The role of cattle husbandry in the development of a sustainable policy to control *M. bovis* infection in cattle

Report to the Ministry of Agriculture, Fisheries and Food

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1. EXECUTIVE SUMMARY

An Independent Husbandry Panel was convened by MAFF to conduct a fast-track review of scientific evidence on the effects of cattle husbandry on bovine tuberculosis and to suggest means of limiting the disease by improved husbandry.

The prevalence of *M. bovis* infection in cattle is increasing rapidly and extending to new districts in England and Wales. Because of the absence of a definitive knowledge of the transmission routes of this disease, the panel assessed a wide range of potential routes, using the scientific literature together with oral and written evidence from experts in the field. For those routes considered to be of potential importance the role of husbandry practices in disease control was then assessed.

The evidence relating to the transmission of the disease from wildlife to cattle, including direct cattle contact with wildlife in the field, their excreta, and wildlife contamination of buildings and cattle foods, suggests that badgers are likely to be the main wildlife reservoir of the disease in the UK. Therefore, attempts should be made to limit contact between cattle and badgers, for example by fencing off badger setts and burying any dead badgers found on farms. Cattle grazing field boundaries or short swards are at particularly high risk, since the chance of contact with badger excreta is increased. Farmers should consider altering grazing patterns to avoid such high risk situations. Wildlife, and particularly badgers, should be prevented from gaining access to cattle food, drinking troughs, farm buildings and silage pits (especially maize), all of which may be particularly attractive to some badgers.

There is potential for the transmission of the disease between cattle, especially following cattle purchase and by transmission across farm boundaries. Farmers should be encouraged to limit stocking densities in cattle housing to recommended maxima and adequate ventilation must be provided. They may wish to consider testing their cattle on housing to reduce the risk of within-house transmission. Milk from confirmed or inconclusive reactor cows should not be fed to calves. Spreading cattle slurry on the fields may be a potential risk on farms that have, or have recently had, infected cattle, but this can be minimized by prolonged storage of the slurry before spreading or by spreading it on fields not used for cattle grazing. Cattle should be kept well away from slurry spreading to avoid transmission by aerosol. Closed herds should be encouraged to reduce transfer through cattle movement, and farmers purchasing cattle should consider pre- or post-purchase tuberculin testing and confining the cattle to quarantine quarters when they arrive on the farm.

Contact with soil, water and arthropods (especially ticks) are also potential means of transmission. The provision of mineral supplements is likely to be beneficial as soil consumption will be reduced and improved mineral status may improve the resistance of cattle to the disease. Cattle should be fenced away from natural water sources and their drinking troughs regularly cleaned, particularly following a breakdown. Further information on transmission by arthropods is required before preventive measures can be recommended.
It may be possible to reduce the potential susceptibility of cattle to infection by selective breeding and by the maintenance of a high status of herd health to avoid concurrent disease. Old cattle are most at risk and should be given particular attention.

Further research is required to better understand the relative importance of different transmission routes before more precise husbandry recommendations can be made. Specific topics that should be investigated include assessing the genetic base of resistance in cattle, quantifying badger use of farm buildings and cattle-wildlife contacts, assessing *M. bovis* distribution and survival on farms and the role of aerosols in disease transmission. There should be better quantification of the timing and epidemiology of outbreaks of the disease in the UK.

The Panel conclude that, although the mechanisms of transmission of the disease are not precisely known, and should be the focus of future research, there are many precautionary husbandry measures that can be taken to limit the extent of this and other infectious diseases in cattle.

### 2. TERMS OF REFERENCE

1) To assess the scientific evidence on the effects of cattle husbandry in relation to bovine tuberculosis (TB) (by reviewing existing literature and receiving evidence and expert opinion from interested parties on current best practice).

2) To produce a report summarising the state of knowledge on the effects of husbandry on controlling bovine TB. This report will also a) identify research in progress that will address gaps in knowledge and its timescale, b) make recommendations on the steps and responsibility which needs to be taken to encourage careful management practices relating to bovine TB and c) make recommendations on whether further husbandry research is justified and if so, which experiments would be most promising.

### 3. INTRODUCTION

The Panel collected evidence from the scientific literature, written submissions and verbal presentations from a range of bodies. A database of nearly 3000 references was assembled and provided the basis for scientific examination of previous research on transmission mechanisms and husbandry practices used to contain the disease. It became evident that much remains unknown about transmission routes of the disease organism to cattle, so the Panel considered a wide range of potential transmission routes before recommending husbandry measures. These are explained in detail in the text of the report. Given the uncertainties regarding routes of transmission, the panel concentrated on reviewing published scientific evidence pertaining to each and then considered associated husbandry methods. In some cases the Panel was able to conclude that there was little likelihood of a particular transmission route being of major importance under current UK conditions, and no husbandry measures are therefore recommended. In many others, the Panel were unable to determine the relative
importance of a particular route, but relevant husbandry advice is given until further research can better determine the importance of each route of transmission. Finally, the Panel considered ways in which advice might be disseminated to farmers, while recognising that this was not their particular area of expertise. The report was finalised in a relatively short-time period, this being a fast-track review, and we apologize in advance for any errors that may remain in the document.

The bacillus *M. bovis* was discovered in 1882 by Robert Koch (1843-1910), who first identified that different organisms cause tuberculosis in cattle and man. It has a wide range of both target organs (lungs, gastrointestinal tract, mammary gland, kidney and reproductive organs) and mammalian hosts. Bovine tuberculosis was a significant problem in British cattle production in the early part of the last century (Smith, 1905) and probably long before that. In the 1920s a control strategy was initiated which included cattle testing and slaughter of infected cattle combined with the following regulations:

1) Double fencing of attested farms to ensure adequate isolation from non-tested cattle

2) Movement of attested cattle to shows or sales governed by movement permits issued by local Veterinary Officer of the Ministry of Agriculture.

3) Only attested cattle introduced directly into attested herds without being isolated (if from attested herds they had to be retested after isolation for not less than 60 days) (Fishwick, 1952).

This strategy led to a reduction in prevalence of the disease to less than 0.05% of total herds in England and Wales in the late 1970s. Since this time, the incidence of *M. bovis* infection in England and Wales has steadily increased, so that by 1999 the rate of new infection was 2.4% of the number of unrestricted herds tested (Ministry of Agriculture, 2000).

In the current situation increasing the testing frequency of cattle is not likely to reduce the TB prevalence in the UK (Barlow *et al.*, 1997). However, in the United States, where there is no extensive wildlife reservoir in contact with cattle, the disease has been reduced to a low level at a cost of 450 million dollars (Nelson, 1999) by an intensive programme of testing, removal and slaughter of infected cattle, begun in the first half of the twentieth century (Yapp, 1944). Most American cases of *M. bovis* infection are now in imported cattle from Mexico (Essey and Koller, 1994).

### 3.1 INTRODUCTION TO TRANSMISSION OF *M. BOVIS* BACILLI TO CATTLE

The most probable transmission route can be determined from the pattern of lesions observed in slaughtered cattle. In the UK most TB reactor cattle show post-mortem lesions involving the lymph nodes and associated organs of the head and chest cavity suggesting that the route of infection is respiratory. The transfer of organisms from the
rumen to the respiratory tract is theoretically possible as a sequel to eructation (Mullenax et al., 1964), but in the case of ingested organisms some gastrointestinal tubercular lymph lesions would also be expected, but are not normally observed. Early transmission experiments involving oral challenge of cattle with *M. bovis* revealed a very different lesion pattern post-mortem to that observed in the UK today, with many animals showing lesions involving the alimentary tract and abdomen (McFadyean, 1910; Francis 1972). The pasture contamination experiments of Maddock (1934) and Schellner (1956) also showed a different pattern of lesion distribution, with many infected animals having lesions in the alimentary tract only.

In Australia the greater proportion of cattle showing only abdominal lesions in the temperate area of Victoria has been attributed to the greater likelihood of transmission of *M. bovis* via contaminated pasture in this climate, compared with the semi-arid Northern region where most lesions are thoracic (Lepper & Pearson, 1973).

*M. bovis* infection in UK cattle is, therefore, primarily a pulmonary disease and the main route of transmission is likely to be by way of aerosols. The infective dose by this route is very low, perhaps as little as one organism when delivered to the correct location (Chausse, 1913; Griffin and Dolan, 1995), compared with the oral route, where several million bacilli may be required to establish infection (O’Reilly and Daborn, 1995). In some situations infection by both routes may be possible. For the respiratory route, the size of aerosol droplets is more critical than absolute numbers of bacilli and is likely to vary between animals and between incidents. Cattle do not excrete large numbers of *M. bovis* in the early stages of infection and most will be detected and slaughtered before they do so.

There is increasing recognition that eradication of the disease may be difficult if there is a widespread reservoir of the disease in the wildlife population – these could include the European badger (*Meles meles*) in the United Kingdom and Ireland, the brush-tailed possum (*Trichosurus vulpecula*) and ferret (*Mustela furo*) in New Zealand and ungulates in a limited number of other countries.

The prevalence of *M. bovis* infection in humans, whilst a potential health risk, is not currently a widespread problem in the UK due to milk pasteurisation and slaughter of infected cattle. It is responsible for only 1% of new tuberculosis infections in humans (Yates and Grange, 1988), but is a cause for concern in individuals at high risk, such as abattoir workers (Grange and Yates, 1994; Robinson et al., 1988), and infected humans may form a reservoir of infection for cattle (Hardie and Watson, 1992). Latent *M. bovis* infections may re-emerge in humans at a time of stress (Sjogren and Hillerdal, 1978), and it is an increasing problem in AIDS-infected humans, mainly in developing countries (Daborn and Grange, 1993) and in poverty-stricken areas such as Romania (Golli and Eliescu, 1976). An outbreak of multi-drug resistant *M. bovis* infection resulted in the death of 19 HIV-infected patients in Madrid in 1993-5 (Guerrero et al., 1997), although it was possibly due to *M. tuberculosis* mimicking *M. bovis* (Gutierrez et al., 1999).

An introduction to the pathology and immunology of *M. bovis* infection is given in Appendix 1.
4. TRANSMISSION OF INFECTION FROM WILDLIFE TO CATTLE

*M. bovis* infection of animals exhibits some host specificity. Among farm animals, it rarely infects equids or sheep, but occurs regularly in pigs, domestic llamas and deer, which exhibit severe lesions discharging infectious material (Krebs et al., 1997). However, although deer are carriers of *M. bovis*, their presence was not thought to be a significant risk factor in an epidemiological survey in Northern Ireland (Denny and Wilesmith, 1999), where they are less prevalent than in much of the UK. It is not clear whether *M. bovis* infection is self-sustaining in UK deer populations and is probably less likely to be so among roe (*Capreolus capreolus*) and muntjac deer (*Muntiacus reevesi*) as these tend to occur as scattered individuals. By contrast, fallow deer (*Dama dama*) are gregarious and often feed in significant numbers on farms, but their current distribution does not closely match that of bovine TB occurrence. In the last quarter of the twentieth century, the prevalence of clinical *M. bovis* infection in UK deer was estimated to be just over 1%, although a small Irish survey at this time recorded the prevalence as 4% (Dodd, 1984) and the prevalence in farmed deer can be as high as 40% (Whiting and Tessaro, 1994). In Britain, all species of deer are, like the badger, increasing in numbers (Harris, 1995) and their distribution ranges are extending. As a consequence of both, there is a possibility that deer (particularly fallow) may become implicated further.

Ferrets in New Zealand have been found with severe *M. bovis* infections (Lugton et al., 1997). They are believed to be a potential source of tuberculosis infection in cattle there (Ragg et al., 1995), but their infrequent contact with cattle makes them a less likely suspect compared with the possum (Sauter and Morris, 1995a). Of the few ferrets that have been examined in Britain nearly 4% were infected (Krebs et al., 1997), and both they and their wild progenitor (the polecat, *Mustela putorius*) are scarce or absent over most of the areas affected by bovine TB. However, polecats have been shown to frequent farm buildings regularly, especially in winter (Birks and Kitchener, 1999), posing a potential threat to cattle. Hedgehogs (*Erinaceus europaeus*) also live on pastureland and around farm buildings, and in New Zealand 5% of a sample of 79 taken in bovine TB areas were found to be carrying significant infections of *M. bovis*, perhaps as an incidental effect of eating infected carrion from dead possums (Lugton, 1995). In Britain this is unlikely to occur and hedgehogs are anyway liable to be scarce or even eliminated through badger predation where densities of the latter are high (Micol et al., 1994). Rats (*Rattus norvegicus*) and foxes (*Vulpes vulpes*) have been shown to be carriers of *M. bovis* infection but not to show progressive disease (Little et al., 1982b).

In New Zealand, the possum population acts as a vector of *M. bovis* infection for cattle (O’Neil and Pharo, 1995). The research in that country has illuminated a possible explanation as to why sheep are rarely infected. They show little exploratory behaviour to possums (Sauter and Morris, 1995a), whereas cattle, especially dominant individuals, show much exploratory behaviour (Sauter and Morris, 1995b) and are particularly interested in moving possums in the field, but not dead possums (Paterson and Morris, 1995).
Pigs are susceptible to mycobacterioses, but usually these are of the avian type in farmed pigs (Cvetnic et al., 1998), rather than *M. bovis*. Wild boar (*Sus scrofa*) are more likely to be exposed to and readily contract *M. bovis* infection, and interspecies transmission of the disease is believed to occur in Northern Italy (Serraino et al., 1999). In the UK the contact between cattle and pigs or wild boar is currently rare, although wild boar populations are established and are increasing (Goulding et al., 1998), so more regular contact could be possible in future.

Any of these animals could potentially act as a disease reservoir, but the likely candidate in most UK outbreaks is the badger (Denny and Wilesmith, 1999; Hutchings and Harris, 1997; Krebs et al., 1997). The badger is an ideal host for *M. bovis* infection and the UK population is endemically infected (Cheeseman et al., 1989). It is capable of excreting *M. bovis* in sputum, urine, faeces and pus. In experimental conditions transmission has occurred from badgers to cattle (Little et al., 1982a), and in the UK the risk of cattle herd infection is positively related to badger sett density (Wilesmith, 1983). Badgers have increased in numbers in recent years in the UK, with a population increase of more than 70% between the first and second national badger surveys in 1988 and 1997 (Cresswell et al., 1990; Wilson et al., 1997). This not only makes more badgers available for transmission of *M. bovis* to cattle, but increased densities may also have forced badgers to spread and feed increasingly in sub-optimal habitats (Rogers et al., 1998), including inside farm buildings. Small increases in population density may therefore result in large increases in disease prevalence (Swinton et al., 1997).

Britain forms about 4.7% of the land area of western Europe, but has 17-20% of Europe’s badgers (Harris, 1995). The population is at its greatest density in the south west where bovine TB prevalence is high (Krebs et al., 1997). The only country believed to have a greater density of badgers than Britain is Ireland (Smal, 1993), where a substantial *M. bovis* problem also exists in cattle. Some areas of Europe, such as Jura district, do have a significant prevalence of *M. bovis* infection but a much lower badger density than in the British Isles (Burnens et al., 1999). The persistence of the disease in such cases is attributable to imported cattle (Burnens et al., 1999).

In the UK cattle to cattle infection is minimised because infected cattle are regularly removed following a positive tuberculin test (Wilesmith and Williams, 1986). However, diseased badgers are not often removed from farms, thus there is an asymmetry in the probability of transmission direction. Where badgers are removed systematically, as occurred experimentally at East Offaly in Ireland, it is likely that a reduction in the rate of breakdowns will occur (Martin et al., 1997). It should be noted that the East Offaly experiment had inadequate controls, and MAFF research in progress will confirm this finding, or otherwise (ISG, 1999).

*M. bovis* infection may reach an advanced stage of infectivity in badgers, which then excrete bacilli in aerosols, faeces and urine. When *M. bovis* infection was widespread in cattle in the early part of the last century, there was less opportunity for transmission to badgers as they were far less abundant, perhaps too few to sustain the disease within the badger population. More recently the situation has been reversed, especially in the south-west, where badgers are numerous but numbers of infected cattle are kept minimal by the slaughter policy.
Badgers live longer than rodents, hedgehogs and most other farmland mammals (except deer and sometimes foxes), allowing more time for *M. bovis* infection to progress to a highly infectious stage. About 20-25% of badgers removed from control areas are infected with *M. bovis* (Krebs et al., 1997), but in only a small proportion of these does the disease reach the fulminating stage in which the animals are highly infectious (Cheeseman et al., 1989).

### 4.1. CLOSE CONTACT BETWEEN WILDLIFE AND CATTLE IN THE FIELD

Little is known about interactions between wild mammals and domestic cattle, although cows will sniff hedgehogs extensively and hedgehogs commonly feed in cattle pastures. Most studies have focused on interactions between cattle and badgers. The presence of an infected badger sett within one km of a cattle herd will increase the risk of *M. bovis* infection (Martin et al., 1997). However, badger contact with cattle is infrequent, cattle normally move away from badgers and stay at least 2-3 m distant, preferring to remain at a distance of 15-20 m (Benham and Broom, 1989). Sick badgers show aberrant behaviour that may bring them into more direct contact with cattle in the field, in cowsheds and through use of feeding troughs (Cheeseman and Mallinson, 1981; Nolan and Wilesmith, 1994). Cattle are naturally inquisitive and may actively investigate dead badgers (Richardson, G., Rowe, J., oral evidence), a high risk situation paralleled in New Zealand with cattle muzzling dead and dying infected possums (Morris, 1995). It has been proposed (Cheeseman, C., personal communication) that explosive snorting of cattle during investigative behaviour may mobilise bacteria in contaminated badgers, leading to infection via the nasal route, assisted by the wet nasal mucosa. Dominant cattle are probably more adventurous in their exploration of potentially infectious material, such as a dead badger, and are more likely to become infected (Sauter and Morris, 1995b). However, low ranking cattle are more likely to have to graze around a badger latrine (Hutchings and Harris, 1997), because they do not have access to the best quality grazing.

#### 4.1.1. Potential husbandry practices to reduce this risk

- Badger setts should be fenced off to prevent access by cattle.
- Farmers should be vigilant in removing dead badgers. Carcasses should preferably be disposed of by burial, although this is labour intensive. A ‘disposal kit’ could be made available, comprising a suitable ‘body bag’, a mask and disposable gloves. Sick badgers may be removed by the RSPCA.
- Farmers should consider adapting their grazing management to minimise the risk of contact. This could include alternative use of high risk fields and avoiding grazing at high risk periods, such as at night. Rotational grazing will help to concentrate cattle in one part of the farm and could potentially reduce the risk of close contact, compared to set stocking (Benham and Broom, 1989).
- Badgers could be fenced out of high risk areas. Benham (1985) examined electric fencing and found ‘flexinetting’, normally used for sheep containment, to be more effective than double-strand electric fencing. It is particularly suitable for small-scale use, such as excluding badgers from buildings or silage pits or separating setts from an adjacent field. Badgers sometimes burrow under...
it (Benham, 1985), and buried badger-proof fencing may be appropriate in some instances.

4.2. CONTACT BETWEEN CATTLE AND WILDLIFE EXCRETA IN THE FIELD

*M. bovis* is deposited in dung from infected badgers in small pits in the ground or as isolated faeces (Neal and Cheeseman, 1996). Faecal deposits are often sited at territorial boundaries and are numerous in areas of high badger density. Badgers occasionally also defecate in cattle troughs which may be licked clean by the cattle. Cattle typically avoid badger faeces for up to about one month after deposition (Benham, 1985). Faeces are less likely than urine to be a danger to cattle (Griffin et al., 1993), being more localised, containing fewer *M. bovis* bacteria and being avoided by cattle (Benham, 1985).

The badger urinates whilst walking so urine trails of more than 0.5 m long are sometimes left (Brown, 1993). If *M. bovis* infects the badgers kidneys, large quantities of bacteria can be excreted in the urine, which can contain 300,000 *M. bovis* bacilli per millilitre (MAFF, 1979). The badger kidney is the preferred site for *M. bovis* infection following haematogenous spread, with almost 20% of badgers with gross tuberculous lesions having infected kidneys (Gallagher et al., 1976). Cattle avoid consuming herbage contaminated with badger urine for up to 14 days (Benham and Broom, 1991), when they are given the choice, but high stocking densities may prevent such avoidance (Hutchings and Harris, 1997; Benham and Broom, 1991) (the organism remains infectious on herbage for several weeks, see section 5.2.1). Cattle avoidance decreases with time since excretion, especially in dry weather. Some cattle respond to the presence of badger urine, and to a lesser extent faeces by sniffing (Benham and Broom, 1991).

Badgers sometimes leave dung and urine markers as they cross fences and other linear features in the landscape (White et al., 1993). Gaps in walls and fences probably pose a particular risk as these are places where territorial (faeces and urine) marking by badgers is most likely to occur (White et al., 1993). The high density of field boundaries is a distinctive and significant characteristic of the *M. bovis* infected areas of the south west of Britain. These field boundaries may be preferentially grazed following silage making and may be visited more often by bulls. Cattle will explore badger setts (Benham, 1985), particularly where there is disturbed soil, and may eat bedding ejected from the sett. Disturbed soil may be consumed or cattle may rub their heads in it, potentially exposing themselves to infection.

The pus from bite wounds of infected badgers is potentially a source for disease spread (Rogers et al., 2000). The frequency of bite wounding has been found to decrease as a badger population increased to its carrying capacity, suggesting that territorial aggression may be less common as the territories become more stable (Rogers et al., 2000).

Although transmission by the oral route cannot be proven, by analogy it appears likely since it is believed to occur in other species. For example, *M. bovis* infection is believed to be acquired by wild boar through feeding on pastures contaminated by
cattle (Serraino et al., 1999). However, the dominance of respiratory tract infection in badgers and cattle (O’Reilly, 1995) suggests that the infection of cattle through consumption of contaminated herbage is less common than through inhalation. Nevertheless, as cattle can acquire M. bovis infection on pasture previously grazed by infectious cattle and contaminated with their excreta (see section 5.2.2), they are likely to be able to acquire the infection from badger excreta.

4.2.1. Potential husbandry practices to reduce this risk

- Cattle should ideally not be fed in the field, but if they have to be then feeding troughs could be raised out of reach of badgers (80cm), which are known to feed from and defecate in low troughs. Feeding from raised troughs may, however, cause abnormal cattle feeding behaviour (feed tossing, (Phillips, 1993)). Scattering cattle feed on the ground should be discouraged as it may attract badgers and increase contact between this species, its excretions and cattle.

- In high risk areas, if badger latrines are found in a field and it is suspected that cattle will graze near them, farmers should consider avoiding grazing in that area until the herbage has grown or converting the field to other uses, such as the production of hay. Hay making was a significant negative risk element in a multivariate analysis of factors implicated in the acquisition of infection in Irish herds (Griffin, 1992). It is possible that silage making does not destroy the M. bovis bacillus (see section 6.1). However, it is accepted that such measures may be impossible on many farms and could seriously impinge on normal husbandry practice.

- Large badger latrines should be fenced off to prevent cattle grazing. Fencing off all latrines is impractical but farmers should focus their attention on areas with high concentrations. Concentrations of latrines often occur quite close to the sett and also at field boundaries.

- If herbage availability is low, for example following strip grazing or a cut for silage, the area will be especially attractive to badgers and there will be less opportunity for cattle to avoid badger excreta. Farmers should be aware of such high risk situations and consider using a back fence with strip grazing. The marking behaviour of badgers at field boundaries may present a particular risk to cattle grazing those areas after a cut of silage.

- Measures to reduce the number of badger crossing points at field boundaries (for example by repairing walls) may reduce the number of marking sites and hence the risk of contact between badger excreta and cattle.

- If seasonal patterns of infection risk are identified by future research, measures should be taken to reduce exposure of grazing cattle during these periods.
4.3. CONTAMINATION OF CATTLE HOUSES AND FEEDING TROUGHS BY WILDLIFE

The badgers’ principal requirements are for shelter and a food source. They are opportunistic, generalist foragers, with an omnivorous diet. They prefer barley and cattle concentrate to maize silage, molassed dairy meals and feed blocks, with evidence of individual variation in preferences (Benham, 1985). The increased badger density in Britain may have forced some badgers, particularly those in terminal stages of disease, to seek refuge in farm buildings where there is food and shelter. No quantitative surveys of badger use of farm buildings have been conducted, but there is evidence of badgers frequenting buildings, which farmers may not be aware of (Cheeseman and Mallinson, 1981). There are several reasons why farm premises may have become more attractive to badgers in recent years: more farmers are mixing their own food, which often involves storing food on the floor of buildings, more maize silage is grown and stored in readily-accessible clamps, and housed cattle are increasingly fed mixtures of silage and concentrate feeds ad libitum, rather than silage in their accommodation and a fixed quantity of concentrates in the parlour.

If infected badgers do enter farm premises, they could transmit their infection to cattle, since badger to cattle transfer has been shown experimentally to be possible, but not frequent or inevitable (Little et al., 1982a).

4.3.1. Potential husbandry practices to reduce this risk

- If there is evidence that badgers are visiting a particular farm building, the farmer should try to prevent their entry. This could be achieved by a combination of electric netting placed around maize silage pits and access points to buildings, sheeting the lower part of gates to prevent badgers entering, the storage of food in covered bins, perhaps elevated, and the regular removal of waste food from the farmyard. Any dead badgers discovered on the farm premises should be dealt with as described in section 4.1.1.

- Feeding and drinking troughs in the field are likely to be attractive to badgers and pose a risk. Where possible they should be raised to the height suggested in the MAFF advisory leaflet (80cm). Furthermore, a design with a rotating lip might prevent badgers climbing into even quite low troughs. Even if badgers could still reach in to feed, stopping them from climbing in would at least prevent contamination of the trough and fodder with faeces or urine. The Forestry Commission has devised selective feeding hoppers that will make food available for small-bodied red squirrels, but deny access to heavier greys. A similar principle could be applied to cattle feeding troughs (e.g. have a rotating cover that only opens when something the weight or shape of a calf stands on a treadle in front). This would enable size-selective feeders to be deployed on farms where provision is needed for calves to be fed in the fields. This would have the additional advantage of preventing food losses to birds and rodents, also preventing contamination by their droppings. These advantages might somewhat offset the additional cost.
• Supplying concentrates by scattering pellets on the ground attracts badgers and creates a high risk situation in which badgers and cattle will frequently come into close contact. This practice should be discouraged. Similarly, mineral licks should be provided in ways that do not allow access by badgers or attract them to cattle concentration points. For example, suspending lick blocks from trees may be possible on some farms. Also filling a steel drum with soil to create a solid pillar, and topping it up with mineral licks or concentrates allows them to be made available more than 80 cm off the ground, out of reach of badgers. These are low-cost options that might significantly reduce risk.

• Routine disinfection of equipment and buildings where reactor cattle are housed is essential (Ministry of Agriculture, 1999a).

• Action to prevent badgers being a problem may often, but not always, reduce the risk from other potentially infected species, such as hedgehogs, polecats and rats. Action to prevent the entry of smaller mammals may be justified.

These measures should be part of an integrated disease management strategy based on good practice. Focusing on badgers may be ineffective unless combined with other management practices, including control of other potential vectors. The effect of other potential carriers, such as rats, is currently unknown. Despite the low prevalence of the disease in rats, they may pose a risk because of the large numbers present in farm buildings. It is important that mammal distributions, population densities and TB prevalence is better known than at present. It is also important that larger samples of a spectrum of species should be examined for *M. bovis* infections, as is currently being undertaken as part of MAFF sponsored research.

5. TRANSMISSION OF INFECTION BETWEEN CATTLE

5.1. WITHIN-HERD TRANSMISSION DURING INDOOR HOUSING

Studies of cattle housed with reactors have confirmed that indoor cattle-to-cattle transmission is possible, but not inevitable. In one study, transmission from artificially-infected calves to housed in-contact cattle occurred in most cattle within 28 days (Cassidy *et al.*, 1999b). In another study, 40% of steers that were initially negative reactors to the single intradermal comparative tuberculin test developed tuberculosis when housed with reactors for one year (Costello *et al.*, 1998). The authors of books on cattle in the first half of the last century, when tuberculosis was common in British cattle, did not doubt that transmission between cattle was much more likely indoors than at pasture (Garner, 1946; Smith, 1905; Francis, 1947). Nowadays, it is also recognised that housing type and quality are significant risk factors for both tuberculosis in humans (LoBue *et al.*, 1999) and other cattle mycobacterial diseases such as paratuberculosis in calves (Collins *et al.*, 1994).
Whilst the evidence that between cattle transmission can occur indoors is convincing, the fact that many herds only experience one reactor suggests that it is not common. Indeed, between 1972 and 1978 85% of UK herds experienced one reactor only (Wilesmith and Williams, 1986), and in Ireland the transmission of *M. bovis* from reactors that were imported onto a farm is at a low level (Flanagan and Kelly, 1996). The apparent lack of indoor transmission may be because cattle kept in good husbandry conditions are unlikely to excrete significant numbers of *M. bovis* bacilli for 4-9 months (O’Reilly and Costello 1988), so where herds are tested annually many reactors will be detected before they become infectious. However, some cows become anergic, where the cellular immune mechanism is suppressed, both in peripheral blood and at the site of the disease (Lepper *et al.*, 1977; Rhodes *et al.*, 2000). These cattle may be a persistent source of infection, leading to the repeated breakdowns experienced by some herds. The use of a supplementary humoral test may detect the presence of some anergic cows (Plackett *et al.*, 1989; Hanna *et al.*, 1992) and be worthy of consideration for such herds.

Poorly-ventilated cattle housing provides an ideal environment for the spread of pathogens (Smith, 1905). Surface-exposed *M. bovis* bacilli will survive for at least 74 days when they are protected from sunlight, probably longer when they are contained in faeces (Soparker, 1917) and the high density of cattle and high humidity provide an ideal environment for transmission of the organism, when compared with cattle at pasture. Tuberculosis outbreaks have been directly linked to old-fashioned housing (Hejlicek and Chloupek, 1982), but a survey of risk factors to tuberculosis in Irish cattle herds did not find that poor quality housing was a risk factor in tuberculosis outbreaks (Griffin *et al.*, 1993). This survey did however, suggest that herds where the cows were housed in cubicles were more likely to have recurrent tuberculosis, but this might be due to associated factors. Conceivably the close contact between cows’ heads in end-to-end cubicles could increase the risk of transmission.

Transmission is most likely when the cattle stocking density is high (Neill *et al.*, 1989). Since *M. bovis* can be isolated from nasal and, or, tracheal mucus of almost 20% of reactor cattle in the UK, and in the lungs of most reactors with respiratory lymph node lesions (McIlroy *et al.*, 1986), infected cattle should be regarded as potentially able to transmit the disease by aerosol. It has been estimated that *M. bovis* can be transmitted up to 1.5 m in aerosols produced by possums when transmitting the disease to deer (Sauter and Morris, 1995a).

Cattle consuming silage, concentrate or mixtures of the two can be observed nosing and sampling their food while they eat, perhaps because the food is contaminated with saliva from animals previously feeding at that point (Phillips, 1993). The provision of fresh food that has not been sampled by other cows usually provokes intense feeding activity, even if food is available *ad libitum* (Rind, 1994), which may reflect the animals’ desire to select uncontaminated food.

### 5.1.1. Potential husbandry practices to reduce this risk

- Adequate ventilation of cattle buildings may be important, but is of little use if the stocking density is too high (Webster, 1987). Strict adherence to recommended stocking densities (see review by Phillips, 2000) is likely to be important. These will be influenced by ventilation, the configuration of the
accommodation and the diet of the cows. For cows in straw yards a total of 7.5-9 m\(^2\)/cow is recommended, depending on cow size, and just under one third of this should be hard standing, with the remainder being a bedded area. In cubicle housing there should be at least one cubicle/cow.

- As respiratory disease is most likely to be transmitted indoors, farmers with high risk herds may wish to consider having their cattle privately tuberculin tested at housing. This may reduce the number of reactors per breakdown.

5.2. WITHIN-HERD TRANSMISSION IN THE FIELD

In the first half of the twentieth century it was recognised that *M. bovis* infection was much less likely at pasture, and beef cattle that were permanently at pasture were rarely infected (Garner, 1946). This may have been partly due to the long incubation period in comparison with the short lifespan of beef cattle. Cattle are most likely to be severely infectious 6-20 months after the acquisition of the disease, although the range in latency to the severely infectious state is reported to be 87 days to 7 years (Barlow *et al.*, 1997), depending on the nutritional state, physiological demands and the presence of other diseases. Cattle infected by the respiratory route may be mildly infectious initially, before they have mounted an immune response, during the period when they have *M. bovis* bacteria resident in the nasal mucus (Neill *et al.*, 1992). The period to maximum immune response is from 8-65 days following infection (Kleeberg, 1960).

As a result of the long incubation period before cattle become severely infectious, most cattle harbouring *M. bovis* in the British Isles are only likely to be mildly or non-infectious under the present testing regime. Hence it has been found that there is little transmission of tuberculosis from bought-in cattle to other herd members in Ireland (Flanagan *et al.*, 1999; Griffin, 1993).

5.2.1. Infection via herbage

When uninfected cattle graze after infected cattle, the weather will largely determine the longevity of the *M. bovis* bacilli on the pasture. Samples of bacilli taken from lung tissue are destroyed by exposure to the UV wavelengths in direct sunlight within about 12 h, but diffuse sunlight takes much longer (c. 30 d) (Soparker, 1917). This dose is determined by the duration, not the frequency of exposure - many short doses have the same impact as one long dose of the same total duration (Mitserlich and Marth, 1984). *M. bovis* appears to be more resistant to sunlight than *M. tuberculosis* (Soparker, 1917). The pathogenicity of *M. bovis* exposed to a noxious agent does not decline linearly, but close to death the organism shows an increased virulence (Saxer and Vonarburg, 1951). Under suitable weather conditions for maintenance of the bacteria (warm, wet and overcast), pasture is capable of transmitting the infection for several weeks after being grazed by manifestly infected cattle (Schellner, 1956). Grass laminae are typically defoliated by dairy cows at frequencies ranging from once every three days (youngest leaves) to once every five days (oldest live leaves) (Chiy and Phillips, 1999), when continuously stocked at 5 cows/ha. During grazing, cattle wrap their tongue around a sleeve of grass laminae, compress it against the upper palate and tear off the top c. 5 cm by jerking the head upwards and backwards (Phillips, 1993).
The remaining upper regions of the herbage are likely to be contaminated with saliva during this process. This laminar region may be exposed to sunlight, but it may be several days before the lethal dose of short wavelength light (12 h) is received.

After one week of resting pasture following grazing by heavily infected cows, Schellner (1956) found that there was approximately a 6% chance of a non-infected cow acquiring the infection each day, but after two weeks rest this had declined to 2% per day. In these experiments the most likely sites for the infection to reside were, in declining order, the bronchial, intestinal, mediastinal and pulmonary lymph nodes, suggesting that infection had occurred by both the oro-pharangeal and respiratory routes. Infection via the respiratory route could occur following eructation or by aerosol inhalation during grazing. It is not clear whether the retro-pharangeal lymph nodes were examined. The infective dose on pasture was less than $10^5$ cfu/cm$^2$.

Maddock (1934) found that pasture infected with 1.2, 12 and 1200 x $10^6$ M. bovis organisms/m$^2$ remained infectious to guinea pigs for 14, 28 and 49 days, respectively. (Schellner, 1956). In the situation where cattle are grazed concurrently with an infectious animal the risk of transmission will be even greater. These transmission experiments broadly agree with research that has shown that M. bovis can survive on pasture land for 7-28 days, less at high temperatures (Jackson et al., 1995).

Under conditions where infected cattle are detected in the early stages of the disease and removed from the herd, other cattle do not commonly become infected with M. bovis in the field. With a predicted rate of potentially infectious contacts made per infectious cow per day of only 0.007 in a large herd with a single reactor cow, the ensuing low rate of infection would probably not sustain the disease in the absence of a wildlife reservoir or anergic cows (Barlow et al., 1997). However, where the disease is allowed to progress to the advanced stages, it may perpetuate within a herd even in extensive grazing conditions, as evidenced by an isolated incident in Texas, in which 27% of a closed rangeland herd of 331 suckler cows were found to be caudal fold skin test positive, with the majority having gross tuberculous lesions of a single M. bovis spoligotype (Perumaalla et al., 1999). In this case the infection was believed to have been introduced 15 years previously, when the herd was established, but it is possible that it was perpetuated by winter feeding practices (T.A. Ficht, personal communication).

5.2.2. Infection via faeces deposited in the field by cattle

The proportion of cattle heavily infected with M. bovis that are excreting the organism in their faeces is typically 10%, but it can be as high as 80% (Reuss, 1955). This is likely to be much reduced for cattle in the early stages of infection, but in the absence of further information, all infected cattle should be considered as potential excretors. Testing at long intervals will increase the risk of significant M. bovis contamination of slurry, by allowing the disease to progress to a mature stage, where the muco-ciliary escalator can convey bacilli from the respiratory to the gastrointestinal tract.

Following faecal deposition at pasture, M. bovis survival depends on the amount of sunlight and the thickness of the deposit. Typically, the faeces will remain infective for up to six months when deposited in winter but only one to two months in
the summer (Mitserlich and Marth, 1984), after which most of the deposit will have been broken down by arthropods and micro-organisms (Phillips, 1991).

Cattle avoid grazing close to faeces initially because of its smell (Marten, 1966) and later because the herbage in the vicinity of the faeces is mature (Broom et al., 1975). The area around each deposit that is rejected increases with herbage availability and varies from 3 (Petersen et al., 1956) to 12 (Greenhalgh, 1968) times the area of the deposit (c. 0.07 m$^2$, (Phillips, 1991)), making it unlikely that there is any acquisition of $M. bovis$ infection directly from the faeces deposited by grazing cattle. If pastureland is chain harrowed there is a greater chance of cattle coming into contact with the faeces.

5.2.3. Potential husbandry practices to reduce these risks

We confirm that transmission between cattle at pasture can occur under experimental conditions, but this does not appear to be a high risk factor, given current testing and removal procedures.

- The major factor influencing the likelihood of transmission at pasture is the stocking density of the cattle. Reducing the stocking density will proportionately reduce the chance that cattle will come into contact with infected grass before the $M. bovis$ bacteria have been destroyed by sunlight. It will also reduce soil consumption.

5.3. VERTICAL (CONGENITAL) TRANSMISSION OF $M. BOVIS$ BETWEEN CATTLE

Vertical transmission of $M. bovis$ has been shown to occur congenitally via the umbilical vessels, as a sequel to uterine infection of the dam (Pritchard, 1988; O’Reilly and Daborn 1995). Calves are generally believed to be congenitally infected if lesions are confined to the liver and portal system only (Stamp and Wilson, 1946). However, the prevalence of cows in the UK with uterine TB is very rare. It has been calculated that approximately 1% of calves born from tuberculous cows are likely to be congenitally infected with $M. bovis$ (Francis 1947). During the period 1986 - 1994 there were no confirmed isolations of $M. bovis$ from uterine tissue samples submitted to the UK Veterinary Laboratories Agency (Richard Clifton-Hadley - Personal communication). Thus although possible, it is unlikely that this route of transmission has any significant role in the incidence of TB breakdowns in the UK.

5.3.1. Potential husbandry practices to reduce this risk

None warranted.

5.4. PSEUDO-VERTICAL TRANSMISSION (VIA MILK) OF $M. BOVIS$ BETWEEN CATTLE.

Pseudo-vertical transmission is possible via the ingestion of tuberculous milk, with sub-clinically infected cows typically excreting $10^3$ cfu/ml (Zanini et al., 1998). In a suckler herd the dam’s own calf is principally at risk, whilst in a dairy herd several
calves can be put at risk by the pooling/sharing of colostrum and milk in the calf pens. The prevalence of cows with tuberculous involvement of the udders is believed to be low in the UK due to the testing regime, the sporadic nature of outbreaks and the high proportion of single reactors, few of which are youngstock. During the period 1986 - 1994 presumptive tuberculous lesions from supramammary lymph nodes constituted only 0.5% of tissue samples received by the UK Veterinary Laboratories Agency for mycobacterial culture (Richard Clifton-Hadley - Personal communication). The risk of this route of transmission is probably very small.

5.4.1. Potential husbandry practices to reduce this risk

• Milk taken from either inconclusive reactors or reactors awaiting slaughter should not be fed to calves.

• Culling dairy cows vigorously on the basis of high somatic cell counts should speed up the removal of infected cows with tuberculous mastitis that may arise in-between tuberculin tests.

5.5. TRANSMISSION THROUGH THE SPREADING OF BOVINE EXCRETA ON FIELDS

5.5.1. Field evidence

As reported in section 5.2.2, a significant proportion, probably between 10 and 40% of cattle that are infected with M. bovis, excrete the organism in their faeces. The Tuberculosis Investigation Unit in Dublin has examined factors affecting the rate of failure rate of farms undergoing a six-month check (SMC) following tuberculosis outbreaks (Christiansen et al., 1992). A total of 118 farms that failed the SMC were compared with the same number of control farms that passed. The study identified the grazing of pasture which had received slurry that had been stored less than two months as the most significant risk factor. However, the storage of slurry for less than two months was not a risk factor in a multivariate analysis of sample of recurrent outbreaks in a sample of 160 Irish dairy herds (Griffin et al., 1993), even though a univariate analysis identified it as significant. These equivocal findings suggest that slurry disposal on fields merits serious consideration.

5.5.2. Survival of M. bovis in excreta

The low frequency and irregular excretion of organisms in faeces, even from heavily infected cattle, suggests that faecal excretion may be a less important mode of transport of M. bovis than direct respiratory spread (Neill et al., 1988a). The rate of excretion depends upon the site and level of infection, and amount of time the animal has been infected or severity of infection. Infected material has been recovered from faeces exposed to the elements for 178 days (Maddock, 1933), but in particularly hot and dry weather this was reduced to 152 days, and there was a decrease in virulence after 61 days. Maddock concluded that faeces was only safe after approximately 7 months storage and that the retention of infectivity was related to the retention of organic matter in the faeces. In further work he demonstrated 63 days survival in the autumn (Maddock, 1934). Others have demonstrated M. bovis survival in faeces for 2 months in summer and 5 months in a wet winter (Rudolfs, 1950), for 5 months in
summer, 4 months in autumn and 2 months in winter (Williams, 1930), for 32 days in dry faeces and 54 in moist (Schellner, 1956). However, *M. bovis* could not be recovered from the manure of infected cattle after twenty years storage (Deutrich and Pioch, 1991).

Survival in slurry shows a considerable variation as well, e.g. 10 weeks to 6 months (Hahesy *et al.*, 1992). Storage temperature is a key factor determining survival, which was for 17 months at 40 - 45°C (Vera, 1988) but only 30 days at 54°C (Hahesy, 1996). Waste water is contaminated with cattle excreta on most UK dairy farms (Brewer *et al.*, 1999) and should also be regarded as potentially infected.

Farm yard (composted) manure must be exposed to a mean temperature of 60 - 70°C for 3 weeks during composting to destroy *M. bovis* bacilli, and the majority of solid dung heaps do not reach this high a temperature (Hahesy, 1996). Thus composted manure cannot necessarily be considered safe. In Ireland, manure from *M. bovis* infected cattle or those that are suspected to be infected is not permitted to be spread on grazing land.

**5.5.3. Methods of application and associated risks**

The spreading of slurry, but not solid manure, can create aerosols which are carried for several hundred metres, particularly on a windy day (Hahesy, 1995). Slurry tankers may also travel to other farms, with the danger of spreading infection, a practice which was highlighted in an outbreak of *M. bovis* infection at West Penwith in Cornwall 30 years ago (Richards, 1972). Rainguns that are widely used to spread dirty water on pasture propel the aerosolised infectious droplets the furthest. Cattle have also become infected with *M. bovis* where grazing pasture was sprayed two days previously with contaminated dirty water (Schellner, 1956). The contamination of water and pasture with Salmonella and Brucella can also occur through the aerosol method (Hoflund, 1961).

The disinfection of slurry before spreading is mandatory in Germany (Hahesy *et al.*, 1992). Possible methods include the use of calcium hydroxide to increase the pH to 12 for at least 24 - 48 hours (Hoflund, 1961). In Ireland, farmers with reactor cattle are advised to store it for at least two months before spreading, which should not be on grazing land (Hahesy *et al.*, 1992). In addition to the direct risk to cattle, spreading potentially infected slurry on the land increases the risk of establishing a local wildlife reservoir of *M. bovis* infection, with consequent dangers of transmission to cattle.

**5.5.4. Potential husbandry practices to reduce this risk**

We conclude that slurry spreading without adequate storage may be a risk factor in repeat breakdowns, but conclusive evidence is not yet available.

- For reactor herds we recommend that slurry should be spread on, in order of priority, arable crops, crops for hay, crops for silage and finally, grazing land. In high risk situations, where slurry can only be spread on pasture, it should be stored for at least six months before spreading.
• Slurry injection could be encouraged to avoid aerosolisation and the accumulation of *M. bovis* on herbage and in surface soil that may be consumed by cattle.

• *M. bovis* can be effectively destroyed by alkalinisation with a mixture of calcium hydroxide and water at a rate equivalent to 20 kg hydrated lime/m$^3$ of slurry (Hahesy and Heneghan, 1998), but this would impose a high cost and labour requirement (c. 10 t/slurry store).

• When using plate spreaders, cattle should ideally be kept upwind or several hundred metres away (Hahesy and Heneghan, 1998), due to the risk of cattle or even humans inhaling aerosolised bacteria.

• Dirty water from the farm premises is potentially contaminated and could spread infection to cattle, or wildlife particularly if operated at night. An umbilical system rather than rainguns would be preferable for spreading, unless the water has been treated. Separation ponds are likely to reduce the concentration of viable organisms, but further research is needed to confirm this.

**5.6. BETWEEN-HERD TRANSMISSION THROUGH CATTLE MOVEMENTS**

In areas devoid of a wildlife reservoir, most transmission is thought to derive from cattle movement (Barlow *et al.*, 1998). Present investigations of herd breakdowns in the UK attribute 15% to cattle movement (Richard Clifton-Hadley – oral evidence). The figure is higher outwith the south-west region. It must be appreciated that this is a dynamic statistic and will alter with time as both cattle movement and the prevalence of disease in cattle varies. *M. bovis* infection has not spread gradually across Britain, as once predicted (Zuckerman, 1980), but there have been localised occurrences of the problem in disparate regions, suggesting the involvement of cattle movement (or re-emergence from latent sources) rather than gradual spread through a wildlife vector. In Ireland, herd breakdowns due to cattle movements have been calculated at 7%, 10% and 15% in various situations (Griffin, 1993; Griffin, 1992). This figure is more likely to be accurate than attribution to contiguous spread or wildlife transmission since it is calculated from the results of retrospective cattle movement back-tracing. The purchase of bulls introduces a particularly high risk of a breakdown (Griffin *et al.*, 1993), indicating greater susceptibility. This may be because of increased contact with other cattle or more investigative behaviour, head muddying in soil (Benham, 1985) or bellowing, which could create aerosols containing *M. bovis* bacilli, or perhaps the greater stress susceptibility of bulls to transport and therefore chance of succumbing to the disease.

Studies in Ireland showed that imported cattle often do not transfer the disease on to the rest of a herd (Flanagan and Kelly, 1996; Griffin, 1993; Griffin and Dolan, 1995). Research in New Zealand suggested that some horizontal transmission does occur, but not enough to maintain the disease in the absence of a wildlife vector, providing an appropriate system of testing and removal of reactors is in place (Kean *et al.*, 1999). In Italy it has been identified in a small sample of herd breakdowns that importing of cattle is one of the main risk factors, not a wildlife vector (Marargon *et
al., 1998b). Where a region is importing a significant number of cattle, some of which come from infected herds, a sustained level of TB infection can therefore be maintained in an area free of a wildlife vector (Marangon et al., 1998a; Pillai et al., 2000).

5.6.1. Potential husbandry practices to reduce this risk

The following options are appropriate to farmers today to control importation of the disease:

- **Closed herds.** If animal replacements are bred on the farm and a closed herd is maintained the risk of cattle-cattle transmission is restricted to lateral spread from contiguous herds. This practice is recommended where possible.

- **Minimal replacement.** Where the production system does not allow a closed herd it is still recommended that cattle purchase should be minimised to reduce the risk of introduction of infection.

- **Bull hire/share arrangements.** The practice of hiring and sharing of bulls should be discouraged.

- **Pre-movement/post-movement private tuberculin testing.** The mechanism already exists for a cattle owner to arrange for the private tuberculin test of one or more of his stock. This must be done by a MAFF-approved local veterinary inspector using the standard procedure for single comparative intradermal test. The Ministry must approve the timing of the tuberculin test so that it does not interfere with the interpretation of any routine herd test by repeat-testing suppression of skin responsiveness (Radunz and Lepper, 1985). The result of any such test must also be notified to the local Divisional Veterinary Manager.

If cattle purchase is necessary, a farmer may reduce the risk of introducing *M. bovis* into his herd either by pre-movement testing of cattle at the vendor’s premises, or post-movement isolation of bought-in cattle and testing at the purchaser’s own premises. The former arrangement has the advantage that any reactor animals disclosed would not be moved from their original premises and the purchaser’s premises are not subject to movement restrictions. This would also have the advantage of minimising the risk of spreading the disease to a non-infected area. However, post-movement testing is likely to be more practical since many cattle are purchased through markets.

This precautionary approach will not eliminate the risk of acquiring infection through purchase entirely, since a recently infected animal may typically take 3-6 weeks before it will produce a positive reaction to the tuberculin test (Francis 1947). There is considerable range in the sensitivity of the tuberculin test, which can be as low as 68% for a cervical single intradermal comparative test, in which case up to 30% of infected animals could pass the test undetected (Francis et al., 1978). However, the test normally has a higher sensitivity (Monaghan et al.1994) and if one assumes a sensitivity of.
91% (Costello et al.1997), almost one in ten infected animals will pass a test undetected.

A farmer’s decision whether to perform a private test will be influenced by the perceived risk. It could be encouraged (or even made mandatory) that cattle sold through markets should declare the date of the last tuberculin test performed on the farm of origin.

- **Facility for MAFF-recognised quarantine and isolation of bought-in animals prior to private tuberculin test.** According to present legislation, a reactor disclosed at a private post-movement test will result in the purchasing herd being subject to the same status and movement restriction as any other reactor herd (even if the animals have been kept in strict isolation from the purchaser’s herd). MAFF is presently considering alteration to the regulations to facilitate a mechanism whereby bought-in animals can be isolated and quarantined pending a post-movement private tuberculin test. The isolation must be sufficient that in the case of failure of one or more of the purchased animals, movement restriction can be limited to the isolated group and not to the whole herd. The British Cattle Veterinary Association has produced a discussion document for the TB Forum on a recommendation for “TB Isolation and Test Protocol”. (BCVA – written evidence) Although some aspects may be difficult to implement, these recommendations are to be encouraged as they will make it easier for farmers to institute their own biosecurity. Quarantine only isolates infected animals from other cattle and may not prevent contact with wildlife.

### 5.7. BETWEEN-HERD TRANSMISSION ACROSS FARM BOUNDARIES

The risk of a farm having a reactor animal is increased in contiguous herds (Denny and Wilesmith, 1999), but this could be due to common access to a wildlife reservoir, rather than between-herd transmission. Badger territories are large and often encompass several farms (O’CorryCrowe *et al.*, 1996). Griffin and Dolan (1995) also reported that 23% of 504 breakdowns in Ireland were attributable to ‘lateral spread’, i.e. cattle-cattle or badger-cattle transmission in contiguous herds, and 25% in an earlier study of 3,975 breakdowns. A Canadian study established that cattle farms with a common boundary with an infected herd are more likely to have a breakdown than cattle farms within the locality of a breakdown herd but without fence-line contact, suggesting that cross boundary transmission between cattle can occur (Munroe *et al.*, 1999). Collapsed walls and damaged fences are routes by which cattle can mix in an uncontrolled way, potentially spreading *M. bovis* infection (Richards, 1972).

#### 5.7.1. Potential husbandry practices to reduce this risk

- Double fencing of boundary fields will prevent physical contact between cattle on neighbouring farms, but cattle may still lean over and exchange aerosols. An advisory leaflet (ERAD, 1990) suggests use of double fencing to prevent the spread of bovine *M. bovis* infection between cattle. Fencing with shrubs between the two fence lines will keep cattle apart and may reduce risk of cattle-cattle transmission. Such fencing might also help to keep cattle off the
peripheral field areas used by badgers for defecation. However, this may only be short term, since growth and thickening of the shrubbery could displace the badgers out into the fields once more. Nevertheless, separation of cattle in contiguous farms should be seen as good management practice to be encouraged wherever possible. A cattle-proof hedge of sufficient height to prevent them leaning over is likely to be most effective.

- Farmers should be aware of potential aerosol drift from slurry spreading between neighbouring farms.

6. OTHER POTENTIAL ROUTES OF TRANSMISSION

6.1. TRANSMISSION FROM SOIL AND SILAGE

*M. bovis* may enter the soil from cattle or wildlife saliva, nasal secretions, urine or faeces. The most reliable evidence suggests that the organism remains pathogenic in soil for about 6 months (Maddock, 1933; Saxer and Vonarburg, 1951). Some studies reported that it survived for less than this but experienced difficulties in culturing *M. bovis* from soil (Duffield, 1985; Portaels *et al.*, 1988).

Soil ingestion, either directly consumed or from splashings onto herbage or poaching damage, may comprise 5-10% of the freshweight intake and 10-15% of the dry weight intake of grazing cattle (Dewes, 1996), thus forming a significant part of the diet (Beyer *et al.*, 1994). The movement of fodder contaminated with soil between farms may also spread the disease. Cattle are particularly attracted to consume soil, such as exists around a badgers’ sett, to reduce mineral deficiencies. They also use soil for head rubbing, during which they create dust that could potentially lead to respiratory infection (Phillips, 1993). Soil will be consumed in greatest quantities when the herbage is short (Healy, 1968). Soil may also contaminate silage if the fields are not rolled, if there are mole hills or if the grass mower is set too low.

There is little information on the survival of *M. bovis* in silage. Reuss (1955) found that samples of faeces containing *M. bovis* were not infectious to guinea-pigs after being ensiled with grass for ten weeks in a mini-silo. Theoretically *M. bovis* organisms face two potential hazards in silage - lack of oxygen and acidity. The oxygen concentration in grass silage is reduced to zero within a day of ensiling, which would cause *M. bovis* to enter a state of non-replicating persistence or dormancy, with thickened cell walls (Hutter and Dick, 1999; Cunningham and Spreadbury, 1998). The pH of silage declines to approximately 4. The optimum pH for *M. bovis* culture is 5.8-6.9 (Mitserlich and Marth, 1984) and it will survive for 20 days at pH 4-5 in yoghurt (Mitserlich and Marth, 1984). The temperature during ensiling and storage of grass increases to c. 30°C (Williams, 1997), which is close to the optimum (37°C) for *M. bovis* survival (Mitserlich and Marth, 1984). There is, therefore, little evidence that *M. bovis* bacilli on herbage are killed by ensiling, although they are likely to become dormant as a result of anoxia, which may explain the lack of infectivity in the short-term trial conducted with guinea-pigs by Reuss (1955).
Maize silage is no less likely to maintain the *M. bovis* population and maize cobs are particularly palatable to badgers both in the field and the silage clamp. Normally the badger has an omnivorous diet including cereals, fruits, invertebrates, small mammals (Moore *et al.*, 1999; Roper and Lups, 1995), but in areas where maize is commonly grown, the cob forms a major seasonal part of the badger diet, together with invertebrates (Lanszki *et al.*, 1999). In the south-west of England badgers do a significant amount of damage to the maize crop in the field (Moore *et al.*, 1999). Circumstantial evidence, therefore, suggests that maize may be contaminated by *M. bovis* from diseased badgers, and the contamination transferred to a silage clamp, or the clamp may be contaminated directly.

### 6.1.1. Potential husbandry practices to reduce this risk

- Providing cattle with mineral supplements in the field may reduce the attractiveness of soil. Badger setts and their surroundings presenting potentially contaminated soil to cattle should be fenced off to prevent cattle becoming infected.

- Until further research is carried out, we cannot confirm that silage is not a risk factor. Meanwhile steps should be taken to avoid contaminating silage fields with slurry and badgers should be kept away from silage pits, particularly maize.

### 6.2. INFECTION BY DRINKING WATER

Mycobacteria survive readily in water and this is the major source of human mycobacterioses (Dailloux *et al.*, 1999). *M. bovis* bacilli survive for 400 days in running water (Briscoe, 1912) (cultures in physiological saline solution and egg-based media have been demonstrated to survive for 300-400 days and at least six years, respectively (Feldman, 1932; Gloyne, 1920)). Some loss of virulence was noted in all experiments after about one year.

When cattle drink, the splashing could provide a means of entry of bacilli into the respiratory tract. Running water can also be directly contaminated with cattle or wildlife excreta (Sheffield *et al.*, 1997), which may contain *M. bovis* organisms, and this was believed in the first half of the twentieth century to be a means of transmitting the disease (Garner, 1946). However, the availability of natural water supplies was not a significant risk factor in a recent Irish epidemiological study of bovine tuberculosis risk factors (Griffin *et al.*, 1993). Water troughs may be contaminated with *M. bovis* organisms from cattle sputum, or wildlife if natural water supplies are unavailable.

### 6.2.1. Potential husbandry practices to reduce this risk

- It is unwise to rely on cattle gaining their water supply from a stream or pond, as they will pollute the water with excreta.

- It is prudent to clean out all water troughs at regular intervals, especially if infected cattle are detected on a farm, in which case disinfection may also be
warranted. Cattle drink mainly from the surface of the troughs, and in large capacity troughs the turnover of water at the bottom of the trough may be negligible.

- If there is no obvious water source for badgers on a farm, or if there are signs of badgers accessing water troughs (such as scratch marks on the sides of the trough), consideration should be given to preventing badgers from using troughs. This can be done by raising them to a height of at least 80 cm or by using troughs that in some way make it difficult for them to access the water.

6.3. ARTHROPOD VECTORS OF INFECTION

Arthropod ectoparasites are rare on badgers in the UK and appear unlikely to be vectors of the disease between badgers and cattle (Barrow and Gallagher, 1981). However, an association between tick-borne encephalitis and tuberculosis has been demonstrated (Meyerova, 1991). However, this was believed to be due to the suppression of immunity by mixed infections, rather than the ticks acting as a reservoir of the disease. *M. bovis* has been found in ticks taken from the skin of infected hosts, and studies in Armenia confirm that ticks can carry viable Mycobacteria for many months. *M. bovis* may be transmitted through inoculation during tick feeding activities or through the tick being eaten by a new host (Blagodarny *et al*., 1971). There is therefore a possibility that ticks may transmit *M. bovis* between wild mammals and cattle.

6.3.1. Potential husbandry practices to reduce this risk

Until ongoing research has been completed, we have seen no evidence to suggest that adjusting husbandry practices will be effective in the UK.

7. FACTORS INFLUENCING SUSCEPTIBILITY OR RESISTANCE TO *M. BOVIS* IN CATTLE

There is a combination of management and genotypic factors that may influence cattle susceptibility to *M. bovis* infection. These are reviewed here to ascertain which, if any, might be amenable to incorporation into husbandry measures to contain the spread of the disease.

7.1. GENETIC VARIATION AND SELECTION PRESSURES

Genetic variation is manifest at three levels, genus, breeds and families. Initially, following the introduction of the tuberculosis eradication scheme when the prevalence of infection was relatively high, tuberculin testing and slaughter must have produced a significant selection pressure for disease resistance. However, in the last three decades the prevalence of disease has been very low and removal of infected
animals will have had much less impact on the genetics of the national herd. This may
have been compounded by the importation of semen from overseas.

7.1.1. Genus

*Bos indicus* cattle appear to be less susceptible than *Bos taurus* to *M. bovis*
infection (Carmichael 1941; Ram and Sharma 1955), however this is of little relevance
to the present UK cattle industry where *Bos taurus* cattle predominate.

7.1.2. Breed

Current epidemiological data available in Great Britain and Northern Ireland
does not show any significant differences in incidence of reactors between breeds
commonly used in the UK (O. Denny, oral evidence). Differences may exist however if
the distribution of breeds in *M. bovis* exposed herds does not match the national herd
breed distribution.

Benham (1985) found no evidence of breed differences in susceptibility to *M.
bovis* infection in the UK. There is some evidence of differences in susceptibility
between Zebu and Zebu crosses in Malawi (Ellwood and Waddington, 1972). However
(Petukhov, 1981) found no difference in the incidence of bovine tuberculosis in brown
and mottled black breeds in Latvia.

7.1.3. Familial genotypic variation

There is anecdotal evidence that certain familial lines of cattle show particular
susceptibility to bovine tuberculosis (Stella Beavan – oral evidence, George
Richardson – oral evidence, Rachel Teverson – panel member, Maddock, 1934).
Petukhov (1981) investigated two cattle farms with 2742 animals in Latvia, where 23
% were infected, and noted that some families had 80% of its members infected,
whereas others had none.

If significant variation exists between familial lines this would not be
surprising. In experimental animals, strains of disease resistant and susceptible mice
and rabbits have long been recognised and utilised for research purposes (Wright and
Lewis 1921; Lurie, 1941; Anderson et al. 1991). Furthermore, in mice, there is a
specific single dominant autosomal gene (Bcg), the presence of which results in
increased macrophage action and increased Interleukin 2 secretion. (Skamene, 1989;
Schurr et al. 1991). In humans both racial and ethnic variation in susceptibility to
tuberculosis has been recognised (O’Reilly and Daborn, 1995), and it has been shown
that genetic differences in macrophage protein expression partially determine the
resistance shown by humans to *M. tuberculosis* infection (Agranoff et al., 1999). In deer
the heritability of resistance to tuberculosis has been estimated as 0.48, and both innate
and acquired mechanisms of immunity are believed to be involved (Mackintosh et al.,
2000).

Established infection with *M. bovis* is a relatively rare event in the UK since
most herd breakdowns involve only very few reactor animals (Wilesmith and Williams,
1986). Natural infection however is thought to be frequently derived from the
multiplication of a single bacillus (Neill et al., 1991). The exposure rate of cattle in high-risk herds remains unknown. Low dose exposure may be common, with the animals’ non-specific immune mechanisms eliminating the mycobacteria before infection becomes established. It has been estimated that an antigenic load of approximately 1000 mycobacterial organisms are required before cell mediated immunity is activated (Smith and Wiegeshaus, 1989, Dannenberg, 1991). Therefore, it cannot be assumed that a skin-test negative animal has not been exposed to *M. bovis* infection. Such an animal may have received a low dose challenge from *M. bovis* bacilli and successfully eliminated the organisms by non-specific immune mechanisms before they multiplied. Neill et al. (1992) reported an interesting case of transient nasal excretion of *M. bovis* from an in-contact calf, which showed no skin test response and no lesions at slaughter. If low dose exposure to *M. bovis* is widespread when a reservoir of infection exists, the efficiency of non-specific immune responses may be critical in determining whether an animal develops infection or not.

Hypothetically, many mechanisms of non-specific immunity may be effective in eliminating a low dose *M. bovis* challenge. Mechanisms under genetic influence might be the chemical nature of the bronchial mucus, the efficiency of the muco-ciliary escalator, the number of active non-specific macrophages in the lungs and the destructive efficiency of those macrophages’ lysosomal enzymes.

Other genetically controlled factors influencing susceptibility to bovine tuberculosis may be behavioural. For example, the animals grazing habits with respect to avoidance of excretory products may be under genetic influence. The amount of social behaviour that might facilitate cattle-to-cattle transmission, or investigatory behaviour towards badgers or their excreta, may also be under genetic influence.

Specific mechanisms of immunity will almost certainly be genetically influenced. The type of immune response effected in human tuberculosis depends largely on the way mycobacterial antigen is presented by the genetically controlled MHC class II molecule. The mycobacterial epitopes presented will determine the classes and proportion of lymphocytes recruited. The predominant classes of lymphocytes recruited will greatly influence whether the disease progresses to the fulminating stage or is effectively limited (see Appendix 1).

### 7.2. ACQUIRED SPECIFIC IMMUNITY

Specific active immunity to *M. bovis* through the generation of appropriate classes of sensitised lymphocytes and memory cells may theoretically be generated by three mechanisms, natural exposure to *M. bovis*, exposure to other mycobacteria and vaccination.

#### 7.2.1. Natural exposure to *M. bovis*

Francis (1947) takes the pessimistic view that unlike man, the primary lesions in cattle are rarely if ever arrested. However, in natural infection in the field, infection rates rarely exceed 50% within a group (Waddington and Ellwood, 1972). This suggests that in the field when disease prevalence is at its highest (and cattle to cattle exposure to *M. bovis* is almost inevitable), a substantial proportion of animals are able to mount an effective protective response to *M. bovis* exposure. The fact that many of
these animals remain tuberculin test negative raises an interesting question. Did these animals mount an effective but non-specific immune response (e.g. through powerful microbicidal macrophages) or were they never exposed at all? If specific cellular immunity was generated by natural exposure to M. bovis, one would expect such animals to be positive to the tuberculin test (yet reveal no visible lesions or positive culture) at slaughter. Interestingly Willesmith & Williams (1987) showed that for the period 1979-1983, 70% of non-visible lesioned tuberculin test reactors in south-west England were probably related to exposure to M. bovis. Undoubtedly, a proportion of these animals will have had lesions present at slaughter which remained undetected (Corner et al. 1990). It remains unclear whether some of these animals mounted a successful specific immune response to M. bovis.

In New Zealand an experimental model has been developed in Red Deer in which M. bovis infection indistinguishable from natural infection is produced by very low dose tonsillar crypt challenge with M. bovis (Mackintosh et al. 1995). Experimentally-challenged animals have produced a spectrum of immune responses and clinical disease ranging from no disease to severely disseminated tuberculosis (Mackintosh 2000). This spectrum of immune response has been predicted by many other workers (see Appendix 1).

It has been proposed that pre-exposure to environmental mycobacteria may, by mechanisms of immunological cross-reactivity, alter the course by which the disease progresses when an individual is challenged with a mycobacterial pathogen (Stanford et al. 1976; Shield 1983; Pallen 1984; Grange 1986, 1987) Other, naturally occurring mycobacteria grow well in soil (Iivanainen et al., 1999) and saprophytic vegetation, particularly bryophytes (Cooney et al., 1997). Mycobacterium avium-intracellularare-scrofulaceum complex predominates in water, dust, and human sputum samples and Mycobacterium fortuitum complex organisms in soil (Kamala et al., 1994). Environmental mycobacteria are also ubiquitous in natural water supplies (Dailloux et al., 1999), where they inhabit the surface biofilm (HallStoodley and LappinScott, 1998).

Most environmental mycobacteria are capable of inducing non-specific reactions to bovine and avian tuberculin (Cooney et al., 1997; Corner, 1979), which may influence the susceptibility of cattle to M. bovis infection. Guinea-pigs that have been immunised with M. fortuitum show a modulated protective response with the BCG vaccine (Kamala et al., 1996). There is no direct evidence for this in cattle, but the immunological priming of humans and other animals by exposure to environmental mycobacteria is well established (Donoghue et al., 1997). A study in south west England found that a change in the distribution of predominating mycobacteria coincided with an introduction of organic farming practices, which, it is suggested, could increase the potential immunity afforded by exposure to non-pathogenic mycobacteria (Donoghue et al. 1997). However, in their review of the epidemiology of M. bovis infection, Morris et al. (1994) conclude that “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.”

In summary, the possibility that the consumption of environmental mycobacteria enhances the immune response to M. bovis cannot be dismissed, but it is unclear why such immunological priming was not effective in the early part of the last century, when
M. bovis infection was even more common than today. It is possible that any effect was not sufficient to overcome major challenges from other highly infectious cattle at that time. Cattle in those days were poorly nourished and they were kept in underventilated and highly stocked cattle houses.

7.2.2 Vaccination

A vaccine is a possible solution to the current escalation of the disease in the UK, but it should be noted that similar pathogenic mycobacteria affecting cattle, such as M. avium subsp. paratuberculosis, cannot be entirely controlled by vaccination, even after one hundred years of research (Johnson et al., 1997). In New Zealand some protection of possums from M. bovis infection has been possible by injecting them with the BCG vaccine (Aldwell et al., 1995). However, assuming that an extensive wildlife reservoir exists in the UK, any cattle vaccine would have to have an efficacy of more than 97% (Kao et al., 1997).

The development of a cattle vaccine against M. bovis infection is at present a priority research objective in the control of bovine tuberculosis in the UK and is reviewed by Krebs et al. (1997). An effective vaccine would prove a most practical and useful husbandry tool in the control of M. bovis infection in cattle. The feasibility of developing an effective vaccine to protect cattle against bovine tuberculosis was reviewed by Foster (1992), who concluded that “any live tuberculosis vaccine is unlikely to confer 100% protection within a population and should be seen as a tool of disease control not eradication. ... Since non-specific mechanisms have an important role in protective immunity, it may be that vaccination will at best have less effect than say improving nutrition or selecting for disease resistance.”

Recent studies using a BCG and Red Deer model in New Zealand have been more encouraging, with low dose vaccination showing an ability to protect a proportion of vaccinates against infection and lessen the disease severity in others (Griffin et al. 1995). The research workers suggest, however, that "genetically susceptible deer may be incapable of developing a protective immune response to M. bovis BCG vaccine". The difficulties in developing an effective vaccine to an organism such as M. bovis, where the disease process is a direct result of the immune response, should not be underestimated.

7.3. CATTLE ENTERPRISE TYPE

Cattle farming systems have increased in the intensity of production in recent decades, with increased milk yield and growth rates of cattle. However, in Irish studies of the risk of herd breakdown by enterprise type (dairy, suckler and drystock units), no differences in breakdown rate were observed between these very different enterprise types (Fallon, 1994; Mairtin, 1994). A smaller study in Italy identified that mixed dairy and beef enterprises were at greatest risk of breakdown (Marangon et al., 1998b). There therefore appears to be no evidence yet that changes in cattle enterprise type following a breakdown would be beneficial.

7.4. AGE
An increase in disease prevalence with the age of cows has been recorded both in Latvia, where the mean age of onset was six years (Petukhov, 1981), and in the UK, where the relative risk to cows over eight years of age was twelve times the risk to one to two year old cows (Benham, 1985). In Mexico, where there is a significant proportion of infected cattle, most are adult females in fair to good body condition (Milian-Suazo et al., 2000). However, Irish researchers did not record a reduced frequency of breakdowns in beef enterprises, where the mean age of slaughter is less than dairy cows (Fallon, 1994; Mairtin, 1994). Francis (1947) writes “the evidence suggests that even when young cattle are pastured with heavily infected old stock, the incidence in the former remains low until they enter the cow shed.”

7.5. PHYSIOLOGICAL STATE (e.g. Pregnancy, Lactation and Parturition)

Pregnancy has been implicated in anergy to the tuberculin test. There is a suppression of skin reactivity for about 15 days around parturition (5 days pre-calving to 10 days post-calving) (Kerr, 1949). A similar reduction in skin reactivity after calving was observed by Buddle et al. (1994), together with a temporary reduction in response to the γ-IFN immunoassay. There was no effect, however, of pregnancy on disease susceptibility. If an effect does occur, this could be associated with the well recognised peri-parturient immunosuppression in dairy cows, which is believed to be partly related to nutrient deficiencies (Kehrli, 1998).

7.6. EXOGENOUS CORTICOSTEROIDS

Corticosteroids are well known for their immunosuppressive effects, and the corticosteroid production by the calf at parturition may be associated with the peri-parturient immunosuppression referred to above. Kerr et al. (1949) record the suppressive effects of corticosteroids on the tuberculin test. Corticosteroids are used in medicine to prevent foreign tissue graft rejection and in treatment of allergic disease, and they may be used therapeutically (e.g. for induction of parturition or the treatment of ketosis). Their use may increase the animal’s susceptibility to infection. With the recent arrival of licensed non-steroidal anti-inflammatory drugs for cattle, corticosteroids are now used much less commonly in general practice.

Corticosteroids could theoretically be used by unscrupulous cattle owners to conceal tuberculous animals, but this might be ineffective and counterproductive.

7.7. CONCURRENT DISEASES

7.7.1. Immunosuppressive disease

The effect of concurrent immunosuppressive disease on M. bovis infection in cattle does not appear to have been investigated. However, the major influence of HIV infection in humans on subsequent infection with either M. tuberculosi or other mycobacteria is well documented (O’Reilly and Daborn, 1995). Recently there has been a report in Cornwall of a severe outbreak of M. bovis infection in housed calves when concurrent infection with Bovine Viral Diarrhoea (BVD) was identified (Monies and Head, 1999). BVD is a disease organism capable of producing immunosuppression (Potgeiter et al., 1984). There has also been a report of concurrent FIV (Feline
Immunodeficiency Virus) and *M. bovis* infection in farm cats (Monies et al., 2000). It is to be expected that immunosuppressive diseases will increase susceptibility to infection. Examples are BVD, Enzootic Bovine Leukosis, although this is no longer present in the UK, Bovine Immunodeficiency-like Virus, even though this has not been isolated in the UK and may not produce an immunodeficiency syndrome like HIV or FIV, or haemolytic diseases such as Babesiosis or Tick Borne Fever.

Diseases that are not intrinsically immunosuppressive may also affect susceptibility to *M. bovis* infection, such as those affecting vascular permeability or serum protein levels, which may indirectly affect cell-mediated immune responses (e.g. protein-losing enteropathies/nephropathies, fasciolosis, haemonchosis and ostertagiasis).

### 7.7.2. Respiratory disease

*Dictyocaulus viviparous* (Husk), *Pasteurella* spp., *Mycoplasma* spp., *Haemophilus* spp., IBR, BVD, PI3 and RSV are all pathogens responsible for causing respiratory disease in cattle in the UK. Their influence on susceptibility to infection with *M. bovis* remains unclear. Clinical changes may include pneumonia, bronchitis, tracheitis and altered bronchial mucus and secretions. Not only is it feasible that they make the respiratory membrane more susceptible to infection with *M. bovis*, but those agents which induce coughing may also facilitate increased dissemination of *M. bovis* in an aerosol form.

Conversely, it has been demonstrated that *M. bovis* infection can predispose cattle to enzootic infectious bronchopneumonia (LeGrand et al., 1997).

### 7.8. MINERAL SUPPLEMENTS

There is epidemiological evidence of an association between mineral licks and *M. bovis* infection. An Irish study found that the provision of mineral licks reduced the risk of a herd acquiring *M. bovis* infection in a study of breakdown herds, with an odds ratio of 2.7 (Griffin, 1992; Griffin et al., 1993). The risk was also greater in farms with rough grazing, agreeing with early twentieth century experience that *M. bovis* survives best in rough grazing (Garner, 1946), perhaps due to protection from sunlight, although it could also have derived from nutrient deficiencies when a farm has only rough grazing. In the study by Griffin et al. the risk of not providing a mineral lick was much greater in the farms with rough grazing, which they attributed to inadequate mineral supply from low quality pasture. This led to the conclusion that mineral deficiencies predispose cattle to the disease. However, later studies in Ireland found no relationship between three of the minerals which are most likely to be deficient in cattle - copper, selenium and iodine - and the prevalence of *M. bovis* infection (Fallon, 1993). This was a widespread survey of almost 2000 herds, both housed and at pasture, many of which showed evidence of being deficient in these elements. However, it is possible that the provision of other minerals commonly provided in mineral licks, sodium, magnesium, iron or cobalt, were responsible for the observed reduction in breakdowns in the work of Griffin (loc. cit.). Published requirements for sodium for dairy cows are now believed to be too low, and there is evidence that increased sodium intakes can reduce mastitis in dairy cows (Phillips et al., 2000). This may be due to enhanced magnesium absorption, as the potassium inhibition of magnesium absorption in the
rumen is negated by the presence of sodium (Chiy and Phillips, 1993). In laboratory animals at least, magnesium status is an important factor in the immune response, with magnesium deficiency leading to reduced antibody concentrations and activity (McCoy, 1992). Magnesium is commonly deficient in grazing cattle and in another pathogenic mycobacterial disease, leprosy, the magnesium status of the host is reduced (Jain et al., 1995).

There is evidence that specific mineral deficiencies play an important role in predisposing animals to other mycobacterial infections. The low iron status of rodents increases their susceptibility to paratuberculosis, however, the high susceptibility of cattle to copper deficiency has led researchers to suspect that a reduction in copper status by competition from iron could predispose cattle to paratuberculosis (Lepper et al., 1989). Copper and zinc superoxide dismutases protect against exogenous superoxide radicals and thereby may determine the virulence of pathogenic mycobacteria (Wu et al., 1998). Alternatively, cadmium is a well known antagonist of zinc and there is some evidence that badgers are susceptible to the increased levels of cadmium in pasture in recent years, which reduces their reproductive rate and could reduce their health (VandenBrink and Ma, 1998). The possibility that mineral licks at pasture (most of which contain zinc that could offset high cadmium intakes) benefit badger health more than cattle could explain the relationship observed between the provision of mineral licks and tuberculosis breakdown rate (Griffin, 1992; Griffin et al., 1993).

The distribution of mycobacteria in the environment is evidence for their susceptibility to mineral supply, particularly iron. Most mycobacteria are particularly tolerant of acid soil conditions and are inhibited by the reduced iron availability in alkaline soils (Mitserlich and Marth, 1984). Mycobacteria are not good at chelating iron and secrete siderophores to sequester this externally (Johnson et al., 1997). In a review of the effects of soil type on the prevalence of paratuberculosis in cattle, (Johnson et al., 1997) noted many studies reporting that the disease is more prevalent in areas with acidic soils, which increase mineral availability. The prevalence of other diseases, most notably anthrax which is caused by Bacillus anthracis and Fusarium wilt which is caused by Fusarium oxysporum, has been demonstrated to vary directly with soil pH (Johnson et al., 1997). This is due, in the case of Fusarium wilt to the restriction of iron availability at high pH. An association between the prevalence of paratuberculosis in cattle and soil pH, while not proven empirically, is supported by evidence from the geographical distribution of the disease and the bacterial iron requirements (Johnson et al., 1997).

Another pathogenic mycobacterium, M. leprae, reduces the zinc and iron status of hosts (Jain et al., 1995), and in M. bovis infection the differentiation of macrophages increases their iron-acquiring properties, creating an intracellular environment unsuitable for mycobacterial multiplication (Lepper and Wilks, 1988). Whilst it might be suspected that this was due to localised effects in the affected region, and in particular the high zinc content of the bacteria, the same changes in mineral status have been observed in humans with pulmonary tuberculosis (Narang et al., 1995). Changes in biometal levels in the sera of leprosy patients may be due to a systemic effect, in particular the release of interleukin-1. This product of inflammatory cells causes hypercupremic, hypozincemic and hypoferremic responses in the hosts (Jain et al., 1995).
In summary, there is evidence that other pathogenic mycobacterial diseases alter the mineral status of animals, but it is unlikely that the trace elements most commonly believed to be deficient in cattle are related to *M. bovis* infection. Some of these elements affect disease predisposition by protecting the host from oxidative damage, which now seems to be an unlikely component of immunocompetence to explain differences in susceptibility. Some other macro-minerals commonly believed to be in deficit may explain why the presence of mineral licks reduces the risk of *M. bovis* infection.

7.9. GENERAL NUTRITIONAL STATUS OF CATTLE

In relation to the impact of general cattle nutrition on the risk of transmitting *M. bovis* infection, low food intake did not increase the risk of transmitting *M. bovis* infection between steers in a study by Costello *et al.* (1998), but replication of the experimental unit was low. In a study in Mexico, cattle that were infected with *M. bovis* were reported to be mostly in fair to good body condition (Milian-Suazo *et al.*, 2000). However, since protein deficiency has been shown to reduce immunocompetence in guinea pigs (McMurray *et al.*, 1989; Appendix 1), it is possible that there are nutritional effects in cattle that have not been elucidated. Experience from collective farms in Czechoslovakia suggests that deficiencies in vitamins A and C, calcium and protein, as well as carbohydrate excesses, are likely to increase the risk of cattle acquiring *M. bovis* infection (Kabrt, 1962).

7.10. WEATHER

It is likely that the transmission of *M. bovis* is affected by weather conditions (see section 5.2.1). King *et al.* (1999) found that the annual prevalence of *M. bovis* increased in relation to rainfall in the previous year, but this association is based on only a single study area. They also examined seasonal weather effects but the large number of possible associations tested meant that those demonstrated may be spurious. Climate may help to explain the geographical localisation of *M. bovis* infection in the south-west region of the UK. According to King *et al.* (1999), the link with climate also suggests that infection is more likely to be field based than through infection indoors. If cattle were infected in early summer, disease could spread to others during confinement the following winter, leading to high numbers of infected animals being detected early in the following next year. However, testing is more intensive in spring, obscuring seasonal patterns, and annual testing is probably insufficiently frequent to determine within year patterns of infection. As well as affecting cattle management and *M. bovis* survival, climatic factors may also affect behaviour of cattle (Phillips, 1993), badgers (Cresswell, 1988) and badger excretory activities (Brown, 1993), which could influence the likelihood of transmission.

7.11. POTENTIAL HUSBANDRY PRACTICES TO REDUCE PREDISPOSING FACTORS TO *M. BOVIS* INFECTION

We found evidence to suggest that a range of factors may predispose cattle to *M. bovis* infection. Although none provided clear evidence of their relevant importance, we believe that the following husbandry advice may be recommended on the strength of current knowledge.
• There is a clear need to further examine the extent of genetic resistance to *M. bovis* infection in cattle. The pursuit of a breeding programme to identify resistant sires may be beneficial, but changes in cattle breed are unlikely to offer any improved resistance.

• Farmers should be aware that old cows are particularly susceptible to infection by *M. bovis* and in some cases may be able to adopt strategies to reduce risk in these cows. For example, they could be kept away from high risk areas of the farm.

• The provision of mineral licks is associated with a reduced risk of cattle acquiring *M. bovis* infection, but supplementary copper, selenium or iodine is unlikely to affect the risk. The potential exists that badgers visiting mineral licks may transmit the disease via their sputum, but this could be avoided by raising mineral blocks out of the reach of badgers and other relevant wildlife.

• Further evidence on the seasonal risks of cattle acquiring *M. bovis* infection may enable farmers to reduce the risk, by for example altering grazing patterns to avoid high risk parts of the farm or practices during these periods.

• Changes in cattle enterprise type are unlikely to reduce the risk of *M. bovis* infection.

• Farmers should be aware that concurrent disease, particularly of the respiratory tract and possibly BVD, may increase the susceptibility of their cattle. Effective treatment or vaccination is likely to reduce susceptibility to *M. bovis* infection.

8. PRIORITIES FOR RESEARCH

We have identified a wide range of husbandry practices that may benefit the control of *M. bovis* in cattle. However, in the absence of a more detailed understanding of the importance of different transmission routes, it is difficult to prioritise the practices which most merit further attention. As a consequence, we do not propose extensive experiments or field trials of particular husbandry practices at this stage. The following research suggestions focus on clarifying predisposing factors, routes of transmission and risk to refine future husbandry practice.

8.1. HIGH PRIORITY TOPICS FOR RESEARCH

1. To determine the genetic base of resistance in cattle to enable it to be incorporated into breeding programmes. This should be a high priority for at least a desk-top study to investigate the likelihood of research being successful. It has been shown that genotype influences susceptibility and research in this area would directly
complement other current lines of research. Recent work on heritability of resistance in deer (MacKintosh et al., 2000) suggests that breeding for disease resistance could provide some answers to the problem of \( M. \text{bovis} \) infection. Investigation could be made into the genetic basis of resistance by sire and by breed, both by data analysis and experimentally-infected animals. Close liaison with New Zealand research would be beneficial. The potential advantages of breeding for decreased disease susceptibility are manifold:-

- Such a policy lends itself to involvement of the cattle industry (via Breed Societies & Genetics/Semen companies) since the results may produce significant commercial opportunities.
- No impact is made on badgers or wildlife populations.
- Rapid genetic turnover in the UK dairy herd could make it possible to obtain benefits reasonably quickly
- Reducing the disease susceptibility of at risk herds may prove synergistically beneficial to a future cattle vaccination policy (particularly if the vaccine does not confer complete protection).
- Having a strategy of decreasing herd susceptibility to bovine tuberculosis may prove a useful fallback policy if an effective vaccine proves elusive.

2. To quantify the extent and nature of badger visitation of farm buildings and food stores, including silage clamps, and to determine the extent of close contact between badgers and cattle in the field. Although the link between badgers and tuberculosis in cattle is strong, there is little evidence of whether transmission mainly occurs indoors or outdoors. Determining the extent of badger occupation of farms, and other high risk situations will allow exclusion methods to be effectively put in place. It is desirable that this research should include behavioural comparisons between diseased and healthy badgers.

Studies of the costs and effectiveness of potential badger exclusion methods should also be conducted.

3. To quantify the survival and distribution of \( M. \text{bovis} \) on repeat breakdown farms. This should include silage (with different additives), slurry, badger excretory products and other potential transmission media. Further development of PCR tests for these substances could be useful for farmers to assess individual risk. A modelling approach might also be beneficial.

4. The generation and transfer of aerosols from different farm sources, with special emphasis on cattle investigatory behaviour, cattle coughing and eructation, badger sneezing, slurry spreading and dust. There is evidence that \( M. \text{bovis} \) is most effectively transmitted through aerosols and the majority of cattle appear to be infected by the respiratory route. There is a need to examine whether cattle exploratory behaviour, eructation, cattle and badger sneezing and certain farm practices produce aerosols of the appropriate droplet size and the distances over which these may carry. This is required to determine the relative risks of different potential transmission routes.
5. **Epidemiology of *M. bovis* infection.** A better understanding of the epidemiology of *M. bovis* infection would significantly help the development of targeted husbandry practices. Topics worthy of investigation include, in order of priority:

- The seasonality of infection and its implications for husbandry.
- Reactor lesion distribution - it is possible that feedback from abattoirs on lesion distribution could assist farms in determining the transmission mechanism. A feasibility study to assess the practicalities of returning such information may be useful.
- The effects of stress and health status of cattle on susceptibility to *M. bovis* infection.
- The use of spoligotyping to further investigate different transmission routes.

8.2. **LOW PRIORITY TOPICS FOR RESEARCH**

Research in this sector may assume a higher priority when transmission mechanisms are better understood.

1. Evaluating the effects of removing cattle from field margins on the behaviour of wildlife.

2. Investigations of the efficacy of using additional testing methods to improve the detection of *M. bovis* infection in reactor herds.

9. **DISSEMINATING ADVICE TO FARMERS**

Husbandry advice to farmers to reduce the risk of TB has been disseminated through a series of leaflets. An early (1990) advisory leaflet was replaced in July 1999 (MAFF, 1999b). Nearly 100,000 copies of the 1999 leaflet were distributed via local Veterinary Inspectors, practising vets, livestock markets and Animal Health Offices, but not directly sent to individual farmers. This distribution system relies partly on farmers taking initiative (e.g. visit a vet or market and collect a leaflet) and partly on dissemination by mechanisms cited. There is no formal mechanism for monitoring the effectiveness of this procedure. Informal feedback suggests the advisory information has been ‘well received’ although it is viewed as rather general in nature (R.A. Hathaway, written evidence).

Relatively little emphasis has been placed on husbandry issues, yet the suggestions in the MAFF leaflet appear eminently sensible. The suggested measures cannot possibly do any harm, and in many cases would cost little to implement. Conversely, if such measures are not taken, then the risk of *M. bovis* infection must be increased, albeit to only a small degree, even if there is no direct scientific proof of their effectiveness. One potential avenue to encourage a greater uptake of appropriate husbandry practices would be to provide compensatory payments linked to improved management, but we recognise that under EU regulations and the CAP, member states are only allowed to pay ‘state aids’ in exceptional circumstances.
Evidence from the RSPCA draws attention to the need for monitoring the effectiveness of current guidelines, otherwise their purpose remains equivocal and their effectiveness in doubt. Without monitoring, there is no way of learning by experience with a view to improving the advice or the manner of its dissemination.

The MAFF leaflet “TB in cattle: Reducing the risk” (MAFF, 1999a) advises:

- walk your farm to see where badgers might be active
- keep wildlife out of buildings
- raise troughs so that the lip is at least 80 cm above the ground
- keep molasses blocks out of reach of badgers
- fence off badger dung pits
- fence off setts
- avoid grass near badger latrines and field edges when cutting silage
- dispose of any dead badgers found

These are to be encouraged and should be seen as good farming practice. In addition to these points we suggest that further advice should be given, including advice given in this report. Surprisingly, the potential dangers posed by slurry are not mentioned in the latest MAFF advisory leaflet (MAFF, 1999a) on control of bovine *M. bovis* infection. However, good advice (including storage of slurry for 4 months before use, spread on arable land not pasture etc.) is included in a different MAFF advisory leaflet entitled ‘Farm Biosecurity’ (MAFF, 1999b). It is not clear why slurry management is included in the one but not the other. Nor is it clear whether many farmers would understand the term “Biosecurity” to mean anything relevant to the *M. bovis* infection issue. There is evidence that advice to Irish farmers not to graze a field within two months of spreading slurry has been heeded (Christiansen et al., 1992). Such advice could be given to British farmers as a precaution, although it must be made clear that conclusive evidence that slurry presents a risk to cattle is not yet available.

It was made clear to us that farmers often need help, being unaware of the potential infection hazards within their buildings or that badgers may be visiting them frequently, creating a high risk situation. However, the cost of special advisers may constrain what can be done. After a breakdown, a farm and its buildings should be checked for signs of badgers and appropriate advice given to the farmer, but vets who visit the farm anyway could be trained to do this. Alternatively, ‘Farm Assurance Visits’ are already made by trained personnel, for the purpose of advising on farm management and FWAG personnel also visit to advise on wildlife issues. An inspection by a professional that already visits farms regularly does allow an opportunity to provide badger management and husbandry advice without the need for additional costly personnel, with the proviso that whoever makes these assurance visits should be suitably trained.

Farmers may also need help to identify badger setts, latrines and other potential risk factors. Such assistance could be made locally available. The Panel heard evidence that farmers can, and do, adjust their management practices to accommodate the restrictions and difficulties caused by *M. bovis*. They should be assisted to be flexible, in whatever ways are feasible.
We recommend that MAFF consider establishing a demonstration farm for biosecurity issues on cattle farms, which can be visited by farmers to see how good husbandry can contribute to reducing the problem of *M. bovis* infection and other infectious diseases.

**10. CONCLUSIONS**

All farmers strive, to varying degrees, to employ good husbandry on their farms. However, the lack of information on transmission of *M. bovis* presents a serious obstacle to reducing the disease through improved husbandry. Some avenues can be profitably explored regardless of the mechanism, for example the genotypic variance in disease resistance, which could be investigated in conjunction with breeding companies. Others require farmers to estimate the risk in their particular circumstances, for example the extent of wildlife presence on the farm, and to manufacture their own husbandry measures to meet the perceived risk.

Further information on risk factors will soon become available from analysis of the TB 99 questionnaire, and may enable transmission experiments to be more effectively targeted at high risk situations. Effective monitoring of the spread of the disease is of major importance. Data on the pattern of herd breakdowns, both temporal and by region, will be of greater value if the prevalence of the disease continues to increase. Analysis of breakdown statistics by herd size, cattle age and enterprise type could be more widely published and would provide additional information to farmers in high risk situations. Analysing road kill badgers for *M. bovis* infection is an important tool for monitoring spread and assessing risk in areas outside the south west. This has a bearing on the way farmers perceive the threat of *M. bovis* infection in their own area and the need, or otherwise, for them to respond and adjust their own husbandry.

**11. ACKNOWLEDGMENTS**

The panel acknowledges the support of the secretariat (Dr Pete Robertson and Mr Angus Barrett) from the Central Science Laboratory, MAFF, and the evidence received from the bodies and persons listed in Appendix 2.

**12. REFERENCES**


Gutierrez, M.C., Galan, J.C., Blazquez, J., Bouvet, E., and Vincent, V. (1999). Molecular markers demonstrate that the first described multidrug- resistant Mycobacterium bovis outbreak was due to Mycobacterium tuberculosis. Journal of Clinical Microbiology 37, 971-975.


Ministry of Agriculture, Fisheries and Food. (1999a). “List of disinfectants approved by the Minister of Agriculture, Fisheries and Food and the Secretaries of State for Scotland and for Wales for the purposes of the diseases of animals (Approved Disinfectants) Order (1968) (as amended) specifying the dilutions at which they are respectively used”, London.


Sauter, C.M., and Morris, R.S. (1995a). Behavioural studies on the potential for direct transmission of tuberculosis from feral ferrets (Mustela furo) and possums (Trichosurus vulpecula) to farmed livestock. New Zealand Veterinary Journal 43, 294-300.


Schellner, H. (1956). Risk of infection in cattle grazing pastures contaminated with tubercle bacilli. die Rindertuberkulose 5, 179-188.


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INTRODUCTION TO THE PATHOLOGY AND IMMUNOLOGY OF M. BOVIS INFECTION

_M. bovis_ is a slow growing organism closely related to _M. tuberculosis_. It produces no recognized exotoxin and clinical disease is largely mediated by the host's immune response. It is the host's cellular immune response which gives rise to the initial granuloma and later the tubercle that characterises tuberculosis (Turk, 1982). For this reason it is necessary to review the pathology and immunology together.

Mycobacteria have a thick protective capsule and cell wall. The composition of the cell wall is largely lipopolysaccharide. Some of the cell wall constituents have potent immuno-modulating effects. Some components (e.g D-arabino D-galactin and the phenolic glycolipids) produce lymphocyte suppression (Ellner and Wallis, 1989; Fournie et al., 1989), but most dramatic is the immunostimulatory effect of muramyl dipeptide, lipoarabinomannan and possibly cord factor (Rook and Al Attiyah, 1991; Brennan, 1989). These compounds cause a profuse cellular inflammatory response and set up the conditions for cell mediated immunity (CMI).

It is this quality of the mycobacteria which has led to their incorporation in vaccine preparations as adjuvants. Freund's complete adjuvant contains mycobacteria and is still widely used. Mycobacteria, it may be said, have their own 'built-in' adjuvant.

The source of infection

In experimental infections, cattle have been shown to excrete _M. bovis_ in both nasal secretions and faeces (Neill et al., 1989). However, faecal excretion has been shown to be common only with the use of a very large experimental challenge (Neill et al., 1988a). Transmission has been demonstrated from infected to non-infected cattle kept in close contact (Neill et al., 1989).

It is an error to regard only animals with obvious lung lesions as open cases and effective transmitters of _M. bovis_. Recently Neill et al. (1992) have isolated _M. bovis_ from the upper respiratory tract of animals without any macroscopic lung lesions. They suggest that _M. bovis_ excretion is normal, rather than exceptional, for _M. bovis_ infected cattle. Moreover, excretion may be intermittent and may even occur from animals with no visible lesions at slaughter (Neill et al., 1988b). Animals that progress to fulminating disease may excrete large numbers of bacilli. The mammary glands and kidneys can become infected by haematogenous spread (Pritchard, 1988).

The route of infection

A respiratory route of infection is generally accepted by most workers. Some debate continues whether an oral route is involved with mycobacteria being eructated in an aerosolized form from the rumen prior to inspiration into the upper respiratory tract (reviewed by Pritchard, 1988).
It has been hypothesized that cattle may aerosolize *M. bovis* from fomites during investigatory olfactory behaviour (Cheeseman, C. oral evidence). Cattle first exhale strongly prior to inhalation and it is suggested that the exhalation may be sufficient to aerosolise *M. bovis*.

A slaughter survey of tuberculous cattle in Australia showed that 85% of lesions were found in either the lungs or in the bronchial, mediastinal or retropharyngeal lymph nodes (Corner *et al*., 1990). A study of tuberculous cattle in Northern Ireland with bronchial/mediastinal lymph node involvement showed that almost all of these animals had small lesions in the lung parenchyma, detected by careful 5mm sections of the whole lung fields (McIlroy *et al*., 1986).

Any inhaled mycobacteria lodging in the upper respiratory tract are likely to be removed by the muco-ciliary escalator and swallowed. It is therefore suspected that most infections start from mycobacteria lodging in the alveoli or lower respiratory tract (Dannenberg, 1989).

Lesions that occur in the head gland may be the consequence of oropharyngeal or nasopharyngeal infection. Recent studies of experimentally infected animals have revealed lesions within the palatine tonsils (Cassidy *et al*., 1999a).

**The infectious dose**

For experimental infection of bovines, $10^3$-$10^5$ colony forming units are commonly instilled intranasally. Such doses often lead to atypical lesion development. Lesions may develop in the upper respiratory tract of such animals and many more lesions may be seen in the lung fields than in typical natural infection (Neill *et al*., 1988b). More recently a low dose (~100cfu) intra-tonsillar experimental infection technique has been developed in deer in New Zealand which seems to produce a disease picture very similar to natural infection (Mackintosh *et al*., 1995). Analogous experimental models are currently being trialled in cattle in the U.S.A and buffalo in R.S.A. (Frank Griffin., personal communication).

It has been calculated that only inhaled droplets of very small size are likely to reach the alveoli and avoid the muco-ciliary escalator. Estimates have been made that such a droplet could contain between 1-3 mycobacterial bacilli (Dannenberg, 1989; Wiegshaus *et al*., 1989; Dannenberg, 1991).

This is supported by experiments using laboratory animal models with aerosol inoculation (O’Grady and Riley, 1963) and a mathematical model by Neill *et al*., (1990). These authors compared periods of infection to excretion, demonstrating that single nuclei infection is common in cattle and is probably the normal mechanism in natural infection where excretion follows infection by an average of 87 days.

This is not to say that every bacillus that enters the alveoli is capable of causing infection, but instead that most natural infections derive from a single bacillus. The establishment of infection probably depends upon the scenario of a phenotypically hardy and virile bacillus being ingested by a relatively weak alveolar macrophage with poor microbicidal activity. It is suggested that this combination might occur only once in many bacillus/macrophage interactions (Dannenberg, 1991).
The significance of infection arising from a single bacillus, is that such a small amount of antigen is insufficient to generate either a primary or a secondary immune response. There will therefore be an intrinsic latent period of mycobacterial multiplication before any immune response is detected in natural infection. Also, antigens present in greatest quantity rather than naturally immunogenic antigens are likely to be recognised first by the immune system.

**The time scale of infection**

Human and animal models show four distinct stages in the progression of pulmonary tuberculosis (Dannenberg, 1991).

Stage 1 follows initial infection of the host. The bacillus is typically ingested by an alveolar macrophage, the bacillus will either be destroyed, arrested or it may start to multiply, ultimately destroying the alveolar macrophage.

Stage 2 is the subsequent period of logarithmic growth of the bacilli. In experimentally infected rabbits this lasts from 7-21 days post infection. The entire bacilli are relatively non-toxic to the macrophages which engulf them. Many blood-borne monocytes enter the lesion attracted by chemotactic factors. Cytokines released by the macrophages already present and bacillary products can act as chemotactic factors, increasing the cellular reaction.

Stage 3 This stage is manifest by a cessation of logarithimic growth of the bacilli. In cattle this stage occurs typically between 3-6 weeks post infection (Francis, 1947) and is marked by the animal being able to generate a positive reaction to the intradermal tuberculin test. Animal models suggest that for typical individuals, naive to infection, this occurs when the number of bacilli reaches approximately $10^4$ organisms. In sensitized individuals this seems to occur at a smaller antigen load of $10^3$ organisms whereas in highly susceptible individuals CMI and DTH may be delayed until the bacilli load reaches $10^5$ organisms (Smith and Wiegeshaus, 1989; Dannenberg, 1991).

The fourth recognised stage of the disease is liquefaction. This involves a biochemical change of the caseous material to a liquid which provides an excellent growth medium for extracellular bacilli (Dannenberg, 1991). Very high bacilli loads can then occur. This stage does not inevitably occur in bovine tuberculosis. More frequently in the bovine, aged lesions have a tendency to become calcified.

**THE IMMUNE RESPONSE TO M. BOVIS INFECTION**

**The role of macrophages**

Alveolar macrophages form one of the host's first line of defence against inhaled tubercle bacilli. Alveolar macrophages have fairly non specific activity, engulfing a whole range of foreign inhaled particles. Macrophages are equipped with microbicidal mechanisms, including lysosomes containing proteases, lipases, reactive oxygen intermediates and other agents (Dannenberg, 1991). Some work suggest that mycobacteriocidal activity involves vitamin D metabolites (Rook, 1987).
Some types of macrophages are better equipped to control or destroy tubercle bacilli than others. Experimentally mouse macrophages can be stimulated with γ-IFN (a lymphokine) resulting in increased ability to inhibit growth of tubercle bacilli (Kaufmann, 1990; Breschin et al., 1991). Kaufmann (1989) has shown that there are differences in nonspecific bacterial resistance of macrophages between strains of mice. He also showed that *M. tuberculosis* resistant strains of mice have less necrosis and greater macrophage activity than susceptible strains (Dannenberg, 1991).

It is widely accepted that the responsibility for killing tubercle bacilli lies with the macrophage. However, in vitro experiments have shown that this process is at best inefficient (Young et al., 1991). Hahn and Kaufmann (1981) proved the ability of tubercle bacilli to survive and replicate in specialized phagocytes. Monocytes are thought to be the key population which can be activated by sensitized lymphocytes to kill mycobacteria but there is some evidence that neutrophils may also play a role (Rook and Al Attiyah, 1991).

**The role of lymphocytes**

Although both cell-mediated and humoral mechanisms of immunity are generally mounted against *M. bovis*, most current opinion suggests that cell-mediated mechanisms are the most important, as far as initial response and protective immunity are concerned (Pritchard, 1988; Young et al., 1991b). An intracellular organism is evidently not susceptible to extracellular antibody.

T-cells are thus the main focus of research into the immune response to *M. bovis* and other tubercle bacilli. They can be classified into several subsets by either function or surface markers. The pattern of predominating T-cell population produced after infection appears to be strongly influential in determining the course of the disease process.

**The role of lymphokines**

As mentioned earlier, γ-IFN released by lymphocytes has been shown to be able to prime macrophages prior to mycobacterial infection to greater microbicidal activity. Interestingly, after ingestion of mycobacteria γ-IFN has less effect but IL4 and IL6 will exert some effect on macrophages at this stage (Kaufmann, 1990). TNFβ is thought to be an important mediator of DTH and cytotoxicity (Dannenberg, 1991). IL2 has a key role in stimulating lymphoblastogenesis (McMurray et al., 1989).

**Possible mechanisms of protective immunity to *M. bovis* infection**

Infection with a single bacillus does not provide a sufficient antigenic stimulus to evoke a specific primary or secondary immune response. There will be a latent period until at least $10^3$ organisms are present before a specific cell mediated immune response can be activated in a sensitized animal. Antigen loads of $10^4$ or $10^5$ organisms may be required in naive or very susceptible hosts.

The period from infection to the onset of DTH is typically 3–6 weeks in cattle (Francis, 1947). Obviously, prior to this event the bovine is reliant on non-specific
mechanisms of protection (i.e. the efficiency of the muco-cilliary escalator and most importantly the intrinsic microbicidal properties of its alveolar macrophages).

The fact that vaccinates mount a specific immune response at a lower antigen load (i.e. earlier after infection) suggests that the ability to mount an early immune response may be an important protection mechanism.

It would seem feasible that the earlier the specific immune response, the smaller the granuloma produced. Microscopic lesions may avoid ischaemic central necrosis and complete resolution may take place. Even small caseous foci (0.1-3.0 mm) may possibly be eliminated by the progressive phagocytosis of peripheral macrophages (Dannenberg, 1989). A larger granuloma may inevitably result in ischaemic central necrosis, caseation and the persistence of the pathogen within a permanent lesion.

If granuloma size is important in determining the eventual persistence or clearance of the bacilli, it is interesting to speculate whether the immunoreactive components of mycobacteria may have a pathogenic role to play. By increasing the size of cellular reaction they may increase the likelihood of extensive central necrosis and pathogen persistence.

Experiments with BCG vaccination in malnourished guinea pigs showed that protein deficiency caused a reduced ability of BCG vaccine to protect guinea pigs against tuberculosis infection and also a reduced ability of protein malnourished guinea pigs to undergo lymphoblastogenesis in LTT (McMurray et al., 1989). Further experiments have shown a direct effect of protein/energy malnutrition on the ability of macrophages to inhibit mycobacterial growth (Redmond et al., 1991).

Protein insufficiency may have affected two mechanisms of protection.

- Inhibiting specific CMI by suppressing the clonal expansion of sensitized T-lymphocytes.
- Aiding establishment of infection by weakening non-specific alveolar macrophages.

Lurie (cited by Smith and Wiegeshaus, 1989) showed that genetic factors were involved in protective immunity, by demonstrating that certain inbred strains of rabbits were particularly susceptible to *M. bovis*.

In mice, there is a specific single dominant autosomal gene (BCG), the presence of which results in increased macrophage action and increased IL2 secretion (Skamene, 1989; Schurr, et al., 1991).

Anderson et al. (1991) demonstrated much variation in natural resistance of mice of different strains and emphasised the importance of correct strain selection for experimental purposes.

The control of the MHC haplotype in man involves the most complex genetic interaction yet recognised. Hence there is tremendous pleomorphism in MHC haplotype (de Vries, 1989, 1991). The MHC has been shown by crystallography to have a central cleft where antigen is held in association. It is probable that certain
MHC haplotypes are predisposed to bind some antigens in favour of others. If a protective antigen is bound in preference, this haplotype may confer innate protective resistance and vice versa, i.e. individuals are effectively predestined to be susceptible or resistant (Orme, 1991). In human leprosy infection susceptibility associated with one MHC haplotype has been proven (de Vries, 1991).

Differences in MHC haplotype may therefore result in:-

- The presentation of different epitopes.
- The selection of different lymphocyte clones
- A different course of disease.

In guinea pigs it has been demonstrated that vaccination does not prevent infection. For the first two weeks post challenge there is no difference between tissue bacillary load in vaccinates and non-vaccinates. The onset of bacteriostasis in the primary lesions is earlier however in vaccinates (Smith and Wiegeshaus 1989).

In summary it would seem that protective immunity depends on :-

1. Healthy macrophages with good non-specific microbicidal properties. This may be associated with:

- Genetic make-up.
- Nutritional status (affects microbicidal activity).

2. An early specific early response before the antigenic load is great enough to create a permanent caseous lesion. This may be associated with:

- Nutritional status (affects lymphoblastogenesis).
- Memory cells.

3. A lymphocyte response consisting of a blend of lymphocyte populations which specifically activate macrophages, but cause only limited cytotoxicity and relatively little DTH. This may be associated with:

- The existence of memory T-cells to specific antigens.
- Not too much cellular response due to mycobacterial elements (cord factor and muramyl dipeptide)
- MHC haplotypes that preferentially associate with and present protective epitopes.

The concept of a spectrum of immune response to mycobacteria

Work by Ridley and Jopling (1966), with human leprosy sufferers, showed a wide range of clinical presentations depending on the nature of the immune response mounted by the patient against the mycobacterium (M. leprae).
Many workers now accept that a similar spectrum of immune response is shown by cattle against *M. bovis*. Put simply, the theory suggests that a strong cell mediated immune response results in either cure or local tissue damage and the effective "walling-off" of the tuberculous lesion. Bacterial growth is thus restricted and such animals would have a low antigen load and low humoral response. Conversely, animals that produce only a weak cellular response may allow unrestricted mycobacterial growth. Bacterial and antigen load would be high stimulating such animals to mount a larger humoral response. These animals may manifest rapidly progressive or even generalised tuberculous lesions. They may also be anergic to the tuberculin test.

Plackett, Ripper, Corner, Small and de Witte et al. (1989) identified a group of cattle with high levels of antibody against *M. bovis* but negative to the S.I.D. tuberculin test. Harboe, Wilker, Duncan, Garcia, Dukes, Brooks, Turcotte and Nagai (1990) and Ritacco, Lopez, de Kantor, Barrera, Errico and Nader (1991), were able to demonstrate an inverse relationship between titres of specific *M. bovis* antibody and cellular responses in experimental cattle.

Other workers also seem to accept this concept of a spectrum of immunological response to mycobacteria (Buchan and Griffin, 1990; Buchan, Grimmett and Griffin, 1990; Lepper and Corner, 1983; Neill, Pollack, Bryson and Hanna, 1994).

**References in Appendix 1**


LIST OF EVIDENCE AND INFORMATION PROVIDED

(excluding scientific papers obtained via literature searches)

Background information supplied by MAFF/CSL to Panel:

- Bovine Tuberculosis in Cattle and Badgers, Report by Professor John R Krebs FRS and the Independent Scientific Review Group (the Krebs report) 1999
- Agriculture Select Committee, Fifth Report - Badgers and Bovine Tuberculosis (April 1999)
- Government Response to Agriculture Select Committee Fifth Report (June 1999)
- Unpublished Paper on Pathogenesis and Diagnosis of Infections with *M. bovis* in Cattle (November 1999) by Ivan Morrison
- Details of MAFF’s TB Research Programme - Factsheet C14 (taken from MAFF website)
- List of MAFF TB research project codes/titles.
- MAFF Bovine Tuberculosis Research Workshop Background Papers (24/11/99), Chief Scientist’s Group
- MAFF booklets ‘TB in Cattle - Reducing the Risk’ and ‘Farm Biodiversity - Protecting Herd Health’ (1999)
- MAFF booklet ‘Dealing with TB in your herd’ (January 2000)
- MAFF News Releases:
  - Scientific study will investigate TB in cattle, 17 August 1998
  - Farmers asked to help in TB investigation, 27 October 1998
  - New boost for TB research, 31 March 1999
  - Jeff Rooker encourages common sense approach to keeping cattle in herds healthy, 21 July 1999
- MAFF monthly statistics on TB incidents in Great Britain for September 1999
- TB survey questionnaires for TB99, and projects on multivariate analysis of risk factors affecting incidence of TB in cattle herds (projects SE3003 and SE3004)
- Extracts of written evidence received by Agriculture Select Committee:
  - Memorandum submitted by the National Federation of badger Groups
  - Extract from NFBG Supplementary Memorandum
  - Memorandum submitted by National Farmers Union
  - Supplementary Memorandum submitted by NFU
  - Memorandum submitted by Trace Element Services Ltd
  - Memorandum submitted by the British Veterinary Association
  - Memorandum submitted by Helen Fullerton PhD
  - Memorandum submitted by the Soil Association
- Hansard extract of Westminster Hall debate on Badgers and Bovine Tuberculosis, Fifth Report of Agriculture Select Committee (10.2.00)
- Protection of Badgers Act 1992
• RSPCA booklet ‘Problems with badgers?’
# Written Evidence Received by Panel

<table>
<thead>
<tr>
<th>Sender</th>
<th>Date</th>
<th>Summary</th>
</tr>
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<tbody>
<tr>
<td>Martin Hancox</td>
<td>20.1.00</td>
<td>Letter and press extracts on cattle to cattle transmission suggesting the need for more frequent testing.</td>
</tr>
<tr>
<td>Leslie Price MRCVS</td>
<td>29.1.00</td>
<td>Letter suggesting that cats and dogs should be BCG tested, a scheme to reduce the rodent population and that animal feeding stuffs are exposed to ultraviolet radiation to kill TB and other pathogens.</td>
</tr>
<tr>
<td>Bristol University Mammal Group</td>
<td>2.2.00</td>
<td>Letter enclosing project proposal for evaluating and improving husbandry methods for separating cattle and badgers.</td>
</tr>
<tr>
<td>RSPCA</td>
<td>9.2.00</td>
<td>Letter discussing the benefits of management practices.</td>
</tr>
<tr>
<td>Alick Simmons, MAFF SVS DVM</td>
<td>10.2.00</td>
<td>Letter commenting on aspects of cattle husbandry that may have a bearing on the incidence of TB in cattle.</td>
</tr>
<tr>
<td>Soil Association</td>
<td>17.2.00</td>
<td>Letter suggesting a number of environmental (and other) factors that may be important in increasing susceptibility of herds to TB.</td>
</tr>
<tr>
<td>British Cattle Veterinary Association</td>
<td>19.2.00</td>
<td>Letter commenting on the dearth of evidence on risk factors, suggesting that isolation and testing of bought-in cattle, avoidance of reservoirs of infection. Also noting lack of evidence on relative importance of concurrent disease (BVD and Tick Borne Fever). Number of BCVA papers enclosed.</td>
</tr>
<tr>
<td>Leslie Price MRCVS</td>
<td>20.2.00</td>
<td>Letter discussing possible outside sources of infection such as feed.</td>
</tr>
<tr>
<td>George Richardson</td>
<td>21.2.00</td>
<td>Letter/e-mail describing a number of practical measures taken on the farm to reduce the risk of cattle contracting TB and flagging up factors thought to add to test failures.</td>
</tr>
<tr>
<td>University of Bristol, Department of Animal Health and Husbandry</td>
<td>22.2.00</td>
<td>Letter making the case for research to be targeted at methods of transmission.</td>
</tr>
<tr>
<td>Soil Association</td>
<td>22.2.00</td>
<td>Letter pointing out drawbacks in TB99 and enclosing scientific papers by Donoughue et al, Goulding et al and Wilson et al.</td>
</tr>
<tr>
<td>Leslie Price MRCVS</td>
<td>22.2.00</td>
<td>Letter mentioning various aspects such as bacterial resistance.</td>
</tr>
<tr>
<td>Roy Hathaway,</td>
<td>23.2.00</td>
<td>Responding to Panel’s enquiry on the effectiveness of isolation and testing of bought-in cattle, avoidance of reservoirs of infection.</td>
</tr>
<tr>
<td>Date</td>
<td>Organization/Entity</td>
<td>Description</td>
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<tr>
<td>24.2.00</td>
<td>Royal Veterinary College National Federation of Badger Groups</td>
<td>Letter summarising MAFF funded project (SE3003) being undertaken.</td>
</tr>
<tr>
<td>24.2.00</td>
<td>University of Glasgow Veterinary Faculty National Federation of Badger Groups</td>
<td>Letter enclosing a joint paper on a sustainable policy to control TB in cattle which flags up a number of environmental pressures, transmission opportunities and weakness in the cattle testing system.</td>
</tr>
<tr>
<td>25.2.00</td>
<td>University of Glasgow Veterinary Faculty National Federation of Badger Groups</td>
<td>Submission by e-mail on using fencing or zero grazing to separate badgers and cattle.</td>
</tr>
<tr>
<td>29.2.00</td>
<td>National Farmers’ Union of Wales</td>
<td>Letter discussing various aspects including relevance of adoption of silage and cubicle housing systems. Copy of evidence submitted to the Agriculture Select Committee also enclosed.</td>
</tr>
<tr>
<td>2.00</td>
<td>National Forest of Dean Badger Patrol Chris Kilner, MAFF</td>
<td>Submitting a copy of the evidence provided to the Agriculture Select Committee to the Panel.</td>
</tr>
<tr>
<td>8.3.00</td>
<td>Dr Richard Clifton-Hadley, VLA National Trust J.A. Smith, MAFF</td>
<td>Responding to Panel’s enquiry on seasonality of TB Infection</td>
</tr>
<tr>
<td>23.3.00</td>
<td>Dr Richard Clifton-Hadley, VLA National Trust J.A. Smith, MAFF</td>
<td>Responding to Panel’s enquiry on seasonality of TB Infection</td>
</tr>
<tr>
<td>22.3.00</td>
<td>National Trust J.A. Smith, MAFF SVS</td>
<td>Letter discussing sward height and other issues</td>
</tr>
<tr>
<td>29.2.00</td>
<td>National Trust J.A. Smith, MAFF SVS</td>
<td>Discussing possible link between selenium deficiency and susceptibility to TB</td>
</tr>
<tr>
<td>3.3.00</td>
<td>National Trust J.A. Smith, MAFF SVS</td>
<td>Letter discussing genetic susceptibility to TB infection</td>
</tr>
<tr>
<td>2.3.00</td>
<td>National Cattle Association (Dairy)</td>
<td>Letter confirming no further points to add to the written evidence submitted to the Agricultural Select Committee.</td>
</tr>
</tbody>
</table>
## PRESENTATIONS RECEIVED BY PANEL

<table>
<thead>
<tr>
<th>Name/Organisation</th>
<th>Date</th>
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</thead>
<tbody>
<tr>
<td>Mr Roy Hathaway, MAFF TBZ Division</td>
<td>18.1.00</td>
</tr>
<tr>
<td>Dr Chris Cheeseman, CSL</td>
<td>10.2.00</td>
</tr>
<tr>
<td>Dr Richard Clifton-Hadley, VLA</td>
<td>10.2.00</td>
</tr>
<tr>
<td>Dr Debby Reynolds, MAFF</td>
<td>25.2.00</td>
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</table>

## ORAL EVIDENCE RECEIVED BY PANEL FROM:

<table>
<thead>
<tr>
<th>Name/Organisation</th>
<th>Date</th>
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</thead>
<tbody>
<tr>
<td>Dr Owen Denny, DANI</td>
<td>15.3.00</td>
</tr>
<tr>
<td>Dr John Griffin, Dublin Veterinary College</td>
<td>15.3.00</td>
</tr>
<tr>
<td>Mr Richard Young, Soil Association</td>
<td>15.3.00</td>
</tr>
<tr>
<td>Ms Stella Beavan, MAFF SVS</td>
<td>16.3.00</td>
</tr>
<tr>
<td>Mr Brian Jennings, Mr Jan Rowe and Mr Peter Rudman, NFU</td>
<td>16.3.00</td>
</tr>
<tr>
<td>Mr George Richardson, Derbyshire Farmer</td>
<td>16.3.00</td>
</tr>
<tr>
<td>Dr Elaine King and Mr Guy James, NFBG</td>
<td>6.4.00</td>
</tr>
</tbody>
</table>