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SPECIAL ARTICLE

Bovine tubercle bacilli and disease in animals and man

In 1882 Robert Koch reported the isolation of *tuberkelbazillen* from human and bovine sources. Sixteen years later, Theobald Smith (1898) demonstrated that strains of Koch's tubercle bacilli from these two hosts differed in cell morphology, cultural characteristics and virulence in rabbits. He did not believe that these variants were limited to the hosts from which they were isolated nor that the differences resulted from adaptations to a given host. Indeed, he remarked that 'It might be better to omit the host designation of such varieties in order to anticipate assumptions that they are necessarily limited to the host whose name they bear.' Nevertheless, heedless of his own misgivings he termed them the 'human' and 'bovine' types.

Remarkably, the first person to make the very assumption that Smith was so anxious to avoid was none other than Robert Koch who, prior to Smith's work, had firmly maintained that tubercle bacilli from whatever source were identical. At the British Congress on Tuberculosis in 1901 (Report, 1902) Koch stated categorically that the bovine tubercle bacillus was of very limited danger to man and that steps to eradicate the disease in cattle were unnecessary. This statement from one so famous and revered as Koch had two important and opposing effects. First, it so astounded the many eminent veterinary surgeons in the audience that they immediately called upon the Government to appoint a Royal Commission to study the matter. Over a 10-year period this Royal Commission performed many important microbiological, pathological and epidemiological studies which incontrovertibly demonstrated the fallacy of Koch's views (Report, 1911; Francis, 1959). Secondly, it generated misassumptions that still, to some extent, affect our thinking today. To take just one example: although there is no doubt that bovine tubercle bacilli cause open pulmonary disease in man that is as serious as that caused by human strains, the idea that such disease may be transmitted from person to person regularly meets with strong opposition.

Bovine tubercle bacilli

The properties of bovine tubercle bacilli have been described in detail elsewhere (Collins, Yates & Grange, 1982; Collins & Grange, 1983). In brief, a bovine strain differs from the human strain in being microaerophilic, nitratase-negative, sensitive to thiophen-2-carboxylic acid and resistant to pyrazinamide. It is stressed that the term 'bovine tubercle bacilli' is only a label for strains with certain cultural and biochemical properties and does not imply that they bear any particular relationship to cattle (Grange & Collins, 1982). Until 1970, the bovine tubercle bacillus was regarded as being a type or variant of *Mycobacterium tuberculosis* and was termed *M. tuberculosis* var. *bovis* or *M. tuberculosis* subsp. *bovis*. The separate species name *Mycobacterium bovis* was proposed by Karlson &

Lessel (1970). Although this, and two other species, *M. africanum* and *M. microti*, were included in the 'Approved lists of bacterial species' (Skerman, McGowan & Sneath, 1980), there is strong taxonomic evidence that these are really members of a single species (Grange, 1982). *Mycobacterium africanum* is the name given to a heterogeneous group of tubercle bacilli isolated from man in equatorial Africa (Castets, Rist & Boisvert, 1969). These bridge the phenetic gap between the human and bovine types; those from West Africa resembling the bovine type and those from more easterly regions having much in common with the human type. These types have been isolated in other countries, principally but not exclusively from immigrants from Africa. Confusion with the bovine type may arise as pyrazinamide-sensitive strains that otherwise resemble bovine strains have been termed the 'Afro-Asian variety of *M. bovis*' (Marks, 1976). Indeed we (Collins, Yates & Grange, 1981) originally adopted this practice although we later abandoned it (Collins, Yates & Grange, 1982; Collins, Grange & Yates, 1984).

'*Bovine tuberculosis*'

Strictly, this term should only be used to refer to tuberculosis in cattle, but it is often used to denominate tuberculosis caused by bovine strains irrespective of the host as, for example, in the series of reports issued by the Ministry of Agriculture, Fishers and Food entitled *Bovine Tuberculosis in Badgers* (see for example Report 1984*a*).

In fact, the bovine tubercle bacillus has one of the broadest host ranges of all known pathogens: disease has been reported in domesticated cattle, bison, buffaloes, marsupials, hares, equines, camels, pigs, sheep, goats, deer, antelopes, elephants, cats, dogs, foxes, mink, badgers, moles, ferrets, rats and primates including man (Francis, 1947, 1958; Lepper & Corner, 1983; Report, 1984*a*).

From the anthropocentric point of view, the most important host is cattle, and there is evidence that the disease was present in cattle from the earliest days of their domestication. Disease and lesions termed 'wens', 'clivers', 'grape disease', 'pearl disease', 'wasters', 'piners' and 'snorters' were almost certainly manifestations of tuberculosis. A link between tuberculous cervical adenitis (scrofula) and drinking milk from diseased cows was established by Klenke in 1846 (see Moore, 1913). In 1866 Villemin demonstrated the transmissible nature of human and bovine tuberculosis by passage in rabbits and in 1868 Chaveau transmitted the disease experimentally to calves. At the time of Koch's discovery of the aetiological agent in 1882, between 20 and 40% of cattle in many European countries had tuberculosis (Francis, 1947). As in man, the lung is the usual site of disease, and spread of infection occurs by the respiratory route. Although milk is the principal vector for transmission of infection to man, disease of the udder is relatively uncommon, effecting only about 1% of tuberculous cows.

The clear establishment of the enormity of the problem, the serious threat to human health, the introduction of meat inspection, pasteurization of milk and the eradication programmes based on slaughter of tuberculin reactors have been reviewed in great detail by many authors, notably by Francis (1958). Despite the obvious health hazard, the eradication programme did not commence in Great Britain until 1935 and was then delayed by the Second World War. When it commenced again in 1950 about 18% of herds contained infected animals. This

was reduced to 3·5% by 1960 and 1% by 1965. In 1979 only 0·18% of herds contained infected animals (Collins & Grange, 1983). This residual low incidence of disease is, with little doubt, the result of infection from wild animals and also, possibly, man.

The benefits of the bovine tuberculosis eradication programmes have been enormous. Apart from preventing suffering and death in the human population, it has been estimated that such control programmes in the USA have an annual economic benefit of between 30 and 300 million dollars (Roberts, 1986).

Reservoirs of bovine tubercle bacilli in wild animals

In the early 1970s two important wild animal reservoirs of bovine tubercle bacilli were discovered on opposite sides of the world. In 1970 infection was reported in the brush-tailed possum (*Trichosurus vulpecula*) in New Zealand (Eckdahl, Smith & Money, 1970). Many more infected animals were discovered during the ensuing decade (Julian, 1981). Tuberculosis in the badger (*Meles meles*) was first reported in Switzerland by Bouvier, Burgisser & Schneider (1959) and was detected in Great Britain in 1971 when the carcass of an infected badger was found on a farm in Gloucestershire. Several other diseased animals were found soon afterwards (Muirhead, Gallagher & Burn, 1974).

While the badger is indigenous to Great Britain, the possum was introduced into New Zealand from Australia. As tuberculosis appears not to occur amongst Australian possums, it is generally assumed that this animal acquired the disease from infected cattle or feral animals after its arrival in New Zealand. Endemic bovine tuberculosis and infected possums occur principally in the King Country and Wairarapa districts of the North Island and in the West Coast region of the South Island (Collins, DeLisle & Goldrie, 1986). Studies in the latter region showed that although tuberculosis due to bovine strains occurred in many feral animals, the incidence was particularly high among possums – ranging from 5 to 20%. In 1970 about 4·5% of cattle in this region were positive reactors; considerably higher than the average of 0·85% for the remainder of the country. A programme of possum elimination by poisoning, carried out between 1972 and 1974, reduced the incidence of positive reactors to 0·85%, the same as the national average. Although this, beyond doubt, implicated the possum as a major source of bovine tuberculosis, the reduction in incidence of the disease had been achieved at a very high cost, and it was considered neither feasible nor possible to eliminate the source completely.

In Great Britain infected badgers have been found throughout the South-west counties (Avon, Cornwall, Devon, Dorset, Gloucester and Wiltshire), some other counties, especially Surrey, Sussex, Hertfordshire and Shropshire, and in Wales (Figure 1). Cases have also been reported in Ireland (J. D. Collins, 1985). Between 1971 and 1983, 13% of 6000 badger carcasses examined at Ministry of Agriculture, Fisheries and Food laboratories were tuberculous (Chown, 1984). The fact that infected badgers were found in areas where the disease continued to occur in cattle inevitably led to the implication of the former as a reservoir of the disease. Badgers live in social groups in which infection spreads by mother-to-cub transmission and by inhalation of aerosols: spread of disease between social groups is slow and restricted (Cheeseman *et al.* 1987). In addition, between 11 and 17%

Fig. 1. The principal regions of England and Wales where badger tuberculosis has been confirmed.

of tuberculous badgers appear to have been infected through bite wounds (Gallagher & Nelson, 1979; Wilesmith *et al.* 1986*b*). The disease tends to develop more slowly in badgers than in possums and appears to go through a 'closed' or non-infectious stage. Some infected badgers have survived for long periods. Ultimately widespread disease develops and bacilli are shed in sputum, faeces, urine and pus from infected bite wounds. Badgers are thus well able to contaminate the environment. Accordingly, in 1975 the Ministry of Agriculture, Fisheries and Food commenced a programme of badger destruction by pumping hydrogen cyanide gas into their setts. This in turn led to a controversy that became a major public issue and was termed 'The Great Badger Debate'. Unfortunately not all opinions voiced were compatible with logic or reason. On the one hand, some farmers wanted the threat to their livelihood removed at all costs, while on the other hand certain conservationists were determined that the badger should remain unmolested, irrespective of the threat of tuberculosis to cattle, man and indeed to the badger population itself.

The Minister of Agriculture, under pressure from both lobbies, requested the then president of the Zoological Society, Lord Zuckerman, to prepare a report on the matter. This report (Zuckerman, 1980) concluded that the badger was a threat to cattle and to other, uninfected badgers, and recommended that gassing should be resumed. On re-evaluation, however, gassing was considered to be both ineffective and inhumane, and in 1982 it was replaced by selective cage trapping (Report, 1983). An obvious advantage of cage trapping is that healthy animals can be released: for this reason attempts are being made to develop simple serological tests for the disease (Cheeseman *et al.* 1987).

Much of the evidence linking tuberculosis in cattle to disease in badgers, as well as information on the nature of disease in the latter, comes from two major epidemiological programmes conducted by staff of the Central Veterinary Laboratory at Weybridge, Surrey, and their collaborators. The first of these

programmes was conducted in Dorset (Little *et al.* 1982*a*, *b*, *c*; Wilesmith *et al.* 1982) and the second on the South Downs of East Sussex (Pritchard *et al.* 1986; Wilesmith *et al.* 1986*a*, *b*, *c*). The evidence, though indirect, was compelling. For example, a major outbreak of bovine tuberculosis on a farm in Dorset was resolved by the destruction of the badger population (Little *et al.* 1982*b*). Furthermore, the area was recolonized by uninfected badgers and no bovine tuberculosis occurred during the ensuing 5-year period.

One interesting finding was the rarity of tuberculosis in feral animals other than badgers in the two regions. In Dorset bovine tubercle bacilli were isolated from pooled lymph nodes from 2 of 90 rats and from 1 of 7 foxes, but none of the animals had visible tuberculous lesions (Little *et al.* 1982*c*). In East Sussex no infected animals (other than badgers) were detected, although sufficient animals were sampled for there to be a 95% chance of detecting disease if its prevalence was at least 5% in a given species (Wilesmith *et al.* 1986*c*). (Surprisingly, no disease was found in rabbits, despite the susceptibility of this animal to infection under experimental conditions.) Additional studies in South-West England have shown that the prevalence of infection in the deer, mole, rat, fox and mink were 1·9%, 1·3%, 1·2%, 0·9% and 0·6% respectively (Report, 1984*a*). Although these studies show that feral animals, other than badgers, have a low incidence of tuberculosis, their role in the transmission of disease cannot be ruled out, especially where the population of such animals is high. Furthermore, badgers may become infected from other animals: in Switzerland it was thought that they acquired the disease from roe deer (Bouvier, 1963).

Although absolute confirmation of transmission of tubercle bacilli from badgers to cattle is lacking, it is very likely that there is an epidemiological link between disease in these two animals. 'The case against badgers is strong but not watertight' (Chown, 1984). Badgers frequent cattle-grazing land, where they burrow for earthworms. Infected badgers excrete the bacilli in their faeces, urine and sputum, as well as pus from infected sores, and these may contaminate the pasture. There is experimental evidence that bovine tubercle bacilli can survive for several weeks in the inanimate environment provided they are not directly exposed to ultraviolet radiation (Duffield & Young, 1984/85), and viable bacilli could therefore be ingested by cattle. Against this route of transmission is the fact that most cattle develop pulmonary rather than alimentary tract tuberculosis. Cattle are, however, much more susceptible to infection by the lung than by the alimentary route: very few bacilli are required for the establishment of disease following inhalation. The concept that badgers are, at least in part, responsible for tuberculosis in cattle is, therefore, a reasonable one and is accepted by the Wildlife Link Badger Working Group of the World Wildlife Fund (Report, 1984*b*). Even so, there is no guarantee that the problems could be solved by a policy of widespread and indiscriminate destruction of the badger population. It is also important to note that infected badgers and cattle can co-exist for long periods of time without the disease necessarily being transmitted to the latter (Cheeseman *et al.* 1987). The Wildlife Link Badger Working Group (Report, 1984*b*) has stressed the need for further studies on the mode of transmission and investigations into other ways of controlling the disease such as alterations in animal husbandry technique and vaccination of badgers.

Prevention of tuberculosis in animals by vaccination

There have been a number of attempts to control cattle tuberculosis by vaccination. Koch and von Behring both marketed vaccines prepared from human tubercle bacilli which usually cause only benign and self-limiting disease in cattle. Subsequently, Griffith (1913) found viable and virulent human tubercle bacilli in the milk of a vaccinated cow and such vaccination was accordingly abandoned. Later, limited trials were made with the vole tubercle bacillus and BCG (see Francis, 1947). Although apparently partially effective, vaccination had the serious disadvantage of inducing tuberculin reactivity, thereby rendering the tuberculin test useless. Vaccination was therefore not introduced in those countries where and eradication policy based on slaughter of reactors was adopted. It was used in Malawi but, despite initial optimism, no overall benefit could be demonstrated (Berggren, 1977).

Considerable interest has been shown in the possibility of preventing infectious tuberculosis in badgers by vaccination. Two aspects must be considered. First, whether badgers have an immune system that could, if appropriately primed, induce a level of protection able to destroy or contain the bacilli. Second, whether it is possible or realistic to administer a living vaccine to a population of wild animals.

It was found that diseased badgers failed to respond positively to skin testing with tuberculin and in other tests for cell-mediated immune reactions (Morris *et al.* 1978), and this raised doubts as to whether protective immunity could be induced by vaccination. It is, however, important to note that tuberculin may induce a marked cellular reaction in the absence of clinically evident dermal swelling (Beck *et al.* 1986) and that there is no proven relation between tuberculin reactivity and protective immunity. In fact, it was subsequently shown by antibody assay, skin testing and lymphocyte transformation tests that some naturally and experimentally infected badgers responded to mycobacterial antigens (Mahmood *et al.* 1987*a*, *b*), although there is no proof that the responses are protective. (It is noteworthy that even in high-prevalence areas only a minority of badgers develop disease – the highest recorded incidence is 34·5% – suggesting that the others effectively limit the infection.) As in the case of man, there is no known *in vitro* correlate of *protective* immunity, and the only way to find out whether a vaccine will be effective is to conduct a trial.

If a vaccine is used, it will probably have to be living BCG. Vaccines prepared from killed tubercle bacilli or from environmental mycobacteria, whether living or killed, are of no proven efficacy. This raises the fear that the vaccine might revert to virulence, although numerous early studies on BCG and very extensive experience in man suggest that this is extremely unlikely. Although nowadays usually given by injection, BCG was originally introduced as an oral vaccine for infants and, in that form, appeared safe and effective. Accordingly it would appear feasible in principle to attempt vaccination of badgers by adding BCG to food placed near badger setts. (This technique has been used, apparently with success, to vaccinate wild foxes against rabies.) In practice, the great danger of such a vaccination programme is that the living BCG, which is an attenuated strain of bovine origin, could be disseminated to cattle and thereby induce tuberculin

reactivity that, by present methods, would be indistinguishable from that induced by infection by virulent bovine bacilli.

Human disease due to bovine tubercle bacilli

The concluding comment of the final report of the Royal Commission on Tuberculosis (Report, 1911) is that man must be added to the list of animals notably susceptible to bovine tubercle bacilli.

Probably as a result of Koch's pontifications at the British Congress on Tuberculosis, it was widely believed that disease in man due to bovine strains was only acquired from cattle, usually by drinking infected milk. It was also assumed that the resulting disease involved the cervical or abdominal nodes rather than the lung, and that it was often mild and transient. The suggestion was even made that no control measures should be taken against tuberculosis in cattle, as a benign infection by bovine tubercle bacilli in childhood would protect against more severe forms of disease due to human strains later in life. This notion received some support from Calmette who, in line with Koch, considered that bovine bacilli were of low virulence for man. (It was probably for this reason he developed BCG vaccine from a bovine strain rather than from one of human origin.) Calmette cited 'Marfan's Law', proposed in 1886, which states that 'one almost never finds pulmonary tuberculosis, at least pulmonary tuberculosis which is evident and progressive, in persons who, during childhood, have had suppurative tuberculous adenitis of the neck and have been completely cured of it before the age of fifteen years, the cure having taken place before any other focus of tuberculosis was appreciable'. There is some truth in this, and evidence for the immunizing effect of childhood tuberculosis of bovine origin was found during an eradication campaign (Sjogren & Sutherland, 1974). This, like variolation for smallpox, was a most hazardous approach to immunization: fortunately the same protective effect can be achieved much more safely and reliably by BCG vaccination.

By 1908 Koch had modified his views, but he remarked that pulmonary tuberculosis was of overwhelming importance relative to other forms of the disease and that no cases of pulmonary disease due to bovine strains had been reported. Indeed by 1922 there were only four reported cases, all from Great Britain. Nevertheless, when Griffith (1937) actively looked for cases, many were discovered, and opened up 'a new era in pulmonary tuberculosis research'. The percentages of cases of pulmonary tuberculosis due to bovine strains in Great Britain were 0·5–0·6 (South England), 0·9 (Central England), 1·5–1·6 (North England), 1·0 (Wales), 5·0 (Scottish cities) and 4·6–8·5 (rural Scotland). Similar incidences were reported from other European countries, and in general cases were relatively more frequent in rural than urban regions.

In a detailed study by Griffith (1937) of 163 cases of human pulmonary disease due to bovine strains, no clinical, radiological or pathological differences were demonstrated between these and disease due to human bacilli. In 62 cases there was evidence of a primary alimentary focus, but in the other 101 the disease appeared to be limited to the lung. Twenty-five of these patients were occupationally exposed to cattle and 21 had a family history of tuberculosis, although bacterial confirmation of bovine disease in the source case was only possible in one instance. Nevertheless, Griffith remarked that it would be very

Table 1. *Nature of the organisms isolated from tuberculosis of various anatomical sites from patients in England in* 1927*

Lesion	No of cases	Causative organism		Percentage due to bovine
		Human	Bovine	
Cervical	112	62	50	45
Skin	176	86	90	51
Scrofuloderma	54	37	17	32
Meningeal	30	22	8	27
Bone and joint	511	418	93	18
Genito-urinary	23	19	4	17
Pulmonary	202	200	2	1

*Data from Savage (1929).

surprising if man-to-man transmission of bovine tubercle bacilli did not occur frequently, and that it would be very difficult to prove transmissibility of human strains in most cases. In addition, he found definite evidence of primary pulmonary tuberculous complexes caused by bovine strains in children, thereby dispelling the notion that lung lesions were always the result of dissemination from an alimentary focus. Despite the fact that transmission of tuberculosis, whether due to bovine or human strains, is notoriously difficult to prove, there have been a number of cases where there has been little doubt that the source of disease due to a bovine strain was another human being (for case reports and brief reviews see Griffith & Munro, 1935; Ruys, 1939; Sigurdsson, 1945; Wigle *et al.* 1972; Kubin *et al.* 1984; Collins & Grange, 1987).

As mentioned above, it was widely believed that pulmonary tuberculosis was due to human bacilli while non-pulmonary disease, often termed 'surgical tuberculosis', resulted from infection via the alimentary route by bovine strains. Table 1 shows data compiled by Savage (1929) on the number of cases due to the human and bovine types. This shows that, although almost all lesions due to the bovine type were extrapulmonary, human strains were nevertheless the predominant cause of such disease. Although classically associated with cervical adenitis, bovine strains only caused about half the cases. It is now appreciated that the variety of lesions as well as the clinical course and extent of disease is the same whether caused by human or bovine strains (Roberts, 1986).

The bovine tuberculosis eradication campaigns appeared to coincide with a marked decrease in the incidence of human tuberculosis due to bovine strains. The observed decline was, however, partly artifactual owing to a failure or unwillingness of laboratories to distinguish between the human and bovine types, or to report the latter. Commenting on the claims that eradication campaigns had eliminated infection by bovine bacilli in man, Habib & Warring (1966) remarked that 'A more likely explanation is that confidence in the effectiveness of present-day chemotherapy against all types of tuberculosis has resulted in a loss of interest in differentiation of bovine and human strains.' Nowadays, in fact, there is a good reason for making this differentiation as pyrazinamide, to which bovine strains are uniformly resistant, is now regularly used as a first-line antituberculous drug.

At present, human tuberculosis due to bovine strains in Great Britain occurs

principally amongst the indigenous white population: in contrast to disease due to human strains it is relatively uncommon amongst those of Asian and African ethnic origin (Collins, Yates & Grange, 1981; Yates, Grange & Collins, 1986). About 1·5% of tubercle bacilli isolated in South-east England are of the bovine type: just under half (44%) were from patients with pulmonary disease and a remarkably high number (26%) were from patients with urinary tract disease. These figures are similar to those for England and Wales for 1962–6: 65 of 102 cases of known anatomical site were pulmonary and, of the 37 non-pulmonary cases, 25 involved the urinary tract (Kataria, 1969). A survey of 31 cases in the Ontario province of Canada between 1964 and 1970 revealed a similar anatomical distribution of infections: 13 were pulmonary and 18 non-pulmonary, of which 12 involved the urinary tract (Wigle *et al.* 1972). These data indicate that urinary tract infection which, as described below, is of relevance to the transmission of disease to cattle, is becoming a relatively more frequent form of the disease.

It is notable that at present about 0·6% of pulmonary tuberculosis in South-East England is due to bovine strains and this, remarkably, is identical to the relative incidence reported in this region by Griffith in 1937 (see above), when cattle tuberculosis was rife! The age distribution of cases is very similar to that of disease due to human strains, and several cases of pulmonary infection have occurred amongst young town dwellers with no known exposure to infected cattle or milk. The possibility that pulmonary and non-pulmonary tuberculosis due to bovine strains might differ markedly in their epidemiology must be seriously considered.

Proven cases of transmission of bovine tubercle bacilli from man to cattle have been reported (Leslie, 1968; Lepper & Corner, 1983). Although transmission is often via the respiratory route, a number of cases of cross-infection resulted from attendants urinating on the hay. This, we are credibly informed, is a common practice and is said to provide a source of salt in the cows' diet! Huitema (1969) reported 50 examples of herds infected from human sources and in 24 cases the responsible individual had renal tuberculosis. Many of these patients, especially the older ones, had clear chest radiographs and only vague symptoms. Diagnosis was thus often delayed until many animals had been infected. As noted above, urinary tract infection is nowadays one of the more frequent manifestations of human tuberculosis due to bovine strains.

These reports and the continuing occurrence of human tuberculosis due to bovine strains led us to stress that man might still be an important source of disease in cattle in Ireland (Collins & Grange, 1987). On the other hand, a review of 1002 consecutive culture-positive cases of tuberculosis at a hospital in Newcastle, Co. Dublin, Ireland (Collins *et al.* 1987) showed that only nine (0·9%) were due to bovine strains. Only four of these patients had definite exposure to cattle, and the characteristics of the disease suggested reactivation of infection acquired decades ago. It was therefore concluded that man was not a significant reservoir for bovine tubercle bacilli in that region. The sites of the lesions were, however, not given, and the possibility remains that urinary tract disease may be a hidden source of infection in that region. For want of better diagnostic and epidemiological tools, the danger of man to cattle remains an unresolved and debatable issue. Nevertheless, veterinary surgeons are well aware of the possible

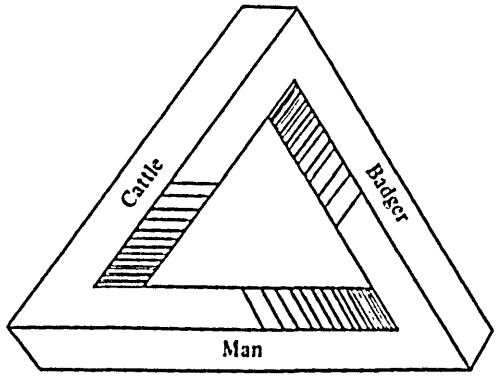

Fig. 2. The transmission of bovine tubercle bacilli – not as straightforward as it first appears.

risks and have stressed the continuing importance of vigilance and cooperation between the veterinary and medical professions (Torning, 1965; Huitema, 1969).

Epidemiological advances

Many of the barriers to our understanding of the nature and the mechanism of transmission of bovine strains arise from the fact that these bacilli are very uniform in their properties, and that until very recently there has been no reliable technique for the subdivision of these bacilli for epidemiological purposes. Bacteriophage typing is of very limited value for epidemiological studies on human tubercle bacilli and of virtually no value for bovine strains, as almost all appear to belong to just one of the three reliable phage types. A biotyping technique for mycobacteria based on the utilization of amino acids was introduced by Grange (1976) and successfully applied to bovine strains by Barrow (1981, 1986). Barrow (1986) also demonstrated differences amongst six strains from badgers by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) of extracted bacterial proteins. Although promising, these techniques have not yet been widely applied to epidemiological problems.

An even more sensitive typing technique is DNA restriction endonuclease analysis. This method is based on the ability of enzymes termed restriction endonucleases to cut DNA at points where there is a certain short sequence of bases. This process generates a number of fragments of DNA of different sizes, which can be separated according to their molecular weight by high-resolution agar-gel electrophoresis. If one of the relevant base sequences is altered by mutation, enzymic cutting will not occur at that point and the fragment sizes will differ. As many restriction endonucleases of differing specificities are now available it is possible to make very detailed comparisons of the genomes of bacteria, as well as those of other organisms.

This highly discriminative technique was adapted for the study of mycobacteria by Desmond Collins and his colleagues at the Animal Health Reference Laboratory in New Zealand (Collins & DeLisle, 1984, 1985). Although the differences between strains were very small, sometimes only one fragment difference, they were reproducibly demonstrable, and patterns from different cultures of the same strain

were identical. The technique was then used to type bovine tubercle bacilli isolated from 83 possums from three different regions (Collins, DeLisle & Gabric, 1986). Digestion of the DNA by three endonucleases – BstEII, PvuII and BclI – allowed 21, 14 and 14 restriction types respectively to be identified. By combining the data a total of 33 types were identified. None of the types was found in more than one of the three regions and many types showed a limited geographical distribution within each region. This technique could well revolutionize epidemiological studies on bovine and other types of tubercle bacilli.

CONCLUSIONS

Disease of cattle and man due to bovine tubercle bacilli, although greatly diminished in prevalence in the industrially developed world, still remain a public health problem. There is no doubt that the disease is present among badgers, possums and other wild animals and little doubt that transmission of bacilli from these sources to cattle occurs. Almost nothing, however, is known about the route of transmission of infection (Figure 2). The nature and extent of man-to-man and man-to-cattle and badger-to-cattle transmission remain unresolved and controversial issues, but modern typing systems have recently been developed and may soon shed light on these issues.

In the Western world there is a remarkable degree of apathy towards tuberculosis, and many people firmly believe that the disease has been 'conquered'. Unfortunately, control is not synonymous with eradication, a fact well appreciated by the veterinary profession but alas not by the medical profession. Dr J. S. Sodhy, a greatly respected tuberculosis specialist remarked, in reference to that disease, that 'no one is safe until all are safe'. This applies equally to animals as to man.

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