as a fresh, refrigerated sample.
- Discard used scalpel blades into a safety tin.

(It is acceptable to collect successive groups of nodes with the one set of forceps and scissors provided that they are not contaminated by dirt or hair, by cutting a lymph node, etc. If instruments become contaminated, discard them and use a fresh, sterilised set.)

- For swamp buffalo in remote areas with poor facilities, submission of lesions in formalin only is acceptable for diagnostic purposes. An analysis of Northern Territory data shows that lesions confirmed on histology to be consistent with TB infection are highly likely (95%) to culture *M. bovis*. It is rare to obtain either substantiated, alternative mycobacterial cultures from lesions consistent with TB on histology, or indeterminate (equivocal) diagnosis with culture-negative results. A formalin-preserved sample and a fresh sample for culture and polymerase chain reaction (PCR) are the preferred diagnostic materials. However, collection of formalin-preserved samples only, is a reasonable diagnostic approach for remote field activities. Tissue PCR sensitivity declines with the amount of time the lesion is exposed to formalin. There are minor alternative causes of granulomas in Northern Territory swamp buffalo that are readily distinguished on histopathology.

Sample processing and dispatch

The departmental officer is responsible for packaging samples and arranging their prompt dispatch to the state or territory veterinary laboratory.

Before dispatch, the laboratory should be advised of the dispatch time, the name of the carrier and the method of transport so that the samples can be collected immediately they arrive.

Samples must be processed and packaged in accordance with International Air Transport Association (IATA) regulations.

Note that most laboratories will not accept specimens unless:

- they have been properly preserved in accordance with this advice
- they are clearly identified
- they are accompanied by appropriate documentation.

Samples that are clearly contaminated or autolysing will also not be accepted. This may result in a major disruption to a testing program, with associated costs and possible legal action.

Appendix 3 Animal Health Committee Property Program Group

Members

The core members²⁰ of the Animal Health Committee (AHC) Property Program Group (PPG) will be:

- a national cattle industry representative
- a state or territory cattle industry representative in respect of the applicable state or territory
- the CVO of the applicable state or territory or his or her representative
- the state or territory TB Incident Manager if he or she is not included as the CVO's delegate
- the Animal Health Australia TB Surveillance Program Manager
- an AHC-appointed chairperson.

Terms of Reference

- Review Proposed Property Programs using the Standard Definitions and Rules and collective knowledge and experience as a guide to assess the likely effectiveness of the measures proposed, including consideration of any additional information on previous property activity.
- Ensure that the Proposed Property Program contains appropriately identified milestones against which its progress may be monitored.
- Endorse the proposed property program, consulting if necessary with AHC and the national cattle industry (NCI), without undue delay.
- Review progress with implementation of the Approved Property Program against milestones.

Method of operation

The AHC Property Program Group will generally meet by teleconference but may, where appropriate, meet in person.

Costs of individual participation in the PPG will be met by each member, with the teleconference or meeting venue costs met by the affected jurisdiction. The host CVO of the affected jurisdiction, in consultation with other PPG members, will prepare the written report to the AHC and the NCI.

²⁰ Appropriate epidemiological expertise should be included in the membership.

Appendix 4 Information for the media on bovine tuberculosis

Tuberculosis in cattle

- Australia has been internationally recognised as TB Free since 1997, with no evidence of the disease since 2000.
- When a case is found, all suspect cattle or even the complete herd will be promptly sent to slaughter.
- Bovine TB is caused by the bacterium, *Mycobacterium bovis*. In Australia, this organism has rarely been found in species other than cattle and buffalo. Pigs were infected when milk from tuberculous cattle was fed to them, but this has not occurred for many years. Sheep and horses have a high natural immunity. *Mycobacterium avium* can cause TB lesions in pigs, and findings of this disease are investigated to exclude bovine and human strains of TB from the diagnosis. Disease caused by *M. avium* is unrelated to bovine TB in either cattle or pigs.
- The disease is currently recognised as being present in many countries. A few countries have conducted successful programs to eradicate TB from cattle.
- A formal surveillance program for TB in cattle — the National Granuloma Submission Program — operated in all major Australian abattoirs for many years and has been followed by a program of careful scrutiny by Australia's meat inspection authorities.
- Infected animals are the main source of infection in endemic areas. Infection is usually via respiratory inhalation of discharges from an infected animal. Infection by ingestion of infected discharges can also occur.
- Cattle density is an important factor in spread, and infection in dairies was therefore once a very common feature of the disease in Australia.
- TB in cattle results in long-term illness in infected animals, often with few clinical signs.
- TB lesions are commonly found in the chest and associated lymph nodes but may be spread throughout the body and involve the liver and intestinal system.
- Lesions may range in size from pinpoint to 10–50 cm in diameter.

The eradication of bovine tuberculosis from Australia

- A formal Brucellosis and Tuberculosis Eradication Campaign (BTEC) commenced in 1970 after many years of efforts by each state and territory to control both bovine brucellosis and TB.
- A declaration of 'Impending Free Area' status for TB was achieved in Australia in 1992, and a declaration of 'Free Area' status was made on 31 December 1997.
- The size and scope of BTEC provided a major challenge to Australia's veterinary services. Eradication of TB (and brucellosis) is a major Australian achievement in animal disease control. It resulted from the combined commitment of the Australian

cattle industry, the seven state and territory governments and the Australian Government.

- BTEC was implemented because of a belief by beef exporters that international markets were at risk of nontariff barriers being imposed by international trading partners. Both diseases led to decreased productivity of cattle and a risk to human health.
- The northern pastoral areas of Australia presented special problems to the eradication of bovine TB because of the harsh and varied environment in which cattle and buffalo grazed.
- In these areas, a structured approach to on-property planning of disease programs was developed, using guidelines to assist the state or territory animal disease control authorities to develop 'Approved Property Programs' with the owner of the property. Testing services and funding only became available after a realistic eradication program was agreed to by both the owner and the disease control authority.
- Other important features of the campaign in these areas included the use of destocking of all or part of a property. Sometimes only young cattle were segregated and retained for testing and the older groups of cattle were sent to an abattoir for slaughter.
- Diagnosis of TB in cattle was primarily by use of the single intradermal tuberculin test applied to the caudal fold (the fold of skin between the head of the tail and the rump/pelvis). The program was based on whole herd tests, or tests of identifiable groups that were managed separately and kept separate from other animals. All tuberculin test reactors were slaughtered.
- Surveillance for evidence of TB by post-mortem examination of cattle sent to an abattoir for slaughter was a critical element of TB eradication. Effective surveillance requires the use of a tail tag identification system to identify the herd of origin, diligence by meat inspectors and a network of veterinary diagnostic laboratories that can examine lesions for evidence of TB. Surveillance for TB continues based upon this system.
- The total cost of BTEC is difficult to calculate but it is known that the official government and industry contributions were more than \$1000 million. The costs incurred by individual producers were also considerable, especially in the extensively grazed areas.
- BTEC was followed by the Tuberculosis Freedom Assurance Program (TFAP), which commenced on 1 January 1998 and operated until 31 December 2002, and TFAP2, which operated during 2003–2006. These were primarily surveillance programs to ensure that any resurgence of TB in Australian cattle was promptly and effectively eliminated.

Some common questions about mycobacteria

Question: Is there a human health risk arising from a case of bovine TB?

Answer: There is no significant risk. Muscle tissue, which is consumed as meat by people, is not affected by bovine TB. When evidence of a case is detected, the affected carcass is condemned as unsuitable for human consumption if there is any evidence of spread throughout the animal. The affected property is immediately quarantined and all recent movements off the property are traced. Extensive testing and removal for slaughter, and

intensive abattoir inspection of potentially infected animals, ensure that the likelihood of meat from an infected animal reaching the consumer is negligible.

Question: Is there any relationship between TB in cattle (caused by *Mycobacterium bovis*) and TB in humans (caused by *Mycobacterium tuberculosis*)?

Answer: Each of these organisms can affect both cattle and humans. Early programs to eradicate TB from cattle were a response to concern over the transfer of TB from cattle to humans via milk. However, the rarity of infection in Australian cattle means that there is now negligible risk to humans from TB in cattle.

Question: What are mycobacteria?

Answer:

- Mycobacteria are a large group of bacteria that are common in the environment and in animals.
- Most mycobacteria are harmless and do not cause disease.
- A small number cause specific diseases, including:
 - *Mycobacterium bovis* — causes TB in cattle
 - *Mycobacterium tuberculosis* — causes TB in humans
 - *Mycobacterium paratuberculosis* — causes Johne's disease in a range of animals
 - *Mycobacterium avium* — causes TB in pigs and birds.

Mycobacterium bovis:

- is the bacterium that causes bovine TB
- grows in many tissues, including lung and lymph glands associated with respiratory and gut tissue
- is shed in large numbers via exhaled air, sputum, faeces, and milk in advanced cases
- has limited survival in the environment; in moist sheltered sites, it seldom survives for more than 4 weeks.

Question: How is *M. bovis* detected?

Answer: There are three ways to differentiate between the types of mycobacteria that may be isolated from an animal:

- grow the mycobacteria and identify the particular mycobacteria to species level using conventional biochemical tests (see 'Growing *M. bovis*' below)
- undertake polymerase chain reaction (PCR) testing to detect *M. bovis* DNA directly in tissue samples and culture products (see 'Testing for specific genetic material' below)
- DNA fingerprint confirmed *M. tuberculosis*-complex isolates using spoligotyping, RFLP and/or VNTR typing.

Growing *M. bovis*

Mycobacteria are grown in a laboratory from specimens taken from infected animals. There are difficulties with this method because it may take several months to get a result and the test must be done using special methods. *M. bovis* is identified by its growth characteristics and by its genetic (DNA) make-up.

Testing for specific genetic material

- Polymerase chain reaction (PCR) tests can detect minute amounts of sections of genetic material (DNA) that are unique to particular organisms:
 - multiplex PCR can differentiate *M. tuberculosis* complex, *M. avium*/*M. paratuberculosis* and *M. intracellulare* species from other mycobacteria
 - *M. tuberculosis* complex multiplex PCR can differentiate *M. bovis* from other *M. tuberculosis*-complex organisms.

PCR is a very useful test to confirm the identity of bacteria in laboratory cultures and may prove useful for direct testing of infected tissues.

Appendix 5 Retesting with the Comparative Cervical Test

The comparative cervical intradermal tuberculin test (CCT) is an official test when performed by an Approved Person and carried out using the test procedure described below.

Where a retest of cattle with a comparative cervical intradermal tuberculin test is deemed necessary the retest may be performed not less than 60 days (and preferably 90 days) after the previous tuberculin test.

The animal must be kept isolated until the retest is completed. While the positive predictive value of reactors to the single intradermal caudal fold tuberculin test will be very low in cattle where tuberculosis is unlikely to exist, the reactor must be treated as suspect due to the tuberculin reaction.

Equipment

- **Syringes**

As for the single intradermal caudal fold test, a single or multi-dose syringe with the means of accurately dispensing 0.1ml of tuberculin is required. Two different syringes must be identified and each used only for one type of tuberculin.

- **Needles**

As for the single intradermal caudal fold test, a 22 gauge needle or finer is required. The unsheathed portion of the needle, when attached to the syringe, is required to be no less than 2mm and no more than 5mm in length.

- **Tuberculin**

- bovine tuberculin purified protein derivative (PPD) at a potency of 1mg/ml and avian tuberculin PPD at a potency of 2500IU/ml. [I.e. CSL Tuberculin PPD (bovine) 1mg/ml; CSL Tuberculin PPD (avian)].

- **Electric clippers or curved scissors**

- **Skin callipers** – of approved design

- **Ruler** – calibrated in centimetres and millimetres.

- **Record sheets**

Method

The injection sites should be in a line in front of, and parallel with, the line of the shoulder. The upper site, used only for the avian tuberculin, should be at least 10cm below the crest of the neck. The lower site, used only for the bovine tuberculin, should be not less than 19 cm from the upper site.

For young cattle, in which the two sites cannot be separated sufficiently, an injection should be made on each side of the neck at an equivalent site.

The selected site is clipped and cleaned. A fold of skin within the clipped area is taken up between the finger and thumb and measured to the nearest millimetre, using callipers, before injection. The measurements are recorded. The Intradermal injection is made in a similar manner to the single intradermal caudal fold test. If a bleb is not raised then a further injection is made preferably on the other side of the neck and at a similar site.

Reading and interpretation

The test is read by picking up the skin fold so that the swelling or injection site is at the apex. The thickness of the fold is then measured to the nearest millimetre with callipers and recorded. It is important that the nature of the swelling is noted and the presence of even a minimum amount of either diffuse or discrete oedema is considered as highly significant. The result of the test is interpreted according to the rules set down.

Reactor

A reactor is an animal showing a visible or palpable swelling at the injection sites, which when measured is interpreted as positive or suspect on reference to Figure 1 and Figure 2 below (Australian Animal Health Council Ltd, TFAP2 Standard Definitions and Rules 2003).

Standard Interpretation (Figure 1)

- a) To be used as the normal interpretation.
- b) Used for Herds where the history is not suggestive of Tuberculosis or Herds with a non-specific sensitisation.

Severe Interpretation (Figure 2)

- a) To be used only on the instruction of the CVO.
- b) May be used for Herds with a recent history of Tuberculosis or with an inadequate history.

Figure 1 Standard Interpretation of the Comparative Cervical Intradermal Tuberculin Test

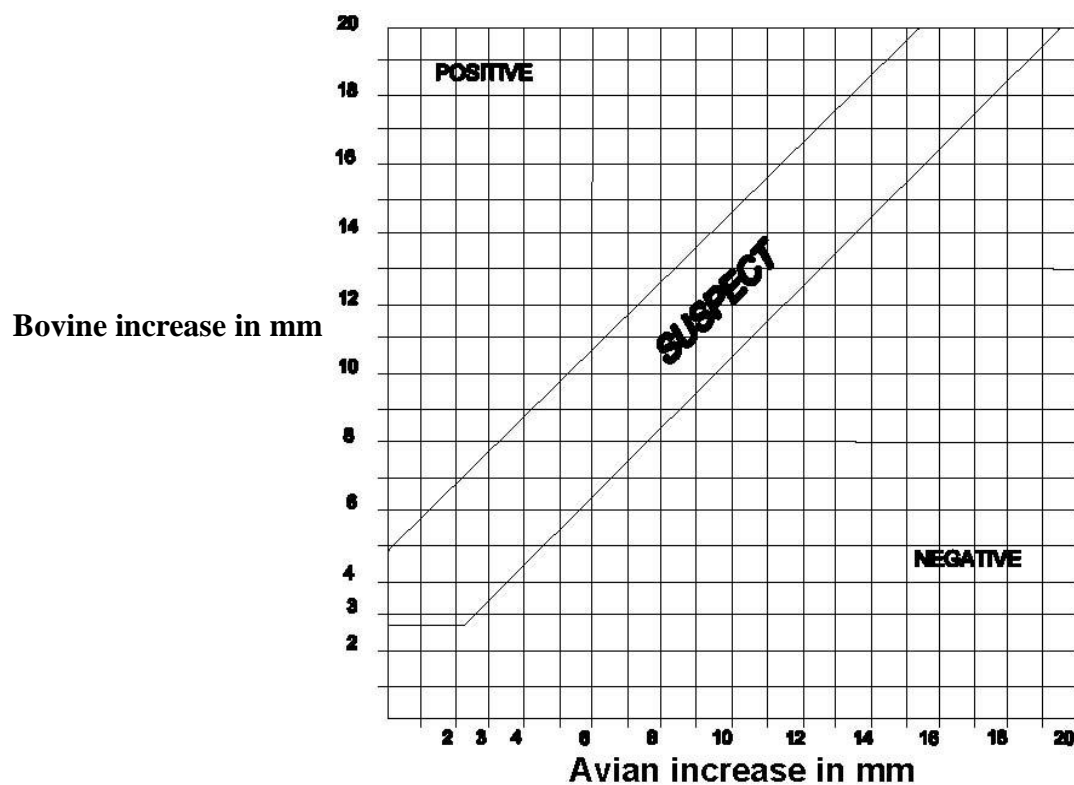
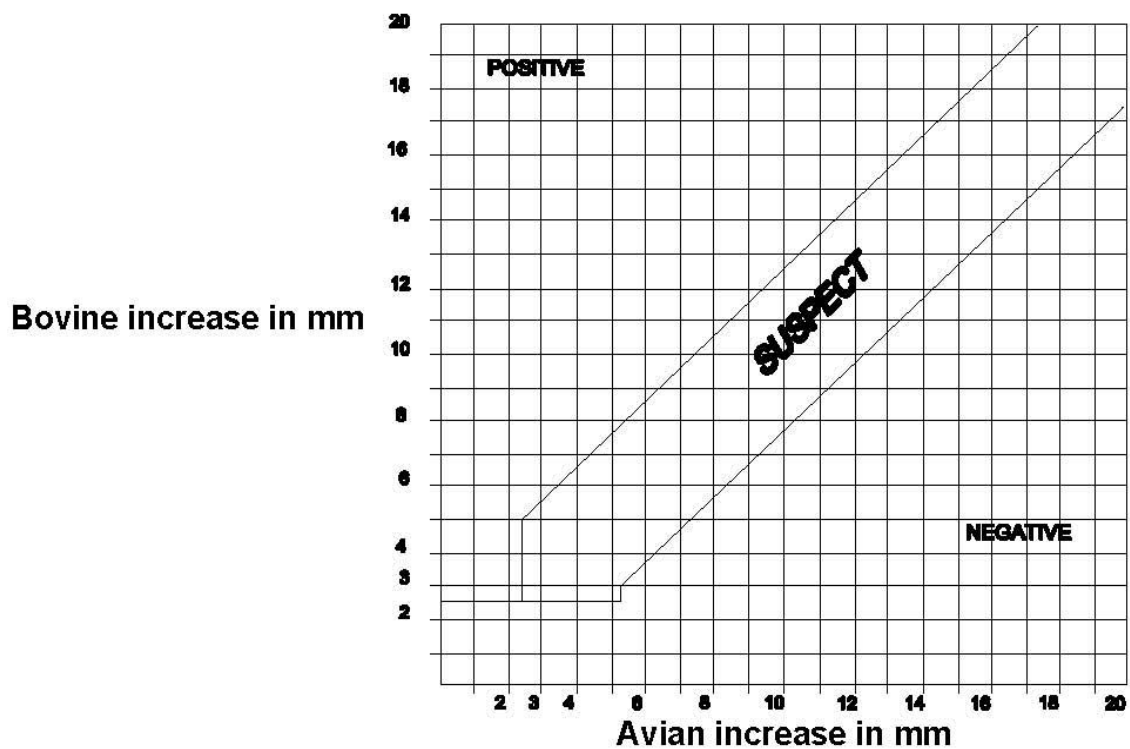


Figure 2 Severe Interpretation of the Comparative Cervical Intradermal Tuberculin Test



Glossary

Animal Health Committee	A committee comprising the CVOs of Australia and New Zealand, Australian state and territory CVOs, Animal Health Australia, and a CSIRO representative. The committee provides advice to PIMC on animal health matters, focusing on technical issues and regulatory policy (formerly called the Veterinary Committee).
Approved Person	A person approved by the Chief Veterinary Officer to carry out bovine tuberculosis tests.
Approved Property Program	A program for investigating or eradicating TB from a property
AUSVETPLAN	Australian Veterinary Emergency Plan. A series of technical response plans that describe the proposed Australian approach to an emergency animal disease incident. The documents provide guidance based on sound analysis, linking policy, strategies, implementation, coordination and emergency-management plans.
Carrier	An animal recovered from a disease, or not showing clinical signs, but capable of passing on the infection to another animal.
Cattle	Animals of the genus <i>Bos</i> (taken to include animals of the genus <i>Bubalus</i> — buffalo).
Chief veterinary officer	The senior veterinarian of the animal health authority in each jurisdiction (Commonwealth, state or territory) who has responsibility for animal disease control in that jurisdiction.
Compensation	The sum of money paid by government to an owner for stock and property that are compulsorily destroyed because of an emergency animal disease.
Destock, destocked, destocking	The process of, or outcome from, removal of all specified cattle from an area.
Emergency Animal Disease Response Agreement	Agreement between government (Commonwealth and states/territories) and livestock industries on the management of emergency animal disease responses. Provisions include funding mechanisms, the use of appropriately trained personnel and existing standards such as AUSVETPLAN.
Feral animals	Domestic animals that have become wild (e.g. cats, horses, pigs).
In-contact animals	Animals that have had close contact with infected animals, such as non-infected in the same group as infected animals.

Incubation period	The period that elapses between the introduction of the pathogen into the animal and the first clinical signs of the disease.
Interferon	Protein with antiviral activity released from cells in response to virus infection or other immunological stimuli.
Judas animals	Animals carrying radio transmitters that are released into an area and join up with local wild animals, allowing the entire group to be tracked.
Monitoring	Routine collection of data for assessing the health status of a population.
Owner	Person responsible for a premises (includes an agent of the owner, such as a manager or other controlling officer).
Polymerase chain reaction	A method of amplifying and analysing DNA sequences that can be used to detect the presence of a specific DNA.
Premises	A self-contained unit, with secure defined boundaries, and run separately from all other premises.
Prevalence	The proportion (or percentage) of animals in a particular population affected by a particular disease (or infection or positive antibody titre) at a given point in time.
Quarantine	Legal restrictions imposed on a place or a tract of land by the serving of a notice limiting access or egress of specified animals, persons or things.
Reactor	An animal judged to have a significant swelling at the site of tuberculin injection with the tuberculin test, or an animal having a positive reaction to a test.
Sensitivity	The probability that a test will correctly identify animals that have been exposed to the disease (true positives). Exposed animals that do not give a positive test response are referred to as false negatives.
Specificity	The probability that a test will correctly identify animals not exposed to the disease (true negatives). Non-exposed animals that test positive are referred to as false positives.
Spreader animal	An animal that is discharging mycobacteria, usually by the respiratory/salivary (oronasal) routes, and far less commonly via milk or other routes. Such discharges can lead to the exposure and infection of other susceptible animals.
Surveillance	A systematic program of investigation designed to establish the presence, extent of, or absence of a disease, or of infection or contamination with the causative organism. It includes the examination of animals for clinical signs, antibodies or the causative organism.

Susceptible animals	Animals that can be infected with a particular disease
Suspect TB herd	A herd where there is evidence that TB may be present.
TB case	A confirmed occurrence of TB
TB case herd	A herd of cattle in which TB has been found.
TB incident	A detection of a granuloma or lesion that is suspect for TB
Tracing, trace-back, trace-forward	The process of locating animals, persons or other items that may be implicated in the spread of disease, so that appropriate action can be taken.
Vaccination	Inoculation of healthy individuals with weakened or attenuated strains of disease-causing agents to provide protection from disease.
Zoonosis	A disease of animals that can be transmitted to humans.

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