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Tuberculosis in free-ranging wildlife: detection, diagnosis and management

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Summary

Mycobacterium bovis is emerging as an important pathogen of free-ranging wildlife in which it is a potential source of infection for domestic animals and a threat to valuable wildlife species. This review examines the procedures for the detection, diagnosis and management of *M. bovis* in wildlife populations. The ante-mortem detection of *M. bovis* infection in wildlife is difficult, due to the common occurrence of subclinical infections and the deficiencies of the currently available diagnostic tests. Serological tests are insensitive, while tests measuring cell-mediated immune responses show promise, but have not been sufficiently developed for routine use in most species. The diagnosis of *M. bovis* in free-ranging wildlife relies on post-mortem examination supported by histopathology and microbiology. A feature of *M. bovis* infections is the variation in the appearance and distribution of lesions in the different host species. Bacterial culture remains the gold standard for diagnosis of tuberculosis, while histopathology is limited by the frequent inability to distinguish lesions caused by *M. bovis* from those produced by other mycobacterial species. Deoxyribonucleic acid (DNA) fingerprinting and advanced typing techniques are increasingly being used to unravel the epidemiology of mycobacterial infections, including tuberculosis in free-ranging wildlife. An understanding of the epidemiology is essential if procedures are to be developed for the management of tuberculosis in wildlife. Few management options are currently available, especially for protected wildlife. Vaccination is the subject of much research, but further developments are required before it can be used to control tuberculosis in any animals, let alone in free-ranging wildlife.

Keywords

Bovine tuberculosis – Diagnosis – Mycobacterium bovis – Vaccination – Wildlife.

Introduction

Tuberculosis caused by *Mycobacterium bovis* has been identified in a wide variety of free-ranging wildlife as well as domestic animals (35). In recent years, awareness of the importance of tuberculosis in wildlife has increased, not only as a potential reservoir of infection for domestic animals, but also as a threat to valuable wildlife species. This review will concentrate on the detection, diagnosis and management of tuberculosis in free-ranging wildlife. However, knowledge of the epidemiology of tuberculosis in free-ranging wildlife is also an important factor in understanding the challenges associated with the diagnosis and management of bovine tuberculosis in this group of animals. Detailed information on the epidemiology of tuberculosis in wildlife has been summarised in Volume **20** (1), 2001 of the *Scientific and Technical Review* of the Office International des Epizooties on mycobacterial infections in domestic and wild animals. Only a small proportion of wildlife species that become infected with *M. bovis* can act as maintenance hosts of this organism. In these species, the infection can persist through horizontal transmission between individuals in the absence of any other source of *M. bovis*. Examples of free-ranging wildlife that can act as maintenance hosts include the brushtail possum (*Trichosurus vulpecula*), badger (*Meles meles*), bison (*Bison bison*), African buffalo (*Syncerus caffer*) and white-tailed deer (*Odocoileus virginianus*). In contrast, a number of species referred to as 'spill-over' hosts

become infected with *M. bovis*, but the infection will only occur sporadically or persist within these populations if a maintenance host is present in the ecosystem.

Detection of tuberculosis in wildlife

A number of significant challenges arise in detecting tuberculosis in free-ranging wildlife. Even in susceptible species, the majority of infected animals show no clinical signs of disease. The realisation that wildlife may be infected with *M. bovis* may result from the apparent failure of programmes to eradicate the infection from cattle. In Great Britain in the late 1960s and early 1970s, incidence data clearly demonstrated that the proportion of infected cattle herds in south-west England was markedly higher than the rest of the country. A wildlife reservoir of infection was suspected and the first cases of *M. bovis* infection in badgers in Great Britain were found in 1971 on a farm where tuberculin reactors with lesions had recently been detected (67). In Michigan, United States of America (USA), *M. bovis* was initially discovered in wildlife upon diagnosis of grossly lesioned white-tailed deer presented by hunters to the Department of Natural Resources for examination. Since recording the index cases in wild deer in 1975 and 1994, sixteen domestic cattle herds in four counties in the north-eastern part of this state have been found to harbour infected animals. Following depopulation of a focus of livestock infection in the area of El Paso in Texas, Michigan will remain the only state in the USA not accredited free of bovine tuberculosis (S.M. Schmitt and D.J. O'Brien, unpublished observations).

Ante-mortem diagnosis

Where wildlife are indigenous protected species and post-mortem samples are not frequently available, consideration may be given to trying to establish an ante-mortem diagnosis of tuberculosis. All the ante-mortem diagnostic procedures have limitations, but have the potential for determining not only the existence of tuberculosis within a population but also the prevalence of infection in that population. Specific and sensitive ante-mortem tests for a large range of species are increasingly required to ensure that wildlife moved to new game parks or zoological gardens are free of bovine tuberculosis and are not a source of infection for other animals. In particular, few tests can be used in pachyderms.

Clinical signs

Tuberculosis caused by *M. bovis* is a chronic, progressive disease and even in the most susceptible species, the time course of infection/disease may last for several weeks; more commonly, disease will last for many months, if not years. For the majority of the course of infection, animals will be clinically normal. The most common clinical sign of disease is weight loss and this only occurs in the advanced stage of the disease. Other clinical signs include swollen lymph nodes, especially of the head, discharging lymph node abscesses and signs associated with a tuberculous pneumonia such as coughing. Skeletal and synovial (elbow hygromata) lesions with associated lameness may be observed in lion (51, 53). Swollen head nodes with draining fistulae are almost pathognomonic in greater kudu (5). Table I is a summary of the clinical signs of tuberculosis observed in a selection of different wildlife species. The severity of clinical signs may be exacerbated by environmental factors such as lack of grazing during droughts (46, 48). In some animal species, a change in behaviour may occur in the advanced stages of tuberculosis. For example, brushtail possums that are normally nocturnal may be observed moving about during daylight. Baboons, which are normally social, become depressed and solitary. These behavioural changes may be important in the spread of infection to other animals. Experimental studies have indicated that cattle and farmed deer may become infected by direct or very close contact on pasture with brushtail possums that are terminally ill with tuberculosis (72, 81).

Table I
Clinical signs of tuberculosis in free-ranging wildlife

Species	Clinical signs
Badger (*Meles meles*)	Weight loss, open lesions, behavioural changes
Wood bison (*Bison bison)*	Weight loss, dull coat and dry coughing in advanced cases
Possum (*Trichosurus vulpecula*)	Weight loss, discharging abscesses, behavioural changes
African buffalo (*Syncerus caffer*)	Weight loss, hoarse, dry coughing, dyspnoea, dull coat, arched back, depression
Lion (*Panthera leo*)	Weight loss, swollen joints, elbow hygromas, lameness, corneal opacities, dull coat, poorly healing, skin wounds, depression
Deer, white-tailed (*Odocoileus virginianus*)	Weight loss (±)
Greater kudu (*Tragelaphus strepsiceros*)	Swollen parotid, retropharyngeal and cervical lymph nodes discharging fistulae, terminal weight loss, coughing and depression
Baboon, chacma (*Papio ursinus*)	Weight loss, coughing, dyspnoea, dull moth-eaten coat, behavioural changes, swollen peripheral lymph nodes
Leopard *(Panthera pardus*)	Weight loss, dull coat, poorly healing skin wounds
Cheetah (*Acinonyx jubatus*)	Weight loss, dull coat, alopecia and poorly healing skin wounds
Hyaena (*Crocuta crocuta*)	Slight weight loss
Warthog (*Phacochoerus aethiopicus*)	Weight loss, dyspnoea, swollen peripheral lymph nodes

Immunologically-based diagnostic tests

An understanding of the immune responses to mycobacterial infection serves as a good guide for assessing the potential usefulness of immune-based tests for the detection of tuberculosis in wildlife (83). The immune responses in tuberculosis are those centred on containing an intra-cellular bacterium that does not produce toxins. In the early, preclinical stages of tuberculosis, cell-mediated immune responses predominate. These responses enhance the ability of macrophages to restrict the multiplication of this intracellular parasite. In contrast, antibody does not affect the survival of the bacterium within the host. High levels of antibody to mycobacteria usually only occur in the advanced stages of disease when large numbers of organisms are present. The specificity of immune-based tests depends on the antigens used in the various tests. Until recently, the only available antigens used in tests for the diagnosis of tuberculosis were crude, multi-component preparations, such as the purified protein derivatives (PPDs) used for skin testing. These preparations contain some antigens that are found in a range of different mycobacterial species and others that are more species specific. False positive reactions occur as a result of exposure to bacterial species that have some identical antigens to those found in *M. bovis*. These reactions can be caused by a range of different mycobacterial species, such as members of the *Mycobacterium avium* complex, but in most cases the cause of false positive responses is not determined. The specificity of immune-based tests can be improved by comparing the responses to antigen preparations from different mycobacterial species, such as PPDs from *M. bovis* and *M. avium*, or the use of purified mycobacterial antigens.

Cell-mediated immune-based tests

The intradermal tuberculin test has proved to be a very valuable test for the diagnosis of bovine tuberculosis in cattle and has been used as the principal tool for identifying infected cattle herds in all the control programmes that have led to the eradication of *M. bovis*. While intradermal tuberculin testing has been attempted in a number of different wildlife species, it has severe limitations when applied to free-ranging wildlife. Skin testing has practical disadvantages because animals must be re-examined 48 h to 96 h after the injection of tuberculin. An important consideration is that intradermal testing has to be standardised for each species. The optimal dose of tuberculin is very low, in the order of 1 to 10 units for some species such as guinea pigs and humans, while the dose required for cattle is in the order of 3,000-5,000 units. For most wildlife species, the optimal dose of PPD required for skin testing is unknown. Raath *et al.* found a marked difference in the sensitivity of the intradermal test in African buffalo, depending on the manufacturer and dose of tuberculin used (77). Both brushtail possums and badgers produce only weak responses in the intradermal skin test (47, 57, 76). Potentially confounding effects on cell-mediated based tests, including skin testing, is the immunosuppressive effect resulting from the stress of capture as well as the effect of dehydration on skin measurement. Evidence for immunosuppression has been recorded in recently caught brushtail possums and wild ferrets (10, 31).

A diagnostic test which could be performed over 24 h would be very useful as this would be rapid enough for a wild animal to be caught and held until the results of the test are known. *In vitro* cell-mediated tests for wildlife have the distinct advantages of requiring only one handling of the animal and detecting cell-mediated immune responses that are likely to predominate in subclinical cases of tuberculosis. Lymphocyte proliferation assays do not require species-specific reagents and have been applied to a number of different wildlife species including brushtail possums, badgers and oryx (14, 31, 32, 39). The sensitivity of lymphocyte transformation tests for detecting tuberculosis in both brushtail possums and badgers is significantly greater than that obtained with serological tests. Technical difficulties in performing lymphocyte transformation tests coupled with the major limitation that blood samples for *in vitro* tests of cell-mediated immunity have to be processed within 24 h-30 h of collection, largely restricts the use of this test to research purposes.

An assay has been developed based on the detection of interferon-γ in antigen-stimulated blood cultures for the detection of cattle infected with *M. bovis* (79). The levels of interferon-γ in the blood cultures are measured using a capture enzyme-linked immunosorbent assay (ELISA), based on the use of monoclonal antibodies. A limitation of this test developed for cattle is that the monoclonal antibodies used in the ELISA will only recognise the interferon-γ of a limited range of species including cattle, sheep and goats. Preliminary investigations have shown that the interferon-γ test also has potential as an ante-mortem test for detecting African buffalo with bovine tuberculosis (77). The success of the interferon-γ test for detecting tuberculosis in cattle has prompted interest in the development of a similar test for badgers, deer and pachyderms. However, species-specific reagents are necessary and expensive to produce.

Recently, the purified antigens ESAT6 and CFP10 from *M. tuberculosis* have been used in the interferon-γ test for cattle, and this resulted in an increase in specificity without markedly compromising the sensitivity of the test (15, 90). The results of these studies contrast with those that used other purified antigens such as MPB70, where many infected animals were not identified (97). Selected purified antigens are also likely to be useful for improving the specificity of cell-mediated tests for wildlife where non-specific responses have been recognised as a problem.

Serological tests

Serological tests have been used to detect tuberculosis in a range of different host species, including wildlife, but none have proven to be as useful as intradermal skin testing. The combination of both cellular immune based and serological tests has been advocated as a means of increasing the sensitivity

of detecting tuberculous animals (45). Some animals with advanced tuberculosis are anergic and may have poor cellular immune responses, but have moderate to high levels of specific antibody. Griffin *et al.* demonstrated that high levels of sensitivity and specificity can be obtained for the detection of bovine tuberculosis in farmed deer through the combined use of a lymphocyte transformation test and an ELISA to detect antibody, with a combination of different antigens (45). Acceptable levels of sensitivity in the ELISA for deer were only achieved if the testing was undertaken ten days following an intradermal skin test that significantly boosted the levels of specific antibody.

In the United Kingdom, an extensive research programme was performed to develop a serological test for identifying bovine tuberculosis in badgers. The goal of this work was to have a test that could be used as the basis for a control programme based on the culling of only those badgers that are infected. Initial studies used crude, multi-component antigens that resulted in tests with poor specificity. Subsequently, a blocking ELISA (the Brock test) was developed which used a monoclonal antibody to a 25 kDa antigen of *M. bovis* that was found to be immunodominant in badgers (42, 43). The Brock test was found to be 98% specific but only 37% sensitive. This level of sensitivity was too low to be useful for a control programme based on a test and cull strategy (54). Similar low levels of sensitivity have also been observed in serological tests developed for detecting tuberculosis in brushtail possums (11). Serological responses were more commonly found in possums in the terminal stages of disease but very few possums with subclinical *M. bovis* infection produced positive responses. In African buffalo, serological tests (ELISA) were found to have little correlation with the disease status of the animal, except in terminal anergic cases (77).

Post-mortem diagnosis

Gross pathology

A presumptive diagnosis of tuberculosis is often made on the basis of finding characteristic macroscopic lesions. In cattle, these lesions are typically caseous, yellow and mineralised (26). The appearance, nature and distribution of lesions in other hosts, including wildlife, are sometimes different from those observed in cattle. For example, the lesions in some hosts, such as the brushtail possum and the badger, have little or no fibrosis, and mineralisation is very rarely observed (25, 41). The macroscopic appearance of the lesions in these hosts may resemble pyogenic abscesses caused by other bacterial genera, such as *Corynebacterium*.

The pathological characteristics of lesions in free-living white-tailed deer are quite variable (38, 69, 82). The predominant lesions occur in lymph nodes which are often grossly enlarged, containing one or more areas of pale, viscid purulent material. The central zones of these areas are sometimes partially mineralised. A smaller percentage of lesions present as solid caseogranulomas. In tuberculous African buffalo, solid caseogranulomas are generally found in lymph nodes, whereas in the lungs the lesions vary from focal tuberculous pneumonia with pin-point areas of caseation, to solid confluent caseogranulomas. Whether focal or disseminated, these lung lesions may progress to cavitation with liquefaction (4, 48), and such cases are extremely infectious.

In greater kudu infected with *M. bovis*, the enlarged head lymph nodes are usually visible externally, and one or both of the parotid lymph nodes are frequently abscessed and fistulated to the exterior, draining viscous mucoid pus. The other head nodes generally have more solid caseous lesions. In this species, the pulmonary lesions are generally raised and plaque-like on the surface of the lung and may be focal disseminated or confluent, suggestive of secondary haematogenous spread. The mediastinal and bronchial lymph nodes are frequently grossly enlarged. Pyogranulomatous lesions of the mesenteric lymph nodes are not infrequent, and peripheral nodes are also occasionally affected (5, 7, 52). With this plethora of lesion sites, including draining lymph node abscesses, intestinal lesions and miliary pulmonary lesions, kudu should be considered 'super shedders' of infectious material.

The most common site of infection in maintenance hosts of *M. bovis*, including Kafue lechwe (*Kobus leche kafuensis*) (5), is the thoracic cavity (Table II). Lesions are also commonly found in the retropharyngeal lymph nodes in some species such as wood bison and red deer (60, 87). Generalised infections, with lesions present in a number of different organs, have been observed in these three hosts.

Interestingly, while baboons appear to be spillover hosts for *M. bovis*, every infected baboon examined in an outbreak in the Kruger National Park had generalised infections with macroscopic granulomas in the spleen, lungs and mesenteric lymph nodes. Other incidental sites were head nodes, inguinal, mammary and axilliary lymph nodes, kidneys, epicardium and spine (50). Generalised lesions have also been observed in tuberculous lion and leopard. These lesions do not resemble those typically found in herbivores and omnivores. No abscessation, caseation or calcification is present, and lesions appear proliferative or occasionally cavitational in nature, with scanty mucoid exudate. Lesions generally involve abdominal and peripheral lymph nodes in early cases, but also spread to involve lungs, bones and synovial structures in advanced cases. The pulmonary lesions are frequently bronchiectatic in nature, and renal amyloidosis is common in generalised cases (51, 53).

In the two cheetahs with confirmed bovine tuberculosis, the most significant macroscopic lesions were found in the lungs. These consisted of numerous scattered granulomas, many of which contained liquefied, caseous necrotic exudates. Some lesions were cavitated and the rupture of one of these in one of the victims resulted in a fatal pneumothorax (49).

Table II
Free-ranging wildlife affected by *Mycobacterium bovis*
Most common sites for gross tuberculous lesions and routes of excretion

Host species	Most common site of gross lesions	Route of transmission	Epidemiological status
Possum (*Trichosurus vulpecula*)	Lungs, superficial lymph nodes	Respiratory	Maintenance host
Badger (*Meles meles*)	Lungs	Respiratory	Maintenance host
Bison (*Bison bison*)	Head and thoracic lymph nodes and lungs	Respiratory	Maintenance host
African buffalo (*Syncerus caffer*)	Lungs, thoracic and head lymph nodes	Respiratory	Maintenance host
Deer, white-tailed (*Odocoileus virginianus*)	Head lymph nodes costal/pleural	Respiratory/oral	Maintenance host
Lechwe antelope (*Kobus leche*)	Thoracic lymph nodes and lungs	Respiratory	Maintenance host
Deer, red (*Cervus elaphus*)	Head lymph nodes/thoracic lymph nodes	Respiratory/oral	Spillover host [a]
Greater kudu (*Tragelaphus strepsiceros*)	Head lymph nodes and lungs	Scarification, oral	Spillover host [a]
Warthog (*Phacochoerus aethiopicus*)	Head lymph nodes and lungs	Oral/respiratory	Spillover host [a]
Ferret (*Mustela putorius*)	Mesenteric lymph nodes	Oral	Spillover host [a]
Baboon, chacma (*Papio ursinus*)	Mesenteric lymph nodes, spleen and lungs	Oral/respiratory	Spillover host
Feral pig (*Sus scrofa*)	Head lymph nodes	Oral	Spillover host
Black bear (*Ursus americanus*)	No gross lesions	Oral	Spillover host
Bobcat (*Felis rufus*)	Mesenteric lymph nodes	Oral	Spillover host
Coyote (*Canis latrans*)	Mesenteric lymph nodes	Oral	Spillover host
Racoon (*Procyon lotor*)	No gross lesions	Oral	Spillover host
Red fox (*Vulpes vulpes*)	No gross lesions	Oral	Spillover host
Lion (*Panthera leo*)	Mesenteric and peripheral nodes, skin, lungs, bones and joints	Oral/respiratory [b]	Spillover host
Leopard (*Panthera pardus*)	Mesenteric and peripheral nodes, skin and lungs	Oral/respiratory	Spillover host
Cheetah (*Acinonyx jubatus*)	Lungs and skin	Oral/respiratory [b]	Spillover host
Hyaena (*Crocuta crocuta*)	No gross lesions	Oral	Spillover host
Common genet (*Genetta genetta*)	Thoracic lymph nodes	Oral	Spillover host

a) in high densities, ferrets, greater kudu, warthogs and wild red deer may be maintenance hosts of *M. bovis*
b) possible alternative route of transmission

Calcified abscesses of the sub-maxilliary lymph node are a consistent finding in tuberculous warthogs. Pulmonary lesions also frequently occur and consist of caseo-calcific consolidation of the entire lung substance, due to the coalescence of masses of small haematogenous nodules. Other incidental sites include pleura, peritoneum, mesenteric lymph nodes, gastro/hepatic lymph nodes and peripheral lymph nodes (98).

In some spillover hosts infected with *M. bovis*, lesions may be absent or very small. No lesions were observed in culture positive hyaenas (D.F. Keet and R.G. Bengis, unpublished observation) and only a single lesion in a thoracic lymph node was observed in a common genet (*Genetta genetta*) in a park adjoining the Kruger National Park (A. Michel, personal communication).

The distribution of lesions in tuberculosis will depend on the route by which the animal was infected and whether or not secondary spread of infection has occurred within the body. Examples of the most common sites for gross tuberculous lesions in free-ranging wildlife are listed in Table II. Animals with lesions restricted to the thoracic cavity are presumed to have been infected by the inhalation of aerosols of *M. bovis*, while those with lesions in mesenteric lymph nodes are thought to have acquired the infection by ingestion. In those animals with lesions in the head lymph nodes, especially the retropharyngeal nodes, determination of whether the infection was acquired through ingestion or inhalation may be difficult. The location of the lesion may also provide an indication of the probable means of spread of infection. For example, kidney lesions occur in badgers with bovine tuberculosis and contaminated urine has been identified as a possible means of spread of infection. Lesions in sites other than the cranial lymph nodes and thorax are infrequently encountered in white-tailed deer. The rarity of lesions of the urogenital tract and mammary gland suggest that these are uncommon routes for dissemination of the organism in this species (69). Variations in the susceptibility of the host will be reflected in the prevalence of generalised lesions and the number of organisms in these lesions.

Histopathology

The examination of histological sections of suspect tuberculous lesions is a powerful tool for establishing a diagnosis following the detection of macroscopic lesions. The fixation of samples in formalin is particularly important for the examination of

suspect lesions from wildlife because maintaining samples in a suitable condition for subsequent bacterial culture may be difficult. The classical histological features of *M. bovis* lesions in cattle are the presence of central necrosis with mineralisation surrounded by a granulomatous inflammatory response of macrophages and an outer walling off with fibrous connective tissue. Variations of the histological picture are observed between different hosts (Table III). In very susceptible hosts such as the brushtail possum and the badger, lesions due to *M. bovis* are poorly organised, neutrophils are more prominent, and giant cells and fibrosis are absent (25, 40). In lion and leopard, little necrosis is evident, and the lesions consist of amorphous, multifocal to coalescing, non-encapsulated granulomatous inflammatory reactions, with an absence of giant cells and mineralisation (49).

In baboons, areas of central caseation containing aggregates of necrotic neutrophils are characteristic of the multifocal to confluent necrogranulomatous pneumonia present. Macrophages, epithelioid cells and multinucleated giant cells interspersed with lymphocytes, surround the necrotic debris. Characteristically, many acid-fast organisms are present, the lesions are non-encapsulated and miliary spread is common (49).

Histopathology can be used not only to identify suspect cases of tuberculosis, but also to exclude such a diagnosis. Lesions which macroscopically may be mistaken for tuberculosis include those caused by helminth parasites, bacteria including the genera *Rhodococcus*, *Actinomyces*, *Actinobacillus*, *Arcanobacterium* and *Nocardia*, and fungi such as *Aspergillus*, *Cryptococcus*, *Blastomyces* and *Histoplasma*, as well as some neoplasms (adenocarcinoma, anaplastic carcinoma and malignant lymphomas); these can all be correctly diagnosed by histology. One of the limitations of histopathology is that the tissue changes in lesions caused by *M. bovis* are often indistinguishable from those caused by other mycobacterial species. Table IV is a list of mycobacterial species other than *M. bovis* that have been isolated from free-ranging wildlife. In many of these infections, the histological appearance will be identical to that caused by *M. bovis*. Furthermore, the histological appearance of lesions caused by some of these other pathogenic mycobacteria differs between hosts. In cattle and sheep, the histological appearance of paratuberculosis caused by *M. paratuberculosis* can easily be distinguished from lesions caused by *M. bovis* because these paratuberculosis lesions do not have the classical features of necrosis and mineralisation present in lesions caused by *M. bovis*. However, histological distinction of lesions caused by *M. bovis* and *M. paratuberculosis* in deer is usually impossible, as necrosis and mineralisation are present in lesions caused by either bacterial species (34).

Table III
Histological features of tuberculous lesions (14)

Host species	Giant cells	Necrosis with neutrophil infiltration	Mineral-isation	Fibrous encapsu-lation	Acid-fast organisms
Possum	±	+++	–	–	+++
Badger	±	+++	–	–	+++
Buffalo, African	++	+++	±	–	+
Bison	++	+++	+	+	+
Deer, white-tailed	++	+	+	+	++
Deer, red	+++	+	–	++	++
Ferret	±	+++	–	–	+++
Cattle	+++	+	–	++	+
Lion	–	+	–	–	+++
Leopard	–	+	–	–	++
Cheetah	–	++	+	–	+
Baboon	++	+++	+	–	+++
Warthog		++	++	++	+

Frequency of detection in a lesion:
– : absent
± : rarely found
+ : low
++ : medium
+++ : high

Table IV
Free-ranging wildlife affected by mycobacterial species other than *Mycobacterium bovis**

Mycobacterial species	Host species (reference)
M. avium	Birds, various species (86)
	Deer, white-tailed (*Odocoileus virginianus*) (RLWLDL, unpublished data)
	Elk (*Cervus elaphus*) (RLWLDL, unpublished data)
	Racoon (*Procyon lotor*) (RLWLDL, unpublished data)
M. paratuberculosis	Rabbit (*Oryctolagus cuniculus cuniculus*) (44)
	Fox (*Vulpes vulpes*) (2)
	Bighorn sheep (*Ovis canadensis*) (96)
	Rocky Mountain goats (*Oreamnos americanus*) (96)
	Ferret (*Mustela putorius furo*) (G.W. de Lisle, R.H. Montgomery and B.M. Paterson, unpublished findings)
M. leprae	Nine-banded armadillo (*Dasypus novemcinctus*) (95) Chimpanzee (*Pan troglodytes*) (37) Mangabey monkey (*Cercocebus atys*) (63) Cynomolgus macaques (*Macaca fascicularis*) (89)
M. fortuitum	Deer, white-tailed (*Odocoileus virginianus*) (RLWLDL, unpublished data)
	African buffalo (*Syncerus caffer*) (A. Michel, personal communication)
M. lepraemurium	Rat (*Rattus ratttus*) (33)
M. marinum	European hedgehog (*Erinaceus europaeus*) (84)
M. microti	Vole (*Microtus arvalus*) (93)
M. ulcerans	Koala (*Phascolarctos cinereus*) (65) Ringtail possum (*Pseudocheirus peregrinus*) (75)

* this list includes only cases in mammals and birds in which mycobacterial lesions are associated with the bacterial isolations
RLWLDL: Rose Lake Wildlife Disease Laboratory

Microbiology

A presumptive diagnosis can be performed by the detection of acid-fast staining bacilli in a smear of the suspect tuberculous lesions. Unlike bacterial culture, the examination of smears is inexpensive and does not require a specialised laboratory with expensive equipment such as biohazard cabinets. While valuable information can be obtained from the examination of smears, the results should be interpreted with caution. The failure to see acid-fast organisms in a smear does not rule out the diagnosis of tuberculosis because this is an insensitive procedure and some lesions contain very few organisms. The sensitivity of acid-fast staining procedures can be increased by examining tissue sections or concentrated homogenised tissues rather than impression smears of lesions (38). Lesions containing acid-fast bacteria may also be caused by species of mycobacteria other than *M. bovis* (Table IV).

Bacterial culture is the gold standard for establishing a diagnosis of tuberculosis. Specialised facilities and methods are required for the isolation and identification of mycobacteria. Particular attention must be placed on the use of good laboratory practices and safety equipment in order to prevent infections of laboratory staff. Considerable care must be taken when collecting samples for mycobacterial culture to avoid contaminating the samples with environmental bacteria. Once collected, samples should either be cultured immediately or chilled to prevent multiplication of environmental bacteria in the samples. If samples can reach the culture laboratory within 48 h, chilling to 2°C-8°C is sufficient to prevent problems with contaminants. If longer delays are likely to occur in the delivery of samples to a laboratory, samples for culture should be frozen.

The critical aspects of bacterial culture are the procedures used for processing and decontaminating samples, the culture media employed and the methods used for identifying mycobacteria (68). Chemicals used to decontaminate samples prior to culture include cetyl pyridinium chloride, sodium hydroxide, sulphuric acid and oxalic acid. Cetyl pyridinium chloride has been demonstrated to be particularly good at killing contaminating microbes without markedly affecting the viability of *M. bovis* (28). The use of both an agar-based medium (7H11) and an egg-based medium (Stonebrink or Löwenstein Jensen supplemented with pyruvate) is recommended for the isolation of *M. bovis*. Media used for the isolation of *M. tuberculosis*, such as Löwenstein Jensen supplemented with glycerol, do not support the growth of *M. bovis*. Liquid culture based systems, especially BACTEC, are rapid, but are subject to overgrowth with contaminating organisms.

Mycobacterial isolates can be identified by classical microbiological procedures using a panel of tests such as acid-fast staining, growth characteristics and selected biochemical tests (68). While classical *M. bovis* is the member of the *M. tuberculosis* complex most often found in free-ranging wildlife, other members of the complex have been isolated from this group of animals. The *M. tuberculosis* complex previously was comprised of *M. tuberculosis*, *M. africanum*, *M. bovis* and *M. microti* (19), but the recent application of DNA-based tests has been pivotal in the identification of the additional members *M. canetti* (91), *M. caprae* (1) and a distinct group of strains from seals (29, 99) (Table V). Tuberculosis has been recognised in free-ranging marine mammals from South America and Australasia and was initially thought to be caused by a group of highly related strains of *M. bovis* (29). Subsequently, the mycobacteria isolated from marine mammals were shown to have similar molecular and phenotypic characteristics and cluster into a group that was distinct from *M. bovis* and *M. tuberculosis* (99). Interestingly, members of this distinct group have also been recovered from a seal trainer (20, 88). The importance of the identification of new members of the *M. tuberculosis* complex lies in providing the opportunity to obtain a clearer understanding of the epidemiology of mycobacterial infections. This information should not be lost in arguments as to whether or not these new members of the *M. tuberculosis* complex should be designated as full species or subspecies of the genus *Mycobacterium*.

Rapid DNA amplification procedures

Although bacterial isolation is a sensitive and specific method for obtaining a diagnosis of tuberculosis, the technique has the significant disadvantage of requiring several weeks to months to isolate and then speciate a mycobacterium. Procedures based on DNA amplification, including the polymerase chain reaction (PCR), have the potential to be sensitive, specific and rapid. The DNA amplification tests used for the rapid identification of *M. tuberculosis* and *M. bovis* have used DNA sequences such as IS*6110*, hsp65 and IS*1081* which are present in all members of the *M. tuberculosis* complex (Table V). This is not seen as a disadvantage because all members of the complex are either pathogenic in the hosts for which these tests are applied or have a very restricted host distribution. Different commercially-available tests produced for the rapid identification of *M. tuberculosis* in humans with suspected tuberculosis have been based on PCR, ligase-chain reaction and strand-displacement amplification (68). These tests work well for sputum samples that are smear positive for acid-fast organisms, but are sometimes less sensitive than culture when applied to smear-negative sputum samples.

A number of different investigators have used DNA amplification methods for the detection of *M. bovis* in tissue samples (16, 55, 94). None of these tests have achieved the sensitivity, specificity and reliability of bacterial culture. The principal technical challenges facing DNA amplification tests on tissue samples are the difficulties of extracting DNA from a tiny mass of bacteria in a very much larger complex biological matrix and purifying the DNA in such a way that it does not contain inhibitors to the amplification of DNA. These challenges are in addition to the common challenges facing all

Table V
Differential characteristics of the *Mycobacterium tuberculosis* complex

Mycobacterial species	IS*6110*	IS*1081*	MPB70	Accumulation reduction *mtp40*	Niacin	Nitrate	TCH	PZA
M. tuberculosis								
Classical	+[a]	+	+	+[a]	+	+	R	S
Asian	+[a]	+	+	+	+	+	S	S
M. africanum								
Type 1	+	+	+	+[a]	V	–	S	S
Type 2	+	+	+	+[a]	V	+	S	S
M. canetti	+	+	ND	ND	+	+	R	S
M. tuberculosis								
Seal	+	+	–[b]	+			S	S
M. bovis	+	+	+	–	–	–	S	R
M. bovis BCG	+	+	+	–	–	–	S	R
M. microti	+	+	+	–	+	–	S	S
M. caprae	+	+	+	–	–	–	S[c]	S

+ : positive
– : negative
ND : not done
R : resistant
S : sensitive
V : variable
IS : insertion sequence
MPB : mycobacterial protein band
Mtp : mycobacterial protein tuberculosis
PZA : pyrazinamide
TCH : thiophene 2 carboxylic acid hydrazide

a) occasionally members lack these genetic elements
b) status uncertain
c) resistant to 1 and 2 µg/ml TCH ml^{-1}, but sensitive to 5 and 10 µg/ml TCH ml^{-1}

diagnostic DNA amplification tests which include the need to prevent contamination of samples with amplified DNA and the requirement for a robust test that can be reliably used in a diagnostic laboratory rather than in a research facility. Although current amplification methods are not ideal, they can be a valuable diagnostic method when used on selected samples, especially those containing moderate to large numbers of acid-fast organisms.

In addition, PCR tests have been developed for use on formalin-fixed tissues, and are useful when unpreserved tissues are not available for culture (64), especially in the case of museum specimens. The limitation of procedures developed for examining formalin-fixed tissues is the lack of sensitivity, and care should be taken when interpreting negative PCR results on samples containing few acid-fast organisms.

DNA fingerprinting

The DNA typing procedure is now widely used for studying the epidemiology of bacterial infections. The ideal typing procedure should be rapid, easy to perform, amenable to computerised recording and able to identify sufficient genetic variation to yield useful epidemiological information. Many of these criteria have been met for DNA typing of the human tuberculosis organism, *M. tuberculosis*, using the procedure based on the DNA insertion element IS*6110*. While a number of different DNA typing systems have been used for examining strains of *M. bovis*, none of the typing methods is ideal (20).

The first useful typing system for *M. bovis* was restriction endonuclease analysis (REA) (22). This procedure has been used extensively in New Zealand to study the epidemiology of bovine tuberculosis, in particular, the complex cycle of infection involving wildlife and domestic animals. Initial studies showed that *M. bovis* isolates from possums from different areas of New Zealand had different REA types (23). Subsequently, it was demonstrated that brushtail possums, other wildlife species and domestic animals in the same area were often infected with the same REA types, indicating a cycle of infection between wildlife and domestic animals (24, 20). When a single REA type is found among animals in a previously disease-free area, this is an indication of a single source of infection. If that type has been seen before, this can direct attention to the possible sources of infection and exclude others. Currently, DNA typing of *M. bovis* by REA is used routinely as an epidemiological tool as part of the bovine tuberculosis control programme. While REA has the sensitivity to yield valuable epidemiological information, the method has the disadvantages of being technically difficult to perform, not easily computerised and slow, requiring eight weeks to examine strains.

Other DNA typing methods that have been used to examine strains of *M. bovis* include spoligotyping and restriction fragment polymorphisms based on the polymorphic guanine and cytosine (GC)-rich repetitive sequences (PGRS), a region of direct repeat sequences and IS*6110* (20). Spoligotyping, which utilises the variation in the number of direct repeat sequences

has the advantage of being able to rapidly examine strains. The principal limitation is the relatively modest ability to discriminate between different strains of *M. bovis*. In a study by Collins, representatives of twenty-four REA types found in a small area of New Zealand were differentiated into only seven spoligotypes and eleven PGRS types (21). However, spoligotyping has proved to be very useful for differentiating into subgroups members of the *M. tuberculosis* complex (Table V) (91). Furthermore, spoligotyping has been recommended as a preliminary typing screen (30). In the Michigan outbreak, restriction fragment length polymorphism (RFLP)/IS*6110* has proven useful for identifying isolates from *M. bovis* infected wild deer, farmed deer, domestic cattle and various non-cervid species as belonging to a common strain. The techniques have also distinguished the Michigan wildlife strain from *M. bovis* strains isolated from human cases diagnosed in the state over the past decade, thus far ruling out zoonotic transmission from infected wildlife. In the Kruger National Park in South Africa an RFLP technique using IS*6110* has been used to type the isolates from the various maintenance and spillover hosts (92). A single dominant genotype (identified as ZA-01) was found to be the cause of infection in most buffalo and spillover hosts, although a cluster of cases in kudu appears to be due to a slightly different genotype with relatively close homology. The dominant ZA-01 genotype was also isolated from cattle south of the Kruger National Park, from whence the initial source of infection of buffalo was suspected to have originated (36). The development of new molecular biological techniques and the increasing availability of genomic DNA sequence data will almost certainly form the basis of new DNA typing methods for *M. bovis* (70).

A further application of DNA techniques is the analysis of specific genetic markers (gender specific microsatellite loci and mitochondrial DNA) to investigate genealogical risks of *M. bovis* transmission in white-tailed deer (8). Genetic markers have also been used as a means of verifying ambiguous location data by comparison of the genetic homogeneity of deer from different geographic areas, and as a quality assurance safeguard to link biopsy samples with specific case animals throughout the diagnostic process.

Surveys of wildlife for *Mycobacterium bovis*

The demonstration that wildlife can act as a reservoir of *M. bovis* for domestic animals has led to an increasing number of wild animal populations being examined for this pathogen. Although robust surveys based on statistically sound sampling are ideal, these may be difficult to achieve due to indigenous wildlife being protected and the expense of examining the desired number of animals. If wildlife are protected, consideration may be given to using ante-mortem tests, although all of the available tests lack the required sensitivity and specificity for obtaining accurate data on the prevalence of *M. bovis* in free-ranging wildlife. Feral animals are generally not protected, and robust lethal sampling of feral animals is generally less of a welfare issue, and receives better support from conservationists and the public at large. The post-mortem examination of wildlife is the method most commonly used for surveying wildlife for tuberculosis. In some cases, wildlife are killed for food and sport (red deer, white-tailed deer, feral pigs) or population control (African buffalo, bison) and the carcasses can be examined in the field or in an abattoir. The post-mortem examination of deer shot by hunters has been crucial in Michigan for identifying the counties that contain *M. bovis*-infected wildlife. Similarly, the examination of badgers killed on roads in Great Britain has been an important source of information for defining the distribution and prevalence of tuberculosis in this host (17). In addition, deer obtained from non-hunt sources (crop damage mitigation and disease control permits, deer killed in traffic accidents, and those found dead) comprise an appreciable, and growing, proportion of sampled animals, increasing the representation and power of the survey. Integral to the success of all these surveillance efforts are public education/outreach programmes which inform hunters, farmers, rangers, ecologists and wildlife enthusiasts of the biology of the disease, its potential impact on wildlife, and its potential zoonotic and agro-economic costs. The ability of the public to recognise suspect tuberculosis lesions, and their willingness to report them to wildlife agencies passively, has proven a valuable and cost-effective supplement to active agency surveillance.

A number of different wildlife species may be present in areas where surveys for bovine tuberculosis need to be undertaken. The choice of which species to examine will depend on the likely prevalence of *M. bovis* within each species, their abundance and the ease of sampling the different hosts. Often the likely prevalence will be quite difficult to determine, particularly for species whose unique physiology, natural history and/or behaviour may be poorly characterised. Less abundant species may need to be sampled continuously over a long period of time in order to accumulate a sufficiently representative sample. In many cases, the species sampled will be the principal, maintenance host, such as white-tailed deer in Michigan, badgers in Great Britain and African buffalo in South Africa. In other cases, the choice of which species to survey may be driven solely by the identification of similarly-named species as maintenance hosts elsewhere (e.g. the brushtail possum and the Virginia opossum [*Didelphis virginiana*] or the Eurasian badger and the American badger [*Taxidea taxus*]), even though key biological and behavioural characteristics of the analogous species differ substantially.

In some areas of New Zealand, surveys of wildlife populations for *M. bovis* are conducted on ferrets in preference to the principal maintenance host, the brushtail possum, reflecting the much higher prevalence in the former species (59). The use of feral pigs for survey purposes is also being examined in

New Zealand. Tuberculosis-free feral pigs fitted with radio collars are released to determine the existence of *M. bovis* infection in an area. Several months after release, the pigs are recaptured and examined *post mortem* to determine whether infection with *M. bovis* has occurred by scavenging infected wildlife (G. Nugent, personal communication). In Michigan, attention is being focused on the potential use of coyotes (*Canis latrans*) as sentinels of bovine tuberculosis in white-tailed deer. Of the sixteen non-cervid species tested to date, coyotes have shown the highest apparent prevalence of *M. bovis* infection (13/287 [4.5%]) amongst species for which appreciable sample sizes are available. Moreover, infected coyotes have been found both in counties where apparent prevalence in deer is comparatively high, and in those in which it is probably very low. Such a sentinel, if validated, could prove useful in surveillance, being abundant, widely distributed, publicly maligned, and logistically simpler to sample than deer. Notably, pathological examinations reveal little evidence to suggest that coyotes develop the extensive lesions of *M. bovis* probably necessary to transmit the disease readily, suggesting that these animals, like all of the other non-cervid species surveyed in Michigan to date, act as spillover, rather than maintenance hosts (9).

In the Kruger National Park, sampling of mammalian predators/scavengers at the top of the food chain may also prove to be a sensitive indicator of the presence of infection in different regions of the Park (53). The use of these carnivorous sentinels makes a lot of sense, epidemiologicaly speaking, because these animals prey on weak debilitated animals or scavenge on carcasses, and thus serve as *M. bovis* filters. The carnivores become infected, diseased (see clinical signs, Table I) and progressively debilitated. In addition, acceptable sensitivity (84%) and specificity (96%) using an intradermal tuberculin test in lion, allows for non-lethal sampling of these populations (51, 53).

In addition to these established sites of wildlife tuberculosis, new areas afflicted with the disease continue to emerge. A potential reservoir of *M. bovis* in elk (*Cervus elaphus*) in Riding Mountain National Park, Manitoba, Canada, has recently been identified (61). A small number of cases of bovine tuberculosis have occurred sporadically in cattle herds occupying land adjacent to the park since 1991, and a bull elk was confirmed positive nearby in 1992. A comprehensive wildlife health monitoring survey in the vicinity of this case between 1997 and 2000 has identified a further four free-ranging infected animals out of the 420 elk sampled. While outbreak control programmes are currently under development, land use patterns (by which park areas are surrounded by cattle pasture land) and livestock feed management practices (which fail to exclude elk from hay yards intended for cattle) continue to present formidable challenges (80).

Management

Bovine tuberculosis in free-ranging wildlife may need to be controlled to protect valuable or endangered wildlife species or prevent the spread of infection to domestic animals. The tools for controlling *M. bovis* in wildlife are limited and have recently been reviewed (13, 35, 83). A thorough understanding of the epidemiology of bovine tuberculosis in wildlife is essential for the development and implementation of control programmes. Mathematical modelling has the potential to play an important role in identifying successful control procedures and the most efficient use of these procedures. In some areas where bovine tuberculosis in wildlife needs to be controlled, more than one host may be infected but control measures may need to focus only on the principal or sole maintenance host. For example, in Northern Australia, wildlife control was restricted to culling feral water buffalo, a known maintenance host. Feral pigs were identified as being spillover hosts which did not require culling. The success of this policy is evidenced by the decline in prevalence of bovine tuberculosis in feral pigs which was associated with measures taken to control the infection in cattle and water buffalo. In 1992, a prevalence of 0.25% was recorded in the feral pig population, compared to 19% in 1973 (27, 62). Similar (lethal) major interventions amongst indigenous wildlife maintenance hosts would not be morally or ecologically justifiable in the eyes of many conservationists and the public at large.

In a restricted number of cases, relatively simple measures can be taken to prevent the spread of infection to wildlife species which are not maintenance hosts. In a troop of free-ranging chacma baboons (*Papio ursinus*) in the Kruger National Park, the prevalence of infection with *M. bovis* reached 50% as a result of the baboons sleeping in an unused elevated pump house. After access to this structure was prevented, and the positive baboons were removed from the troop, the disease in these baboons disappeared (50). One possible initial source of infection for this troop was material from a local post-mortem facility. Abattoir material was also suggested as a likely source of infection for olive baboons (*Papio cynocephalus anubis*) in the Masai Mara Game Reserve (85). Since the recognition in 1995 that *M. bovis* had become established as a self-sustaining disease in Michigan white-tailed deer, an ad hoc control programme has been developed and implemented, as no previous, comparable outbreak or intervention programme existed. Wildlife biologists, epidemiologists and veterinarians, working in concert, have implicated two probable principal factors in the establishment and spread of the disease, as follows:

a) high deer population densities

b) focal concentration and aggregation of deer caused by the human practices of baiting (attracting deer with feed for the purpose of hunting) and recreational/supplemental feeding.

Corresponding with these factors, two broad intervention strategies aimed at reducing transmission have been advanced. Firstly, reduction in the size of the deer population by hunters has been encouraged by liberalising regulation, through issuance of unlimited permits for the hunting of deer without antlers. This is expected to decrease the overall number of deer by reducing the reproductive capacity of the population, diminish recruitment of offspring into the breeding population, and shift the age structure of the population towards younger animals. Secondly, the need to stop baiting and supplementary feeding was an important measure for the control of bovine tuberculosis in white-tailed deer (73). Baiting and supplementary feeding brings together large numbers of deer into close proximity, often nose to nose contact over a prolonged period, in contrast to the normal grazing patterns in which animals remain spread out over large areas (82). Moreover, these supplemental sources of nutrition artificially increase the size of the population beyond the natural ability of the landscape to support it. While political officials and some segments of the public have been slow to embrace the necessity of abandoning these long established and popular practices, the scientific community has recognised that their elimination will further reduce the size of the deer population (as herd density approaches biological carrying capacity), and decrease both direct and indirect contacts among deer. Baiting and feeding of deer have been banned since 1998 in counties in which *M. bovis* positive deer have been identified. Additionally, population estimates suggest that the size of the deer herd has been halved in the five counties in which tuberculosis is most prevalent, largely through increased harvest of deer without antlers. Apparent prevalence in the same area has also declined by half since 1997, providing hopeful preliminary evidence that eradication strategies are succeeding. Strikingly, public perceptions of the differential value of favoured species have proven the principal obstacle to aggressive population control efforts. White-tailed deer are widely venerated in Michigan (the species has been proclaimed the official state mammal by legislative action), to the point that even ills directly attributable to high deer densities (habitat damage, fatal vehicular collisions) are largely overlooked by the public. As a result of these attitudes, some of the highly effective strategies (e.g. poisoning, aerial shooting) that have been used in New Zealand and Australia have been impossible to implement thus far in Michigan.

Control of tuberculosis based on test-and-slaughter principles, although very successful for cattle, will have a very limited role for the control of bovine tuberculosis in free-ranging wildlife until improved diagnostic tests are developed. Even if effective ante-mortem tests become available, accessing sufficiently large numbers of animals for testing to be representative and capable of detecting the disease at a low prevalence will remain a formidable task.

Where wildlife species are infected but not protected (usually feral), culling may be used to control bovine tuberculosis. In New Zealand, culling of brushtail possums is a central component of the campaign to control bovine tuberculosis in cattle and farmed deer. Currently, numbers of brushtail possums in New Zealand are being controlled by the use of poisons and trapping over approximately three million hectares, at a cost of US$15 million per year (58). The wildlife control programme has been central in reducing the number of infected cattle and deer herds by over 60% between 1994 and 2001. In some parts of New Zealand, the wildlife control programme has included the culling of ferrets in addition to reducing possum numbers. Some debate has arisen as to whether ferrets are a maintenance host of *M. bovis* (66). Results from a number of surveys indicate that brushtail possums may be the underlying source of infection for most ferrets. A reduction in the prevalence of *M. bovis* infection in ferrets has been observed in areas where numbers of brushtail possums but not ferrets have been reduced (P. Caley, personal communication). While ferrets may not be a true maintenance host of *M. bovis*, the work required to overwhelmingly demonstrate this has not been completed and many people in New Zealand believe that these animals play an important role in the spread of infection to domestic animals. Culling of wildlife, principally brushtail possums, has led to *M. bovis* being eradicated from both domestic animals and wildlife, from six small areas of New Zealand. However, whether the current wildlife control programme will result in the elimination of *M. bovis* from the entire country remains doubtful.

The practice of culling indigenous badgers in the United Kingdom for the control of bovine tuberculosis has been highly controversial. Opposition to the culling of badgers is based on animal welfare grounds, the lack of data to conclusively demonstrate a direct causal link between infected badgers and the occurrence of tuberculosis in cattle, as well as the abhorrence of killing a much treasured wildlife species. The decline in the prevalence of *M. bovis* infection in cattle in areas in which badger control has been undertaken provides the most compelling evidence that badgers are an important reservoir of infection for cattle. For example, all badgers were removed from a 104 km^2 area at Thornbury, in south-east England between 1975 and 1981 (18). The incidence of herds with visible lesion reactors in this area fell from 5.6% prior to badger control, to 0.06% after control. This data needs to be interpreted with some caution because of the lack of information regarding the incidence of tuberculosis in cattle in a comparable area where badgers were not controlled. The role of the badger in the spread of bovine tuberculosis has recently been re-examined by an independent scientific review group, led by John Krebs (49). The results of this analysis were that 'The sum of evidence strongly supports the view that, in Britain, badgers are a significant source of infection in cattle. Most of the evidence is indirect, consisting of correlations rather than demonstrations of cause and effect; but in total the available evidence, including the effects of completely removing badgers from some areas is compelling'. The Krebs review recommended performing a controlled culling trial to provide better data to demonstrate the link between bovine tuberculosis in badgers and cattle. Even if such data is produced, public opinion appears extremely likely to prevent the use of large scale culling of badgers for the control of bovine tuberculosis in the United Kingdom. In many countries,

willingness of the public to accept or allow various control measures is the deciding factor bearing upon which management practices will be used to control bovine tuberculosis in indigenous wildlife.

In South Africa, lethal management of infected buffalo has been considered. Currently in the Hluhluwe/Umfolozi Park, a 'capture-test-and-slaughter' policy is employed, with test negative animals being released. Preliminary data appears to indicate that the capture and holding of the buffalo in a confined 'boma' for 72 h, before the intradermal tuberculin test can be read, may significantly increase transmission rate between infected and uninfected individuals (D. Cooper, personal communication).

In the Kruger National Park lethal management of certain high prevalence herds or infected herds geographically located within a relatively low prevalence area of the Park, has been considered. The use of buffalo depopulated or fenced buffalo-free buffer zones to separate the high/medium tuberculosis prevalence zones from the low/zero prevalence zones has also been suggested. Central to the problem are the following:

– buffalo are indigenous wild bovids with an important 'bulk feeding' role in the grazing hierarchy of herbivores in this ecosystem

– the Kruger National Park comprises an effective area of 22,000 km^2, and a buffalo population of 22,000, consisting of approximately 100 fairly evenly scattered herds. A dynamic exchange of individuals or small groups regularly occurs between herds, therefore determination of the northernmost point of infection in order to delineate a control option is difficult (35)

– the Kruger National Park has a relatively high biodiversity, including 147 mammalian species of which 65% can be considered 'small mammals', including many rodents, lagomorphs, mustelids and viverrids (74). Amongst the other 35%, comprising the larger mammals, are several gregarious species other than buffalo, such as impala, wildebeest, zebra, elephant, waterbuck, greater kudu, warthogs, hippopotamus, white rhino, sable antelope and lion. A mustelid (badger) is known to be an important tuberculosis maintenance host in Britain and Ireland, and most gregarious ruminants theoretically have maintenance host potential. With this background knowledge, the launch of a major buffalo depopulation exercise may be considered irresponsible if other maintenance hosts are present in the system. In South Africa at least, historical and current findings are pointing increasingly to greater kudu as a maintenance host

– if major destruction of infected buffalo herds is initiated, there must be reasonable certainty that entire herds and eventually entire sub-populations can be effectively extirpated. Herds in the Kruger National Park average approximately 270 individuals, and the task will be technically difficult, if not impossible to achieve, given the remoteness and inaccessibility of large areas of the Park, and given the fact that when buffalo herds are frequently harassed, they tend to 'bombshell', and splinter groups will then join up with other herds.

Thus, in the Kruger National Park, containment or control exercises designed to reduce prevalence, such as test-and-slaughter, selective depopulation of high prevalence herds, or buffalo exclusion zone, may only be appropriate in the interim management of the disease (3).

In the interim, the Kruger National Park has instituted a successful breeding programme which produces buffalo calves free from tuberculosis, brucella, foot and mouth disease and theileriosis. These animals are removed from the Park to set up a 'disease-free' breeding herd of Kruger National Park genotype buffalo in a remote location, for re-stocking conservation areas at a later stage (6).

Vaccination has been recognised as a possible means of controlling bovine tuberculosis in free-ranging wildlife (13, 83). Initially, interest in the vaccination of wildlife was focused on halting the spread of infection to domestic animals. More recently, vaccination for the protection of endangered or valuable wildlife species has also received attention. Successful vaccination of wildlife against bovine tuberculosis requires a safe and efficacious vaccine which is inexpensive, requires a single administration, and is easy to deliver. Currently, the only available vaccine that could be used for vaccinating wildlife is the live attenuated *M. bovis*, bacillus Calmette-Guérin (BCG) which has been used extensively in humans. Even though BCG has been more widely used in humans than any other vaccine, its efficacy has been variable. In controlled trials, the level of protection in humans has varied from 0% to over 70%. Moderate levels of protection have been observed in brushtail possums, badgers and ferrets which have been vaccinated with BCG and experimentally challenged with virulent *M. bovis* (83). The variable efficacy of BCG and the importance of tuberculosis in humans has been the stimulus for a number of research groups to initiate programmes to develop new tuberculosis vaccines. While most of these studies have the goal of producing improved tuberculosis vaccines for humans, many of the findings of these studies will be directly applicable to the development of vaccination strategies for wildlife. Approaches being used to develop new vaccines include the inactivation of virulence or 'house keeping' genes of *M. bovis* or *M. tuberculosis*, and the investigation of a range of different non-living vaccines, including killed mycobacteria, subunits of mycobacterial proteins and DNA vaccines (83). A major challenge to overcome in the vaccination of free-ranging wildlife is the development of cost-effective strategies to immunise animals. Vaccination through the use of baits which are eaten by the target animal have been used successfully to deliver rabies vaccines to semi-domesticated dogs, wild foxes, jackals and racoons (56, 71, 78). Oral baits are readily accepted by brushtail possums and badgers, indicating that this would be a practical means of delivering a tuberculosis vaccine to these species. Delivery of vaccines by this route does have some significant limitations.

Levels of immune response induced by non-living vaccines delivered by the oral route are usually poor, even with the addition of mucosal adjuvants. While live vaccines can induce immune responses when administered orally, the bactericidal effects of the stomach can markedly affect the efficacy of the vaccine. Buddle *et al.* demonstrated that BCG produced higher levels of protection in the brushtail possum when introduced directly into the duodenum rather than being administered orally (12). Strategies to overcome this problem of gastric inactivation include micro-encapsulation of live tuberculosis vaccines to protect the vaccine during transit of the stomach. In Africa, certain species such as buffalo and kudu in large extensive conservation areas are never artificially fed, and an appropriate bait-vehicled vaccine that might work is difficult to conceive. However, predators should prove no problem for bait-based vaccination. Other future alternatives may include helicopter-delivered aerosolised vaccines which work via the respiratory mucosa, or self-replicating recombinants that incorporate important mycobacterial antigens.

Unquestionably, the development of effective vaccines holds great promise for control of wildlife outbreaks of *M. bovis* in the future, and should continue to be pursued actively. However, the logistical and economic challenges of instituting vaccination programmes in the field on a large scale should not be underestimated. An unforeseen danger of placing unrealistic faith in the pace of vaccine development is that more aggressive and less popular, but nonetheless essential, intervention efforts may be postponed in the hope that a 'magic' vaccine will be found. History has proven control of tuberculosis in wildlife to be sufficiently onerous even when control efforts are instituted promptly, and unwarranted delays in implementation may make the task yet more difficult.

Future directions

As countries embark on programmes to control bovine tuberculosis in domestic animals, determination of the role of wildlife species as sylvatic reservoirs of *M. bovis* will become increasingly necessary. This will require the use of the appropriate diagnostic procedures to perform robust epidemiological investigations of different wildlife species. While improvements in diagnostic tests for tuberculosis are likely in the medium term, the challenges of sampling wildlife populations will remain. Where control of *M. bovis* in wildlife is necessary, especially in those countries with infected wildlife that are protected, vaccination is, in the long term, the most promising and acceptable control method. Recent advances in molecular biology and an improved understanding of the immunology of mycobacterial infections are likely to result in the development of more effective tuberculosis vaccines, as well as methods to cost-effectively deliver these vaccines to wildlife.

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La tuberculose chez les animaux sauvages vivant en liberté : détection, diagnostic et gestion

G.W. de Lisle, R.G. Bengis, S.M. Schmitt & D.J. O'Brien

Résumé

Mycobacterium bovis est un agent pathogène dont l'importance ne cesse de croître chez les animaux sauvages vivant en liberté ; il y constitue une source d'infection potentielle pour les animaux domestiques et une menace pour les espèces sauvages considérées de grande valeur. Les auteurs décrivent les procédures de détection, de diagnostic et de maîtrise de *M. bovis* dans les populations d'animaux sauvages. La détection *ante mortem* de l'infection due à *M. bovis* chez les animaux sauvages est difficile en raison de la fréquence d'infections inapparentes et des imperfections des épreuves de diagnostic actuellement disponibles. Les tests sérologiques sont peu sensibles alors que ceux mesurant la réponse à médiation cellulaire, tout en étant prometteurs, ne sont pas encore au point pour une utilisation de routine chez la plupart des espèces. Le diagnostic de *M. bovis* chez les animaux vivant en liberté repose sur l'examen *post mortem* ainsi que sur des études histopathologiques et microbiologiques. L'une des caractéristiques des infections dues à *M. bovis* est la différence d'aspect et de

répartition des lésions chez les diverses espèces hôtes. La mise en culture bactérienne reste la méthode de référence pour le diagnostic de la tuberculose alors que l'histopathologie se heurte souvent à la difficulté de distinguer les lésions dues à *M. bovis* de celles provoquées par d'autres espèces mycobactériennes. L'identification à l'aide de l'empreinte de l'acide désoxyribonucléique (ADN) et les techniques modernes de typage sont de plus en plus utilisées pour élucider l'épidémiologie des mycobactérioses, y compris la tuberculose chez les animaux sauvages vivant en liberté. La compréhension de l'épidémiologie est indispensable à l'élaboration de procédures de maîtrise de la tuberculose dans la faune sauvage. Les méthodes prophylactiques actuellement disponibles sont peu nombreuses, notamment pour les espèces sauvages protégées. De nombreux travaux de recherche sont en cours sur la vaccination mais il faudra encore du temps avant qu'elle puisse être utilisée pour lutter contre la tuberculose chez les animaux, notamment les animaux sauvages vivant en liberté.

Mots-clés
Diagnostic – Faune sauvage – Mycobacterium bovis – Tuberculose bovine – Vaccination.

Detección, diagnóstico y gestión de la tuberculosis en animales salvajes en libertad

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Resumen
Mycobacterium bovis está cobrando cada vez más importancia como patógeno de los animales salvajes en libertad, desde los cuales puede propagarse no sólo a la fauna doméstica sino también a especies salvajes particularmente valiosas por una u otra razón. Los autores pasan revista a los procedimientos de detección, diagnóstico y gestión de *M. bovis* en poblaciones salvajes. La detección *ante-mortem* del patógeno en la fauna salvaje resulta difícil por la frecuencia con que aparecen infecciones subclínicas y por las deficiencias de las pruebas de diagnóstico disponibles. Los ensayos serológicos carecen de sensibilidad, y las pruebas que determinan la intensidad de la respuesta inmunitaria celular, aunque resultan prometedoras, no están lo bastante contrastadas como para aplicarlas sistemáticamente a la mayoría de las especies. El diagnóstico de *M. bovis* en animales salvajes en libertad sigue dependiendo de la realización de una autopsia y los subsiguientes análisis histopatológicos y microbiológicos. Uno de los rasgos característicos de las infecciones por *M. bovis* es la variabilidad en la apariencia externa y la distribución de las lesiones según la especie de que se trate. El cultivo en medio bacteriano sigue siendo el método de referencia para diagnosticar la tuberculosis, mientras que los análisis histopatológicos resultan de poca utilidad en los casos, bastante frecuentes, en que no es posible distinguir entre las lesiones debidas a *M. bovis* y las causadas por otras especies micobacterianas. Cabe señalar el uso cada vez más extendido de la identificación por huella del ADN (ácido desoxirribonucleico) y otras técnicas avanzadas de tipificación para desentrañar la epidemiología de las infecciones micobacterianas (entre ellas la tuberculosis en animales salvajes en libertad). Para elaborar protocolos de gestión de la tuberculosis en la fauna salvaje es indispensable entender previamente la epidemiología de la enfermedad. Por ahora no están muy claros los procedimientos de gestión que resultan más adecuados, sobre todo en el caso de especies salvajes protegidas. Aunque abundan las investigaciones sobre el tema de las vacunas, las técnicas no están a punto para controlar la tuberculosis en animales de cualquier tipo, y todavía menos en la fauna salvaje en libertad.

Palabras clave
Diagnóstico – Fauna salvaje – Mycobacterium bovis – Tuberculosis bovina – Vacunación.

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