

## **Bovine tuberculosis in domestic and wild mammals in an area of Dorset. II. The badger population, its ecology and tuberculosis status**

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### SUMMARY

Following a major outbreak of tuberculosis in cattle on a farm in Dorset, badgers were discovered to be infected with *Mycobacterium bovis*. Two hundred and forty sets were found in the 1200 hectares of the study area. The sets were found predominantly in areas of Portland Sand. A high prevalence of tuberculosis was found in the badger population which was removed and repopulation prevented for 3 years. The removal of the infected badgers led to the resolution of the problem in cattle. Re-colonization of the area has progressed slowly and the cattle have remained free from infection for a period of 5 years.

### INTRODUCTION

A major outbreak of tuberculosis in cattle on a farm in Dorset has been described by Wilesmith *et al.* (1982). No origin for the infection was found until badgers (*Meles meles*) on the farm were discovered to be infected with *Mycobacterium bovis*.

The occurrence of bovine tuberculosis in badgers was first described in a region of Basle in Switzerland (Bouvier, Burgisser & Schneider, 1957, 1959, 1962; Bouvier, 1963) where it was thought that the badgers were becoming infected from roe deer.

After the original discovery of infection in Gloucestershire (Muirhead, Gallagher & Burn, 1974) it was subsequently found in other parts of the south west of England particularly in Cornwall but also in Devon, Avon and Wiltshire. Infected badgers have also been found in East and West Sussex, Surrey, Hereford, Worcester and South Wales (Reports, 1976, 1977, 1979). An infected badger has been discovered in County Cork in Ireland (Noonan *et al.* 1975) and *M. bovis* was isolated from 18 out of 50 badgers examined in Northern Ireland (Report, 1978*a*). There would thus appear to be a number of natural foci of bovine tuberculosis in badgers in Britain.

These widely separated foci of tuberculosis in badgers indicate the susceptibility

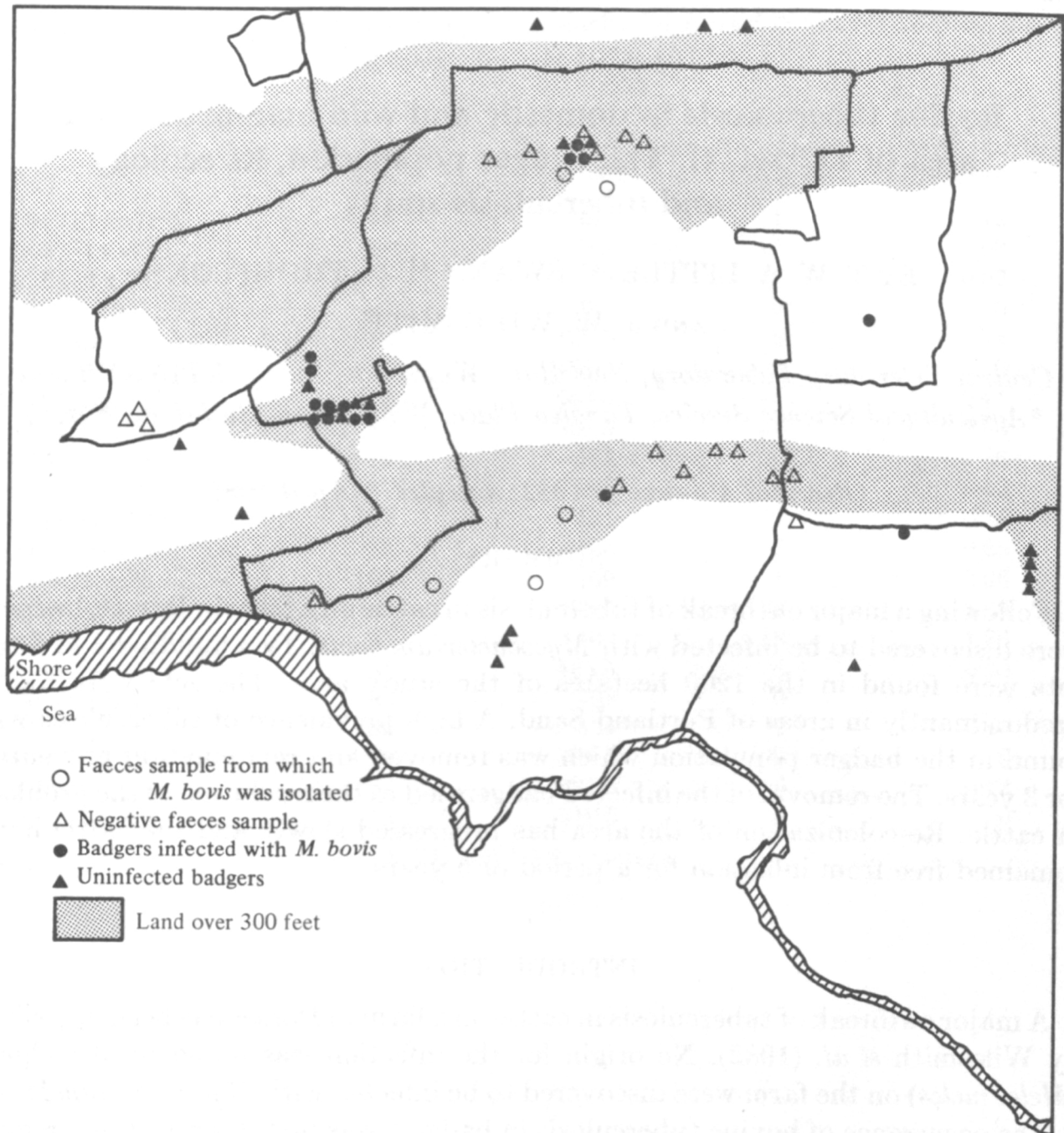


Fig. 1. Distribution of infected badgers and faeces samples obtained during preliminary survey 1974, and the location and infection status of badgers trapped during 1974/5.

of badgers to this infection and suggest that badgers are not just occasional accidental hosts but are acting as a reservoir or maintenance host.

An extensive wildlife reservoir of *M. bovis* infection has also been discovered in opossums (*Trichosurus vulpecula*) in New Zealand and there is evidence linking infection in opossums and cattle (Julian, 1981).

The badger population described in this paper was first investigated for the presence of tuberculosis in 1974. Fourteen badger faeces samples were collected in February from the central ridge (Fig. 1) and *M. bovis* isolated from four gathered at the western end. A further two out of eleven faeces samples collected in May 1974 and two out of five badgers obtained by the farmer were infected with

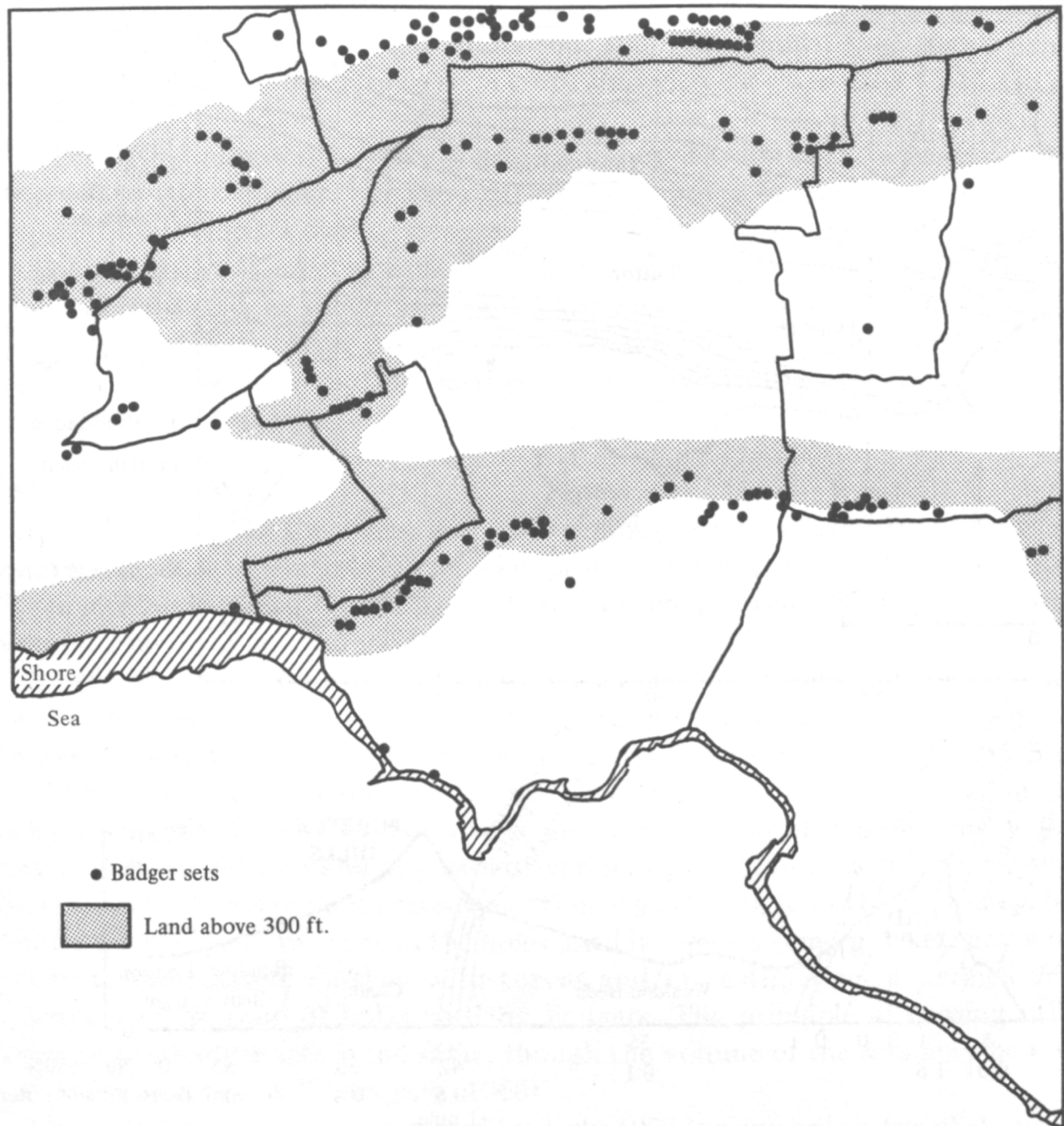


Fig. 2. Distribution of badger sets within the control area.

*M. bovis*. These discoveries prompted a survey of badger sets in the area, the further investigation of the tuberculosis status of the population by capturing and post-mortem examination of badgers, and eventually the total removal of the badger population.

## MATERIALS AND METHODS

### *The survey of sets*

Initially the whole area of 1200 hectares was surveyed and the location of the sets marked on 1:10560 Ordnance Survey maps (Fig. 2). As the results of other surveys of the distribution of badger sets have demonstrated there is a close

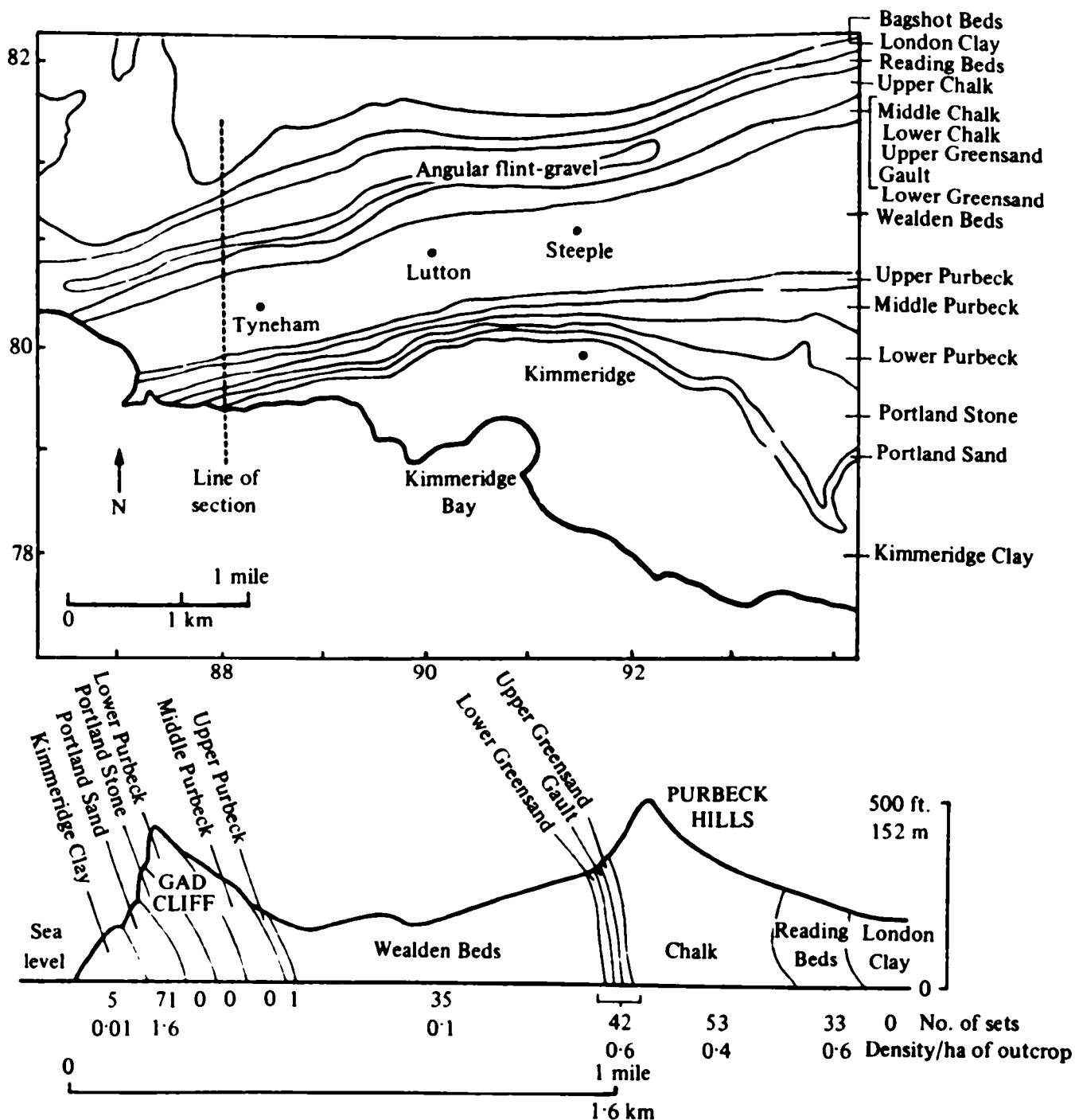


Fig. 3. Geological map of the Steeple area in Dorset with section to show distribution and density of badger sets.

relationship between locality and geology (Report, 1975), the location of the sets was transferred to 1:63360 Geological Survey maps and assessments were made of the area of exposure of each geological formation and a set density figure (number of sets per hectare) calculated (Fig. 3).

*The capture of badgers*

*Live trapping*

Surveys in the central farm area to locate active sets were completed by September 1974. In consultation with the Universities Federation for Animal Welfare (UFAW) cage traps for individual badgers were designed that were both efficient and humane (Cheeseman & Mallinson, 1980). Trapping commenced

immediately and the first badger was caught in a cage trap on 18/19 September. Subsequent work (Cheeseman & Mallinson, 1980) has shown that snares can be more efficient and humane than cage traps provided they are used with skill and care.

The original plan was to use a limited number of traps in the area most likely to produce quick results, but the restrictions on entry to two thirds of the area imposed by the MOD during army exercises, made for slow progress. Following the identification of infected animals in the first group of badgers caught, the traps were concentrated as much as possible in those areas known to have yielded diseased badgers. By June 1975, 33 badgers had been caught and examined at the Central Veterinary Laboratory (CVL). The sites where the badgers were caught are marked on Fig. 1.

### *Gassing*

In 1975 the Wild Creatures and Wild Plants Act permitted the use of 'any specified means' to control (kill or take) protected wild creatures 'for the purpose of preventing the spread of disease'. It was decided to remove all badgers in the area outlined in Fig. 2 by gassing the sets.

Gassing was carried out using cyanide powder, which generated hydrocyanic-acid gas (HCN) when in contact with moisture in the air. This compound has been used for rabbit destruction since 1900. The use of HCN for rabbit control owes much to the UFAW who have encouraged the development of the technique since the early 1930s. The most effective and economical method of dealing with a large warren is to use either hand or petrol driven pumps to blow sufficient powder into the warren to generate a lethal concentration of gas throughout the burrow system. The powder is blown into one of the holes until it is seen to emerge at others, which are then immediately blocked with turves and/or earth. A set is defined as an interconnected group of holes used by badgers. The principle of gassing rabbit warrens and badger sets is the same, though the volume of the sets may be much greater (Thompson & Thompson, 1966).

Most of the gassing was completed by early 1976 leaving only a few of the earlier derelict sets, now occupied, to be gassed. At intervals, sets showing signs of reoccupation were gassed.

### *Post gassing surveillance of badgers*

During 1976 to 1979 badgers were caught as they attempted to enter the area and taken to the CVL for examination; badgers found dead or killed by keepers in this period and in 1979–81 were also examined; faeces samples were tested for the presence of *M. bovis*.

### *Natural recolonization*

No cattle on the central farm have reacted in the tuberculin test since 1978 and reactors with visible lesions of tuberculosis have not been found since 1975. Natural recolonization of the area by badgers from outside the control area was allowed from February 1979.

Table 1. *Laboratory results of live trapped badgers in the control area*

Badger no.	Date of capture	Weight in lb	Sex	CFT result on arrival at CVL	Post-mortem findings	Bact. exam. of faeces on arrival at CVL	Bact. exam. of tissues
1	18/19 Sept. 1974	14	M	Positive	NVL	Negative	Negative
2	25/26 Sept.	24	M	Negative	NVL	Negative	Negative
3	29/30 Sept.	18½	M	Positive	NVL	Negative	Negative
4	1/2 Oct.	15½	M	Positive	Lesions in retropharyngeal precapular and thoracic lymph nodes, large abscess in right axilla. A few large lesions in lungs	Positive	Positive
5	2/3 Oct.	13½	F	A/C	Multiple lesions in lungs, lesions in thoracic lymph nodes, large lesions in both kidneys	Positive	Positive
6	25/26 Oct.	21½	M	Positive	Lesions in bronchial lymph nodes	Negative	Positive
7	25/26 Oct.	16½	M	Positive	Tiny lung lesion, lesion in bronchial lymph node	Negative	Positive
8	27/28 Oct.	23	M	Positive	NVL	Negative	Negative
9	28/29 Oct.	14	F	Positive	NVL	Negative	Negative
10	1/2 Nov.	17½	M	Positive	Tiny lung lesion	Negative	Positive
11	2/3 Nov.	23	F	Positive	Small lung lesions and lesions in bronchial and mediastinal lymph nodes	Negative	Positive
12	3/4 Nov.	17	F	Positive	Lesions in bronchial lymph node	Negative	Negative
14	17/18 Dec.	25	M	Positive	NVL	Negative	Negative
15	18/19 Dec.	17	F	Positive	Lesion in inguinal lymph node	Negative	Positive
24	4 June 1975	7	M	Negative	NVL	Negative	Positive
25	12 June	17	M	Negative	Small lesions in lung and thoracic lymph nodes	Negative	Positive
26	18 June	9½	F	Positive	NVL	Negative	Negative
27	19 June	8½	F	Negative	NVL	Negative	Positive
28	20 June	7	F	Negative	Right axillary lymph node enlarged and nodular	Negative	Positive
32	25 June	18	F	Positive	NVL	Negative	Positive

NVL = no visible lesions; A/C = anticomplementary.

*The examination of badgers*

Thirty-three badgers were live-trapped and transported to CVL. Before handling the badgers, they were immobilized as described by MacKintosh *et al.* (1976). The badgers were then weighed and examined for signs of bite wounds or any other abnormality. A blood sample was collected from the cephalic vein for a complement fixation test and other tests. An intradermal comparative tuberculin test was performed by clipping an area of the left shoulder and injecting intradermally 0.1 ml of Weybridge avian and bovine tuberculins. The site was inspected at 24 and 48 h and then regularly for a period up to 7 days. A faeces sample was collected from each badger on arrival.

Twenty of the 33 badgers (see Table 1) were kept separately in isolation for several days and then killed. The carcasses were subjected to a detailed post-mortem examination and portions of any lesions found were fixed in buffered formalin for histological examination. Sections were stained with Ziehl-Neelsen's stain and haematoxylin and eosin. Parts of the lesions and associated lymph nodes or, in the absence of any lesions, a collection of pharyngeal, bronchial, mediastinal and mesenteric lymph nodes and organs were decontaminated using 5% oxalic acid and examined by both cultural examination and biological tests in guinea pigs for the presence of *M. bovis*.

The remaining thirteen badgers were kept at the CVL for up to 4 years to study the course of *M. bovis* infection in badgers. Calves were exposed to these badgers for various periods of time and the results have been reported in detail by Little, Naylor & Wilesmith (1982).

Badger and faeces samples from the post-gassing surveillance were similarly examined, as was material from reactor cattle.

*Bacteriological examination*

The culture media used for primary isolation from either badger material or guinea pigs were Stonebrink medium (Lesslie, 1959), Lowenstein-Jensen medium with and without glycerol and a blood medium (Birn, 1965). The isolates were identified as *M. bovis* providing they met the following criteria.

The colonies were of typical appearance and were composed of strongly acid alcohol fast rods. There was dysgonic or no growth on medium containing glycerol. The organisms sensitized guinea pigs to mammalian PPD and produced generalized tuberculosis (and were pathogenic to rabbits – but this test was rarely used).

Further confirmatory tests were used to demonstrate

- (1) Strict mesophilic growth.
- (2) Microaerophilic growth.
- (3) Lack of pigmentation.
- (4) Sensitivity to 500 µg/ml of *p*-nitrobenzoic acid.
- (5) No reduction of nitrate.
- (6) Utilization of urea.

## RESULTS

*The survey of sets*

Two hundred and forty active sets were found in the area (Fig. 2), an overall density of 0.2 sets/ha. Twenty-four additional sets were reopened up to 31 December 1978.

Most of the control area consisted of Kimmeridge Clay (31 %) and Wealden Beds (33 %) but these formations contained only 17 % of the sets. A high density 1.6 sets/ha was found in Portland Sand which, whilst being exposed over only 45 hectares (4 % of area) contained 30 % of the sets. The overlying Portland Stone provided a very solid roof for the tunnel systems. Another highly favoured formation exposed as in the case of Portland Sand as a steeply rising hillside was Upper Greensand. This occupied 2 % of the control area but had a set density of about 1.9/ha. After these the most frequently selected were Reading Beds (0.6 sets/ha) and Chalk (mostly Upper) with density of 0.4/ha (Fig. 3) (Report 1978b).

*Natural recolonization of sets*

Since this was first allowed in February 1979 it has progressed slowly. Fifty-nine of the gassed sets have been re-opened and 27 of these have been reoccupied for periods exceeding 2 weeks during the past 2 years. Of the 53 badgers caught and marked by ear-tag and tattoo, 13 were outside the control area in late 1978. Twenty-six were caught from March to December 1979 (9 male, 7 female and 10 cubs) while 14 were caught in 1980 (four male, eight female and two cubs). Seven of these have carried radio-transmitters for varying periods and three are still providing information, one being located in an area 6 km to the west of the study area. Two of the marked badgers are known to have been killed.

Some of the badgers showed injuries resulting from fighting; missing ears and facial scars were commonplace. The largest area known to be used by a radio-carrying badger (a male) is some 300 hectares. Results from bait-marking (as described by Kruuk, 1978) have been limited and there is as yet little indication of clearly defined territories. The greatest distance recorded by trapping and re-trapping that a marked badger has travelled was some 3½ km and the longest distance by bait-marking was 2½ km.

*The examination of badgers*

Five badgers were caught by the farmer in 1974, two had lesions of tuberculosis from which *M. bovis* was isolated. One of these had extensive lesions in the lungs, liver and kidneys while the other had lesions only in its lungs. One of these badgers came from above the cliffs at the western end of the downs and the other further along the downs.

The results for the 20 live trapped badgers which were post-mortemed shortly after arrival at the laboratory are presented in Table 1. *M. bovis* was isolated from 12 of these animals.

The badgers were all adults except for four (numbers 24, 26, 27, 28). All the



badgers were active without any obvious signs of illness. However, no. 5 was light in weight and had worn and broken teeth.

None of the tuberculin tests gave any sign of redness or swelling but most of the badgers had antibodies against mycobacteria which could be detected in the complement fixation test.

At post-mortem examination only badgers no. 4 and 5 showed extensive lesions. In three no lesions were observed and the remainder of the badgers from which *M. bovis* was isolated the lesions were trivial.

Most of the lesions were confined to the thoracic cavity but in no. 5 the kidneys were also involved. However, the abscess in the axilla of no. 4 was probably the result of a bite wound.

Histologically, both early and more advanced lesions were seen in the lungs; giant cells of Langhans were not observed. Large numbers of acid-fast bacilli were present in the kidney lesions of no. 5. *M. bovis* was isolated from pooled lymph node collections of three badgers (nos. 24, 27 and 32) in which no lesions were seen. One badger (no. 12) had lesions in the bronchial lymph nodes which were considered to be tuberculous but yielded no mycobacteria by cultural and biological examination.

None of the 41 badgers or 92 badger faeces samples collected after gassing were found to contain *M. bovis*.

*M. bovis* was isolated from the faeces of the two badgers with obvious lung lesions and from one (no. 22) of the badgers retained for other work on arrival.

All the strains of *M. bovis* isolated from the badgers fulfilled the basic requirement of the species. These strains, along with some of the strains isolated from cattle on this farm, have been examined using a number of other techniques, such as phage typing, thin layer chromatography of the mycolic esters and pyrolysis gas chromatography. The results will be reported separately.

## DISCUSSION

The normal reservoir of *M. bovis* is cattle and it is possible that the infection of badgers in Britain is a recent evolutionary event. Francis (1947) suggests that although the Romans imported cattle into Britain, bovine tuberculosis was probably introduced into Britain from Holland in the seventeenth century. With the coming of the industrial revolution and the widespread increase in the number of town dairies the disease rapidly spread until in 1847 Hunting reported 20% of the cattle in Durham to be infected. During the period before attempts were made to control tuberculosis in cattle, when advanced cases of the disease were common, McFadyean & Knowles (1915) found *M. bovis* in the faeces of 54% of infected cattle. Although the earthworm is the preferred diet of the badger, at times of scarcity badgers will search for other food including dung beetles (*Geotrupes* sp) in cow pats and during May and August may eat considerable numbers. Also, Francis (1947) stated that about 5% of tuberculous cows had tuberculous metritis. Some of these cows would abort producing infected placentae and foetuses which badgers may

have eaten. Thus, in the period when tuberculosis was widespread in cattle, badgers must have frequently been exposed to *M. bovis*.

Although it is possible to postulate an efficient mechanism for cattle to infect badgers in the past this source of infection has to a very large extent been cut off by the virtual eradication of tuberculosis in cattle. It remains now to be seen how efficient badgers are as maintenance hosts of *M. bovis*, but they appear to be susceptible and yet survive long enough for infection to be passed from one generation to another. Thus, a new cycle of infection may have become established, the prevalence and severity of which may depend on the density of the badger population. The mechanism by which badgers infect cattle may be much less efficient. However, the preferred habitat of the badger is near the edge of deciduous woodland, usually within 25 m of the edge (Neal, 1977). The most significant item in the badger's diet is the earthworm (*Lumbricus terrestris*) which is commonly found in pastures. It has been shown (Kruuk *et al.* 1979) that badgers selectively feed on short grass rather than long grass as they have greater difficulty finding and catching worms in longer grass, and the foraging areas of badgers and of cattle clearly overlap and the two species come into close proximity. In this part of Dorset virtually all the cattle grazing areas were within 50 m of the sets and the badgers spend a large part of their time searching for earthworms in such fields.

Badgers will contaminate pasture by infected dung, urine, sputum or pus from infected bites and the length of time any excreted *M. bovis* survive on pasture is an important factor in transmission of infection from badgers to cattle. Early workers such as Stenhouse-Williams & Hoy (1930) concluded that *M. bovis* remained alive and virulent in cows' faeces on pasture for at least five months in winter but less than two months in summer unless protected from direct sunlight. More recent work (Report, 1979) suggests that *M. bovis* does not remain viable for more than a few days in badger urine or pus during the summer months but for longer periods during winter. However, the constant presence of infected badgers in the environment of cattle would result in *M. bovis* being deposited frequently on pasture via for example infected urine. Other organisms such as leptospira, which are very much more fragile than *M. bovis* will pass from infected wild animal hosts to domestic animals via the urine where their two habitats coincide (Sebek, 1965). Some of the infected material may be inhaled by cattle actively sniffing or by chance. Cattle are very susceptible to infection by the respiratory tract. Francis (1971) reviewing the literature suggests that up to a million times more bacilli are required by the alimentary route than by the respiratory route, where perhaps even a single organism can initiate infection and in over 90% of cases of tuberculosis in cattle the lesions are confined to the thoracic cavity. On the farms in this area the majority of lesions in cattle were found in the respiratory tract.

The ease with which tuberculosis was discovered in this badger population (i.e. of the preliminary sampling four out of fourteen badger faeces were found to contain *M. bovis* and two out of five badgers had lesions of tuberculosis) indicates that this population had a high prevalence of infection and disease. Further sampling, where twelve out of twenty badgers were infected, confirmed this.

In his review of the importance of predisposing factors to tuberculosis Francis

(1971) was sceptical of any factor except those tending to increase the weight of infection. Thus, the prevalence of tuberculosis in any given population may be related to the density of that population, a point further discussed by Zuckerman (1980).

Neal (1977) pointed out that the distribution of badgers is to a large extent determined by how well a particular area fulfilled their ecological requirements. In the control area there was 240 sets in just under 12 square km which indicates a very dense badger population, as high as anywhere in Gloucestershire which is regarded as having the densest badger population in Britain. The habitat is obviously ideal for badgers, the Portland Sand and Upper Greensand in particular providing good enough digging areas with solid roofs. The linear deposition of the sets on relatively steep hillsides places all the sets within easy reach of fields in which earthworms are plentiful and some cover is provided by elderberry or gorse. The steepness of the hillside reduces interference from both man and domestic animals and the military activities in the area also increase its relative seclusion. An ideal habitat and an adequate supply of food led to the establishment of a very dense badger population. Neal (1977) suggested that in good badger country between 30 and 50 sets per 10 square km may be found. The density of sets on the farm is at least five times this number and Neal suggests that this level of population density could not be maintained for long.

The existence of a large population living underground in social groups is an ideal situation for the spread of a respiratory infection such as tuberculosis and, in the majority of cases, post-mortem examination suggested that this was so, although two cases were probably infected as a result of bite wounds. In two badgers extensive kidney lesions were found and these badgers were probably excreting infected urine. Three of the badgers were demonstrated to be passing infected faeces and the abscess in the axilla of one of the badgers in time would probably have burst leading to the discharge of infected pus. As the lungs appear to be the predilection site, live tubercle bacilli present in faeces are probably a result of swallowing infected sputum.

It is unfortunate that no tests for detecting infection in live badgers were found to be reliable other than cultural examination of badger excreta. It was not surprising that the skin tests were uniformly negative in view of the mild cellular reaction seen histologically, which Gallagher *et al.* (1976) suggested indicated a low level of hypersensitivity. When the response of badger lymphocytes to bovine tuberculin was studied using lymphocyte transformation tests (Morris *et al.* 1978) the lymphocytes from infected badgers were suppressed rather than stimulated, indicating that tests of cell-mediated immunity were unlikely to be of value.

The detection of humoral immunity by the complement fixation test also was of limited value. Fourteen of the 20 badgers gave a positive reaction whether infected or not. The serum from one badger was anticomplementary. The problem of the relatively non-specific nature of antigens used to detect mycobacterial infection was further demonstrated in badgers using an ELISA test by Morris *et al.* (1979) who concluded that a serological test to detect tuberculous badgers must await the development of more specific antigen preparations.

The gassing strategy in this area was successful in removing the badger population and maintaining the area free of badgers for three years. The density of badgers in the surrounding area was considerably lower and recolonization is proceeding at a relatively slow rate; the new population appears to be free from tuberculosis.

Removal of badgers by gassing plus the detection and removal of infected cattle by tuberculin testing has been followed by freedom from infection in cattle and the herd has now remained free from infection for five years – the longest period without a tuberculin reactor since 1962 (Wilesmith *et al.* 1982).

In conclusion this study indicated that: the area contained an unusually high population of badgers and the prevalence of *M. bovis* infection and disease in this population was also very high; badgers were the only common reservoir of infection for cattle in this area; no other possible origin of infection being detected despite extensive and intensive investigations; other species of mammals were apparently not being infected from the badgers and the badger was the only species acting as a reservoir of infection (Little *et al.* 1982); removal of the infected badger population led to the resolution of the problem in cattle, thus providing very strong evidence for a causal relationship; the control of tuberculosis in cattle has demonstrated that natural foci of infection occur in badgers in Europe, as also in opossums in New Zealand. In considering the control of infectious diseases in domestic animals, the presence of the causal organism in wild mammals must be taken into account.

This work would not have been possible without the active co-operation of many people, both within the Ministry of Agriculture, Fisheries and Food and the agricultural community.

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