

**Mycobacterium bovis** in free-living and captive wildlife, including farmed deer

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**Summary**

*Mycobacterium bovis* has been isolated from a wide range of wildlife species, in addition to domestic animals. This review examines the role played by various species in the maintenance of *M. bovis* in wildlife communities and the spread to domestic animals. Badgers (*Meles meles*), brushtail possums (*Trichosurus vulpecula*), deer (*Odocoileus virginianus*), bison (*Bison bison*) and African buffalo (*Syncerus caffer*) are examples of wildlife that are maintenance hosts of *M. bovis*. The importance of these hosts has been highlighted by the growing realisation that these animals can represent the principal source of infection for both domestic animals and protected wildlife species.

The range of methods for controlling *M. bovis* in wildlife is limited. While population control has been used in some countries, this approach is not applicable in many situations where protected wildlife species are concerned. Vaccination is a potential alternative control method, although as yet, no practical, effective system has been developed for vaccinating wildlife against bovine tuberculosis.

Tuberculosis caused by *M. bovis* has also been a problem in captive wildlife and in recently domesticated animals such as farmed deer. Control of *M. bovis* in this group of animals is dependent on the judicious use of diagnostic tests and the application of sound disease control principles. The advances in the development of bovine tuberculosis vaccines for cattle and farmed deer may offer valuable insights into the use of vaccination for the control of tuberculosis in a range of captive wildlife species.

**Keywords**


**Introduction**

*Mycobacterium bovis*, the cause of bovine tuberculosis, has one of the broadest host ranges of all pathogens, in marked contrast to other members of the Mycobacterium tuberculosis complex (*M. africanum*, *M. bovis*, *M. canetti*, *M. microti* and *M. tuberculosis*), especially *M. tuberculosis*. This paper reviews *M. bovis* infections in free-living wildlife (Table I) and captive wildlife including farmed deer (Table II). Mycobacterium bovis infections in domestic animals are reviewed in a separate paper in this issue of the Review (39). Given the vast amount of literature on *M. bovis* infections, only selected information is referred to in this paper. Additional information can be obtained from two excellent reviews of *M. bovis* infections (58, 112). The section on *M. bovis* infections in free-living wildlife concentrates on the most-studied wildlife populations, principally those in New Zealand, the United Kingdom (UK), Ireland, North America and Africa. Particular attention is given to the relationship between various hosts within a given wildlife population and the effects of these hosts on the maintenance of *M. bovis* and the spread of infection to other wildlife and domestic animals. In a number of countries, the failure to eradicate *M. bovis* from cattle is due to the presence of a wildlife reservoir of *M. bovis*. In addition,
examples are cited of cases of *M. bovis* that may have an impact on protected wildlife species. *Mycobacterium bovis* has also caused major problems in a range of different captive wildlife species, including various species of deer which, over the last thirty years, have been captured from the wild, domesticated and farmed. Some examples of *M. bovis* in captive wildlife colonies are described, together with methods of avoiding or controlling these infections.

Table I

Reports of *Mycobacterium bovis* in free-living wildlife*

<table>
<thead>
<tr>
<th>Host</th>
<th>Country (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antelope, marsh (<em>Kobus leche</em>)</td>
<td>Zambia (27)</td>
</tr>
<tr>
<td>Baboon, olive (<em>Papio cynocephalus anubis</em>)</td>
<td>Kenya (135)</td>
</tr>
<tr>
<td>Baboon, chacma (<em>Papio ursinus</em>)</td>
<td>South Africa (85)</td>
</tr>
<tr>
<td>Badger (<em>Meles meles</em>)</td>
<td>Switzerland (14), England (109), Ireland (111)</td>
</tr>
<tr>
<td>Bear, black (<em>Ursus americanus</em>)</td>
<td>United States of America (4)</td>
</tr>
<tr>
<td>Bison (<em>Bison bison</em>)</td>
<td>Canada (136, 137)</td>
</tr>
<tr>
<td>Bobcat (<em>Lynx rufus</em>)</td>
<td>United States of America (4)</td>
</tr>
<tr>
<td>Buffalo, African (<em>Syncerus caffer</em>)</td>
<td>Uganda (71, 146), South Africa (10, 84)</td>
</tr>
<tr>
<td>Buffalo, water (<em>Bubalus bubalis</em>)</td>
<td>Australia (72, 89)</td>
</tr>
<tr>
<td>Cat, feral (<em>Felis catus</em>)</td>
<td>New Zealand (118)</td>
</tr>
<tr>
<td>Cheetah (<em>Acinonyx jubatus</em>)</td>
<td>South Africa (85)</td>
</tr>
<tr>
<td>Coyote (<em>Canis latrans</em>)</td>
<td>United States of America (4, 16)</td>
</tr>
<tr>
<td>Deer, axis (<em>Axis axis</em>)</td>
<td>Hawaii (127)</td>
</tr>
<tr>
<td>Deer, fallow (<em>Dama dama</em>)</td>
<td>England (68), New Zealand (45)</td>
</tr>
<tr>
<td>Deer, mule (<em>Odocoileus hemionus</em>)</td>
<td>United States of America (123)</td>
</tr>
<tr>
<td>Deer, red (<em>Cervus elaphus</em>)</td>
<td>New Zealand (45), England (88)</td>
</tr>
<tr>
<td>Deer, roe (<em>Capreolus capreolus</em>)</td>
<td>England (88)</td>
</tr>
<tr>
<td>Deer, sika (<em>Cervus nippon</em>)</td>
<td>England (88), Ireland (50), New Zealand (45)</td>
</tr>
<tr>
<td>Deer, white-tailed (<em>Odocoileus virginianus</em>)</td>
<td>Canada (9), United States of America (128)</td>
</tr>
<tr>
<td>Duiker, common (<em>Sylvicapra grimmia</em>)</td>
<td>South Africa (113)</td>
</tr>
<tr>
<td>Ferret (<em>Mustela putorius furo</em>)</td>
<td>New Zealand (47)</td>
</tr>
<tr>
<td>Fox, red (<em>Vulpes vulpes</em>)</td>
<td>England (88), United States of America (4)</td>
</tr>
<tr>
<td>Goat, feral (<em>Capra hircus</em>)</td>
<td>New Zealand (125)</td>
</tr>
<tr>
<td>Hare, European (<em>Lepus europaeus occidentalis</em>)</td>
<td>New Zealand (34)</td>
</tr>
<tr>
<td>Hedgehog (<em>Erinaceus europaeus</em>)</td>
<td>New Zealand (93)</td>
</tr>
<tr>
<td>Kudu, greater (<em>Tragelaphus strepsiceros</em>)</td>
<td>South Africa (11)</td>
</tr>
<tr>
<td>Leopard (<em>Panthera pardus</em>)</td>
<td>South Africa (R.G. Bengis, unpublished findings)</td>
</tr>
<tr>
<td>Lion (<em>Panthera leo</em>)</td>
<td>South Africa (65)</td>
</tr>
<tr>
<td>Lynx, Iberian (<em>Lynx pardinus</em>)</td>
<td>Spain (15)</td>
</tr>
<tr>
<td>Mink, American (<em>Mustela vison</em>)</td>
<td>England (88)</td>
</tr>
<tr>
<td>Mole, European (<em>Talpa europaea</em>)</td>
<td>England (88)</td>
</tr>
<tr>
<td>Pig, feral (<em>Sus scrofa</em>)</td>
<td>Italy (128), Spain (6), Australia (35), Hawaii (53), New Zealand (141)</td>
</tr>
<tr>
<td>Possum, brush-tailed (<em>Trichosurus vulpecula</em>)</td>
<td>New Zealand (52)</td>
</tr>
<tr>
<td>Rabbit, European (<em>Oryctolagus cuniculus cuniculus</em>)</td>
<td>New Zealand (61)</td>
</tr>
<tr>
<td>Raccoon (<em>Procyon lotor</em>)</td>
<td>United States of America (25)</td>
</tr>
<tr>
<td>Rat (<em>Rattus norvegicus</em>)</td>
<td>England (88)</td>
</tr>
<tr>
<td>Seal, Australian fur (<em>Arctocephalus pusillus doriferus</em>)</td>
<td>Australia (148)</td>
</tr>
<tr>
<td>Seal, New Zealand fur (<em>Arctocephalus forsteri</em>)</td>
<td>New Zealand (79)</td>
</tr>
<tr>
<td>Seal, subantarctic fur (<em>Arctocephalus tropicalis</em>)</td>
<td>Argentina (6)</td>
</tr>
<tr>
<td>Saa lion, South American (<em>Otaria flavescens</em>)</td>
<td>Argentina (12)</td>
</tr>
<tr>
<td>Stoat (<em>Mustela erminea</em>)</td>
<td>New Zealand (118)</td>
</tr>
<tr>
<td>Warthog (<em>Phacochoerus aethiopicus</em>)</td>
<td>Uganda (147)</td>
</tr>
</tbody>
</table>

* Only examples where *M. bovis* or a closely-related variant has been isolated are included in this list. Additional cases of tuberculosis have been reported in free-living wildlife, but the causative agent was not identified (58).
number of species referred to as ‘spill-over hosts’ become infected with *M. bovis*, but the infection will only sporadically occur or persist within these populations if a true maintenance host is present in the ecosystem. Examples of spill-over hosts are numerous, including the majority of animal species listed in Table I. If no further transmission of infection occurs from a spill-over host, it is referred to as a dead-end host. A combination of genetic susceptibility, abundance of host and behavioural characteristics will determine whether or not a species is a maintenance or spill-over host for *M. bovis*. Some species, such as rabbits, are very susceptible to *M. bovis* but are not maintenance hosts and are rarely infected naturally. An understanding of the role that each potential host may play in the maintenance and transmission of *M. bovis* within an animal community is essential for the development of successful control schemes. The role played by various hosts may change as a result of efforts to control bovine tuberculosis. For example, the control of brush-tail possums appears to affect the prevalence of tuberculosis in scavenger species such as feral pigs and ferrets (*Mustela putorius furo*).

One of the principal factors affecting the epidemiology of tuberculosis is the mode of transmission and the route of infection within and between species. These are generally indicated by the locations of the tuberculous lesions in that species. For example, the predominance of lesions in the lungs and thorax is evidence of aerosol transmission, while animals with predominantly mesenteric lesions were probably infected orally. The presence of lesions in lymph nodes of the head, as frequently detected in furred deer, may be due to either aerosol or oral transmission because the retropharyngeal lymph node receives lymphatic drainage from both the nasal and oral cavities. In some animals, including some infected brush-tail possums, cattle and lions, lesions are frequently found in the major superficial body lymph nodes, such as the prescapular or precrural nodes. A possible explanation for these cases is transmission of infection through a bite or contamination of a skin wound. Alternatively, an infection in a body node could be the result of spread within the body from a distant primary complex. Pseudo-vertical transmission of *M. bovis* from mother to offspring has been suggested as an important route of transmission in the brush-tail possum (108).

Information about the spread of *M. bovis* among species is limited. Possible means of transmission between species include direct contact with live infected animals, consumption of infected animals in the case of carnivores and omnivores and indirect contact through contamination of the environment by infected animals. A number of studies have used a range of different methods, bacterial strains and environmental conditions to examine the survival of *M. bovis* in the environment (3, 51, 81, 134). The studies are in general agreement that *M. bovis* survives for only a limited period (from three to fourteen days) when directly exposed to

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**Epidemiology of *Mycobacterium bovis* in wildlife**

Knowledge of the epidemiology of bovine tuberculosis provides a valuable insight into the relative importance of different hosts in the maintenance and spread of *M. bovis* within a locality. In the UK, New Zealand and Africa, a number of different wildlife species are infected with *M. bovis*. Many of the infected species may be of little or no consequence in the maintenance of the infection in the wild or the spread of infection to domestic livestock. A maintenance or reservoir host is required if *M. bovis* is to persist within a locality. Infection can persist in a maintenance host through horizontal transmission between individuals, in the absence of any other source of *M. bovis*. Examples of maintenance hosts include cattle, farmed and wild deer, brush-tail possums (*Trichosurus vulpecula*), badgers (*Meles meles*), bison (*Bison bison*) and African buffalo (*Syncerus caffer*). In contrast, a

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**Table II**

Reports of *Mycobacterium bovis* in captive wildlife and farmed deer*

<table>
<thead>
<tr>
<th>Host</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baboons (<em>Papio hamadryas</em>)</td>
<td>140</td>
</tr>
<tr>
<td>Baboons (<em>Papio papio</em>)</td>
<td>139</td>
</tr>
<tr>
<td>Bison (<em>Bison bison</em>)</td>
<td>21</td>
</tr>
<tr>
<td>Camel, bactrian (<em>Camelus bactrianus</em>)</td>
<td>22</td>
</tr>
<tr>
<td>Chimpanteef (<em>Pan troglodytes</em>)</td>
<td>122</td>
</tr>
<tr>
<td>Deer, axis (<em>Axis axis</em>)</td>
<td>82, 121</td>
</tr>
<tr>
<td>Deer, fallow (<em>Dama dama</em>)</td>
<td>13, 124</td>
</tr>
<tr>
<td>Deer, red (<em>Cervus elaphus</em>)</td>
<td>68, 110</td>
</tr>
<tr>
<td>Deer, roe (<em>Capreolus capreolus</em>)</td>
<td>93</td>
</tr>
<tr>
<td>Deer, sika (<em>Cervus nippon</em>)</td>
<td>98, 105</td>
</tr>
<tr>
<td>Dusky langur (<em>Presbytis obscura</em>)</td>
<td>78</td>
</tr>
<tr>
<td>Fox, fennec (<em>Vulpes zerda</em>)</td>
<td>77</td>
</tr>
<tr>
<td>Gibbon, siamang (<em>Symphalangus syndactylus</em>)</td>
<td>145</td>
</tr>
<tr>
<td>Kudu, greater (<em>Tragelaphus strepsiceros</em>)</td>
<td>76</td>
</tr>
<tr>
<td>Lemur, Mayotte (<em>Lemur mayottensis mayottensis</em>)</td>
<td>145</td>
</tr>
<tr>
<td>Leopard (<em>Panthera pardus</em>)</td>
<td>140</td>
</tr>
<tr>
<td>Leopard, snow (<em>Uncia uncia</em>)</td>
<td>73, 140</td>
</tr>
<tr>
<td>Macaque, lion-tailed (<em>Macaca sylvanus</em>)</td>
<td>145</td>
</tr>
<tr>
<td>Macaque, stump-tailed (<em>Macaca arctoides</em>)</td>
<td>122</td>
</tr>
<tr>
<td>Monkey, colobus (<em>Colobus guereza caudatus</em>)</td>
<td>131</td>
</tr>
<tr>
<td>Monkey, rhesus (<em>Macaca mulatta</em>)</td>
<td>151</td>
</tr>
<tr>
<td>Owl, Anime (<em>Gonyx leucopia</em>)</td>
<td>57</td>
</tr>
<tr>
<td>Rhinoceros, black (<em>Diceros bicornis</em>)</td>
<td>104</td>
</tr>
<tr>
<td>Rhinoceros, white (<em>Ceratotherium simum</em>)</td>
<td>131</td>
</tr>
<tr>
<td>Sea lion, Australian (<em>Neophoca cinerea</em>)</td>
<td>38</td>
</tr>
<tr>
<td>Sea lion, South American (<em>Otaria flavescens</em>)</td>
<td>24, 140</td>
</tr>
<tr>
<td>Seal, New Zealand fur (<em>Arctocephalus forsteri</em>)</td>
<td>24</td>
</tr>
<tr>
<td>Tiger (<em>Panthera tigris</em>)</td>
<td>97</td>
</tr>
</tbody>
</table>

*only examples where *M. bovis* or a closely-related variant has been isolated are included in this list. Additional cases of tuberculosis have been reported in captive wildlife, but the causative agent was not identified (58)*
sunlight. Much longer survival times (six weeks) have been recorded when \textit{M. bovis} was present in moist, shady conditions.

\textbf{Mycobacterium bovis} infection in wildlife in New Zealand

Before human settlement, approximately 1,000 years ago, bats and marine mammals were the only mammals present in New Zealand. In the 18th and 19th Centuries, a range of different species was introduced, principally from the UK and Australia, and these now form the mammalian wildlife of New Zealand (86). \textit{Mycobacterium bovis} was not isolated from wildlife in New Zealand until 1967 when the pathogen was recovered from feral pigs and brushtail possums (52). During the following few years, evidence accumulated to suggest that infected wildlife was the principal source of infection for cattle in New Zealand. The most compelling evidence for this was that removal of local brushtail possums, by trapping and poisoning, enabled eradication of \textit{M. bovis} from cattle herds using classical test and slaughter procedures. Numerous investigations since 1970 have clarified the role of various wildlife and domestic animal species in the maintenance and spread of \textit{M. bovis} in New Zealand (107, 108). Furthermore, wildlife surveys and data from cattle tuberculosis testing revealed that wildlife was infected in only some parts of the country (Fig. 1). The importance of wildlife in the spread of infection to domestic animals (cattle and farmed deer) is highlighted by the differences in herd prevalence between those areas that contain tuberculous wildlife and those that do not. In October 1999, the point prevalence of infected farmed herds in areas containing infected wildlife and in areas with no infected wildlife was 5.23\% and 0.14\%, respectively (P.G. Livingstone, personal communication).

\textbf{Australian brushtail possums}

Brushtail possums were introduced into New Zealand from Australia in the 19th Century to establish a fur industry. In the absence of any predators and with a plentiful food supply, the brushtail possum population expanded to over sixty million and colonised all areas of the country where domestic livestock were present. A feature of brushtail possums is a high susceptibility to infection with \textit{M. bovis}. Although this susceptibility has been stated to reflect a deficient cellular immune system (20), generalised cases of infection with other \textit{Mycobacterium} species, especially \textit{Mycobacterium avium}, have not been observed. The lesions of brushtail possums in the advanced stages of infection with \textit{M. bovis} have the characteristics of a very susceptible host. The most common sites for gross lesions are the superficial lymph nodes (superficial, deep axillary and inguinal nodes; 75\%) and the respiratory tract (77\%) (80). The distribution of lesions has been cited as evidence that brushtail possums are infected by either the respiratory or percutaneous routes. Tuberculous skin lesions are rare in brushtail possums, casting doubt on the percutaneous route being a common means of transmission. Affected lymph nodes are enlarged and often contain green pus, while tuberculous lungs have soft cream foci or nodules ranging from 1 mm to 60 mm in diameter. The microscopic features of the lesions in brushtail possums are characteristically poorly organised, consisting of pyogranulomas, often with extensive areas of necrosis. Very large numbers of \textit{M. bovis} are often present in these lesions. Mineralisation and fibrosis are not a feature of tuberculous lesions in brushtail possums.

Based solely on the detection of macroscopic lesions, the prevalence of tuberculosis in brushtail possums is generally low, ranging from 0\% to 3\% (74). Coleman \textit{et al.} reported isolations of \textit{M. bovis} from 53\% (36/68) of brushtail possums, a level that is at least twice the highest previously recorded (30). Although possums were examined in considerable detail and extensive use was made of bacterial culture in this survey, this does not explain the high level of infection detected in these animals. The point prevalence of infection, assessed by cross-sectional surveys, could be an unreliable guide to determining the importance of a species as a wildlife reservoir of \textit{M. bovis}. The point prevalence is influenced by the
incidence in the species and the duration of infection. As brushtail possums die soon after the infection becomes detectable, the annual prevalence of infection has been estimated to be approximately five times higher than the point prevalence estimated from cross sectional surveys (108). Clustering of infected animals and seasonal variation may also affect the prevalence of M. bovis in brushtail possums. The clustering of M. bovis-infected brushtail possums in certain locations has been identified as a central factor in the maintenance of infection in this host.

The combination of abundance of host, susceptibility to tuberculosis and sharing a habitat with domestic animals renders the brushtail possum the most important wildlife host of M. bovis in New Zealand. Interestingly, M. bovis has not been identified in free-living brushtail possums in Australia. Morris and Pfeiffer (107) listed the following evidence to support the theory that brushtail possums are a maintenance host and a reservoir of infection for domestic animals in New Zealand:

a) A clear spatial and temporal association exists between infection in possums and the incidence of infection in domestic stock.

b) Infection can persist continuously in possum populations in the absence of any evidence of infection being transmitted from domestic or other wild animals.

c) The use of control programmes to reduce the spatial density of tuberculous possums produces a prolonged reduction in the incidence of infection in those indicator species studied (principally cattle). Control efforts directed against possums would be unlikely to reduce the numbers or prevalence of tuberculosis in other infected wildlife to a sufficient degree to mimic this effect.

d) The CASTLEPOINT longitudinal study demonstrated a coherent epidemiological pattern of spatial and temporal distribution of infection, using specific restriction endonuclease types of tuberculosis in possums. This pattern is consistent with the long-term maintenance of infection in groups of possums and the transfer of infection to other species, such as cattle, without maintenance in those secondary species.

**Ferrets**

*Mycobacterium bovis* was first isolated from ferrets in New Zealand in 1982 (47). Subsequently, infected ferrets have been found in most of the areas in which other infected wildlife are known to be present. Macroscopic tuberculous lesions in ferrets are most frequently found in lymph nodes which drain the gastrointestinal tract, less commonly in the retropharyngeal and prescapular lymph nodes and rarely in the lungs or associated lymph nodes. Generalised infections involving multiple sites have been found in approximately 20% of infected ferrets (119). Tuberculous lymph nodes are often enlarged and may contain white focal lesions. This distribution of lesions is consistent with an oral route of transmission, as would be expected in a predator and scavenger such as the ferret. Lugton et al. found *M. bovis* in 30.7% (70/228) of ferrets using culture of normal and affected lymph nodes. A high percentage (27.8%) of those ferrets from which *M. bovis* was isolated did not have macroscopic lesions (95). This observation is important in that estimates of the prevalence of tuberculosis based on macroscopic findings are likely to be significantly lower than the true level of infection. In comparison, Ragg et al. found that only 17.9% of ferrets examined had macroscopic evidence of tuberculosis (118). The high prevalence of infection, especially when compared with brushtail possums, has led to much speculation as to the role of ferrets in the maintenance and spread of *M. bovis* in New Zealand. An association has been observed between the presence of *M. bovis* in ferrets and the infection of cattle or deer (120, 142). Furthermore, high levels of infection have been found in ferrets in areas that contain few brushtail possums (118). However, Caley found that the prevalence of *M. bovis* infection in ferrets was positively correlated with brushtail possum abundance, but unrelated to ferret abundance (23). These results highlight the possibility that brushtail possums are the underlying source of most *M. bovis* infections in ferrets. In a subsequent trial, Caley observed a reduction in the prevalence of *M. bovis* infection in ferrets in areas where numbers of brushtail possums, but not ferrets, had been reduced by poisoning and trapping (P. Caley, personal communication). The understanding of the role of ferrets in the maintenance and spread of *M. bovis* in New Zealand remains incomplete. Significant doubts exist as to whether ferrets are a true maintenance host of *M. bovis* (107). Although ferrets may not be a true maintenance host, many people in New Zealand believe that these animals play an important role in the spread of infection to domestic animals. An improved understanding of the role of the ferret in the spread and possible maintenance of bovine tuberculosis is required if cost-effective control schemes are to be developed.

**Wild deer**

The first case of tuberculosis in a wild deer in New Zealand was diagnosed in 1956 in an animal from the West Coast of the South Island. However, isolation of *M. bovis* from wild deer in New Zealand did not occur until 1970 (45). In the 1970s and 1980s, large numbers of wild deer were harvested and the meat was exported. Inspection of the carcasses and lungs of these animals detected tuberculosis in animals from areas in which other wildlife species were known to be infected with *M. bovis*. Prevalence estimates based on meat inspection would have markedly underestimated the true level of infection because the heads were not inspected for tuberculosis. The head is the most common site of lesions caused by *M. bovis* in both farmed and wild deer. Lugton et al. recovered *M. bovis* from 32% (34/106) of wild deer that were examined for tuberculosis (96). This level of infection is greater than that reported from other countries where *M. bovis* is present in wild deer. Such a high level of infection may be an indication of spread of infection from other wildlife, especially from brushtail possums to wild deer. Some evidence to support this hypothesis has been supplied by the
observation that the prevalence of tuberculosis in wild deer declined following the reduction in numbers of brushtail possums in the area containing the deer (G. Nugent, personal communication). While farmed deer can act as maintenance hosts of M. bovis, further evidence is required to determine whether or not the infection can persist in wild deer under the conditions present in New Zealand and in the absence of any other infected wildlife.

Other wildlife

*Mycobacterium bovis* has also been isolated in New Zealand from feral pigs (141), hedgehogs (*Erinaceus europaeus*) (93), feral rabbits (61), a hare (34), feral cats (118), stoats (*Mustela erminea*) (118), feral goats (125), feral sheep and feral cattle. No evidence has been found to suggest that any of these species play a significant role in the maintenance of *M. bovis* in an area or the transmission of *M. bovis* to domestic animals. A cross-sectional survey of feral pigs in the central South Island found evidence of tuberculosis in 31% of 251 animals examined (141). Bacteriological examination of a sample of the tuberculous pigs showed that the animals were infected with *M. bovis*. Members of the *M. avium* complex have rarely been isolated from feral pigs but are the most common cause of mycobacterial infection in domestic pigs in New Zealand (44). The possible role played by infected feral pigs in the maintenance and spread of *M. bovis* in New Zealand has not been determined. A limited amount of information suggests that feral pigs are most probably infected with *M. bovis* by scavenging carcasses of infected wildlife, especially brushtail possums, and that these pigs do not act as a maintenance host of *M. bovis* in New Zealand. Some concern has been raised that hunters may inadvertently spread *M. bovis* by introducing infected pigs to establish new hunting areas. Ferrets may then scavenge these animals. A moderate level of prevalence of *M. bovis* infection (5%) was revealed in a survey of seventy-nine hedgehogs from an area containing other infected wildlife (94). The detection of *M. bovis* in the tonsils or retropharyngeal lymph nodes of five of these deer, together with observational studies, led to the conclusion that these animals most probably became infected through close inspection and investigation of tuberculous brushtail possums. Indirect spread from contaminated pasture is also a possibility, especially considering the very large number of *M. bovis* present in the pus of brushtail possums with discharging lesions.

Transmission of *Mycobacterium bovis* between wildlife and domestic animals

The spread of *M. bovis* between wildlife and domestic animals in New Zealand has been the subject of much speculation. The patchy distribution of infected wildlife in New Zealand supports the hypothesis that spread of infection from domestic animals to wildlife has been a rare event. In marked contrast, spread of infection from wildlife to domestic animals is a very common event. Morris and Pfeiffer suggest that infected deer may have played a central role in the initiation of new areas of wildlife infection (107).

Since the 1980s, on at least three occasions, infected brushtail possums have been found in areas adjacent to deer farms that have reported multiple clinical cases of bovine tuberculosis. Furthermore, possums have recently been observed feeding on deer carcasses (G. Nugent, personal communication). Nevertheless, the presence of infected brushtail possums in at least one area of New Zealand in which deer are absent demonstrates that other species can play a role in the initiation of new areas containing *M. bovis*-infected wildlife.

Direct evidence to demonstrate the way in which *M. bovis* spreads from wildlife to domestic animals is scarce. A series of studies investigated the response of cattle and farmed deer to sedated brushtail possums and ferrets, to determine possible means of transmission of *M. bovis* from wildlife to domestic animals (115, 126). Cattle and farmed deer showed considerable interest in sedated brushtail possums by sniffing and licking the animals, but significantly less interest in sedated ferrets. The hypothesis arising from these studies is that cattle and farmed deer would be likely to become infected by direct or very close contact with brushtail possums that were terminally ill with tuberculosis. Further evidence to support this hypothesis was provided by a study in which six farmed deer were observed on pastures visited by tuberculous brushtail possums (94). The detection of *M. bovis* in the tonsils or retropharyngeal lymph nodes of five of these deer, together with observational studies, led to the conclusion that these animals most probably became infected through close inspection and investigation of tuberculous brushtail possums. Indirect spread from contaminated pasture is also a possibility, especially considering the very large number of *M. bovis* present in the pus of brushtail possums with discharging lesions.

Deoxyribonucleic acid (DNA) fingerprinting of *M. bovis* has been used extensively in New Zealand as an aid to understanding the epidemiology of bovine tuberculosis, and to provide information for planning wildlife control operations. The initial studies of Collins et al. using DNA restriction endonuclease analysis (REA) revealed that the DNA restriction types of *M. bovis* in New Zealand were regionally distributed (31). The distribution of restriction types was related to the different areas in which wildlife infected with *M. bovis* were present (Fig. 1). Additional evidence that these different areas of wildlife infection were established by a rare and independent spread of infection from domestic animals to wildlife was provided by DNA fingerprinting. Subsequent investigations have shown that, within a given locality, different hosts are infected with the same restriction type, indicating a cycle of infection between wildlife and domestic animals (32). No evidence for host-specific strains of *M. bovis* has been found from DNA typing in New Zealand. With one minor exception, all the common restriction types have been found in both domestic animals and wildlife (Table III). Where a single REA type was found among animals in a previously disease-free area, this indicated that a single source of infection existed. If this type had been detected elsewhere, this result directed attention at a possible source to be investigated. If the type had not been previously observed, this information could be used to exclude possible sources. In
Table III  
The host distribution of the most common deoxyribonucleic acid restriction types of strains of Mycobacterium bovis isolated in New Zealand from 1982 to 1999  
(D.M. Collins and G.F. Yates, personal communication)

<table>
<thead>
<tr>
<th>Restriction type</th>
<th>Bovine</th>
<th>Cervine</th>
<th>Possum</th>
<th>Ferret</th>
<th>Porcine (a)</th>
<th>Other (b)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>232</td>
<td>35</td>
<td>26</td>
<td>10</td>
<td>13</td>
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<td>319</td>
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<td>71</td>
<td>7</td>
<td>13</td>
<td>9</td>
<td>136</td>
</tr>
<tr>
<td>C</td>
<td>47</td>
<td>50</td>
<td>5</td>
<td>6</td>
<td>16</td>
<td>3</td>
<td>127</td>
</tr>
<tr>
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<td>2</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>23</td>
</tr>
<tr>
<td>R</td>
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<td>15</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>571</td>
<td>231</td>
<td>267</td>
<td>85</td>
<td>84</td>
<td>27</td>
<td>1,265</td>
</tr>
</tbody>
</table>

(a) includes domestic and feral pigs  
(b) domestic and feral cats, goats, humans, sheep, hedgehogs and a stoat.

In many cases, REA is able to clearly indicate whether an infection in farmed animals originated in infected local wildlife or in infected cattle or farmed deer brought onto the farm.

**Mycobacterium bovis** infection in wildlife in the United Kingdom and the Republic of Ireland

A compulsory programme to eradicate bovine tuberculosis from cattle in Great Britain commenced in 1950, on an area by area basis. By 1960, all cattle were under test and the annual incidence had been reduced to approximately 2%. In the late 1960s and early 1970s, incidence data clearly demonstrated that the proportion of infected herds in south-west England was markedly higher than the rest of the country. A wildlife reservoir of infection was suspected because 'open cases' of *M. bovis* infection were extremely rare among reactor cattle and human sources of infection were absent. The first case of *M. bovis* infection in a badger in Great Britain was found in 1971, on a farm where tuberculin reactors with lesions had recently been detected (109). Three years later, badgers infected with *M. bovis* were found in Ireland (111).

**Badgers**

Tuberculosis in badgers is primarily a chronic respiratory disease. Initially, the badger was thought to have little ability to control an *M. bovis* infection, unlike many other species such as cattle. The early lesions in badgers contain few *M. bovis* bacilli, usually within epithelioid cells or a central area of necrosis. As the lesions progress, the area of necrosis enlarges and the number of bacilli increases markedly. The absence of a prominent fibrous tissue capsule and the rarity of calcification was suggested as evidence of a host with a poor ability to control *M. bovis* (91). Recently, Gallagher et al. reported that some non-diseased badgers infected with *M. bovis* had fibrosed, lightly calcified lesions (60). Using these observations, a model was proposed for the pathogenesis of tuberculosis in the badger. Gallagher et al. suggested that infection in many badgers leads to a slowly progressive disease that eventually advances to generalised disease, with massive excretion of *M. bovis*. Animals with non-visible lesions were said to represent an early containment or latent phase that might later become activated to develop into the progressive condition. Up to 85% of badger carcasses demonstrated by culture to be infected with *M. bovis* have either no lesions at post-mortem examination or very small lung lesions (88). Gallagher et al. raised the possibility that in some cases, containment of the infection may result in partial or complete resolution (60). Alternatively, lesions in the lungs may rupture into the bronchioles, allowing local spread and haematogenous spread...
by extension of lesions into blood vessels. The kidneys are a predilection site following haematogenous spread.

The total population of badgers in Great Britain is between 210,000 and 250,000, with highest densities in the South-West. The population density may be as large as 25 adults/km² and home ranges vary from 35 ha to over 170 ha (88). Between 1971 and 1987, infected badgers were found in sixteen of the sixty-one counties in Great Britain (26). Cheeseman et al. considered that tuberculosis is endemic in the British badger population (26). The lack of evidence of infection in some parts of the country, such as Scotland, was suggested to be due to the small number of badger carcasses examined from these areas. Infections with M. bovis are highly localised within badger populations. Mycobacterium bovis was detected in 22.1% of badgers killed in the course of badger removal operations (88). Some social groups show no sign of infection, even when the territory is immediately adjacent to infected groups.

A number of different approaches have been used to identify M. bovis in living badgers. Bacterial culture of faeces, urine and tracheal aspirates has significant limitations, being a poor indicator of infection and requiring too long a delay to be a practical diagnostic test. These deficiencies prompted the search for a rapid sensitive test to detect badgers infected with M. bovis. The intradermal skin test was impractical because of the need to re-examine animals after three days and because badgers have poor delayed type hypersensitivity responses (91). A serological test was developed using an indirect enzyme-linked immunosorbent assay (ELISA) to detect antibody to antigen MPB83, identified as one of the principal antigens of M. bovis recognised by badgers (63, 64). The sensitivity of this test, known as the Brock test, was only 40% which was too low to be useful for epidemiological studies or control programmes.

The role of badgers in the transmission of M. bovis to cattle has a long and highly controversial history in England and Wales where strong opposition exists to the killing of badgers. This opposition has been 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, and the abhorrence to many of killing a much treasured wildlife species. In response to public concern over the gassing of badgers in the 1970s, Lord Zuckerman was asked to review the evidence on whether badgers were a significant reservoir of bovine tuberculosis (149). He concluded that badgers did constitute a significant reservoir, based on the following key factors:

a) the infecting organism has been experimentally proved to be the same in both species — cultures made from tuberculous lesions in cattle have infected badgers experimentally and vice versa

b) badgers develop 'open lesions' and can spread the organism
c) badgers can transmit the disease when placed in contact with uninfected cattle
d) in areas in which infected badgers are common, the infection rate in cattle is high
e) if infected badger colonies are removed, the incidence of bovine tuberculosis in cattle declines
f) the organism can persist in pasture long enough to allow the spread of infection to cattle.

The most compelling evidence that badgers are an important reservoir of M. bovis infection for cattle is provided by an analysis of the decline in prevalence of M. bovis infection in cattle in areas in which badger control was undertaken. All badgers were removed from a 104 km² area at Thornbury, in south-west England between 1975 and 1981 (28). In this area, the incidence of herds with visible lesion reactors fell from 5.6% prior to badger control, to 0.06% after control. This operation was not designed as a scientific study, but as a means to reduce the spread of M. bovis from badgers to cattle.

No data were available on the incidence of tuberculosis in cattle in a comparable area in which badgers had not been controlled. In 1989, a project commenced in Ireland to investigate the role of badgers as a source of M. bovis for cattle (103). Badgers were removed from a central project area of 528 km² and an outer buffer zone of 210 km². A control area of 1,456 km² was included in the study. Cattle herds in the badger-removal area required a significantly lower proportion of new confirmed tuberculosis movement restrictions compared with cattle in the control area (103). The results of this study suggest that effective control of bovine tuberculosis in cattle in some areas of Ireland will require the removal of infected badgers (55).

The epidemiology of bovine tuberculosis in the British Isles has been studied using DNA typing of M. bovis isolates. In Great Britain, 2,668 isolates of M. bovis, principally from cattle and badgers, were examined by spoligotyping (25). Two spoligotypes were widespread and were found in 70% of the isolates, highlighting the limitations of this typing method for detailed epidemiological studies. However, the great majority of geographically-linked cattle and badger isolates shared the same spoligotype. Restriction fragment length polymorphism (RFLP) analysis with probes derived from the direct repeat sequence and the polymorphic G-C rich sequence, in addition to spoligotyping, were used to examine 452 strains of M. bovis, principally from cattle, badgers and deer in Ireland (38). The RFLP analysis revealed eighty-five different types compared with only twenty types detected by spoligotyping. The same range and geographic distribution of DNA types were found for the majority of isolates of M. bovis from cattle, badger and deer in Ireland. This finding indicates transmission of infection amongst these hosts.

Nearly twenty years after the review by Zuckerman, Krebs et al. stated 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 (88).

The Krebs review identified as a high priority the collection of data to quantitatively determine the contribution of badgers to cattle infection. The review recommended that the Ministry of Agriculture, Fisheries and Food should undertake a controlled experiment to quantify the impact of culling badgers. The experiment recommended comprised the following three treatments:

- proactive culling of badgers
- reactive culling following the identification of bovine tuberculosis in cattle
- no culling

Furthermore, the review suggested that the experiment be performed in a minimum of ten high risk areas, each with three replicates of 10 km by 10 km.

One of the concerns of the Krebs review was the possibility that wildlife other than badgers may also play a role in the spread of M. bovis to cattle. Since the discovery of M. bovis-infected badgers in England in 1971, a wide range of different wildlife species have been examined for the presence of tuberculosis. Between 1971 and 1995, infection with M. bovis was found in moles (Talpa europaea) (2/166), red foxes (Vulpes vulpes) (11/954), mink (Mustela vison) (1/172), rats (5/412), wild deer (19/1,817) and ferrets (1/26). This compares with identification of M. bovis in 880 of 21,731 badgers examined. Whether other wildlife are important in maintaining M. bovis in England and Wales is unknown, but a number of factors will be important, including the susceptibility and abundance of the species, the extent to which the species shares a habitat with cattle, and behavioural characteristics and interactions with other wildlife, especially those infected with M. bovis.

**Mycobacterium bovis** in wildlife in Africa

*Mycobacterium bovis* was probably introduced into Africa during the colonial era of the 1800s or early 1900s, by cattle imported from Europe which became a source of infection for native breeds of cattle (75). Subsequently, infection was transmitted to wildlife that had a shared habitat with infected cattle. For M. bovis to become permanently established in wildlife, infection had to spread to one or more maintenance hosts. Once the infection was established in a maintenance host, M. bovis was able to spread to other animals, especially predators. Determination of the epidemiology of M. bovis infections in wildlife in Africa presents major difficulties. Diagnosis of tuberculosis in the live animal is hampered by a lack of sensitive and specific ante-mortem diagnostic tests that have been validated in wildlife; in addition, significant problems are associated with capturing and restraining animals for testing. The ideal test should not only be sensitive and specific, but should also require a single sampling. In a comprehensive evaluation of various ante-mortem tests for detecting M. bovis infection in African buffalo, the comparative intradermal tuberculin test and the gamma-interferon test performed similarly with sensitivities in the range of 78%-82% and specificities in the range of 94%-96% (117; R.G. Bengis, unpublished findings). The ELISA was found to be totally unsuitable for the routine diagnosis of tuberculosis in this species, but did have some application in the identification of anergic animals. Intradermal tuberculin testing of carnivores (mainly lions) is currently being evaluated with encouraging results (D.F. Keet, personal communication). No satisfactory ante-mortem test is as yet available for pachyderms, which invariably show false positive reactions with most traditional tests. This is a major cause for concern, since elephant, hippopotamus and both species of rhinoceros are frequently translocated to repopulate conservation areas or establish metapopulations in Africa.

Detection of bovine tuberculosis in free-ranging wildlife in Africa is difficult in that most infected animals show no clinical signs until the disease has reached an advanced stage, when progressive emaciation and coughing may be observed. However, an exception is the greater kudu (*Tragelaphus strepsiceros*) which generally demonstrates typical unilateral or bilateral swellings of the parotid lymph nodes which are visible from a distance (11). Surveillance for bovine tuberculosis in most wild species therefore relies heavily on *ad hoc* necropsies, targeting road kills, hunter kills and problem animals that are destroyed. Detailed information on tuberculosis can be gathered from culling operations but this opportunity is only available for a limited range of species in a few countries. Dedicated surveys using the catch, sample and release technique can also be employed in species for which a validated ante-mortem test exists.

Maintenance hosts of *M. bovis* in Africa are generally members of the family Bovidae and characteristically have gregarious habits. They include Kafue lechwe antelope (*Kobus leche kafuensis*) in Zambia (27, 59) and the African buffalo in Uganda (71, 146) and South Africa (10, 84). Greater kudu may also have maintenance host potential, but are generally a lower density species (11). The best-studied maintenance host is the African buffalo. In 1963, Guilbride et al. reported eight cases of tuberculosis in African buffalo shot in the Ruwenzori/Queen Elizabeth National Park in Uganda (71). *Mycobacterium bovis* was isolated from six of these animals. A high prevalence of tuberculosis was detected in cattle in areas adjacent to the park, and these animals were undoubtedly the source of infection for the buffalo. Later investigations by Woodford found *M. bovis* in up to 38% of a sample of thin buffalo (146). Tuberculosis was estimated to cause an annual
mortality of 1% of the buffalo in this National Park. Warthogs (Phacochoerus aethiopicus) were the only other wildlife species in this park found to be infected with M. bovis, and were thought to become sporadically and opportunistically infected by scavenging on infected carcasses or feeding on dung beetles in infected buffalo dung (147).

Tuberculosis was first diagnosed in African buffalo in the southern region of the Kruger National Park, South Africa, in 1990 (10). A follow-up survey for tuberculosis was undertaken between 1991 and 1993 by examining carcasses that became available as part of normal culling procedures (49). An attempt was made to sample some animals from all the herds within the park. Infected buffalo were present in the southern and central areas of the park, whereas no evidence of infection was detected in the northern half of the park. The prevalence of infection ranged from 2% to 67% in different herds. Circumstantial evidence indicates that bovine tuberculosis entered the Kruger Park from infected cattle herds on the southern boundary in the late 1950s, and a gradient of tuberculosis prevalence exists, with prevalence of tuberculosis declining as one moves northwards. Thus, the longer a herd has been infected, the higher the prevalence. Tuberculous lesions in buffalo were principally found in the head nodes, tonsils and thoracic cavity. The lesions ranged from white to grey-yellow nodules of 1 mm-2 mm in the lungs or lymphatic tissue, to an extensive bronchopneumonia and miliary disseminated lesions. The lesions were typically pyogranulomatous with minimal encapsulation or calcification. Occasionally, cavitation disease was observed in advanced cases, and these individuals would be considered highly infectious. The distribution of the lesions indicates that the infection is transmitted among buffalo via the aerosol/respiratory route. Lesions were also occasionally detected in mesenteric lymph nodes and peripheral lymph nodes, in buffalo with advanced disseminated disease. Although the disease was subclinical in most infected buffalo, some animals with extensive lesions, including generalised infections, were emaciated (84). In the herds with a high prevalence of infection, assuming that individuals with advanced/generalised disease would die within six months, then a mortality index due to tuberculosis could be estimated at approximately 10%. At sustained high prevalence, de Vos et al. predicted that the mortality due to tuberculosis may negatively affect buffalo population dynamics (49).

In 1993, no indication was found that M. bovis had spread from infected buffalo to other wildlife in the Kruger National Park (49). Two years later, tuberculosis caused by M. bovis was diagnosed in a cheetah (Acinonyx jubatus), two lions (Panthera leo) and a chacma baboon (Papio ursinus) (85). These animals were assumed to have become infected either directly or indirectly from tuberculous buffalo. Tuberculous granulomatous lesions were observed in the lungs of the lions and cheetah, indicating that these animals were infected by the respiratory route. Possible explanations of transmission by the respiratory route include: direct intraspecific horizontal aerosol transmission from other pride members during grooming, feeding and competitive growling, or infection when killing prey with open pulmonary lesions. More recently, following necropsies on more than thirty M. bovis-infected lions, pulmonary lesions were found to be the exception; infection in this species is generally alimentary, with later spread to distal sites. Peripheral lymph nodes were also frequently affected, probably due to infected fight wounds. Mycobacterium bovis infection of the mammary tissue was also identified in a few lionesses, and lion cubs as young as six months have been found infected (D.F. Keet, personal communication). It must be emphasised that the macroscopic lesions of bovine tuberculosis in predators in no way resemble the classical caseo-necrotic lesions with encapsulation and calcification that are observed in bovids and primates. The lesions of predators are more proliferative in nature, with occasional cavitation, especially in the lungs. In some parts of the Kruger National Park, over 75% of the lions randomly tested had positive skin reactions to M. bovis antigen, and the disease appears to have the potential to disrupt the lion pride structures. In 1997, the first M. bovis-infected leopard (Panthera pardus) was diagnosed in the Kruger National Park (R.G. Bengis, unpublished findings), the animal had lesions typical of alimentary infection. In 1999, a common genet (Genetta tigrina) with thoracic tuberculous lesions was found in a Provincial Reserve adjoining the Kruger National Park (A. Michel, personal communication).

Baboons most probably became infected by scavenging the remains of infected buffalo, either in the veld or at the necropsy facility at Skukuza in the Kruger National Park (85). The distribution of the lesions in baboons was consistent with infection by either the oral or respiratory route. Removal of the source of infected material, testing and slaughter of infected members of the troop, and the closure of a disused pump room on the railway bridge which was opportunistically used as a 'safe' sleeping facility, resulted in a decline in the prevalence of tuberculosis in this troop of baboons (D.F. Keet, personal communication). No further cases of bovine tuberculosis have been diagnosed in this troop for over two years, and adjoining baboon troops that have been sampled are negative. Similarly, tuberculosis has also been observed in olive baboons (Papio cynocephalus anubis) in the Masai Mara Game Reserve. The most likely source of infection for these animals was M. bovis-infected waste material from a cattle abattoir (135).

The greater kudu was the first wildlife species in Africa documented to be infected with tuberculosis (75). The first cases were recognised in the 1920s in the Eastern Cape Province of South Africa, in an area in which a range was shared with infected cattle. Tuberculosis caused by M. bovis was also recognised in the same area in a few common duiker (Sylvicapra grimmia) and in a bushbuck (Tragelaphus scriptus). Subsequently, the infection appears to have spread to springbok (Antidorcas marsupialis), bushpigs
(Polamocherus larvatus) and hares (Lepus spp.), but these cases were not verified by laboratory examinations. In the 1940s, further cases of tuberculosis in greater kudu were reported in the Eastern Cape Province, but the disease appeared to progressively disappear from kudu as eradication from cattle in the area proceeded. More recently, a single case reported in the Eastern Cape Province, but the identity of the Mycobacterium in this animal was not determined (143). This report highlights the possibility that M. bovis may be able to persist in the greater kudu population, be it at low levels, for a considerable time, and that this species may have true maintenance host potential. Bovine tuberculosis has also been confirmed in greater kudu in the Kruger National Park (11; D.F. Keet and R.G. Bengis, unpublished findings). To date, ten cases have been confirmed on necropsy followed by histopathology and culture, and several other cases, with typical "mumps-like" swellings of the parotid lymph node, as well as secondary swellings of the upper cervical lymph nodes, have been reported. Primary infection in kudu appears to occur via ingestion (primary complex in retropharyngeal lymph nodes and tonsils), scarification of the ears by contaminated thorns or scratching with contaminated hind hooves (primary complex in the parotid lymph nodes). In all cases studied to date, one or both of the parotid lymph nodes were abscessed, had fistulated to the exterior, and were draining thick, mucoid, infectious pus. Given that kudus are browsers, infectious purulent material draining from fistulae may quite possibly contaminate leaves and thorns during browsing activities. In kudu, secondary haematogenous spread of infection to distal sites such as lungs, pleura, kidneys, liver and distal lymph nodes has been noted. Mesenteric lymph nodes frequently have lesions that are either associated with primary ingestion, or are a result of ingesting bacilli from pulmonary lesions.

High levels of subclinical and clinical infections with M. bovis have been observed in lechwe antelope in Lochinvar National Park on the Kafue flats in Zambia since 1956 (27, 59). Prior to the creation of the National Park, the area was grazed by over 12,000 cattle, in which bovine tuberculosis was diagnosed and confirmed as early as 1954. Heavy infection rates in cattle were reported in the following years and the first two cases of bovine tuberculosis in lechwe antelope were recorded in 1956 by Le Roux. The shared rangeland was the most likely source of M. bovis infection in the lechwe, which now appear to have become maintenance hosts. Gallagher et al. reported that annual mortality due to M. bovis was 20% in lechwe antelope (59).

The implications of bovine tuberculosis in free-ranging wildlife

Whether or not bovine tuberculosis in free-ranging wildlife in Africa is a cause for concern is a debatable question, to which two divergent answers have been proposed. Since bovine tuberculosis is a slowly progressive disease, most cases are sub-clinical and dramatic episodes of mortality rarely occur. Bovine tuberculosis does not appear to affect reproduction in the prime sylvatic maintenance hosts (buffalo and lechwe), and hence no acute effects are seen in the population dynamics of these species. This has lulled many biologists and some veterinarians into assuming that the disease will not affect free-ranging wildlife populations, and that in time, natural selection will result in adaptation and development of evolutionary tolerance between host and pathogen. These experts therefore advocate a laissez faire approach to the infection.

The opposing opinion is that when a sustained high prevalence of infection occurs in the prime maintenance hosts, young animals will become increasingly exposed at an early age, and may die of disease before reaching reproductive age. Thus, in the medium to long term, a significant impact on these host populations may result. Furthermore, increasing evidence shows that the most serious effects of bovine tuberculosis in natural ecosystems may be on "spill-over" hosts, most notably the predators at the top of the food chain. These predators may target tuberculosis-debilitated animals or may scavenge infected carcasses, thus receiving frequent exposure to M. bovis. The higher the prevalence of tuberculosis in the maintenance hosts, the greater the exposure and infection rate in these predators and scavengers. This may affect these relatively low density populations by reducing longevity and/or altering social structures.

The possibility that cross-over of the infection from wildlife back into domestic cattle will occur, either at the interface of their ranges, or where common range is shared, is also a grave concern. In the subsistence communal farming areas adjoining wildlife conservation areas in Africa, milk is seldom pasteurised, and the potential zoonotic implications should not be underestimated, particularly in the light of the current pandemic of human immunodeficiency virus (HIV) infection.

Finally, in some countries of Africa, tuberculosis eradication schemes in cattle have been implemented and executed at great cost over a number of years. These schemes have resulted in eradication of the disease or have significantly reduced the prevalence rates, and eradication appears attainable in the near future. The spectre of new sylvatic reservoirs of infection in such countries is extremely worrying for veterinary regulatory authorities and public health officials.

**Mycobacterium bovis** infection in wildlife in Australia

In 1997, Australia was declared a bovine tuberculosis free area based on the definition of the Office International des Epizooties (41). Some of the last regions of Australia to...
eradicate M. bovis were those in which infected, feral water buffalo (Bubalus bubalis) were present. The water buffalo were descendants of domestic stock introduced during the early half of the 19th Century. Subsequently, a feral population of up to 250,000 animals became established in the Northern Territory. Inspection of feral buffalo meat between 1959 and 1979 revealed a prevalence of tuberculosis between 1.7% and 16.4% (72). The presence of thoracic lesions and generalised cases of tuberculosis supported the contention that water buffalo were acting as a maintenance host of M. bovis. In recognition of this observation, water buffalo were extensively culled as part of the programme to eradicate bovine tuberculosis from Australia. Between 1958 and 1981, a relatively high level of tuberculosis was identified in feral pigs that were living in close association with water buffalo. Corner et al. concluded that transmission of M. bovis from live, infected pigs to cattle or buffalo was improbable, based on the low prevalence of generalised infection in pigs, the absence of pulmonary lesions, the lack of obvious routes of excretion and infected pigs to cattle or buffalo was improbable, based on the recognition of this observation, water buffalo were extensively culled as part of the programme to eradicate bovine tuberculosis from Australia. Between 1958 and 1981, a relatively high level of tuberculosis was identified in feral pigs that were living in close association with water buffalo. Corner et al. concluded that transmission of M. bovis from live, infected pigs to cattle or buffalo was improbable, based on the low prevalence of generalised infection in pigs, the absence of pulmonary lesions, the lack of obvious routes of excretion and the lack of contact between pigs and other live infected species (35). Further evidence that feral pigs were an end host of M. bovis was provided by the observation of a major decline in prevalence of infection from 19% in 1972 to 0.25% in 1992 (99). This decline in prevalence was associated with a marked reduction of bovine tuberculosis in cottage and the culling of water buffalo.

**Mycobacterium bovis** in wildlife in North America

**White-tailed deer**

*Mycobacterium bovis* infection in cattle in the United States of America (USA) is now very rare, as a result of a successful control programme. In 1994, *M. bovis* was confirmed in free-living white-tailed deer (*Odocoileus virginianus*) in the north-east Lower Peninsula of Michigan.

An extensive surveillance programme was begun to assess the extent of this endemic infection. The same strain of *M. bovis* was isolated from each of the species mentioned below. Cases were detected in eleven counties, three of which are outside the intrastate animal movement restriction zone established by the State Government.

In 1999, fifty-eight wild deer were tested positive. Over five years of surveillance, a total of 285/38,607 deer were culture positive or suspect for bovine tuberculosis. Since the test samples for white-tailed deer were taken from the head only, these data may under-represent the true prevalence of infection in deer. One farmed deer herd was identified as infected in 1997. Eight test-positive cattle herds have been identified in Michigan: seven beef herds and one dairy herd. *Mycobacterium bovis* has also been isolated from five wild carnivore species (six coyotes (*Canis latrans*), two raccoons (*Procyon lotor*), one black bear (*Ursus americanus*), one bobcat (*Lynx rufus*) and one red fox). These species are unlikely to function as reservoirs for the infection. Once eliminated from the white-tailed deer, infection is expected to also disappear from the carnivore species (4).

In Michigan, white-tailed deer can act as a maintenance host for *M. bovis*. The practice of supplementary feeding of deer over winter is thought to have exacerbated the spread of *M. bovis*. This practice brought together large numbers of deer for a prolonged period, in addition to maintaining deer densities above the natural carrying capacity. A bovine tuberculosis eradication programme has been instituted which includes a ban on supplementary feeding to attract free-ranging deer or elk, and the reduction of deer numbers through increased hunting.

**Bison**

The hybrid bison population in and around Wood Buffalo National Park in Alberta, Canada, constitutes the largest wildlife reservoir of bovine tuberculosis in North America. Between 1925 and 1928, over 6,000 plains bison (Bison bison bison) were moved to the Wood Buffalo Park from an infected herd at Buffalo Park near Wainwright, Alberta. These bison interbred with the existing wood bison. Between 1923 and 1937, over 50% of the bison slaughtered near Wainwright had tuberculosis lesions. In 1940, the herd at Wainwright was disbanded. Tuberculosis has been found in the hybrid bison at Wood Buffalo Park since the 1930s, demonstrating that bison are a maintenance host for *M. bovis* (136). In 1990, Tessaro et al. reported tuberculosis in 15/71 (21%) of bison found dead in and around Wood Buffalo National Park (137). Animals with a range of different lesions were found, including some animals with lesions only in the head nodes or bronchial nodes, and others with generalised infections involving multiple lymph nodes and organs.

**Mycobacterium bovis** infection in feral pigs

The domestic pig is an end host for *M. bovis*, as evidenced by the failure to persist in pig herds once the initial source of infection is removed. The decline in the prevalence of *M. bovis* in feral pigs in Australia indicates that in some conditions, the feral pig is also an end host for this pathogen (99). The role of feral pigs in the maintenance of *M. bovis* on the island of Molokai in Hawaii was not well defined. *Mycobacterium bovis* was present in cattle, axis deer (*Axis axis*) and feral pigs. Essey et al. observed that bovine tuberculoceusts appeared to transmit efficiently through the feral pigs of Molokai and that the disease primarily involved the thoracic viscera (53). The problem has subsequently been controlled by depopulating an infected cattle herd and by culling both deer and feral pigs (54). More recently, *M. bovis* has been isolated from fifteen of sixty-three wild boar in Italy (129). These animals came from western Liguria in northern Italy where *M. bovis* is still present.
in domestic cattle. The results of DNA typing of porcine and bovine isolates of M. bovis revealed that the most common DNA type was isolated from both cattle and wild boar. Serraino et al. hypothesised that the feral pigs were most likely to be infected by feeding on pastures contaminated by infected cattle (129). The lesions in the pigs in Italy occurred principally in mandibular lymph nodes and the animals were concluded to be end hosts. The authors do not appear to have investigated the possibility that other wildlife may play a role in the spread of M. bovis. A recent report from Spain used DNA fingerprinting of M. bovis isolates to demonstrate that transmission of infection occurs amongst cattle, deer and wild boar (5).

**Mycobacterium tuberculosis** complex in marine mammals

The first case of tuberculosis in marine mammals caused by an organism belonging to the M. tuberculosis complex was identified in captive Australian sea lions (Neophoca cinerea) and New Zealand fur seals (Arctocephalus forsteri) at a marine park in Western Australia (40). Subsequently, tuberculosis caused by members of the M. tuberculosis complex has been identified in wild fur seals (A. pusillus doriferus) (148) from Australia; sea lions (Otaria flavescens) and fur seals (A. australis and A. tropicalis) from Argentina (6, 12); and fur seals (A. forsteri) from New Zealand (79). Tuberculous lesions in these animals were found in the lungs, spleen and a range of different lymph nodes. In some cases, the infection was generalised and animals were clinically affected. Interestingly, the mycobacterial isolates from marine mammals have similar molecular and phenotypic characteristics and cluster into a group within the M. tuberculosis complex that is distinct from M. bovis and M. tuberculosis (130). An important question is whether or not tuberculous marine mammals can act as a source of infection for other hosts. This type of Mycobacterium has been isolated from a human with pulmonary tuberculosis who was an animal trainer working with infected seals. Collins and de Lisle reported the isolation of a similar strain from lesions in the head lymph nodes of a bovine from a coastal region of New Zealand (33).

Surveys of wildlife for **Mycobacterium bovis**

The realisation that wildlife may be an important reservoir of M. bovis for domestic animals has resulted in an increasing number of wild animal populations being examined for this pathogen. The design of wildlife surveys for M. bovis is dependent on whether or not the host species is protected and the expected prevalence of infection. There are no ideal ante-mortem tests for performing surveys of M. bovis infections in wildlife (19). Bacterial culture has the major limitations of being slow, not being highly sensitive and being restricted by difficulties in obtaining suitable samples from live animals. Culture of faeces has been used with limited success in identifying M. bovis-infected badger sets. Serological tests for tuberculosis also have major limitations of being insensitive and having variable specificity. These tests have been used with limited success in detecting tuberculosis in brushtail possums (17) and badgers (63, 64). Skin testing, being based on cellular immune responses, has the major disadvantage of requiring re-examination of animals. The lymphocyte transformation test is a useful method as a research tool for studying tuberculosis in brushtail possums, but is not a practical procedure for surveys of this host (18). The post-mortem examination of wildlife is the method most commonly-used for surveying wildlife for tuberculosis. In some cases, wildlife are killed for sport (deer and feral pigs) or population control (African buffalo, bison) and the carcasses can be examined in an abattoir.

If the objective of a survey is to determine whether or not any of the wildlife in a given area is infected, a choice of different species to examine may be presented. The choice will depend on the likely prevalence of M. bovis within each species, the abundance and the ease of sampling. For example, in some areas of New Zealand, surveys of wildlife for M. bovis are conducted by examining ferrets in preference to brushtail possums, reflecting the much higher prevalence of infection in the former species. Recently, the release of feral pigs fitted with radio-collars has been proposed as a means of surveying areas of New Zealand for infected wildlife (P.G. Livingstone, personal communication). The proposal is to recapture the pigs a number of months after release and to perform post-mortem examinations to determine whether the animals have become infected with M. bovis by scavenging infected wildlife.

**Control of Mycobacterium bovis** in wildlife

The control of M. bovis in wildlife presents many challenges. The objectives for a control programme may be solely to prevent the spread of infection to domestic animals, as is the case in New Zealand, or may also include the need to preserve protected wildlife. Where wildlife are not protected, such as brushtail possums in New Zealand and water buffalo in Australia, control of M. bovis can be achieved to varying degrees by culling. A good understanding of the epidemiology of M. bovis infections will help identify the species to be controlled and the level of control required. Modelling predictions and analysis of wildlife control operations in New Zealand have demonstrated that maintaining brushtail possum numbers at less than 20% of pre-control levels can
lead to eradication of *M. bovis* from populations of this host (P.G. Livingstone, personal communication). Currently, brushtail possum numbers 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 (92). The wildlife control programme has been central in reducing the number of infected cattle and deer herds by over 50% between 1994 and 2000. Although *M. bovis* has been eradicated from domestic animals and wildlife in six small areas in New Zealand, some doubt exists as to whether the current wildlife control programme will result in the elimination of *M. bovis* from the entire country.

Alternative measures to culling, especially vaccination, are required to control *M. bovis* in protected wildlife species or as an additional tool to control the infection in abundant species such as the brushtail possum. The ideal wildlife vaccine against *M. bovis* should be safe, efficacious, inexpensive and easily delivered to the target species. However, vaccine strategies have not yet been sufficiently developed to allow effective use in wildlife (19). The reason for vaccinating wildlife may be to preserve protected species such as those in game parks in Africa or to prevent the spread of infection from wildlife to domestic animals. Currently, a major research programme is being conducted in New Zealand to develop vaccination as a tool to control *M. bovis* in wildlife. Interestingly, while vaccination has been identified as a possible means of controlling *M. bovis* in badgers, the recent Krebs review recommended the development of cattle vaccines as a much higher priority than producing vaccines for badgers (88).

The most widely-used vaccine in humans, the live attenuated *M. bovis* bacillus Calmette-Guérin (BCG), has been tested in brushtail possums (18), deer (70), ferrets (42, 116) and badgers (132) for ability to protect against bovine tuberculosis (19). High levels of protection against tuberculosis have been observed in farmed deer vaccinated with BCG (70) and a series of studies has shown that BCG can induce a significant level of protection in caged brushtail possums against intratracheal challenge with virulent *M. bovis* (1, 2, 18). When compared with unvaccinated controls, vaccinated brushtail possums had less severe disease, as shown by reduced loss of body weight following challenge, fewer and smaller lung lesions and lower bacterial counts in the lungs and spleen. Whether the level of protection induced by BCG is sufficient to control and eradicate *M. bovis* from free ranging brushtail possums or deer has not been determined. The variable efficacy of BCG in protecting humans against tuberculosis and the less than ideal protection provided for animals has prompted the development of new tuberculosis vaccines. The development of these new vaccines is based on the inactivation of virulence or 'house keeping' genes of *M. bovis* or *M. tuberculosis*, or by the investigation of a range of different non-living vaccines including killed mycobacteria, subunits of mycobacterial proteins and DNA vaccines (19).

A major hurdle to be overcome in vaccinating wildlife is the development of an effective method to deliver the vaccine to the target species. The most practical method of vaccine delivery to wildlife is by bait, a system that has been successfully used to deliver rabies vaccines to foxes and jackals (90, 114). Live attenuated vaccines against tuberculosis, in contrast to killed, subunit or DNA vaccines, are able to induce moderate to high levels of immunity with a single administration. A number of scientific hurdles have to be overcome to successfully incorporate a live tuberculosis vaccine into a bait that will induce the appropriate immune responses when eaten by wildlife. The live bacteria must remain viable in the bait and when consumed by the animal must not be inactivated in the stomach. Buddle et al. found that BCG introduced directly into the duodenum of brushtail possums induced significantly higher levels of protection compared to animals vaccinated intragastrically (18). Non-living vaccines, such as subunit vaccines, are poorly immunogenic when administered orally. Even the use of multiple doses and the incorporation of an appropriate adjuvant, such as cholera toxin, do not stimulate the same levels of immune response that can be obtained with live vaccines.

A system of vaccinating brushtail possums against tuberculosis using a mechanical device that delivers an aerosol of live vaccine to the face of the animal has been suggested (R.S. Morris and L.A. Corner, personal communication). Significant levels of protection against tuberculosis have been achieved in brushtail possums vaccinated by spraying the face with an aerosol of BCG (L.A. Corner and B.M. Buddle, personal communication). Although the use of projectile darts has been suggested for vaccinating large animals such as buffalo, this procedure has yet to be evaluated in free ranging wildlife. A detailed discussion of vaccination against *M. bovis* is presented in the paper by Skinner et al. in this issue (130).

**Control of bovine tuberculosis in free-ranging wildlife in Africa**

Once bovine tuberculosis has been introduced into an ecosystem with free-ranging maintenance hosts, the infection is almost impossible to eradicate without extreme measures. If the free-ranging maintenance host is a 'feral' or introduced non-indigenous species, then the implementation of these extreme measures (such as depopulation) would probably receive some support from conservationists and the public. The ecological considerations and public opinion may be quite different when dealing with infected indigenous populations, as occurs in several ecosystems in Africa.

The available options when dealing with tuberculosis in free-ranging indigenous maintenance hosts in conservation areas in Africa are discussed below.
Laissez faire

Laissez faire is an option that has some support from conservationists, but carries the responsibility of ignoring the unknown future impact of the disease on the maintenance host populations, or any sympatric species in the ecosystem. The possibility that the disease may spread to domestic livestock in neighbouring communities, and the zoonotic implications of this, must also be considered.

Minimal interference

If the option of minimal interference is chosen, no active control efforts are initiated, but active intensive research and monitoring of the disease is performed in an attempt to identify the major epidemiological determinants. Most veterinarians believe that this is the minimum action that should be taken, and that the results of these studies should then indicate which intervention option is the most appropriate. The implication is that an understanding of the infection and, if possible, the identification of all possible maintenance hosts is preferable, before trying to manage the disease. This is the current option being used in Kruger National Park.

Limited intervention

The limited intervention approach reduces spread and spill-over into other species, and can be used to address a finite focus of infection or to prevent any worsening of the situation whilst a solution is sought. Limited intervention includes focal depopulation of maintenance hosts to create a host-free zone which separates diseased and non-diseased herds at the interface. This is possible only when the appropriate spatial distribution of disease is present. Limited intervention may also include depopulation of high prevalence herds to reduce spill-over, or depopulation of finite infected foci. Another technique involves the mass capture of maintenance hosts followed by testing and removal of positive animals. This technique is currently being applied in the Hluhluwe/Umfolosi Park in KwaZulu Natal, South Africa. These techniques help to contain the infection, may assist in retaining the tuberculosis-free status of segments of the population and provide data concerning the pathogenesis of the infection through analysis of the infected animals. These are valuable tools to delay worsening of the situation before an effective vaccine or better option becomes available. These methods all entail the destruction of significant numbers of animals, which although justifiable, would probably be unpopular among some environmentalists and members of the public.

Major intervention

Major intervention is the most drastic option and involves total depopulation of infected herds in the conservation area, with the intention of repopulating the area with tuberculosis-free animals after a suitable time period. This option is not likely to receive significant support from conservationists and the public.

In the long term, the most appealing and popular control measure would be mass vaccination, with progressive reduction in the susceptibility to infection of the target species. Thus, the development of appropriate vaccines and delivery systems remains the long-term solution for controlling tuberculosis in free-ranging populations. In addition, successful pilot projects for breeding disease-free buffalo have been completed in South Africa, so that the genotypes of infected populations can be saved and proliferated in order to restock areas where major intervention may become necessary.

Conclusion

In conclusion, the control of bovine tuberculosis in free-ranging indigenous wildlife is a highly emotional and complex issue, involving animal health regulatory authorities, public health authorities, biologists, conservation authorities, environmentalists and the general public. Decisions on control measures need to be realistic, multi-disciplinary and transparent to all participants.

Mycobacterium bovis in captive wildlife

Tuberculosis caused by M. bovis has been a major problem in collections of wildlife in zoological parks and captive wildlife facilities. Not only can M. bovis affect the well-being of a range of different wildlife species, including endangered animals, tuberculosis is also a zoonosis which can infect animal handlers and the general public (138). Although the most severe cases of tuberculosis have been observed in captive primates, a wide range of other captive wildlife species has been infected with M. bovis (Table II). In some cases, M. bovis has been introduced into a zoo and has spread to other species. An outbreak of M. bovis in the Audubon Zoological Garden in New Orleans resulted in the death of four southern white rhinoceros (Ceratotherium simum) and two colobus monkeys (Colobus guereza caudatus) (131). Positive skin tests in zookeepers attending these animals indicated that these personnel had also been infected. In Dublin Zoo, an outbreak of M. bovis resulted in a Mayotte lemur (Lemur mayottensis mayottensis), a lion-tailed macaque (Macaca silenus), a patas monkey (Erythrocebus patas) and a siamang gibbon (Symphalangus syndactylus) becoming infected (145).

To prevent M. bovis becoming established in collections of captive wildlife, all potentially susceptible animals introduced into zoological parks and captive wildlife facilities should be held in quarantine until a tuberculosis test has been performed. Tuberculin skin tests are the most commonly-used procedure to examine animals for tuberculosis. For many wildlife species, tuberculin skin tests have not been adequately standardised to determine the optimal dose of tuberculin and the preferred site for injection. Other tests for detecting tuberculosis, such as lymphocyte transformation tests...
and ELISA have also been used to examine captive wildlife for the presence of M. bovis. For example, lymphocyte transformation and an ELISA were used to identify infected animals in an outbreak of tuberculosis in Arabian oryx (Oryx leucoryx) (57). Some difficulties were encountered with both false positive and false negative results. The false positive results were thought to be due to sensitisation by environmental mycobacteria.

Once M. bovis has been introduced into a captive wildlife facility, eradication can be difficult. Initially, steps must be taken to define which groups of animals are infected and to ensure that measures are implemented to prevent any further spread to other wildlife and to minimise the risk to animal handlers and the general public. Tuberculin testing may be useful in identifying infected animals. Culling clinically affected animals and those reacting to diagnostic tests is often the most efficient method for dealing with an outbreak of tuberculosis in captive wildlife. Where species are highly endangered, antimicrobial treatment may be considered. A combination of sanitary procedures and antimicrobial treatment was successfully used to establish a group of tuberculosis-free animals from a herd of Arabian oryx that suffered a high level of mortality due to infection with M. bovis (65). Infected oryx were treated with a combination of isoniazid, ethambutol and rifampicin administered daily in the drinking water.

**Mycobacterium bovis in captive primates**

Tuberculosis can be a major problem in captive primate colonies (62). *Mycobacterium tuberculosis* and M. bovis cause the majority of the mycobacterial infections, but other mycobacterial species can also cause clinical disease in primates. Rhesus monkeys (*Macaca mulatta*) are highly susceptible to tuberculosis, whereas baboons, other macaques and Old World monkeys are less susceptible. Mycobacterial infections in New World monkeys are comparatively rare.

Primates are generally infected by the respiratory route. In susceptible primates such as rhesus monkeys, exposure by inhalation to either M. bovis or M. tuberculosis results in a rapidly progressive disease. Highly susceptible primates with tuberculosis often have only a short illness, with weight loss, dull hair coat, cachexia and lethargy. If a rapidly progressive pneumonia or widespread dissemination occurs, primates may be found dead without any overt clinical signs of tuberculosis. Coughing by tuberculous primates can result in the spread of infection both to other primates and animal keepers. Primates may also be infected by oral and subcutaneous routes.

Skin testing is a useful method for detecting tuberculosis in primates. The tuberculin test is performed by injecting intradermally, 10,000 units of mammalian tuberculin in 0.05 ml-0.1 ml into the skin of the eyelid using a 12 mm, 25 to 28 gauge needle. Primates should be chemically sedated before injection of the tuberculin. The skin test is checked 24 h, 48 h and 72 h post injection and a positive reaction is indicated by oedema and erythema of the injected eyelid. False negative reactions may be observed in primates with fulminating tuberculosis or those immunosuppressed with concurrent infections such as measles. Chest X-rays may be a useful ancillary method for diagnosing tuberculosis, but should not be used as the sole criterion for determining the infection status of an animal. Although successful treatment of tuberculosis in primates with antimicrobials has been reported, this course of action is only recommended for rare, endangered species and very valuable experimental animals (62). A screening programme should be used to ensure that tuberculosis does not become established in primate colonies. All new primates should be quarantined for thirty days and skin tested every two weeks before being admitted to the colony. Animals that show a positive skin test should be euthanised and examined post mortem for tuberculosis.

**Mycobacterium bovis in farmed deer**

New Zealand has been a pioneer in farming deer using intensive pastoral farming methods. The first deer farms in New Zealand were established in the 1960s and subsequently the deer population has expanded to over two million animals, reared on around 5,500 properties. Approximately 93% of these are red or red/wapiti hybrid deer (*Cervus elaphus*), 3% are pure wapiti (elk), 2% are fallow deer (*Dama dama*) and the remainder sika (*Cervus nippon*) and various hybrids. Many of the farms were established with free-living, wild deer that were caught using traps or helicopters. In some areas of New Zealand, wild deer are infected with M. bovis, and this was one of the sources of infection for farmed deer. The first case of M. bovis infection in farmed deer was identified in 1978 (7), and by the early 1980s, tuberculosis was recognised as a significant problem. In 1983, a voluntary scheme was introduced in New Zealand to control tuberculosis in farmed deer.

Tuberculosis in farmed deer most commonly presents as a lymphadenitis involving one or more of the lymph nodes of the head, thorax or gastrointestinal tract. The mediastinal retropharyngeal lymph node is the most commonly affected node in deer infected with M. bovis and over 50% of lesions occur in the head. Experimental infection studies support the hypothesis that infection occurs via the palatine and pharyngeal tonsils, by either the oral or respiratory route (101). A study of naturally-acquired tuberculosis in farmed deer supports the experimental evidence, citing the tonsil as a common route of infection (94). A feature of the lymph node lesions is the frequent presence of 'abscesses' containing semi-liquid pus (106). These lesions are difficult to distinguish from abscesses that are caused by other agents. In herds in which tuberculosis was poorly controlled, a high incidence of generalised infections was often reported, with
extensive lesions in multiple sites, including extensive involvement of the lungs. The occurrence of multiple cases of generalised bovine tuberculosis in farmed deer prompted the suggestion that farmed deer were highly susceptible to *M. bovis* (68). A number of different factors affect the susceptibility of deer, including age, environment and genetics (66, 101, 102). Recently, Mackintosh *et al.*, in a series of experimental infection studies, demonstrated the importance of the genetic component in the susceptibility of deer to *M. bovis* (102). Resistance to bovine tuberculosis was demonstrated to have a heritability of 0.46 in a group of red deer stags gathered from a number of properties. These authors hypothesised that the selection of tuberculosis-resistant deer could be used as an additional control strategy. Where the disease is being controlled in herds through implementation of a strict control programme, the number of infected deer per herd is small and infected animals usually have a single affected lymph node. Nevertheless, major outbreaks of tuberculosis affecting up to 50% of a herd still occur sporadically. Annual testing of deer for tuberculosis is recommended, to ensure that infection is detected as early as possible after introduction into a herd, to prevent extensive deer to deer transmission. Removal of the small percentage of highly susceptible deer from herds may reduce the incidence and severity of herd breakdowns (102).

Considerable care is required in establishing a post-mortem diagnosis of *M. bovis* infection in deer. The macroscopic and microscopic appearance of the lesions is not pathognomonic for *M. bovis*. Infection with *M. avium* or *M. paratuberculosis* is not uncommon in deer in New Zealand, and these bacteria can cause lesions indistinguishable from those caused by *M. bovis* (46, 48). All these bacterial species can cause necrotic lesions with calcified centres in lymph nodes. Bacterial culture of affected organs is required to confirm a tentative diagnosis based on the observation and histopathological examination of macroscopic lesions.

When *M. bovis* was first identified in farmed deer in New Zealand, no diagnostic tests for bovine tuberculosis had been standardised for use in this species. Initial investigations concentrated on adapting the tuberculin skin test used in cattle (8). Initial recommendations for the single cervical test (SCT) were that the test be performed in the mid-cervical region at a well shaved (8 cm x 8 cm) site by injecting intradermally, 0.1 ml of 2 mg/ml purified protein derivative from *M. bovis* culture (PPD-B). The SCTs were to be read 72 h following injection, and any visible, palpable or measurable skin thickening was considered a positive reaction. Using this test, Beason *et al.* found gross lesions consistent with tuberculosis in 82 of 107 reactors slaughtered (8). Some deer that were skin-test negative were also found to be infected. The parameters for skin testing were further evaluated using experimentally-infected deer (36). This study confirmed that skin test reactions were maximal at 48 h and 72 h post injection and no difference was observed in the reaction sizes between 1 mg/ml and 2 mg/ml PPD-B. An important finding of this study was that the performance of the tuberculin skin test was impaired by repeat testing at three or six week intervals. This finding led to the recommendation that a minimum interval of sixty days, and preferably ninety days, should be observed between skin tests. Careful skin preparation and adequate restraint of deer are essential to correctly perform skin testing in this species.

The voluntary tuberculosis control scheme achieved some early success, although some farms encountered problems with false positive reactions to the SCT due to exposure to members of the *M. avium* complex. In addition to *M. avium*/*M. intracellularare*, it is assumed that *M. paratuberculosis* may also cause false-positive skin test reactions when using only PPD-B. The lack of compensation for these reactors, the high value of deer and the lack of approved ancillary tests resulted in a large number of deer herds remaining untreated. This problem was overcome in part by the development and official approval of the comparative cervical skin test (CCT) for deer, following experimental and field trials (37). Corrin *et al.* used 1 mg/ml PPD-B and 0.5 mg/ml purified protein derivative from *M. avium* (PPD-A) in experimentally-infected deer and discovered that when the test interval was greater than sixty days, the sensitivity of the test was 91.4% (37). In a field trial involving 1,157 deer in seventeen uninfected herds with a history of false positive skin test reactions, the specificity of the CCT was 98.7%. Lower levels of sensitivity of the CCT are observed in the field where deer have been naturally infected with *M. bovis* and exposed to a range of environmental mycobacteria.

Additional stresses such as pregnancy, adverse environmental factors and possible genetic variation may also affect the sensitivity of the CCT. Considerable caution should be exercised when using the CCT in deer because the test is less sensitive than the SCT. In New Zealand, the use of the CCT is not recommended in herds in which the likelihood of *M. bovis* infection is high. In the UK, Stuart *et al.* reported that the CCT had a sensitivity of 80% in naturally-infected red deer (134).

Each country using diagnostic tests, especially skin tests, to detect tuberculosis in deer, needs to conduct studies to assess the performance of the test under the specific conditions in that country. A number of variables can affect the performance of skin tests, including the species of deer, the degree to which the animals are under stress, the stage of pregnancy, the genetics of the animals, the range of mycobacterial species present in the environment, the type of facilities and restraint used and the level of expertise available (68, 87). For example, when applied to fallow deer in Texas, the SCT and CCT had poor sensitivity and specificity and were of little diagnostic value in determining the infection status of individual fallow (43). On a number of occasions, tuberculosis has crossed international borders by the importation of infected deer that were negative to pre-movement skin tests (13, 133). To minimise the risk of
importation of tuberculosis in deer, individual skin tests should not be relied on, and animals should only be obtained from herds that have been demonstrated to be free of infection over a long period. Although skin tests have some limitations for the diagnosis of tuberculosis in deer, these tests have been very successfully used in New Zealand as the primary screening method in the tuberculosis programme for this species.

Griffin et al. developed a blood test based on lymphocyte transformation for the identification of deer with tuberculosis (69). The test involves the co-culture of separated mononuclear leukocytes with PPD-B and PPD-A. Following culture for five days, the cultures are labelled with tritiated thymidine, harvested 18 h later and the uptake of radiolabel measured. Unstimulated negative controls and mitogen-stimulated positive controls are included in every assay. This test had a sensitivity of 85.9%-90.7% when tested in M. bovis culture positive deer. If the lymphocyte transformation test was run in conjunction with an ELISA, the sensitivity of the combined tests increased to 95.7%-98%. Griffin et al. refer to the combined tests as the ‘blood test for tuberculosis (BTB)’ (69). The ELISA result was based on the combination of three different tests in which PPD-A, PPD-B and the purified antigen from M. bovis, MPB70, are used in separate assays. The MPB70 is only present in a limited range of mycobacterial species and is used in the ELISA to improve the specificity of the test. Prior skin testing has a marked effect on the sensitivity of the ELISA. The sensitivity of the ELISA increased from 45.7% when samples were tested immediately prior to skin testing, to 85.3% when the same animals were tested ten days after skin testing. The comparatively high cost of the BTB has led to this being used as an ancillary test to examine skin test reactors, rather than for whole herd testing. The use of the SCT followed by an ELISA ten days later resulted in a combined test with a sensitivity of 95%. The combination of a skin test and an ELISA is currently being used in New Zealand as a whole-herd test to eradicate M. bovis from selected deer farms. This parallel combination of tests has the advantage of using the ability of the skin test to detect early infections together with the ability of the ELISA to detect antibody in advanced cases of tuberculosis (cases that may be negative to the skin test due to anergy).

Vaccination of farmed species has been proposed as a method for the control of bovine tuberculosis in countries in which eradication is prevented by a wildlife reservoir of infection or in countries that cannot afford the cost of a test and slaughter programme. Over the past decade, a series of experiments has been performed to evaluate and optimise the use of BCG to protect deer against tuberculosis (67, 70). These studies used the infection model developed by Mackintosh et al., in which deer are challenged by inoculating low numbers of virulent M. bovis into the tonsil (101). The experimental variables that have been evaluated include the strain of BCG, dose of the vaccine, effect of booster vaccination, use of live versus killed BCG and route of vaccination. The highest levels of protection were obtained by vaccinating deer subcutaneously, twice at an eight-week interval with a low dose (5 × 10^7 colony forming units) of live BCG. This vaccination regime produced significant levels of protection against both infection and disease. A feature of this vaccination procedure is that predominantly cellular immune responses are produced that are important in the development of protection against tuberculosis. Recent studies indicate that the protection against infection persists for at least twelve months following booster BCG vaccination of deer (J.F. Griffin, personal communication).

Farmed deer are now found in many countries in addition to New Zealand. Various species of deer, especially reindeer and wapiti (elk) are held under extensive ranching conditions in the USA, Canada, Scandinavia and the former Union of Socialist Soviet Republics (USSR). Red, sika, rusa (Cervus timorensis), wapiti, axis and fallow deer are the most common species held under intensive pastoral farming systems in Australia, New Caledonia, Europe, Canada and the USA. Throughout Asia, many deer, especially sika, rusa, sambar (C. unicolor) and red/wapiti deer are kept in outdoor or indoor enclosures throughout the year, under intensive management systems. Many deer are kept in parks in Europe, North America and Australasia. These are not ‘farmed’, but the purpose and style of management includes hunting parks in which the animals are wild and are shot for trophies, zoological parks in which the animals are bred to preserve a range of deer species, and private estates in which deer are kept for aesthetic reasons. Tuberculosis has the potential to cause serious problems in all of these situations, but especially under intensive conditions (100).

**Mycobacterium bovis** infection as a zoonosis

Tuberculosis continues to cause more deaths in humans than any other single infectious agent. Although the majority of cases of human tuberculosis are caused by *M. tuberculosis*, 5%-10% of cases in developing countries may be due to *M. bovis* (112). In humans, cases of pulmonary tuberculosis due to *M. tuberculosis* and *M. bovis* are indistinguishable clinically and pathologically. The most likely source of *M. bovis* infection in humans is infected domestic animals. Prior to the advent of pasteurised milk, *M. bovis* infection was a common cause of extra-pulmonary tuberculosis in children. The role of infected wildlife as a source of infection for humans has received little attention. Weyer et al. found no evidence that *M. bovis* was being transmitted by wildlife to humans in the Kruger National Park (144). Tuberculous farmed deer and zoo animals have caused *M. bovis* infections in humans. A large number of positive skin tests were observed in humans who were in contact with farmed elk infected with *M. bovis* (56). One case of active infection with *M. bovis* was identified in a human exposed to these deer.
Similarly, seven of twenty-four zoo keepers exposed to a southern white rhinoceros that died of severe *M. bovis* pneumonia, developed positive tuberculin skin tests, but not clinical tuberculosis (131).

**Future directions**

Recently, 22% of countries surveyed reported that tuberculosis had been identified in wildlife within the last ten years (92). In many of these countries, the role of infected wildlife in the maintenance and spread of *M. bovis* has not been well defined. As more countries embark on programmes to control bovine tuberculosis in domestic animals, the role of infected wildlife will become more apparent. In both the UK and New Zealand, the discovery of infected wildlife occurred after the implementation of programmes to eradicate bovine tuberculosis from cattle. Successful control of bovine tuberculosis in domestic animals in some countries is likely to depend on the identification of wildlife reservoirs of infection and development of control measures to halt the spread of infection from wildlife. Where control of *M. bovis* in wildlife is necessary, especially in those countries with infected wildlife that are protected, vaccination is the most promising control method. Furthermore, vaccination may also be useful in the control of bovine tuberculosis in captive wildlife, including recently domesticated animals, such as farmed deer. 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 (19, 70).

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*Mycobacterium bovis* chez les animaux sauvages vivant en liberté ou en captivité, y compris chez les cervidés d'élevage

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**Résumé**

*Mycobacterium bovis* a été isolé chez de nombreuses espèces animales autres que domestiques. Les auteurs décrivent les différentes espèces sauvages qui interviennent dans la persistance de *M. bovis* et dans sa transmission aux animaux domestiques. Le blaireau (*Meles meles*), le phalanger-renard (*Trichosurus vulpecula*), le cerf de Virginie (*Odocoileus virginianus*), le bison d'Amérique (*Bison bison*) et le buffle d'Afrique (*Syncerus caffer*) en sont quelques exemples. Le rôle de ces hôtes naturels est d'autant plus déterminant qu'ils sont certainement à l'origine de la plupart des infections affectant les animaux domestiques ainsi que les espèces sauvages protégées.

Les méthodes utilisées pour lutter contre *M. bovis* chez les animaux sauvages ne sont pas très nombreuses. Des programmes visant à contrôler les populations incriminées ont été mis en place dans certains pays, mais cette méthode est souvent inapplicable, notamment lorsqu'il s'agit d'espèces sauvages protégées. La vaccination constitue un autre moyen de lutte potentiel mais, pour l'instant, aucun système pratique et efficace n'a été mis au point pour vacciner les animaux sauvages contre la tuberculose bovine.
La tuberculose due à *M. bovis* a également été observée chez des animaux en captivité et chez des espèces récemment domestiquées comme les cervidés d’élevage. La prophylaxie dans ce groupe d’animaux dépend d’un usage judicieux des tests de diagnostic et de l’application de bons principes de prévention. Les progrès accomplis dans la mise au point de vaccins contre la tuberculose bovine pour les bovins et les cervidés d’élevage pourraient permettre des avancées significatives en matière de vaccination contre la tuberculose chez diverses espèces d’animaux sauvages en captivité.

**Mots-clés**

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*Mycobacterium bovis* en fauna salvaje en libertad y en cautividad, comprendido el ciervo de granja

**Resumen**
Además de infectar a animales domésticos, *Mycobacterium bovis* ha sido aislado en muy diversas especies de fauna salvaje. Los autores repasan la influencia de una serie de especies en el mantenimiento de *M. bovis* en comunidades salvajes y su propagación a animales domésticos. El tejón (*Meles meles*), la zarigüeya australiana (*Trichosurus vulpecula*), el ciervo (*Odocoileus virginianus*), el bisonte (*Bison bison*) o el búfalo africano (*Syncerus caffer*) son otros tantos ejemplos de animales salvajes que ejercen de reservorio natural de *M. bovis*, hecho muy significativo en la medida en que esos animales, según parece cada vez más claro, pueden constituir la principal fuente de infección de animales domésticos y especies salvajes protegidas.

No hay muchos métodos posibles para luchar contra la presencia de *M. bovis* en animales salvajes. Aunque en algunos países se haya intentado controlar las poblaciones, tal procedimiento no es aplicable en circunstancias en que resulta afectada alguna especie animal protegida. La vacunación constituye un posible método alternativo, aunque de momento no existe ningún sistema práctico y eficaz para vacunar a los animales salvajes contra la tuberculosis bovina.

La tuberculosis provocada por *M. bovis* se ha convertido en un problema también entre animales salvajes en cautividad y en animales domesticados hace poco tiempo, como el ciervo de granja. De que se usen atinadamente las pruebas de diagnóstico y se apliquen principios coherentes de control zoosanitario depende el éxito en el control de *M. bovis* en esos animales. El progreso en la elaboración de vacunas aplicables a bovinos y ciervos de granja puede ofrecer pistas interesantes para utilizar la vacunación como método de lucha contra la enfermedad en diversas especies de animales salvajes en cautividad.

**Palabras clave**
References


