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Aspects of the epidemiology of bovine tuberculosis in badgers and cattle. I. The prevalence of infection in two wild animal populations in south-west England

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SUMMARY

The prevalence of infections caused by *Mycobacterium bovis* was estimated in two populations of wild animals in the south west of England. A variety of mammalian species was trapped on farm land where incidents of infection with tuberculosis had occurred in cattle. Bacteriological analysis of lymph node samples and lesions showed that the only animals acting as a reservoir for *M. bovis* were badgers. Examination of arthropod ectoparasites of infected badgers proved negative for *M. bovis* and suggests that these are unlikely to act as vectors in transmission of the disease.

INTRODUCTION

Since 1971 considerable evidence has accumulated implicating the badger (*Meles meles*) as the origin of infection in cases of tuberculosis in cattle in areas in the south west of England (Muirhead, Gallagher & Burn, 1974; Gallagher, Muirhead & Burn, 1976; Report 1976, 1977, 1979). This association was further supported by the demonstration that *Mycobacterium bovis*, isolated from diseased badgers, produced typical lesions when inoculated into cattle (Little, Burn & Stuart, 1975). A close relationship has been found between the spatial distribution of tuberculous badgers and cattle herds experiencing breakdowns to the tuberculin test. This association has led to the Ministry of Agriculture, Fisheries and Food (MAFF) introducing a policy of destruction of infected badgers by gassing their setts where it was considered that they were responsible for local herd breakdowns. An apparent reduction in the incidence of disease in badgers has followed with a corresponding reduction in the incidence in cattle (Report 1979). *M. bovis* has been isolated from other species of mammal notably the brown rat (*Rattus norvegicus*), the mole (*Talpa europaea*) and the fox (*Vulpes vulpes*) but at a much lower

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frequency. The contribution of these species to the wild animals reservoir of bovine tuberculosis was therefore considered to be negligible.

An independent investigation into the significance of bovine tuberculosis for the badger and other species of wild life was commissioned by the Nature Conservancy Council. As part of this project the importance of mammalian species other than the badger in contributing to the disease in cattle was assessed. Although many samples of a variety of species had been examined during MAFF investigations, these were trapped over relatively large areas. In all probability the incidence of disease varies from area to area and extremely large samples would be necessary for statistical significance to be attached to the results obtained. It was therefore considered that the importance of wild animals in tuberculosis outbreaks in cattle could best be assessed by examination of animals in the immediate vicinity of the farms affected.

This paper describes two studies made in the south west of England. The studies involve two farms both of which had recently experienced outbreaks of bovine tuberculosis and in both cases tuberculous badgers were found to be resident on the farm land. Investigations were therefore made to study the mammal populations of the two farms in detail.

MATERIALS AND METHODS

Study areas

The field work was carried out in two areas situated in the Cotswold Hills in the south west of England.

Area 1 (Fig. 1)

This area lies to the north and south of a village at the west edge of the Cotswolds facing the Severn Estuary. The geology of the area consists of Athelstan Oolite overlying Fuller's Earth. The area lies between 120 and 180 m above sea level. Deciduous woodlands cover less than 10% of the land, the remainder being permanent pasture. Dairy and sheep farming are practised here and a number of cattle have been found showing minor lesions of tuberculosis over the last few years. Most of the badger setts were situated in wooded areas as indicated on the map. Sampling was carried out in the spring of 1979.

Area 2 (Fig. 2)

The area consists of a small east facing valley between 120 and 200 m above sea level lying to the west of the village of Woodchester in the heart of the Cotswolds. The geological structure of the area consists of inferior Oolite overlying Cotteswold Sands. The steep sloping sides of the valley are covered with mainly deciduous woodland constituting about 20% of the land area, the remaining land being permanent pasture. Beef and dairy farming are practised here and tuberculosis had been very recently diagnosed in several cattle prior to the sampling period. The badger setts all lay within the wooded areas. Sampling was carried out in November 1979, the weather being comparatively mild. This area constituted

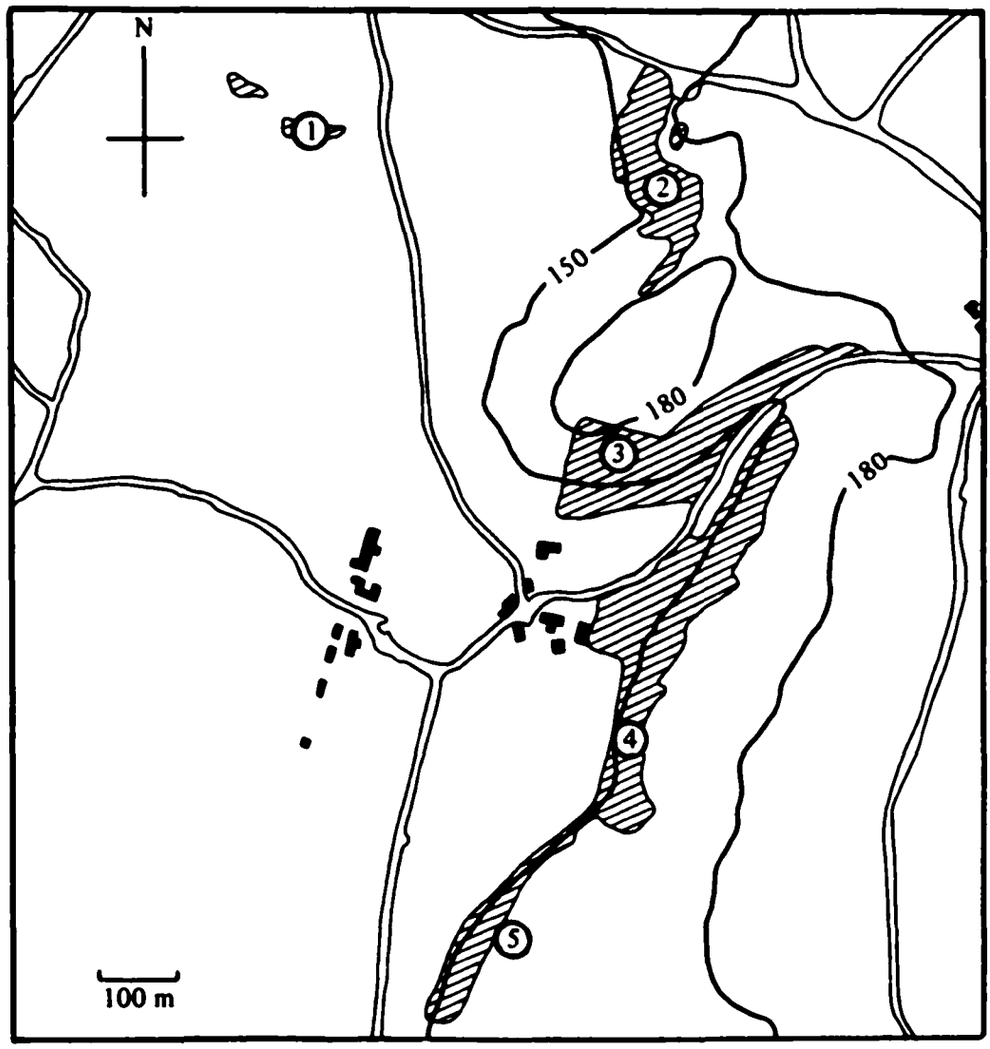

Fig. 1. Sketch map of area 1. Hatching denotes wooded areas. Numbered circles represent the location of badger setts. Height above sea level is in metres.

part of a larger area in which investigations into aspects of badger behaviour and ecology had been studied since 1975. The occurrence of cases of tuberculosis in a cattle herd in the area, however, necessitated removal of a part of the badger population. Further aspects of this removal are described by Cheeseman *et al.* (in preparation).

Sampling techniques

In both areas animals were trapped over a period of several weeks. Badgers were caught using cage traps baited with peanuts. Between 2 and 6 cage traps were laid at each of the badger setts and complete removal was effected as described by Cheeseman *et al.* (in preparation). Mice, voles and shrews were caught using Longworth traps baited with whole oats, and weasels were occasionally caught in this way. Single and double Legg traps and mink traps were used for squirrels and Bledorbury traps were used for rats, all baited with whole maize. Rabbits were caught by setting Imbra and Fenn traps in rabbit holes. Duffas traps were set in mole tunnels. Foxes were snared immediately outside the study areas.

All animals were humanely despatched. Badger carcases were sent to the MAFF Veterinary Investigation Centre, Gloucester, and other species to the Department

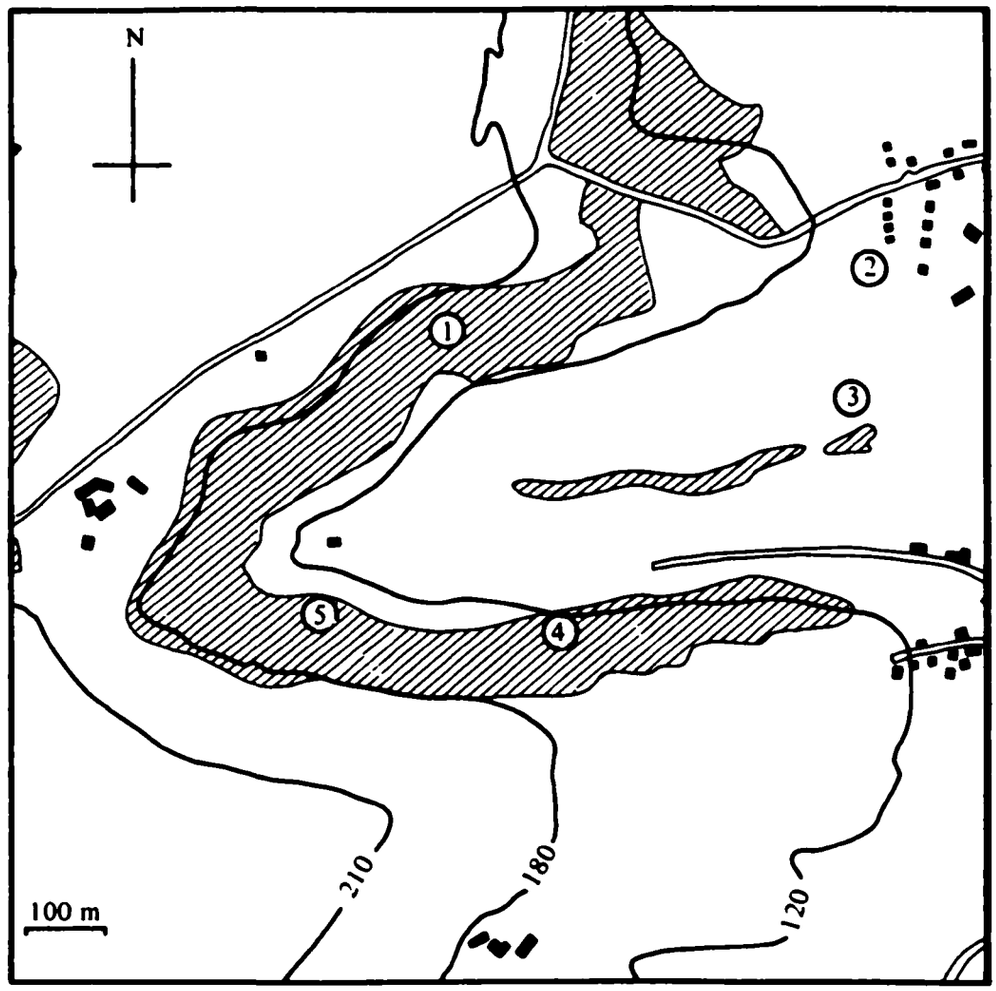

Fig. 2. Sketch map of area 2. Hatching denotes wooded areas. Numbered circles represent the location of badger setts. Height above sea level is in metres.

of Medical Microbiology, London School of Hygiene and Tropical Medicine, London. Arthropod ectoparasites were collected from the carcasses after these had been allowed to cool. In area 1 species other than badgers were sampled for ectoparasites and in area 2 badgers were also sampled.

Bacteriological investigation

At Gloucester full post-mortem examinations were carried out on the badger carcasses as part of a routine screening of large numbers of carcasses from the district for evidence of tuberculosis. A similar autopsy procedure was adopted for other species. Suspect lesions were sampled and, in the absence of any gross abnormalities, a collection was made of pharyngeal, bronchial, mediastinal and mesenteric lymph nodes of each animal. These were then macerated in 2 ml of sterile distilled water. Smears were stained by the Ziehl–Neelsen stain and examined for acid-fast bacilli. Six slopes of modified Middlebrook 7H11 agar (Gallagher & Horwill, 1977) were inoculated with 1·2 ml of macerate. A second portion of the macerate was decontaminated with 5% sulphuric acid (Mitchison & Aber, 1974) and inoculated onto six slopes of modified Middlebrook 7H11 agar and one slope of each of Lowenstein-Jensen medium with and without glycerol. All media were

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Table 1. *Results of animal sampling for* Mycobacterium bovis *in study area* 1

Animal species (Sample)	No. sampled	Sample positive for *M. bovis* by: Microscopy	Culture
Fox (*Vulpes vulpes*)	4	—	—
Long-tailed field mouse (*Apodemus sylvaticus*)	28	—	—
Short-tailed vole (*Microtus agrestis*)	20	—	—
Bank vole (*Clethrionomys glareolus*)	6	—	—
Common shrew (*Sorex araneus*)	2	—	—
Shrew (unidentified species)	4	—	—
House mouse (*Mus musculus*)	4	—	—
Mole (*Talpa europaea*)	12	—	—
Weasel (*Mustela nivalis*)	4	—	—
Rabbit (*Oryctolagus cuniculus*)	12	—	—
Grey squirrel (*Sciurus carolinensis*)	15	—	—
Badger (*Meles meles*)	18	2	2
Arthropod ectoparasites			
Fleas (identity and host not recorded)	43	—	—
Tick (*Ixodes hexagonus*, host not recorded)	1	—	—

Table 2. *Results of animal sampling for* Mycobacterium bovis *in study area* 2

Animal species (Sample)	No. sampled	Sample positive for *M. bovis* by: Microscopy	Culture
Fox (*Vulpes vulpes*)	8	—	—
Brown rat (*Rattus norvegicus*)	30	—	—
Long-tailed field mouse (*Apodemus sylvaticus*)	40	—	—
Short-tailed vole (*Microtus agrestis*)	6	—	—
Shrew (unidentified species)	4	—	—
House mouse (*Mus musculus*)	3	—	—
Mole (*Talpa europaea*)	5	—	—
Weasel (*Mustela nivalis*)	1	—	—
Rabbit (*Oryctolagus cuniculus*)	5	—	—
Grey squirrel (*Sciurus carolinensis*)	5	—	—
Badger (*Meles meles*)	38	12	12
Arthropod ectoparasites			
Badger fleas[1]	137	—	—
Badger lice (*Trichodectes melis*)	222	—	—
Badger ticks (*Ixodes ricinus*)	1	—	—
Rat fleas[2]	61	—	—
Field mice fleas[3]	16	—	—
Moles fleas[4]	6	—	—
Shrew fleas	2	—	—
Squirrel fleas[5]	20	—	—

[1] *Paraceras melis melis* (Walker); [2] *Ctenophthalmus noblis*, *Nosopsyllus fasciatus* (Bosc); [3] *Malaraeus* sp.; [4] *Palaeopsylla minor minor* (Dale), *Ctenophthalmus bisoctodentalis occidentalis*; [5] *Orchopeas* sp., *Nosopsyllus fasciatus* (Bosc).

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Table 3. *Reactions of mycobacteria strains to standard tests*

	Origin of *Mycobacterium bovis* strains	
	Area 1	Area 2
No. of strains tested	2	7
Colonial morphology[1]	D	D
Bacillary morphology[2]	SMR	SMR
Oxygen preference[3]	M	M
Photochromogenicity	−	−
Scotochromogenicity	−	−
Rate of growth[4]	S	S
Growth at 25 °C	−	−
Growth at 37 °C	+	+
Growth at 42 °C	±	±
Growth at 45 °C	−	−
Hydrolysis of Tween 80	−	−
Production of arylsulphatase (2 weeks)	+	+
Production of urease	+	+
Production of niacin	−	−
Production of nitrate reductase	−	−
Production of peroxidase	+	+
Production of catalase	+	+
Production of catalase, resistant to 68 °C	−	−
Stimulation by glycerol	−	−
Reduction of potassium tellurite	−	−
Resistance to pyrazinamide (25 μg/ml, pH 5·5)	+	+
Resistance to T2CH (10 μg/ml)[5]	−	−

[1] Dysgonic growth. [2] Short- to medium-length rods. [3] Microaerophilic. [4] Slow. [5] Thiophene-2-carboxylic acid hydrazide.

incubated at 37 °C for 12 weeks at London and 6 weeks at Gloucester before being discarded.

Arthropod ectoparasites sampled from individuals of the same species were pooled. From the flea and lice collections several individual parasites were taken and preserved in alcohol for the purpose of identification. The number of individuals taken depended on the size of the sample. The remainder were homogenized in 2 ml sterile distilled water with a sterile pestle and mortar, and the samples were tested as for the lymph node samples. All ticks were individually identified prior to homogenization. All ectoparasites were identified using keys described by Smit (1957).

Isolates from Gloucester were sent to London for further investigations and these were subjected to a variety of tests for confirmation of identity (Table 3). The tests carried out are described by Marks (1964, 1976) and Runyon *et al.* (1974).

RESULTS

In both areas the only samples positive for mycobacteria were lymph node samples or lesions from badgers (Tables 1 and 2). These samples were positive by both microscopy and culture.

Badger ectoparasites were not collected in area 1 but those of a substantial sample from the badgers in area 2 were all negative for mycobacteria. The fleas identified were typical for the host species caught.

From the cultural characteristics and the results of biological tests all strains were identified as *Mycobacterium bovis*. The results of further tests carried out on two strains from area 1 and seven from area 2 confirmed this identification (Table 3). No other mycobacterial species was identified.

DISCUSSION

The transmission of bovine tuberculosis between badgers and from badgers to cattle is thought to take place mainly via contaminated secretions and excretions from diseased badgers (Gallagher *et al.* 1976). There is little evidence at present to suggest that arthropod vectors play any part in transmission of tuberculosis as occurs in myxomatosis in rabbits. Ticks and terrestrial arthropods can carry different species of mycobacteria (Beerwerth, Eysing & Kessel, 1979) and it has been suggested that the spread of *Mycobacterium bovis* infection amongst possums in New Zealand may be mediated by mosquitoes (Cook, unpublished results). This presumably involves mechanical uptake or ingestion of bacteria from discharging sinuses that occur with the disease in the possum (Ekdahl, Smith & Money, 1970). In relation to the present problem, however, ticks, fleas and lice have been sampled from tuberculous badgers and from pasture on farms where cases of bovine tuberculosis have occurred but, as in the present investigation, all produced negative results (MAFF, unpublished results). With the exception of cases of very advanced tuberculosis mycobacteria are seldom likely to be present in large numbers in the blood and transmission of *M. bovis* by blood-sucking arthropods is therefore unlikely to be of any significance in comparison with spread by the respiratory route. Contamination of the external surfaces of ectoparasites by infected sputum, pus or urine on the coat of the animal might occur, but again, no evidence of this has been found in association with badger tuberculosis.

Intensive trapping and snaring of the badgers was considered to have removed the whole of that population. Intensive trapping of the small mammals was carried out over a period of several weeks and this would have removed a large proportion of the populations involved although it was not possible to carry out strategic sampling to estimate the population sizes.

The results obtained from this exercise relate directly to the individual farms studied and they cannot necessarily be extrapolated to other situations. This sampling technique is likely to be a more accurate method for examining the role of animal species as potential reservoirs of infection than is random sampling of populations. The latter may lead to extremely large numbers of animals being examined before a statistically accurate result is obtained (Barrow, unpublished results). In addition, random sampling may lead to local pockets of high frequencies of infection being completely missed. It is therefore felt that the finding that badgers were the only species to harbour *M. bovis* does reflect the situation in the areas studied.

It is perhaps suprising that *M. bovis* was the only species of *Mycobacterium*

isolated. *M. avium* has occasionally been isolated from badgers and foxes in the past (Gallagher, in preparation) and Beerwerth and his colleagues isolated a large number of species including *M. fortuitum*, *M. gordonae*, *M. terrae* and the *M. avium–intracellulare* complex from terrestrial arthropods some of which form part of the normal diet of the badger (Beerwerth *et al.* 1979).

The absence of *M. bovis* infections from other wild mammals in an environment contaminated by infected badgers is, at first sight, somewhat surprising. Roe deer infected with *M. bovis* have been found associated with tuberculous badgers in Switzerland (Bouvier, Burgisser & Schmeider, 1957, 1959, 1962). In this case it was suggested that badgers had become infected by consuming deer cadavers. However, in considering the possibility of chains of infection existing between different species, account must be taken of the degree of contact between these and the badger. The wild mammals sharing the badgers' habitat have either a predator–prey relationship or a casual indirect contact from feeding at common sites or habitation in vacant badger setts. Foxes fall into the latter category and, although none of those sampled in this exercise was infected, 3 of 290 examined by MAFF were infected by *M. bovis* (Report, 1979). Those were trapped at two sites in Gloucestershire where badgers with advanced discharging lesions of tuberculosis were trapped concurrently. In contrast the foxes showed no macroscopic lesions and thus it was considered that the foxes were most likely to have acquired their infection from the badgers (Gallagher, in preparation).

Rats have been found infected with *M. bovis* on a farm in Dorset and two infected moles were found on a farm in Cornwall (Report, 1976). Rats and moles are amongst the species preyed upon by the badger but the infections in these cases were latent or incubating and no macroscopic lesions were present. Infection via the alimentary tract in badgers is extremely rare and accounted for only 1·6% of all infections in 185 tuberculous badgers examined (Gallagher, in preparation), indicating that infection via the agency of prey species plays an insignificant role in maintenance of the badger reservoir of infection.

Because of the absence of infection in any of the other wild animal species examined here it is considered that, in the areas studied, the badger is the only species infected. This supports previous findings of the badger being the major wild animal reservoir of *M. bovis* infection in the south west of England (Report, 1976, 1977, 1979) and suggests that the reservoir is self-perpetuating with only occasional overspill to produce infection in other species. The species of which a few individuals have been found infected may thus act only as 'blind end' hosts which would be unlikely to transmit infection to other individuals. Future investigations are desirable to ascertain whether independent cycles of infection become established in other wildlife species.

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