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The epidemiology of *Mycobacterium bovis* infections

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Abstract

Mycobacterium bovis has an exceptionally wide host range, but until recent years there was little concern about infection in species other than cattle and man. Diversification of farming enterprises has led to cognizance of the need for control in other domestic animals, notably deer. There has also been recognition that self-maintaining infection is present in wildlife hosts in some countries – notably the European badger in the United Kingdom and Ireland, the Australian brush-tailed possum in New Zealand, and various species of ungulates in limited areas of a number of countries. Although transmission of *M. bovis* can occur by a number of different routes, control measures imposed on cattle and to a lesser extent on other species have reduced a number of the routes to insignificance. Hence the vast preponderance of transmission within host species is now by the airborne route, and predominantly between species as well. Transmission of infection from badgers to cattle may be an exception, with evidence remaining equivocal about the relative importance of pasture contamination by excretion in badger urine and airborne transmission. In general, contamination of feed and pasture appears to be unimportant in transmission of the disease, because survival times of infective doses of organisms on fomites are relatively short under realistic conditions and because animals are not commonly exposed to a dose high enough to be infective by the alimentary route. Infection through the oro-pharyngeal mucous membrane may be significant, although the infective dose for this route is not known. While many species of animals can become infected with *M. bovis*, only a few act as maintenance hosts and the rest are spillover hosts in which infection is not self-maintaining. With the exception of cattle and deer, other species have become maintenance hosts only within part of their ecological range. For both badgers and possums, maintenance of infection within a local population is due to pseudo-vertical transmission from mother to young, and horizontal transmission linked to breeding activity. Transmission from possums to domestic animals appears to occur mainly during atypical behavioural interactions between the species, and this may well be important for badgers as well. Difficulties in controlling the disease adequately in domestic animals generally result from administrative problems since the necessary technical procedures are available and have been shown to be effective. Where there is interplay between infection in wildlife and domestic animals, eradication of the disease becomes impractical. Although herd prevalence will then be inescapably higher and

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characteristically clustered in patches in both time and space, reduction of the incidence rate to a low level in domestic stock is nevertheless achievable with current methods. Further reduction of incidence in the presence of a wildlife host should be possible through application of ecologically designed management procedures at farm level, but greater gains could be made if new control measures could be developed, especially vaccines.

Key words: Mycobacterium bovis; Cattle; Tuberculosis, bovine; Epidemiology

1. Introduction

Mycobacterium bovis combines one of the widest host ranges of all pathogens (Grange and Collins, 1987) with a complex epidemiological pattern which involves interaction of infection among human beings, domestic animals and wild animals. Yet there have been few reviews which have comprehensively considered the epidemiology of this organism and the epidemiological requirements for effective control. This paper summarises the information which is available on transmission of the disease and the dynamics of infection in major hosts, in order to draw conclusions about the most effective methods of control and the strategies which should be adopted in control programs.

2. Ecology of the organism

2.1. Survival of the organism in the environment

M. bovis is an obligate pathogen but it can survive for substantial periods in the environment under favourable conditions. This ability has been demonstrated in observational studies of survival of organisms following both natural and artificial contamination of various environmental sites. However the issue of epidemiological interest is how long these surviving organisms remain infective, and considerable caution is required in inferring duration of infectivity from survival time measured by indirect methods.

Wray (1975) reviewed factors influencing survival within the environment of pathogenic bacteria in general, and *M. bovis* in particular. Adequate availability of nutrients as organic matter is the most important factor. If nutrients are scarce, organisms become more susceptible to the adverse effects of a rise in temperature, which then causes bacterial death. Sunlight mainly influences survival indirectly by causing desiccation, and ultraviolet light has limited direct effect in temperate zones, although it may influence survival more strongly in the tropics. Adequate levels of moisture assist survival, especially if oxygen is dissolved in the water. Both low pH and certain inorganic ions reduce survival, as can competition with other organisms.

Kelly and Collins (1978) considered that the survival of pathogenic bacteria on pasture may be affected by their location (on the herbage, on the soil, in the soil or in the sub-soil), since in each location there will be differences in the availability of nutrients and the physical structure of the substrate, especially soil. Major factors influencing survival in soil and on pasture include the temperature, moisture and pH, exposure to sunlight, dissolved oxygen,

the presence of naturally occurring antibiotics in the soil, the natural microflora and the types of microfloral associations. The success of recovery of pathogenic bacteria from soil and sub-soil is generally greater than that achieved from the herbage, or from the soil surface, largely because of the desiccating influence of sunlight. Thus in their view *M. bovis* may be expected to persist in slurry-treated soil for up to 2 years.

Genov (1965) mixed *M. avium*, *M. bovis* and *M. tuberculosis* with faeces, blood and urine and found that they survived 150 to 332 days at 12 to 24°C when shielded from direct sunlight. When exposed to sunlight at 24 to 34°C, the length of survival was only 18 to 31 days. When buried at a depth of 5 cm in shaded soil they survived up to 2 years, but only for 11 to 12 months at a depth of 1 cm. In uncontaminated tap water at 18 to 24°C, *M. bovis* survived for 236 days, but addition of faeces and urine to the water extended survival to between 452 and 469 days. Donsel and Larkin (1977) sprayed the BCG strain of *M. bovis* plus effluent or sludge on to plots growing radishes and lettuce. The time to achieve 90% reduction in *M. bovis* on effluent-sprayed soils was 11 days, and on sludge-sprayed soils it was 8 days, slightly longer than survival on radishes. Survival on lettuce was up to 35 days.

Anon (1979) kept naturally and experimentally infected badger faeces and infected badger tissues in a disused badger sett and periodically sampled them for *M. bovis*. *M. bovis* was recovered after 1 week but not after 1 month from naturally infected faeces. *M. bovis* was recovered from the heavily artificially infected faeces after 1 month but not after 3 months. Badger tissue was negative after 1 month. In another test, one infected tissue sample was positive after 1 week, and 2 experimentally infected samples of faeces were also positive. There was no recovery of organisms at 6 weeks or more after deposition. Artificially infected soil and the original culture were stored in a covered yard at ambient temperatures and after 7 months storage, no organisms could be recovered from the soil samples although the culture was still alive and virulent for guinea pigs. When various badger secretions and excretions were cultured after exposure of the material on pasture for varying times, the organism survived in urine for 3 days in summer and 28 days in winter, in bronchial pus for 7 and 70 days respectively and in naturally infected faeces for 14 and 28 days. Little et al. (1982) studied artificially and naturally infected badgers to determine the course of the disease and to investigate transmission to other badgers and to calves. Soil, hay, bedding and water were regularly cultured but only a single culture (from a water trough) was positive. Artificially contaminated faecal samples were regularly found positive.

Duffield and Young (1985), working in tropical Australia, inoculated *M. bovis* into faeces, dry soil and moist soils. It survived for 4 weeks in non-sterile dry and moist soils under 80% shade, in darkness and in the laboratory; and for a similar time in sterile moist soil kept in the shade and in darkness. Re-isolation was not made at 4 weeks from any of the substrates exposed to sunlight or faeces under any conditions. No re-isolation was made at 8 weeks or longer. In contrast, Maddock (1933) recovered *M. bovis* by guinea pig inoculation for up to 7 months after including *M. bovis* in soil, faeces, and mixtures of the two. He also found that pasture sprayed with various concentrations of *M. bovis* remained positive for up to 49 days at the highest concentration used. Williams and Hoy (1930) found that *M. bovis* could remain alive in cattle faeces for up to 5 months after winter application, 2 months for spring or summer application, and 4 months for autumn. Protection from sunlight in summer increased survival to 4 months and protection from insects and

worms took it to 6 months. They were however unable to culture *M. bovis* from soil of an area grazed by a known *M. bovis* excreting cow.

Schellner (1959) experimentally irrigated pasture plots with 10^2 to 10^{12} *M. bovis* per ml of water, and after intervals of 7, 14 and 21 days allowed 56 heifers to graze the plots. Only 2 animals became infected, and these were among 14 which had grazed a plot irrigated 7 days previously. Maddock (1936) was unable to infect calves grazed on naturally-infected pasture at 6 and 10 weeks after contamination, although faeces from the contaminating animals which remained on the pasture contained viable *M. bovis* at 42 days.

It is clear from this literature summary that information on survival of *M. bovis* outside living animals is sparse and patchy, despite the crucial importance of this data for control. However it seems from the available evidence that the duration of infectivity of environmental sources (such as pasture, urine and faeces) for a susceptible species such as cattle is measured in weeks rather than months and is generally well below the period for which organisms can be recovered by artificial isolation. Organisms on naturally contaminated material tend to die more quickly than similar organisms on artificially contaminated but otherwise equivalent material. *M. bovis* in sterile faeces and soil when stored away from daylight lasts for several months, but as natural environmental conditions are progressively approached, survival time decreases rapidly. Thus, given the further fact that the size of the minimum infective dose for oral infection is high, the practical significance of persistence on fomites as a source of infection in most environments appears to be small compared with other mechanisms of transmission, and certainly smaller than is commonly suggested.

2.2. *Survival of organisms in infected animals after death*

For wild reservoir host species of *M. bovis*, persistence in infected animals after death may provide a source of infection for scavengers and possibly later for domestic stock which graze the site of decomposition. The length of time that *M. bovis* can be recovered from carcases of tuberculous possums depends on the speed of putrefaction and decomposition, and hence on the ambient temperature and degree of environmental protection given to the carcase. Livingstone (1979) concluded that recovery was possible if tuberculous lesions were still recognisable in the carcass, but not after decomposition had proceeded beyond that point. Pfeiffer and Morris (1991) were able to recover *M. bovis* from the interior of largely dry carcases for at least a month after death when the moist interior was swabbed. However in this same study, most tuberculous carcases left in accessible locations were scavenged completely within 2 to 3 days at most, thus eliminating the protection otherwise given to the organisms. Recovery from tuberculous badger carcasses varied from 2 weeks when the carcase was lying on pasture to 6 weeks for a buried carcass (Little et al., 1982) while badger tissues in a badger sett were negative after 1 month (Anon, 1979).

2.3. *Possible variation in virulence of the isolate for a host species*

One theoretically attractive explanation for some of the field epidemiological findings in various countries would be that there is considerable variation in virulence among strains of *M. bovis*. However the literature provides no evidence that there are any measurable differences in virulence between strains of *M. bovis*, with the notable exception of the

artificially attenuated strain BCG. It is unlikely that strain differences which can be demonstrated by laboratory methods, such as restriction endonuclease analysis (REA), have any epidemiological significance in relation to the likelihood of disease transmission. It is conceivable that the occurrence of *M. bovis* infection in particular wild animals in some countries and not in others which have the same wild animal present could be due to natural selection of a strain of *M. bovis* adapted physiologically to that host, but there is no evidence to support this as an explanation for the situation. Efforts to identify features which would distinguish bovine and badger strains of *M. bovis* bacteriologically failed (Stuart and Wilesmith, 1988). It is far more likely that ecological factors (population density of various host species, interactions, habitat differences, etc) are entirely responsible for the presence or absence of a wildlife reservoir of tuberculosis in a country, rather than a specific adaptation process to a new host.

2.4. Host resistance

Genetic resistance of hosts to *M. bovis* infection has never been conclusively demonstrated. Wells and Lurie (1941) found evidence of differences among certain families of laboratory rabbits in their resistance to experimental challenge, and in the nature of the disease which developed. Carmichael (1941) produced some weak evidence suggesting that Zebu cattle are more resistant to the effects of the disease than European breeds, but his findings were not conclusive. There is plentiful anecdotal evidence that the physiological and immunological state of an animal or person (including the degree of environmental stress being experienced at the time) strongly influences the course of tuberculosis in various ways, but no experimental evidence has been produced in either cattle or deer to reinforce the observations. Nor is there direct experimental evidence that age, sex, reproductive state and other similar characteristics of the host have a direct influence on transmission or on the course of the disease within an animal. There is however epidemiological evidence from multivariable statistical studies that host factors of this nature are among the explanatory variables associated with the clinical expression of disease in the possum (Pfeiffer and Morris, 1991), and the same would probably be true of other susceptible species, if the relevant data were collected on an adequate sample size for such analyses. There is no data to suggest alterations in susceptibility due to prior or intercurrent exposure to mycobacteria of different species or to other less closely related organisms.

2.5. Environmental and management factors

The large body of field data arising from tuberculosis control programs in cattle shows that there is substantial variation in both incidence and prevalence between geographical regions, and between farms within regions. One simple example of this is that incidence is typically higher in dairy than in beef herds. While some of the variation between herds and regions is purely a consequence of the management system chosen and the opportunity which it offers for transmission of infection and development of disease, there must also be specific environmental and management factors in farms and regions which contribute to this variation. Some of these undoubtedly relate to specific ecological factors which modify

the risk either of transmission between hosts or of the establishment of infection and of disease once transmission has occurred.

Little has been published on the association between variation in specific extrinsic factors and variation in tuberculosis incidence. Since the relationships are clearly multifactorial, involving interactions among risk factors to produce the recorded differences in incidence, multivariable epidemiological methods of investigation can contribute substantially to distinguishing the contribution of various putative causal and contributory factors. Pfeiffer et al. (1991) used logistic path analysis to identify from a much larger initial list, a sub-group of extrinsic factors which appeared to be associated with herd breakdown for tuberculosis. As discussed later in this paper, the same general approach could be used to explore differences in incidence between areas and farms, but does not appear to have been applied to the problem.

3. Transmission of infection and epidemiological patterns in major species

Given the importance of an accurate understanding of both the transmission of tuberculosis and its epidemiological pattern for effective control of the disease, the borderline between solid data and unsupported conviction on the subject is surprisingly fuzzy. Since the situation in cattle has been most fully investigated it will be considered in detail, then specific features will be considered for other species.

3.1. Cattle

Little research effort has been devoted to the subject for some decades, and the summary provided by Francis (1947) remains the most appropriate synthesis of knowledge: "The results of experimental aerogenous and alimentary infection together with the distribution of lesions found in naturally acquired tuberculosis show that about 80 to 90 per cent of all cattle are infected by inhalation. Even in calves this is usually the most important route although occasionally a whole group may be infected by tuberculous milk. Cattle are much more easily infected by the respiratory than alimentary route and although relatively large numbers of bacilli are passed in the faeces, pastures are probably not an important source of infection. The evidence suggests that even when heifers are pastured with heavily infected cows the incidence remains low until they enter the cowshed. The primary lung lesion is a bronchopneumonic focus which may progress rapidly or may remain quiescent for many years. Despite this, however, it would appear that the complete healing of lung lesions which often occurs in man seldom takes place in cattle. This is a fundamental difference and in practice all tuberculin positive cattle are regarded as infectious to other cattle. The most important route of spread in the lung is along the bronchi; sputum passing up the trachea and being swallowed may also infect the intestine and mesenteric nodes, but other organs such as the spleen and kidney are infected by bacilli entering the blood stream from lesions in the lung."

Francis is supported by Collins and Grange (1983) and Pritchard (1988) in their reviews on bovine tuberculosis. Pritchard states "Analysis of a century of necropsy studies clearly indicates that the aerogenous route is the most important route for the transmission of

tuberculosis in cattle''. The experimental studies help explain why this is so. Chaussé (1913) showed that no more than 5 and probably as few as one bacillus would produce a lesion in the lung of sheep when introduced as a fine spray, whereas 13 million bacilli would not always infect sheep by the oral route. The mesenteric nodes of the animals he experimentally infected by the respiratory route were non-infective for guinea pigs. Chaussé further showed that the mesenteric lymph nodes were only infective in one of 20 naturally infected animals, a case with early primary thoracic tuberculosis. Later workers obtained similar results.

The large dose needed for infection by mouth explains the low infectivity of *M. bovis*-contaminated faeces. M'Fadyean (1910) reviewed evidence showing that 10 mg (probably several thousand million tubercle bacilli) was necessary to infect calves by mouth, and as stated above, Chaussé (1913) found that 13 million bacilli fed to sheep would not always produce infection. This is consistent with evidence cited earlier that it is difficult to produce natural infection from contaminated pastures. However, there is no doubt that infection via the alimentary route can occur. Lepper and Pearson (1973) in Australia put forward the suggestion (when comparing 241 animals kept on pasture in Victoria with 166 range animals from the Northern Territory) that *M. bovis* has a better chance of surviving on pasture in a temperate environment. The Northern Territory cattle had a ratio of 20:1 thoracic to abdominal lesions, whereas the Victorian cattle had equal distribution of lesions between the two sites. However, pasture survival time is only one of a number of possible explanations for this variation in lesion distribution, and other evidence would suggest that it is not the most likely one.

The transmission mechanism which produces lesions in the retropharyngeal lymph nodes remains uncertain, despite the high frequency of lesions in this site. It is common to attribute these lesions to respiratory and alimentary infection in equal proportions, but such a simplistic assumption eliminates their epidemiological explanatory value, and has little solid evidence to support it. It seems more plausible that these common lesions have a distinctive mechanism. Thoen and Himes (1986) report that the medial retropharyngeal nodes receive afferent vessels from the floor of the mouth and adjacent parts. It could be that inhalation of organisms which do not penetrate to the lower respiratory tract can initiate multiplication at a site in the oro-pharyngeal area, which drains to the retropharyngeal lymph nodes. From there, infection may spread to additional sites if the animal is not detected by early tuberculin testing. The issue of how these lesions are initiated deserves more definitive resolution as part of the process of clarifying mechanisms of transmission between wildlife and cattle.

Mullenax et al. (1964) used marker organisms which had been introduced to the rumen to show that during eructation those organisms can end up in the lungs. However no evidence has ever been reported which would suggest that this process contributes to the epidemiology of tuberculosis or similar diseases. Organisms which penetrate the upper intestine may move in the circulatory system so that the lesions they cause are in the lung, but it seems unlikely that either of these mechanisms is a significant pathogenetic mechanism overall.

Langmuir (1961) discusses the epidemiology of airborne infection and classifies transmission by contact, droplet, droplet nuclei (residues of small droplets which dry down to a size capable of staying airborne for extended periods and moving away from the source which exhaled them) and dust. Droplet nuclei (and dust under some circumstances) are the principal vehicles which are capable of meeting the three requirements for airborne transmission between animals – they are capable of carrying bacteria of the size of *M. bovis*,

they can persist long enough in the air to be inhaled, and they are small enough to meet the requirements worked out by Wells et al. (1948) and Lurie et al. (1950) to penetrate into the lung and initiate infection.

Langmuir makes the point "Epidemiologically, the dominant concept in tuberculosis control has been, and to a large degree still is, the importance of close contact. Yet, in my opinion, the mass of the evidence is far more consistent with the concept of airborne infection. Household contacts and even marital partners of sputum-positive patients often fail to become infected. At the same time, numerous epidemics of tuberculous infection have been recorded where essentially everyone in a group became instantaneously infected. These observations are much more consistent with the hypothesis that only certain tuberculous individuals act as effective disseminators and these do so probably intermittently and only under certain circumstances." Although the statement is directed at the human disease, it fits very well to experience with tuberculosis in animals.

In considering airborne spread, it is erroneously considered by many farmers and veterinarians involved in bovine tuberculosis control that the dangerous animals which excrete *M. bovis* are those with large clearly identifiable lung lesions. On the contrary, it may well be that animals in the early stages of the disease before the development of readily detectable lesions (no visible lesion animals) contribute substantially to production of infective aerosols. McIlroy et al. (1986) found that 63% of tuberculous lungs had a single lesion in the caudal lobes, and that 70% of these were less than 1 cm in diameter. These findings are fully consistent with the early studies of Stamp (1948). In the study of McIlroy et al. (1986), *M. bovis* was isolated from nasal and/or tracheal mucus of 19% of those with lesions in the lungs.

Neill et al. (1988a) were able to isolate *M bovis* from the anterior respiratory tracts of 4 of 25 reactors and from a nostril lesion in a fifth reactor, all of which were subsequently confirmed as tuberculous at slaughter. Neill et al. (1988b) used 92, 10^4 and 10^6 colony forming units (cfus) of *M bovis* inoculated intranasally in groups of calves to initiate infection. Infection was successfully established with the two higher dose levels, all animals excreted *M bovis* and the largest inoculum gave the shortest time to initial excretion, longest duration of excretion and largest quantity of organisms excreted. Although calves given 92 cfus did not develop disease, *M bovis* was isolated from nasal secretion of one at 100 days after inoculation. Excretion in faeces was not regular, and appeared to be due to swallowing of mucus from the respiratory tract containing organisms. Further trials (Neill et al., 1991) supported by field data, showed an inverse exponential relationship between dose as cfus and time to first excretion. In natural infections, Neill et al. (1992) found that excretion commenced typically at 87 days and in some cases occurred in tuberculin test negative cattle. Once excretion commences (to echo Langmuir), "only certain tuberculous individuals act as effective disseminators and these do so intermittently and only under certain circumstances", a statement which is supported by the studies of Neill et al. (1989 and other cited sources). Physiological and environmental stresses of various kinds may well act as precipitating factors for periods of *M. bovis* excretion.

For disease control purposes, many of the issues which remain unresolved with regard to initiation of infection and excretion of *M. bovis* could be effectively answered by judicious epidemiological analysis of tuberculosis control records from large numbers of infected herds with different management and environmental characteristics, since such data provides

a more realistic representation of the natural course of disease than can ever be obtained from experimental studies. Epidemiologically, patterns of tuberculin reactors in herds do not in many cases fit to the pattern seen in classical contagious diseases, and statistical evaluation for large numbers of herds of the time pattern of incident cases in relation to multiple potential risk factors could give very valuable insights into the number of different temporal infection patterns which occur, their relative frequency, and their relationship to risk factors which may have explanatory value for the spread of disease.

Regular tuberculin testing of cattle herds has changed the relative frequency of different methods of transmission. Some which were important in the past have declined to insignificance in countries which have active bovine tuberculosis control programs, either because they required very large challenge doses or because they usually occurred only late in the course of the disease when clinical signs were beginning to develop. Francis (1947) summarised data which showed that in the absence of effective control measures about 5% of tuberculous cows had tuberculous metritis, originating from peritonitis, the external genitalia, or most commonly from haematogenous spread. Tuberculosis of the penis was fairly common in bulls. Only about 1% of calves from tuberculous cows were congenitally infected. One to 2% of tuberculous cows had tuberculosis of the udder due to haematogenous spread. Alimentary infection of calves was commonly secondary to these cases of udder tuberculosis. Thus, as these other routes have declined under the influence of control programs, airborne infection has become even more dominant.

Transmission from human beings to cattle has been recorded on a number of occasions and the need for co-operation between medical and veterinary personnel was stressed by Collins and Grange (1983, 1987) and Pritchard (1988). Transmission in such cases is probably mainly airborne, but spread via urine from human renal excreters was also thought to be important by Huitema (1969) in Holland.

3.2. Farmed deer

The epidemiology of *M. bovis* infection has not been as fully investigated in deer as in cattle, and some aspects of the disease are as yet poorly understood. The disease in deer has recently been reviewed in detail by Clifton-Hadley and Wilesmith (1991). Typically lesions appear to be most common in the retropharyngeal lymph nodes, lungs and lymph nodes of the thoracic cavity, and mesenteric nodes (Livingstone, 1980, cited by Beatson, 1985; Brooks, 1984; Wilcockson, 1986). However large superficial (often visible) abscesses also occur and may (Robinson et al., 1989) or may not (Fleetwood et al., 1988) discharge to the exterior in particular cases. Mesenteric nodes draining to the lumen of the intestine have also been reported in deer (Bertram, 1986). The distribution of lesions suggests that both inhalation and ingestion are important routes of infection in this species. Lesions found in deer with natural cases of *M. bovis* infection range from caseous and granulomatous to quite liquid lesions, probably related to the much greater role of neurophils in the inflammatory process in deer than in other species.

There is little direct evidence on the epidemiology of the disease in deer, and extrapolation from other species may not be entirely valid. It has been suggested that farmed deer may be more susceptible to *Mycobacterium bovis* infection than cattle. Under suitable circumstances extensive lesions can develop rapidly, producing increased probability of spread

within a herd (Clifton-Hadley and Wilesmith 1991). As in cattle, the pathogenesis of the disease is mainly dependent on the size of the infecting dose and the susceptibility of the host. Susceptibility depends on factors such as genetic constitution, previous exposure to *Mycobacterium* spp., nutrition, social status in the herd, handling stress and endocrine factors (de Liste et al., 1985).

Deer appear to be more infectious for other species than cattle, and possibly fall at the high end of the range as sources of infection for other species. They appear unusually likely to cause human infection compared with cattle and possums (Fanning and Edwards, 1991), for reasons which remain unresolved beyond the level of hypothesis. There is also both clearcut and circumstantial epidemiological evidence from various incidents in New Zealand of their ability to initiate new foci of infection in possums, whereas cattle seem to have done so rarely, considering the relative numbers of tuberculous animals of each species with which possums would have had the opportunity for contact. The first reported infections of badgers (which occurred in Switzerland) were also attributed to transmission from roe deer (Bouvier et al., 1957; Wilesmith, 1983). The possible central role of deer as a reservoir host for *M. bovis* in some countries deserves critical evaluation using the various epidemiological methods which have been employed in other species but not so far in deer.

3.3. Other domestic animals

Prevalence of tuberculosis in small ruminants is low and apparently proportional to that in major hosts such as cattle (Cordes et al., 1981). In general the nature of sheep husbandry and limited exposure to source animals makes the disease a rare one, even where exposure to infected wildlife might be considered a risk (Schliesser, 1985). Very occasionally, prevalences of up to 5% have been observed in sheep flocks in New Zealand (Davidson et al., 1981). Behavioural differences between sheep and cattle may influence this, since sheep in contact with possums sedated to simulate terminally ill animals did not expose themselves to "infection" by close contact, whereas the more curious of a group of cattle exposed themselves liberally (Paterson, 1993). Infection can occur in horses, but is of no epidemiological significance.

Disease levels in pigs also usually reflect those in local cattle populations, and high disease levels in cattle can result in prevalences of up to 20% in local pigs (Blood and Radostits, 1989). Myers and Steele (1969) reported that in 1921 in the USA 12% of market pigs slaughtered under federal inspection were found to have tuberculous lesions. They note that in the mid west of the USA it was possible to trace 96% of swine carcass condemnations for tuberculosis to feeding of unsterilized skim milk or other dairy products or to keeping pigs together with cattle. Prevalence in pigs is thought to increase with age. The principal route of infection in the pig is the digestive tract, by consumption of milk or milk products, kitchen and abattoir scraps, and excreta from tuberculous cattle (Acha and Szyfres, 1989). Transmission between pigs is considered epidemiologically insignificant, as lesions usually remain localized and pigs are slaughtered at an early age.

Lepper and Corner (1983) quote work by Snider and Cohen who found tuberculous lesions in four dogs and 24 cats out of 61 contacts on farms with *M. bovis* infection. The authors concluded that on premises with tuberculosis infection, surveillance of these species is recommended. In another report of cases in two cats in the UK, association with badgers

was suggested as one possible explanation of the source of infection. Yet, although tuberculosis is diagnosed sporadically in cats, it is unlikely that domestic dogs and cats represent a epidemiologically significant factor in the dynamics of tuberculosis infection.

3.4. Epidemiology in wildlife

Tuberculosis has been known as a serious clinical disease in wild mammals in captivity for more than a century. Thoen and Himes (1981) state that it is widely distributed in wild mammal populations in the United States, where outbreaks caused by infection with *M. bovis* have been reported mainly from zoos, game parks and primate colonies. Schliesser (1985) notes that in European countries, sporadic incidents of bovine tuberculosis in wild mammals were mainly reported before eradication of cattle tuberculosis was achieved. Evidence from various countries in the world shows that given conducive epidemiological circumstances, significant levels of tuberculosis infection can be found in feral and free-living wild species such as buffalo, goats, pigs, deer, badgers and brush-tailed possums (Lepper and Corner, 1983). The risk which these reservoirs of infection constitute for infection in domestic animals and man is quite variable depending on the specific epidemiological situation for the species and the environment.

3.5. Badgers

The first report of *M. bovis* infection in the European badger (*Meles meles*) was in Switzerland in 1956 (Bouvier et al., 1957). However while there has not been any evidence of continuing transmission of *M. bovis* between badgers and domestic livestock in the European continent, infection is endemic in badger populations in both the United Kingdom and Ireland, and transmission to cattle is a significant factor in the epidemiology of bovine tuberculosis in both countries. But because of management and other factors which amplify the amount of transmission within the cattle population, Ireland has a much higher national incidence rate and different epidemiological pattern (Downey, 1990).

The disease in badgers has been studied intensively in England (Cheeseman et al., 1988; Stuart and Wilesmith, 1988; Wilesmith, 1991b), and various studies have also been conducted in Ireland. The evidence shows that the prevalence of infection in a badger population shows substantial fluctuation over a series of years, from a low of about 2% to a peak of over 12%. The badger is a long-lived animal, and the pattern of change in prevalence suggests a degree of cyclicity of infection prevalence arising out of the interaction of disease with the dynamics of the total badger population in the area. Transmission among badgers appears to be mainly by the respiratory route, and serological evidence points towards a high component of pseudovertical transmission from mother to cub (Wilesmith, 1991b). This presumably takes place during the rearing period through the respiratory route, rather than in utero. Some transmission occurs as a result of fighting between males, which would include infection of bite wounds as well as aerogenous transmission between the combatants. Cheeseman et al. (1989) recorded that the distribution of lesions was consistent with a respiratory infection in 23 out of 28 badgers examined, and that the other 5 could have been infected from bites. Gallagher et al. (1976) found similarly that 22 of 36 naturally infected

wild badgers had lesions in the lungs or thoracic cavity nodes, but that where haematogenous spread had occurred there was a predilection for the kidney.

Badgers are unusual in that kidney infection with excretion in urine is much more common than in other species (37% of tuberculous badgers excreted *M. bovis* in urine), and it has been proposed that infection of cattle occurs predominantly from urine deposited on pasture by foraging badgers, especially those in which the disease has reached an advanced stage. Cattle do not avoid pasture contaminated with badger urine although they avoid areas around badger faeces. However, maximum survival time of organisms from urine on pasture is quite short (3 days in summer and 28 in winter; Wilesmith, 1991a). The importance of pasture contamination is not strong enough to rule out respiratory transmission as a substantial contributor to the total quantum of transmission from badgers to cattle, and possibly the most important. While normal badgers avoid close contact with cattle, terminally ill tuberculous badgers can exhibit abnormal behaviour which may facilitate transmission (Cheeseman and Mallinson, 1981; Collins, J.D., personal communication, 1992). There are numerous reported instances where large numbers of cattle have become infected over a very short period of time, and such cases are more consistent with respiratory transmission from a heavily infected badger than with pasture contamination by urine, the second mechanism being a better explanation for low incidence rates, with or without persistence of infection in a herd. Tuberculous badgers have been shown to be able to act as a source of infection to calves by contact, using both naturally infected and experimentally infected badgers (Little et al., 1982).

3.6. Brush-tailed possum

In the nineteenth century a fur-bearing marsupial, the Australian common brushtailed possum (*Trichosurus vulpecula* Kerr), was introduced to New Zealand and liberated at numerous locations over several decades (Pracy, 1962). The susceptibility of possums to *M. bovis* infection was first experimentally demonstrated by Bolliger and Bolliger (1948). In 1967 the first possum with tuberculous lesions to be identified in New Zealand was found by a trapper on a farm with a persistent tuberculosis problem. In this and later cases it was reported that in all tissues examined, large numbers of *M. bovis* organisms were present and the animals were discharging organisms through open sinuses or the respiratory tract (Ekdahl et al., 1970).

Since then, various workers (Cook, 1975; Cook, undated; Julian, 1981; Coleman, 1988; Hickling et al., 1991) have demonstrated that infection of this species is present in various areas of New Zealand, and is growing in geographical distribution. Studies have shown point prevalence of cases with visible lesions in affected areas to average about 5%, with seasonal variation typically from 2% to 10%. Lesions are most common in the respiratory tract (about 55 to 70% of animals), although lesions in superficial lymph nodes have been reported in over 50% of tuberculous possums in various studies. It is quite common for these lesions to discharge intermittently or continuously to the skin through sinuses.

The disease is progressive and invasive with large tuberculous nodules which have central necrotic areas containing relatively few polymorphs but a large number of organisms. There is no fibrous capsule, and liquefaction of the central necrotic area produces greenish semi-liquid exudate which discharges to the exterior. The lesions are consistent with a poor host

response to the disease process. Lung lesions are commonly very extensive, involving multiple lesions, from miliary to large abscesses, and provide ample opportunity for airborne excretion in most infected animals. In contrast, although Corner and Presidente (1981) found urine shedding in experimentally infected animals, it does not appear to be common under field conditions. Faecal shedding is also apparently unimportant. However transmission from mothers to their dependent joeys between birth and independence (termed pseudo-vertical) is a very important method of transmission. The close association of mother and joey, with only 18 days in utero but approximately 5 months of pouch rearing followed by a period of back-riding, appears to expose joeys born to tuberculous mothers to a very high risk of infection (O'Hara et al., 1976; Pfeiffer and Morris, 1991).

A longitudinal field study of the epidemiology of tuberculosis in possums and cattle which is still in progress (Pfeiffer and Morris, 1991; Pfeiffer, unpublished results) is clarifying the transmission of infection among possums and to cattle. The current hypothesis from this work is that local transmission is pseudo-vertical between the generations, and that horizontal spread within local possum communities is predominantly associated with courting and mating activities between the sexes, and competition among males. It is not yet certain whether one or other of these two main mechanisms is substantially more important than the other. Outward movement of infection arises principally from the dispersal of juvenile males and relocation of some adult males. The time from infection to development of clinical disease is believed to be very variable, and strongly influenced by the severity of environmental, climatic and other stresses to which a particular infected possum is exposed. The combination of these factors produces the marked clustering of infection seen in cross-sectional and longitudinal studies. Evidence from behavioural research (Paterson, personal communication, 1993) suggests that cattle are highly likely to become infected principally through close contact with terminally ill possums – which commonly behave abnormally and thus attract the attention of cattle. It seems likely that a few cattle and probably almost all of the scavenger species (wild pigs, ferrets, stoats) become infected by contact with possum carcases. It does not appear that surface contamination of pasture or of den sites and other areas frequented by possums is responsible for a significant amount of transmission either among possums or between possums and other species.

3.7. Feral buffalo and bison

Bovine tuberculosis is known to be endemic at low prevalence in feral swamp buffalo populations of the Australian Northern Territory (Hein and Tomasovic, 1981), although evidence 15 years earlier showed a much higher prevalence (Letts, 1964). This reduction in prevalence may have been due to removal of infected animals by selective harvesting of mature animals for meat, or the earlier figure may have come from sampling an unusually high prevalence area. The large proportion of cases with sole or predominant involvement of the thoracic organs suggests that, as in cattle, the respiratory route is the most important transmission path in feral buffalo. McCool and Newton-Tabrett (1979) indicate that 97% of buffalo tuberculosis in northern Australia is contracted via the respiratory route, whereas behavioural work by Tulloch (1978) suggested ample opportunity existed for both alimentary and respiratory infection.

Woodford (1982a) studied the occurrence of tuberculosis in wild Cape buffalo in Ruwenzori National Park, Uganda. He found tuberculous lesions in 10% of 52 buffaloes from a random sample and 38% of 64 animals which were selected based on being in poor condition. *M. bovis* was identified in 12 of 14 cases. Most cases appeared to be infected by respiratory transmission and no lesions were seen which could be ascribed to alimentary infection. The author explains this finding as due to the close herding habits of wild buffaloes and their propensity for wallowing in tight groups in small mud holes which facilitates droplet transmission. Woodford concluded that bovine tuberculosis could cause an annual mortality of about 1% in this particular area.

Choquette et al. (1961) reported that they found tuberculous lesions in 50% of 436 bison from Wood Buffalo National Park, Canada, which had reacted to the tuberculin test. They quote other work by Fuller who wrote that tuberculous lesions had been found in 39% of 1508 bison slaughtered between 1952 and 1956. The same authors refer to a report by Hadwen who during the period between 1923 and 1939 found tuberculous lesions in 53.4% of 12005 bison slaughtered at Wainwright. Choquette et al. examined another 500 bison from Elk Island National Park, which were not tuberculin tested and did not show any lesions on post-mortem. They conclude that in Wood Buffalo National Park current tuberculosis control based on tuberculin testing and slaughter of reactors would be at best a disease reduction program. More recently concern has been expressed again about the levels of tuberculosis infection in bison in Canada. Between 1983 and 1985 during a survey in and around Wood Buffalo National Park, Canada, 21% of 72 bison found dead showed tuberculous lesions on post-mortem. The results suggest that infection occurred primarily via the respiratory route. It was concluded that the disease was endemic within the population and that therefore there was a growing risk of spread to uninfected bison and cattle (Tessaro et al., 1990).

3.8. Feral deer

Infection with *M. bovis* has been reported from a number of free-ranging deer species, as pointed out in the review by Clifton-Hadley and Wilesmith (1991). These authors and others report that there have been some incidents where infected feral deer were suspected of introducing infection into captive deer populations. Mackintosh and Beatson (1985) state that in New Zealand a high proportion of wild deer captured or shot was found to be infected with *M. bovis*. It has been suggested that in New Zealand (where deer had been live-captured in many parts of the country and then traded for breeding purposes) infected animals probably provided a means for introducing infection into areas which previously had been free of tuberculosis.

3.9. Other wild and feral animals

Feral pigs have been found to be infected with *M. bovis* at significant levels in a number of countries. Recently a survey was conducted in Central Otago New Zealand, where 251 feral pigs were post-mortemed and 31% were found to have tuberculous lesions (Wakelin and Churchman, 1991). The authors suggested that the disease possibly had spread between pigs by airborne transmission. Letts (1964) confirmed *M. bovis* infection in 54% of 149

tuberculosis-like lesions from a total of 260 feral pigs in Australia's Northern Territory which were autopsied. He ascribed this relatively high prevalence as due to the pigs living in close association with swamp buffalo. At the end of each dry season hundreds of old buffalo die, thereby providing food and a potential source of infection with *M. bovis* in pigs which scavenge the carcases. Corner et al. (1981) subsequently showed that pigs in the Northern Territory were almost certainly dead-end hosts, which rarely transmitted the disease to other species.

Feral goats were found with tuberculosis prevalences of up to 31% within individual groups in areas with endemic tuberculosis in New Zealand (Sanson, 1988). The epidemiological significance of bovine tuberculosis in goats is generally considered as minimal. In most cases it is related to the presence of a reservoir of infection in another species, such as the brushtailed possum in New Zealand.

Woodford (1982b) found that bovine tuberculosis infection was endemic in the wart hog population of Ruwenzori National Park, Uganda. He concluded that the disease must have been introduced with domestic cattle. Little et al. (1982) examined a range of wild mammals in an area of Dorset where tuberculosis was endemic in badgers, and found a low prevalence of infection with *M. bovis* but no pathology in rats and foxes. Wilesmith et al. (1986b) undertook a very detailed study of wildlife in an area where badgers and cattle were infected, and found no evidence of infection in any of the 15 species of small mammals examined, including rabbits. Rabbits kept under laboratory or domestic conditions are highly susceptible to infection with *M. bovis*. However, only a single case of field disease has ever been recorded in the world (Anon., 1980), despite efforts to find evidence of infection in various places.

Infection in feral carnivorous species has to be expected in areas with endemic tuberculosis in important reservoir hosts such as the brush-tailed possum in New Zealand. Allen (1991) reviewed the evidence available on the occurrence of bovine tuberculosis in feral carnivores in New Zealand. Although cats are relatively resistant to infection with *M. bovis*, the disease is present at a low level in the feral cat population, and the limited evidence available suggests that infection is acquired mainly by predation and scavenging of other affected species, although some transmission among cats could occur, especially associated with fighting among males and mating activity. Mustelids are quite susceptible to *M. bovis*, and the disease has been diagnosed in ferrets, weasels and stoats. However only a few cases have been diagnosed, and it would appear that these result from scavenging. Neither cats nor mustelids are likely to be of primary epidemiological importance in transmission of the disease.

4. A synthesis of the current epidemiological situation

4.1. Maintenance and spillover hosts

In the absence of a wildlife reservoir of infection with *M. bovis*, tuberculosis in cattle is now a readily controllable disease. Test and removal of infected animals at intervals of less than a year can eliminate the infection from herds provided that an epidemiologically sound control policy is followed which leaves no loopholes for persistence of infection. Such a

policy eliminates virtually all transmission mechanisms other than airborne infection, and that can be controlled effectively as well, provided that infected animals are not left in or allowed to enter herds.

Where infection persists under such circumstances, it is entirely due to management procedures allowing infected animals to remain in herds and to move between herds, thus starting new foci of infection. Normally this results from incomplete testing of eligible animals (mustering difficulties, deliberate deception), or the following of various practices which allow infected animals to move between herds and create new foci of infection. There is no evidence that long-term persistence of infection in cattle herds or in areas is due to any unexplained cause, if there is no wildlife reservoir host. The fundamental epidemiology of the disease remains as it was 30 years ago, and if anything has been simplified by the elimination of almost all transmission mechanisms other than airborne infection.

The situation in farmed deer is somewhat more difficult because of the apparent high transmissibility of disease within this species, and the fact that the effectiveness of testing procedures is not as definitively determined as in cattle. In addition, the epidemiology of the disease in deer has not been as well defined as in cattle, especially with regard to the contribution of different transmission mechanisms. However, as in cattle, the answer to more effective control does not rely on finding the elusive ideal test with maximum sensitivity and specificity, but rather on using adequate tests as epidemiological tools to eliminate infection at herd level. The evidence suggests that this is generally achievable in deer with only moderately greater difficulty than in cattle.

However, there are hints in the evidence that both wild and farmed deer may be exceptionally effective in transferring infection to other species, and thus would deserve special attention in control programs. At present, the epidemiological evidence to confirm or refute any hypotheses about the contribution of deer to the disease are very meagre indeed, and this deficiency needs to be rectified.

The major complication of recent years has been the recognition (and probably the emergence as well) of reservoirs of infection in various wildlife and feral species around the world. Once a wild species can maintain infection in an area in the absence of cross-transmission from other species of domestic or wild animals, then total elimination of disease from the population becomes an elusive goal. This status of maintenance host appears to have been reached by the badger, the brush-tailed possum and various species of ungulates in some countries, although it is notable that none of the maintenance hosts have achieved that status in all countries where they occur in significant numbers. If anything, it is the exception rather than the rule for a species to become a true maintenance host in a country, even though it has achieved that status elsewhere.

In addition, there is a long list of spillover hosts which become infected when the challenge level is relatively high, but on current evidence do not seem to maintain infection within the species in the absence of continuing acquisition of infection from maintenance hosts. Man belongs in this category. Yet another group of species is susceptible to experimental infection, but does not reach detectable prevalence under field conditions. The rabbit is a notable and intriguing example of such a species, since all the ingredients for rabbits to become reservoir hosts seem to be present but it has not so far done so anywhere in the world.

It must be remembered that this situation with maintenance and spillover hosts reflects a dynamic equilibrium rather than a fixed state of nature. As early as the 1940s the brush-tailed possum was known to be a susceptible species, but it now seems likely that the disease became established in the New Zealand possum population between about 1950 and 1960, field disease was first seen in 1967, and it was only in the 1970s that it became clear that the species was now a maintenance host in New Zealand, although free of infection in its native Australia. Given the ubiquity of *M. bovis*, it must be seriously asked whether in future *M. bovis* will be endemic in a gradually changing spectrum of wildlife hosts in different parts of the world, with little prospect of eliminating the infection from these species. Control would then need to focus on minimising the establishment of infection in new maintenance host populations and the spillover from maintenance species into animals of economic or environmental note, and possibly into man.

4.2. Transmission pathways

The evidence concerning wildlife tuberculosis points to marked similarities in the transmission mechanisms within maintenance hosts, and from these to other species. In both badgers and possums, the evidence to date suggests strongly that pseudo-vertical transmission from mother to young is a key factor in maintenance of infection within local populations, with local dissemination resulting from competitive interaction among adult males and from courting and mating activity between the sexes. Even in swamp buffalo it would seem that there are comparable features to the epidemiological pattern (W.J. Freeland, personal communication, 1993). Distant spread within wild animal populations is due largely to dispersal of animals seeking new home ranges, although in practice this is intertwined with spread of infection due to commercial trading of infected domestic stock. The vast majority of spread within wildlife is by the airborne route, and elimination of this route of transmission would probably render the species no longer a maintenance host. As in human tuberculosis, transmission by environmental contamination does not contribute significantly to the spread of infection. This fact deserves considerable emphasis because it simplifies the problem greatly.

Transmission from wildlife to domestic animals and among domestic animals appears now to be predominantly also by the airborne route, and occurs principally when there are interactions (usually behaviourally atypical) between an excreting (frequently terminal) wildlife host and domestic animals. The badger is exceptional in excreting organisms extensively in urine and hence contaminating pasture, but the evidence is so far equivocal that transmission from badgers to cattle is more by this method than by airborne spread.

Spillover hosts seem to become infected by routes other than the respiratory one more commonly than do maintenance hosts – for example the infection of predators and scavengers which consume infected animals. By definition these hosts have only limited capacity to transmit infection to other members of their species. This may reflect different location of lesions arising from different entry portals, different susceptibility to development of clinical disease, different excretion routes or quantities of organisms excreted, or different social behaviour patterns.

The maintenance wildlife hosts are characterized by their ability to produce temporally continuous but spatially very patchy distribution of infection in their own population. They

produce much lower incidence in domestic animals than within the maintenance host, with the distribution of wildlife-derived infection being both temporally and spatially patchy, even if infection is continuously prevalent in the maintenance host. Any theory of transmission must be able to explain these discrepant patterns among the hosts, and the theory explained above meets that requirement.

In circumstances where infection in domestic animals does not show both spatial and temporal patchiness, it is almost certain that there is a substantial overlay of additional transmission within the domestic stock, which is being randomly seeded at low incidence with infection by the wildlife host. Transmission both within and between herds due to inadequate control of spread among cattle will then cause the disease to become more uniformly distributed.

5. Control of tuberculosis

The basic measures required for control of bovine tuberculosis in domestic stock are well-defined and the information is readily accessible (Blood and Radostits, 1989). In essence, regular testing of all eligible animals with a test equal to or superior in sensitivity and specificity to the intradermal tuberculin test will, if meticulously carried out in combination with appropriate hygiene measures and restrictions to prevent introduction of infected animals, control bovine tuberculosis very effectively. The disease has been eradicated from countries and large regions using this approach, even under circumstances where some aspects of the control measures are mildly compromised by inescapable limitations. The core of the control program must however be to block pathways of transmission by appropriate epidemiological methods rather than merely to complete testing protocols without regard to epidemiological loopholes which might invalidate the control effort. Where control difficulties have arisen, there has typically been too much concern with test characteristics and with uncontrollable sources of failure to eradicate at herd level, and inadequate concern with identifying and preventing methods of transmission. There has also been undue concern with "singleton reactors" – the herd which has one positive animal at a test. Given that no test for tuberculosis is 100% specific, singleton reactors must be found routinely in the course of testing. Most single positives will be false positives, but a small proportion will be the first in a series of infected reactors. Various strategies are available for dealing with this problem (which is universal in disease eradication programs), and the strategy chosen should represent a careful balance between cost and effectiveness – it is fundamentally an economic problem rather than a disease control problem, and should not be allowed to confuse the situation.

Where herds show reactors at all tests or a substantial proportion of tests, then there are either deficiencies in the implementation of the control procedures at herd level, or more commonly, infected animals are being allowed to enter the herd. A wildlife reservoir in the area may contribute some of these reactors and may be responsible for initiation of an outbreak, but it will not typically produce a steady stream of reactors involving multiple animals at a majority of tests – unless there is also transmission between cattle, which should be a rare event if there is frequent testing and prompt removal of reactors.

The design of a regional control program therefore requires certain basic principles, regardless of whether or not a wildlife reservoir exists. There must be multiple interlocking control procedures which block transmission by each of the pathways known to be operating in the population. In this way, failure of one control measure will not undermine the whole control strategy. The first step is to motivate farmers to cooperate, and convince them that progress is achievable. Unless this can be done, there is little prospect of success with tuberculosis control. Increasingly, use is being made of farmer leadership in programs to ensure that the farming community contributes fully to the design of control measures and their enforcement. Where possible, the control approach chosen should be based on incentives for achievement of program goals rather than on coercion to participate.

Achieving effective control within herds presents no major technical problems, but may fail either due to non-cooperation of the farmer with control procedures or to genuine inability to present all animals for testing on all testing occasions. Achieving effective control between herds relies on minimizing the possibility of infected animals firstly moving between herds and secondly infecting animals in the purchasing herd before being detected as infected. To do this, it is usually necessary to minimize the number of movements from infected to non-infected herds by some form of animal or herd identification and movement control, and to test animals one or more times in association with movements in order to detect any infected animals which move despite the preventive measures. In principle this is not a particularly difficult objective and has been achieved for a variety of diseases in numerous countries. However, in particular livestock industries and particular countries it has usually been the major stumbling block for effective control of tuberculosis, primarily because a proportion of farmers rate tuberculosis control lower than competing motivations and therefore do not comply with measures which would be necessary for tuberculosis control. At this point, the choice lies between various forms of coercion and accepting as inevitable a continuing flow of tuberculin reactors in an epidemiological pattern which arises from frequent movement of infection among herds.

If there is a wildlife reservoir of infection, then the control measures described above will still produce a low prevalence of infection across the livestock population, but eradication will be impossible with current control measures. There will be patchy incidents of herd breakdowns producing a higher herd prevalence of infection within the population than otherwise, combined with a lower than expected within-herd incidence.

At present the only control measure available for wildlife hosts is removal of infected animals. In New Zealand, the possum is considered a pest species on multiple grounds and control has been carried out by large-scale aerial and ground poison baiting on one or repeated occasions in affected areas. This reduces the scale of transmission to cattle for some years, but only under favoured circumstances is there any real prospect of local eradication with current methods. In both Britain and Ireland the badger is a protected species and selective euthanasia or removal of apparently infected badgers based on a diagnostic method is currently the only feasible approach. Linking specific badger setts with a cattle herd breakdown has been used in the past, and individual ELISA testing of captured badgers is currently under consideration. Selective removal may be locally effective in badgers provided that infected animals can be removed at a rate which exceeds incidence. Such methods can work in circumscribed areas surrounded by populations free of the disease, but where wildlife dispersal cross-infects populations regularly, they will at best

reduce the incidence in the livestock population. This may be quite adequate in some circumstances, but certainly will not eliminate the disease in the wildlife population or prevent transmission to domestic stock.

If future progress is to be made, it will depend on broadening the range of available control measures and integrating them into combined programs which jointly reduce transmission. There are strong epidemiological similarities between tuberculosis and wildlife rabies, where oral vaccination of the wildlife reservoir is proving a major advance in control of the disease. In the case of tuberculosis, vaccines could be considered either for the wildlife reservoirs or domestic stock, or both. However this would depend on the development of vaccines which gave cost-effective protection at population level, and in the case of domestic stock did not interfere with detection of infected animals by testing. The use of a test capable of discriminating between infection and vaccination would therefore be an essential complement to the introduction of vaccination of domestic stock. In principle, it seems likely that vaccination would contribute substantially to reducing the seeding of infection from wildlife into livestock, and hence would make control in domestic stock simpler to achieve. It therefore deserves high priority as an area for research.

For animals such as the possum in New Zealand, where it is recognized as a pest species for reasons beyond its transmission of tuberculosis, a mix of population control measures may well offer advantages over area poisoning alone. Mechanisms which deserve consideration are fertility control in adults of one or both sexes, measures to reduce the dispersal of infected juvenile animals and the use of continuous-access poison sources. However all of these measures require major research investment and are unlikely to produce a useable result in under ten years. In the meantime, evidence is accumulating that there is significant scope for reducing transmission between wildlife and domestic stock by farm-based control programs. These are likely to be built around a combination of effective control of the disease in domestic stock, use of stock management procedures which minimize transmission-prone behavioural interactions between stock and wildlife, and in some cases carefully timed local population control measures. Timing of these various measures may turn out to be as important as the nature of the measures taken. However no control policies based on measures other than simple population reduction have yet been attempted, so there is no experience so far to report.

6. Investigation methods

If the answer to better control of *M. bovis* lies both in the development of new control methods and especially in the more precise application of existing control methods, then there is a need for epidemiological methods to be used to define and implement future integrated control strategies. Many of the answers to questions about how to improve control program effectiveness can be garnered from careful scrutiny of properly kept campaign records, combined with specific field studies of issues which arise from these investigations. The first stage lies in defining the dynamics of both animal movement into and out of herds, and the incidence and removal of tuberculin test positive animals. The situation must be examined first in terms of apparent annual inflow and elimination of disease so that the dynamics become clear. Second, the temporal dynamics must be evaluated in terms of the

length of time for which herds continue to have reactors in a single "infection episode" or in a sequence of such episodes over a fixed time period. These processes are then linked to as many "risk factors" as can be readily extracted from campaign records, such as enterprise type, location, perhaps age of animals, purchased/home bred and other variables. Multivariable analytical techniques such as multiple linear regression, multiple logistic regression, the path analysis methods derived from them, and proportional hazards regression for survival analysis can all be used to identify variables which have explanatory value for understanding the dynamics of the infection process within the dynamics of herd management and turnover. This is the risk factor identification process, which in turn should lead on to designed studies which examine specific potentially important risk factors in a more controlled way.

Of the epidemiological study methods, the most useful one to open up issues which may allow the control program to be improved is the case-control study design. In this approach, case herds (or animals) which show a particular outcome of interest (for example, animals becoming infected with tuberculosis or herds taking more than three years to eliminate infection) are compared with equivalent control units which have not shown this undesirable outcome. The selection of herds requires appropriate skill to minimize the risk that invalid conclusions will be drawn, and the technique is a weak one in the degree of support which it provides for an hypothesis. Nevertheless, it is a low cost approach and it can be very powerful in generating hypotheses which open up new control options. These can then be subjected to more critical evaluation through intervention studies where control measures are imposed on one group but not on the other, or cohort studies in which the factor of interest is naturally present in one group but not in the other. There has been inadequate use of these techniques in defining what form of control is likely to produce improved field results, in comparison with efforts to slightly improve diagnostic procedures or some other minor technical aspect of control strategies.

One area which is undoubtedly crucial to effective tuberculosis control but has received very little attention is the influence of farmer attitudes and behaviour, livestock trading practices and herd management in determining how well tuberculosis is controlled in individual herds. Examination of these farmer factors is one of the keys to progress in reducing tuberculosis further, in situations either complicated or uncomplicated by wildlife hosts.

In carrying out further epidemiological investigations, improved laboratory techniques are vital to further progress. It is still not possible to diagnose the disease in important wildlife hosts with sufficient accuracy to allow the full potential of epidemiological research methods to be used on the problem, and it would be of great benefit to have improved diagnostic methods for these species. Secondly, a rapid and inexpensive test of high sensitivity and specificity to detect the presence of the organism in a sample (in lieu of culture) would be a valuable advance which would open up new epidemiological research opportunities. Thirdly, restriction endonuclease (REA) typing has already become an extremely important tool in assessing and refining epidemiological hypotheses. There is scope for this technique to be further enhanced by procedures such as computer analysis of REA gel patterns and extension of the technique to additional and perhaps overlapping systems of classification. This information could be of great value in spatial and temporal epidemiological analysis, but cannot yet be exploited to its full potential.

The use of spatial techniques of analysis based on computer programs termed geographical information systems (GIS) allows aspects of disease spread to be explored which have not until recently been susceptible to objective evaluation. These can provide important new insights into the disease (Pfeiffer and Morris, 1991). Such methods can also be incorporated into computer models of the disease which allow possible variations on current control policies to be evaluated in detail under geographically realistic conditions. The interactions among factors involved in tuberculosis control in overlapping wild and domestic animal populations are complex and controlled studies on a realistic scale are difficult and expensive to conduct. Therefore, computer simulation of possible control methods on genuine geographical surfaces (where the model takes account of actual geographical features such as rivers and mountain ranges) offers considerable potential for improving program effectiveness and comparing field outcomes with model predictions, in order to provide new insights into transmission processes.

These various techniques will in future be incorporated into epidemiologically-based decision support systems (Morris et al., 1993), which will combine herd testing and livestock movement data, local geographical data and information on wildlife host distribution and infection to permit evaluations to be conducted both of past effectiveness (by epidemiological analysis) and of future options (by computer modelling). This in turn will allow veterinarians to make the kind of detailed integrated evaluations of control options at farm and regional level which offer the best hope of making short to medium term progress in control of the disease, pending the development of new control technology.

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