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Tuberculosis in East Sussex

II. Aspects of badger ecology and surveillance for tuberculosis in badger populations (1976–1984)

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SUMMARY

Following the disclosure of *Mycobacterium bovis* infection in badgers in East Sussex in 1976, badgers have been examined from and around farms on which cattle have become infected, but with no other attributable source of infection. These farms are confined to the downland of the south-west of the county and *M. bovis* has been confirmed in badger populations utilising their land. The available evidence indicates that *M. bovis* infection in badgers is also confined to this area. A detailed study in one area on the South Downs suggested that *M. bovis* is endemic in the badger population and therefore presents a continued risk for cattle occupying the area.

INTRODUCTION

Badger sett surveys have been carried out in East Sussex as part of the National Badger Survey organized by the Mammal Society of Great Britain (Clements, 1974; Neal, 1977). This has provided an indication of the relative distribution of badger density throughout the country and the sett survey has been continually updated. Clements (1980) reported the highest average number of setts per 1000 acres, of all counties in Great Britain, in East Sussex. Subsequent more detailed studies have suggested that Gloucestershire supports the highest badger density in Great Britain (Cheeseman *et al.* 1981); the badger density in East Sussex can therefore only be regarded as relatively high. The Mammal Society survey has also examined badger's habitat preferences and ecological requirements, but there are no published reports of detailed ecological studies in the county.

Such ecological studies were prompted by the disclosure of infection in badgers in the county in 1976 (Report, 1976) because of the risk infected badgers pose for cattle herds. The first paper in this series described the epidemiology of tuberculosis in cattle herds in East Sussex (Wilesmith *et al.* 1986). This paper describes ecological studies and the surveillance for tuberculosis in badger populations in the county during the period 1976–84.

MATERIALS AND METHODS

Study areas

The main and project areas are as defined in the previous paper (Wilesmith *et al.* 1986).

Field investigations for badger setts and signs

A systematic field investigation was carried out to obtain initial information on the badger population in the area. This investigation involved experienced field staff walking the entire area to locate and record on a 1:10000 Ordnance Survey (OS) map main and outlying setts, the number of active and inactive holes at each sett, latrines and evidence of badger tracks.

The project area was first surveyed in 1978 following the finding by a farmer of a badger carcass which subsequently proved to be infected with *Mycobacterium bovis*. Since then the maps have been regularly updated, a systematic survey being carried out each spring before and during bait marking described below. The surrounding area to the south was also regularly surveyed.

From 1979 to 1984 bait marking to determine social group territories in the project area was carried out each spring during April and early May using the method described by Kruuk (1978) and Cheeseman *et al.* (1981). From 1979 to 1982 the peanuts and plastic pellets were mixed with English honey (to avoid the risk of spreading Foul Brood Disease of bees presented by imported honey), but in 1983 and 1984 a mixture of peanuts, honey and golden syrup (Tate & Lyle Ltd) was used with equally good results. The technique was modified slightly from 1980 onwards, so that searching for plastic pellets in latrines started on the third day of the initial feeding period and continued for a week after the feeding was stopped. This was because during 1979 apparently empty dung pits had been found to contain plastic markers, probably because sufficient peanut material passes through the badger gut undigested for the faeces to remain attractive to birds and small mammals. Major outlier setts were also bait-marked with separate colours where this was necessary in order to establish to which main sett they belonged. When considered necessary, setts outside the project area were also bait-marked in order to confirm that they were not being used by social groups from the project area. Social group boundaries were determined using recoveries of marked faeces together with other signs such as badger paths and also physical features (Cheeseman *et al.* 1985*a*). The boundaries were marked on the maps of bait-marking returns. Where the boundary could be marked with confidence a solid line was used; a dotted line was used to indicate the best interpretation from available data. The horizontal area of each badger territory was calculated using a planimeter, no transformation being undertaken to allow for sloping ground.

Between January and March 1982 a survey of habitats within the study area was undertaken. Habitats were assigned to one of the following categories.

Unimproved grassland	Chalk downland particularly on the steeper slopes, grazed by cattle and sheep and dominated by sheep fescue (*Festuca ovina*) and upright brome (*Bromus erectus*)
Improved grassland	Predominantly perennial ryegrass (*Lolium perenne*)
Rough grass	Areas where the grass is rough and tussocky and not regularly grazed

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Arable	Arable land situated on the flatter ground
Scrub	Areas dominated by shrubby or scrambling species less than 5 m high
Woodland	Areas dominated by tree species taller than 5 m
Built-up areas	Houses, gardens, roads and farm buildings
Water	Sources of free water: ponds, streams, wet ditches and water troughs
Field boundaries	Recorded as: wire fences, trimmed hedges and overgrown hedges.

Habitats were mapped on the ground by marking on a 1:2500 OS map, and boundaries confirmed by reference to 1:7000 black-and-white aerial photographs taken in July 1981. The horizontal areas of habitat were calculated using a planimeter (no transformation being undertaken to allow for sloping ground).

Acquisition of badger carcasses for laboratory examination

Badgers were acquired throughout the county, for bacteriological examination for *M. bovis* infection, by three means. Animals found dead, e.g. on roads and railway lines, and submitted by members of the public; badgers caught by Ministry staff on and around land grazed by infected cattle herds to investigate the source of infection for cattle; and badgers taken under licence from MAFF to prevent damage.

Faeces sampling

From 1976 to 1981 badger faeces samples were collected on and around farms grazed by infected cattle herds in the main area. An attempt was made to collect only fresh deposits, but these were not always attributable to particular badger social groups. These samples were stored in a refrigerator and despatched by post to the Central Veterinary Laboratory (CVL) as soon as practicable. This method of obtaining faeces samples will be referred to as haphazard faeces sampling.

Samples of fresh badger faeces were collected from the project area at weekly intervals each spring from 1981 to 1984 using a method similar to that described by Cheeseman *et al.* (1985*a*). The day before samples were to be collected every main sett was visited and faeces in latrines associated with the sett were covered with a light sprinkling of soil. On the following day, fresh faeces were easily identified and individual samples were taken by hand using disposable plastic gloves and transferred to a sterile screw-topped plastic pot; a new glove and a new pot were used for each sample. Samples were grouped according to the main sett from which they had been taken so that they could be subsequently treated as individual samples or pooled as necessary and transported by car to the Central Veterinary Laboratory to arrive within 24 h of having been deposited. This method of obtaining faeces samples will be referred to as rigorous faeces sampling.

Two badger social groups in the project area were selected to examine the efficacy of a range of bacteriological techniques for the isolation of *M. bovis* from badger faeces. These two social groups were known to contain infected badgers from investigations in the preceding months.

Individual faeces deposits were obtained during October 1981 from the main sett latrines of the two groups on two occasions at an interval of 14 days. On the first day of each sampling all individual deposits found in the latrines were taken as

separate samples. These samples are referred to as old samples. On the following day these latrines were revisited and the fresh individual deposits of faeces collected. These samples are referred to as fresh samples. This method of obtaining faeces samples will be referred to as intensive faeces sampling.

Six samples were selected at random from each social group for bacteriological examination from each of the four samplings of old faeces. These were examined individually on the day of arrival, then pooled and stored at 4 °C for 4–6 days before further examination. Thus 24 individual samples and 4 pooled samples of old faeces and 25 individual and 4 pooled samples of fresh faeces were examined.

Laboratory methods

At post-mortem examination the badger carcass was weighed, sexed and aged as a cub or adult. A cub was defined as an animal caught in the calendar year of its birth. The general body condition and any bite wounds present were noted. If no visible lesion of tuberculosis was seen, a pool of retropharyngeal, submandibular, axillary, broncho-mediastinal and mesenteric lymph nodes was taken together with a portion of the kidneys. Any lesions suggestive of tuberculosis, including bite wounds, were smeared, stained by the Ziehl–Neelsen method for acid-fast bacilli and examined. They were also examined histologically using paraffin-embedded sections stained with haematoxylin and eosin and the Ziehl–Neelsen method for acid-fast bacilli. Samples examined after 1 January 1983 were stored in 1% cetylpyridinium chloride (Sigma) until they were cultured. Before culture, tissues were suspended in sterile physiological saline (PS), and decontaminated with 5% oxalic acid as described previously (Wilesmith *et al.* 1986). In previous years the tissues were kept frozen at −20 °C if there was a delay before culturing, and biological tests were also used.

Faeces samples were pooled by social group (up to a maximum of five samples per pool), or examined individually. They were emulsified in sterile PS with glass beads and by overnight soaking, and thorough mixing. The supernatant was poured off, treated with a final concentration of 5% oxalic acid and centrifuged. The deposit was then used in biological and cultural tests for *M. bovis* as described previously (Wilesmith *et al.* 1986). The media used for both faeces and tissues were Stonebrink's (Lesslie, 1959), modified 7H11 (Gallagher & Horwill, 1977) and 'Improved Stonebrink's' containing the same antibiotics as modified 7H11. The faeces samples collected as a result of the intensive sampling at two setts were used to examine the isolation rates of *M. bovis* from fresh and old faeces processed as individual and pooled samples. Two methods of decontamination and four methods of isolation were compared. The decontaminants were 5% oxalic acid and 1 M sodium hydroxide. Lowenstein–Jensen with pyruvate and antibiotics as used in 7H11, Stonebrink's and modified 7H11 media and guinea-pig inoculation (Lesslie, 1959) were used as the isolation methods. All isolates were identified as described by Little *et al.* (1982) and Marks (1976).

RESULTS

Badger sett and social group territory studies

Twenty-nine badger setts were located within the project area, but of these 15 were used only occasionally or not at all during the period of the study (Table 1).

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Table 1. *Main setts and principal outliers showing activity, social groups and territory sizes (ha) 1979–1984*

Set no.	Social group	1979	1980	1981	1982	1983	1984	Percentage of area as grassland, 1984
28 / 1	A	40 / —	— / —	58	36	59	43	54
2	B	59	86	46	37	56	48	69
3 and 4	C	49	42	43	43	—	45	53
11 and 29	D	60	24	61	20	29	27	78
13 and 14	E				35	25	40	63
19 and 10	F		61	18	24	31	17	66
18* / 5	G	74	46	— / 47	— / 41	— / 39	45	51
8	H	51	34	45	32	39	41	46
Total territory size		333	293	318	268	278	306	
Total no. social groups		6	6	7	8	7	8	
Mean social group territory size (grand mean: 42·8)		55·5	48·8	45·4	33·6	39·7	38·3	

* Outlier. —, Sett inactive.

Of the remaining 14 setts which were actively used by badgers at least at some time during the study, 8 were considered as permanently paired with one other sett. Sett 10 which was paired with sett 19 conformed well to Kruuk's (1978) definition of an annexe, but in the case of setts 3 and 4, 11 and 29 and 13 and 14, the setts were of approximately equal size although one may have been used more than the other in a particular year. Sett 18 was regarded as an outlier of sett 5 and was not used at all in 1981, 1982 and 1983.

Seven of the 14 setts used were situated on Middle Chalk and 7 on the Lower Chalk. Nine of the remaining 15 rarely used setts were on Chalk, 3 on Lower Greensand and 3 on valley gravels. Eight of 14 active setts were situated in woodland, 1 in an overgrown hedgerow, 2 within light scrub and 3 completely in the open, lacking even the cover of annual plant growth.

The number of social groups of badgers apparently increased from 6 in 1979 and 1980 to 7 in 1981 and 1983 and 8 in 1982 and 1984. The size of social group territories declined as the numbers of groups increased. The changes in social group boundaries as determined by bait-marking are depicted in Fig. 1. The maximum total area of the territories in any one year, was 333 hectares (ha), but the total area exploited over the 6-year study period was 420 ha; 58% of the area was grassland (37% improved, 21% downland or rough grass), 24% was arable, 11% woodland, 2% scrub and less than 1% open water; the remaining 5% was a built-up area. Although the habitat survey was carried out only once there was no major change during the 6-year period.

The percentage of grassland in 1984 is given in Table 1, and varied between 46 and 78% of each social group territory.

A total of 510 badgers was examined from the whole county during the period 1976–84; *M. bovis* infection was confirmed in 27 badgers all from the most southern part of the county. The precise location of one infected badger was not known,

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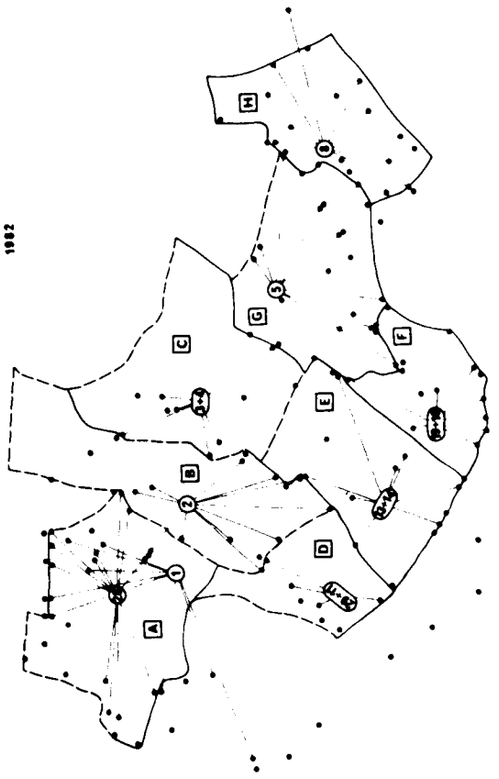

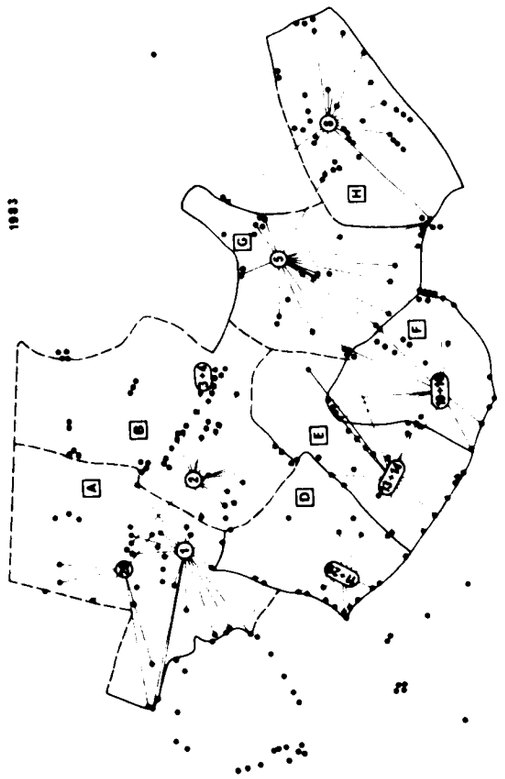

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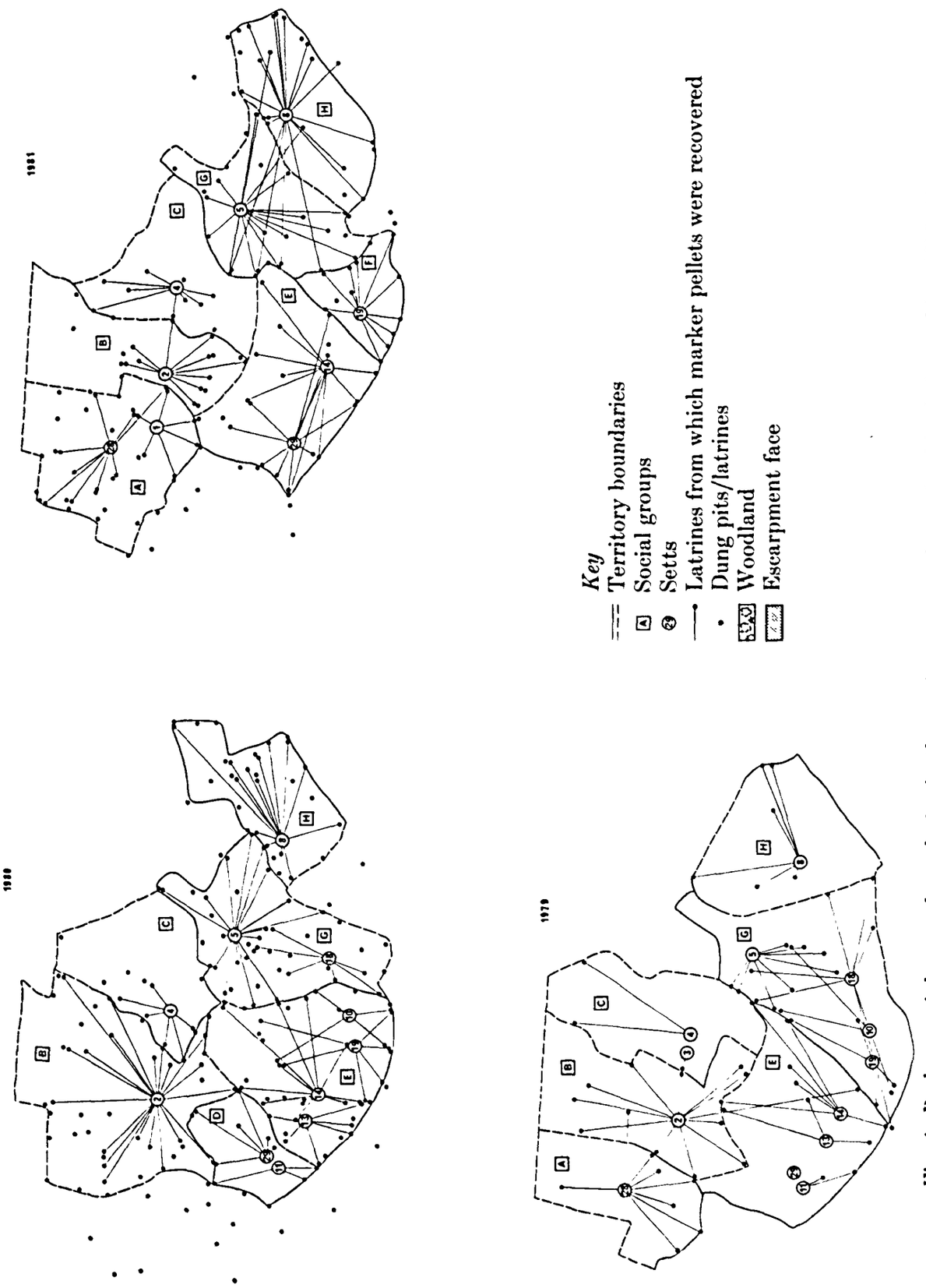

Fig. 1. Badger social group boundaries in the project area as determined by bait marking 1979–1984

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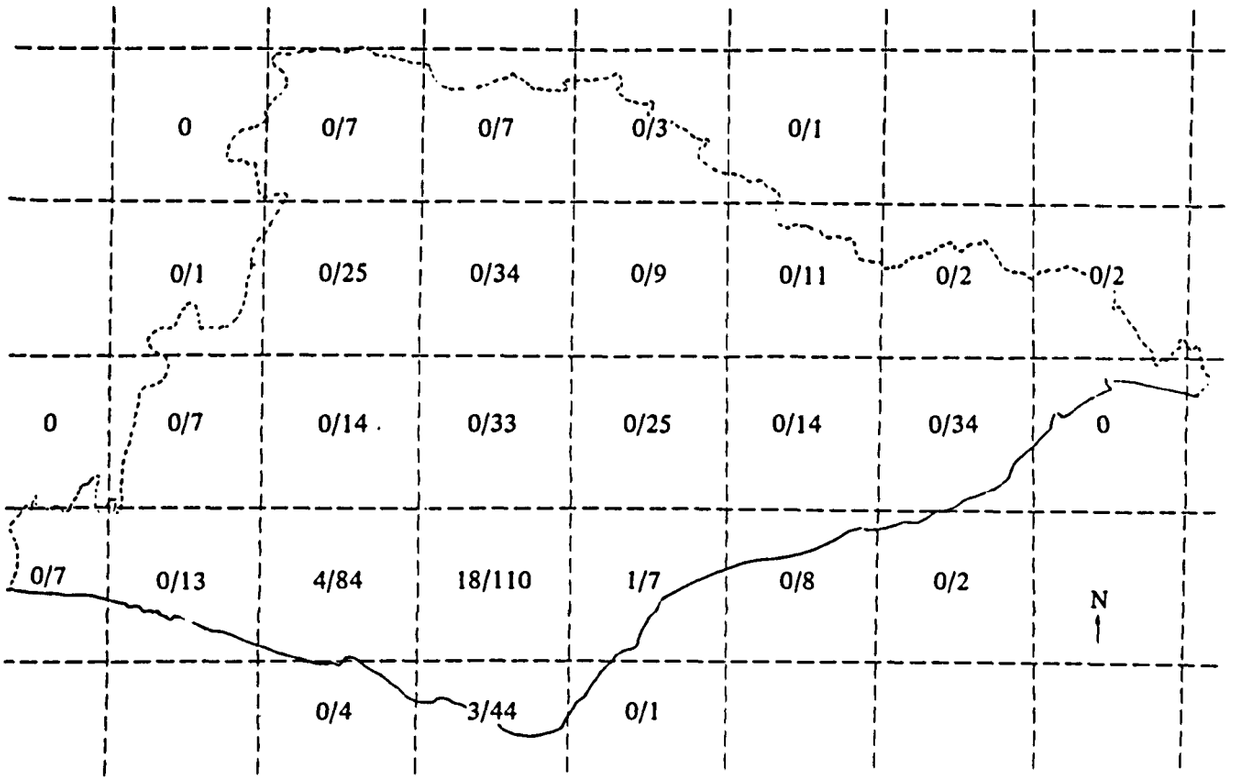

Fig. 2. Geographical distribution of badgers examined from East Sussex (1976–1984)

Key 18/110 = number of badgers infected with *M. bovis*/number of badgers examined in each 10 × 10 km square.

but the broad geographical distribution and infection status of the other 509 badgers is shown in Fig. 2.

The monthly distribution of badgers found dead as a result of road traffic accidents during the period 1979–84 is shown in Fig. 3. Disproportionately large numbers of such casualties were found in February, March, April and October.

One hundred and forty-two badgers were examined from the main area. *M. bovis* infection was confirmed in 23 badgers (16%); 15 of these infected animals had lesions typical of tuberculosis, and evidence of bite wounds was found in 4 (17%), 3 of which were found in 1984.

The annual prevalences of infection in the main area are shown in Table 2. Relatively small numbers were examined in each year. No infected badger was found in 1981 and the highest prevalence was observed in 1984, when 42·1% of badgers (8 out of 19) were infected. Seven of these badgers had lesions typical of tuberculosis; 5 of these were found *in extremis* as a result of tuberculosis and 1 was found dead. This latter badger was found in a field, 3 were found in farm buildings and 2 in gardens of local residents. These badgers were all within the main area, and only one such tuberculous badger had been recorded dead in the area previously, in a field in 1977, the first tuberculous badger found in the county. Three of the badgers with lesions typical of tuberculosis were found within the project area.

Seventy-eight (54%) of the 142 badgers were male and the prevalence of infection was higher in males (19%) than in females (12%). Only 11 cubs were examined, and none was infected.

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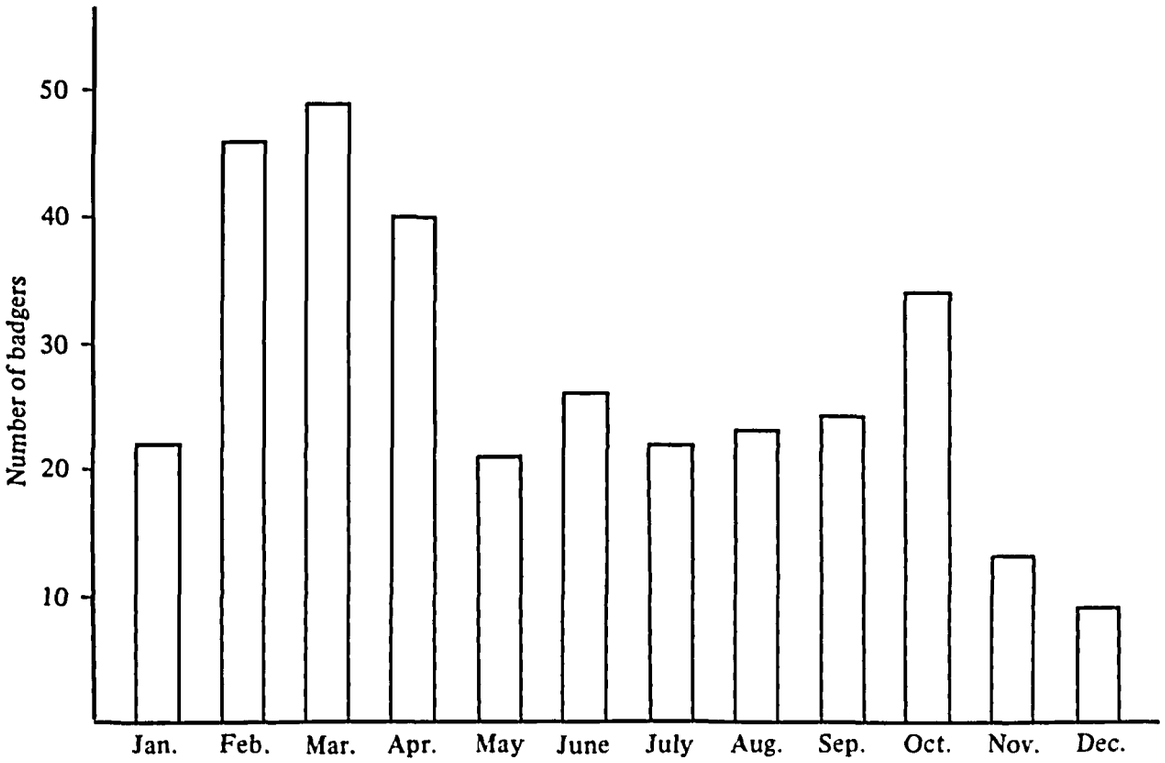

Fig. 3. Monthly distribution of badger road casualties from East Sussex submitted for laboratory examination (1979–1984).

Table 2. *Distribution of badgers from the main area examined post-mortem by year and* M. bovis *infection status from July 1976 to December 1984*

	1976	1977	1978	1979	1980	1981	1982	1983	1984	Total
Male										
Number examined	2	9	9	6	14	8	9	9	12	78
Number *M. bovis*-positive	0	0	2	1	2	0	3	1	6	15
Number with VL of TB*	0	0	0	1	1	0	2	0	5	9
Female										
Number examined	2	12	14	6	4	5	8	6	7	64
Number *M. bovis*-positive	0	2	0	1	1	0	0	2	2	8
Number with VL of TB	0	1	0	1	1	0	0	1	2	6
Total										
Number examined	4	21	23	12	18	13	17	15	19	142
Number *M. bovis*-positive	0	2	2	2	3	0	3	3	8	23
Number with VL of TB	0	1	0	2	2	0	2	1	7	15
Prevalence of *M. bovis* infection (%)	0	9·5	8·7	16·7	16·7	0	17·6	20·0	42·1	16·2
Prevalence of VL of TB (%)	0	4·8	0	16·7	11·1	0	11·8	6·7	36·8	10·6

* VL of TB, visible lesions typical of tuberculosis on gross examination and isolation of *M. bovis*.

Faeces sampling

One thousand and sixty-four faeces samples were obtained by haphazard sampling from the main area and examined bacteriologically; *M. bovis* was isolated from 27 samples. *M. bovis* was isolated from 7 of the 278 samples obtained from the project area. All positive faeces samples were from areas in which infection was subsequently confirmed in badger carcasses.

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Table 3. *Number of weeks in which* M. bovis *was isolated from faeces from badger social groups in the project area obtained by rigorous sampling in the spring of each year 1981–1984*

Social group	Setts	1981 8 weeks sampling	1982 5 weeks sampling	1983 6 weeks sampling	1984 6 weeks sampling	81/84 25 weeks
D	11/29	3	2	0	0	3
E	13/14		4	1	0	7
F	19/10	0	3	2	1	6
H	8	5	3	0	0	8
G	5	2	2	0	0	4
C	3/4	0	1	—	0	1
B	2	2	4	2	1	9
A	1	2	1	1	0	4
	28	4	2	0	0	6
Total		18 (270)*	22 (119)	6 (141)	2 (197)	48 (727)
Minimum samples positive (%)		6·7	18·5	4·3	1	6·6

* Figures in parentheses are the number of faeces samples collected.

The results of the rigorous method of faeces sampling in the project area are summarized in Table 3. *M. bovis* infection was confirmed in all social groups during the 4 years 1981–4.

The results of the bacteriological examination of unstored individual old and fresh faeces obtained as a result of the intensive faeces sampling are summarized in Table 4. Isolation rates from old faeces on culture media were similar to those from fresh faeces following decontamination with oxalic acid, but lower with sodium hydroxide. However, contamination rates were higher with old faeces.

Sodium hydroxide reduced contamination rates but also resulted in a lower isolation rate. The highest rate of isolation was achieved on Stonebrink's medium after oxalic acid decontamination. Biological testing, using guinea-pigs, was the most sensitive means of detecting *M. bovis*.

Pooling samples resulted in higher contamination rates with all cultural and decontamination methods. Again, Stonebrink's medium after oxalic acid decontamination was the most sensitive cultural method for the isolation of *M. bovis*. All pools containing individual positive samples were also positive for *M. bovis* on biological testing.

Storage of the pooled samples at +4 °C for 4–6 days resulted in overgrowth of all culture media by contaminants. Biological testing detected three of the four positive pools.

DISCUSSION

The results of a national badger survey indicated that only 9% of setts are located in open situations (Neal, 1972). The high proportion (21%) of setts in this study area which lacked even the cover of annual plant growth may be due to an insignificant level of interference at these locations. The density of badgers in this area may therefore be somewhat higher than would be expected from an assessment based on geographical and geological features.

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Table 4. *Intensive faeces sampling:* M. bovis *isolation from unstored individual faeces samples*

Method of decontamination...	5% oxalic acid: media										
	Stonebrink's			LJ+P*			7H11			Guinea-pig	
Age of faeces	+	−	C	+	−	C	+	−	C	+	−
Old	2	5	17	1	2	21	0	4	20	7	17
Fresh	2	10	13	1	6	18	1	10	14	5	20
Total	4	15	30	2	8	39	1	14	34	12	37
Positive faeces detected by culture (%)	33			16			8			100	

Method of decontamination...	1 M sodium hydroxide: media										
	Stonebrink's			LJ+P			7H11			Guinea-pig	
Age of faeces	+	−	C	+	−	C	+	−	C	+	−
Old	0	10	14	0	4	20	1	10	13	6	18
Fresh	2	14	9	1	6	18	1	13	11	5	20
Total	2	24	23	1	10	38	2	23	24	11	38
Positive faeces detected by culture (%)	16			4			8			91·6	

+, *M. bovis* isolated; −, *M. bovis* not isolated; C, contaminated.
* LJ+P, Lowenstein–Jensen medium + pyruvate + antibiotics.

The mean size of badger social group territories recorded over the 6-year study period (43 ha) was higher than that recorded in two study areas in Gloucestershire (22 ha and 25 ha), but lower than in one area in Cornwall (75 ha), an area in Avon (73 ha) and one in Staffordshire (104 ha) (Cheeseman *et al.* 1981, 1985*a*). Kruuk (1978) calculated two estimates for social group territories in Wytham Woods, Oxford, a mean of 52 ha for social groups completely surrounded by other social groups and 87 ha for all groups including those on the periphery which had ill-defined outer boundaries. The mean territory size in the present study is somewhat similar to the former estimate. The areas to the north of social groups A, B, C and H were not known to be occupied by badgers, but there appeared to be defined limits to the extent of these four territories in that direction. This was provided by a deep ditch and a major road in the case of social groups A, B and C.

There is no published report of prospective studies of the social structure of badger populations in which variations in territory size and the mutability of social groups have been examined. Kruuk (1978) suggested that in Wytham Woods the amount of movement of individuals between social groups was small and circumscribed, but required further study. One such study is in progress in Gloucestershire and a number of social groups have been found to be associated with one of their neighbouring groups. An apparently new social group has been

formed by individuals from one group, the two groups exhibiting signs of their past relationship for several years after the initial division (Cheeseman, unpublished findings). A similar picture was observed in the present study, but some caution is required in interpretation as there are obvious limitations in the methods used to determine territory boundaries, for example radio tracking studies were not used. Social group D exhibited signs of a relationship with neighbouring group E on the downland, apparently joining in 1979 and 1981. No relationship between social groups A or B on the low ground and social group D was observed, however. It seems likely that group D had been part of group E, an increase in the size of setts 11 and 29 over the period supporting the idea that this social group was emerging in its own right.

The general concluson over the 6-year study period was that neighbouring groups on the downs were apparently more likely to combine with each other, but not with groups on the low ground with which contact was probably limited by the intervening area of arable land. These apparent relationships between particular social groups would obviously assist in the transmission of *M. bovis* within a population, thereby aiding the maintenance of infection. Conversely, the lack of relationship between certain social groups could account in part for any observed discontinuous distribution of infection in a population.

The absence of a non-destructive diagnostic test for *M. bovis* infection in the badger imposes limitations on epidemiological investigations. Clinical sampling of badgers at regular intervals as described by Cheeseman *et al.* (1985*b*) was not possible. The bacteriological examination of faeces as a diagnostic tool has limitations (Cheeseman *et al.* 1985*a*), but the opportunity was taken in the present investigations to determine optimal methods for faeces collection and the isolation of *M. bovis* from badger faeces.

The bacteriological examination of faeces is made difficult by the relatively small number of *M. bovis* bacilli likely to be present and the large numbers of other organisms present which result in contamination and overgrowth of culture media. Methods for the decontamination of specimens to facilitate the selective isolation of mycobacteria have been reviewed by Songer (1981). Sodium hydroxide has been the most commonly used decontaminant, but its toxicity for mycobacteria results in a marked reduction in the isolation rate (Krasnow & Wayne, 1966). In this study the milder decontaminant effect of oxalic acid was apparent, but the degree of contamination was sufficiently low to allow a higher isolation rate than with sodium hydroxide. The advantage in obtaining freshly deposited faeces samples and subjecting them to bacteriological examination without any delay or storage is also evident. The relatively poor performance of modified 7H11 and Lowenstein–Jensen plus pyruvate with added antibiotics, as compared with Stonebrink's medium, may be due to the combination of decontamination procedures and antibiotics in the media. This could result in an inhibition of growth of the low numbers of tubercle bacilli present. Gallagher & Horwill (1977) recommend the use of modified 7H11 for direct culture of *M. bovis* from tissues without prior decontamination, but this would be unsuitable for faeces samples.

Biological testing using guinea-pig inoculation is the most sensitive method for the isolation of *M. bovis* from bovine tissues (Lesslie, 1959) and from badger tissues (Gallagher & Horwill, 1977). The marked difference in the sensitivities of guinea-pigs

and culture media for the isolation of *M. bovis* from badger faeces was therefore not unexpected. Pooling of faeces samples from one social group did not reduce the isolation rate, further indicating the sensitivity of guinea-pig inoculation. The methods described for the collection and bacteriological examination of faeces obtained by the rigorous sampling were therefore based on the results of these investigations, these examinations being used to supplement the information obtained from the post-mortem examination of badgers from the project area.

The examination of badger carcasses provided only a haphazard means of surveillance for *M. bovis* in the county, as badgers were only actively sought after on and around farms where cattle had become infected and other sources of infection had been ruled out. The sample of badger carcasses obtained was therefore biased geographically, a larger sample being acquired from the South Downs where such infection in herds had occurred (Wilesmith *et al.* 1986). Farmers in this part of the county were consequently more aware of badgers, and this resulted in a higher rate of submission and notification of badgers found dead. At present the results of the surveillance of *M. bovis* infection in badgers suggest that infection could be confined to the downland of the south-west of the county. Infection of cattle by badgers has not yet occurred in the rest of the county (Wilesmith *et al.* 1986), and this may be a result of a lower badger density. Muirhead, Gallagher & Burn (1974) observed that infection in badgers was not always associated with infection in cattle, and this has been confirmed in the present studies. Cattle are therefore unreliable indicators of the geographical distribution of infection in badgers. The continued examination of badger carcasses will provide a better indication of this geographical distribution in East Sussex.

Gallagher (1977) observed a seasonal variation in badger road casualties in Gloucestershire during the period 1973–6. Disproportionately larger numbers of road casualties were found in February, March and April with a less marked rise in August and September of each year except 1975, when the summer drought produced greater activity to seek food in July (Neal, 1977). This spring peak in road casualties was also observed in East Sussex, but the autumn peak occurred in October. The seasonal variation recorded reflects precisely the seasonal variation in latrine activity observed in Gloucestershire (Cheeseman & Rose, personal communication). The variation in the use of latrines is associated with changes in mating activity, the monthly distribution of long-duration matings recorded by Neal (1977) mirroring latrine activity. The significance of these seasonal variations in badger activity in the exposure of cattle to *M. bovis* is uncertain at present, but Wilesmith *et al.* (1982) recorded that cattle in one herd were at greatest risk of becoming infected in April and May.

Temporal variations in the prevalence of *M. bovis* have been observed in a naturally infected population (Cheeseman & Wilesmith, unpublished observations). The relatively small number of badgers examined each year precluded the disclosure of temporal variations in the prevalence of infection in East Sussex. The highest prevalence of infection occurred in 1984 and the unusually large number of badgers found dead or *in extremis* as a result of tuberculosis suggests that the prevalence of infection was higher than in previous years. In addition, the highest prevalence of tuberculosis badgers with bite wounds was observed in 1984.

Behavioural changes in tuberculous badgers have been reported (Muirhead,

Gallagher & Burn, 1974), such individuals being seen in daylight and occasionally in and around farm buildings. Cheeseman & Mallinson (1981) also noted that two of three tuberculous badgers, whose movements were closely monitored, had left their own social group. This behaviour could result in agonistic encounters between tuberculous badgers and the residents, resulting in transmission of tuberculosis and an increase in the prevalence of infected animals with bite wounds.

Gallagher & Nelson (1979) considered that 14·1% of their series of infected badgers had become infected by bite wounds. In a study of a naturally infected population in Gloucestershire biting could have accounted for 11% of new infections (Cheeseman & Wilesmith, unpublished observations). The proportion of infected badgers with bite wounds (17·4%) in East Sussex is therefore similar to that found in areas of higher badger density.

The mean crude prevalence of *M. bovis* infection during the period 1976–84 was similar to that recorded in the south-west region of England (14%) from 1973 to 1983 (Reports 1976–83). The proportion of infected animals with lesions typical of tuberculosis was also similar to that found by Gallagher & Nelson (1979) in their series of 1206 badger autopsies in which 71·8% of infected animals had gross lesions. These authors also recorded a higher prevalence of infection in males then females, and noted that this difference could be due to the higher potential contact rate with infected animals for males because of their greater territorial activity and forays to neighbouring social groups when sows are in oestrus. The difference in the immune response between the sexes is an additional explanation, females of most species being immunologically more competent than males (Castro, 1974). This phenomenon has not been examined in badgers, but the previously recorded difference in the prevalence of infection between the sexes is supported by the findings of the present study.

In some local areas the prevalence of infection has been higher in cubs than adults (Cheeseman *et al.* 1981). Only a small number of cubs was examined in the present investigations and none was infected.

A peak in the prevalence of badgers with advanced lesions of tuberculosis has been observed in March and April (Gallagher, personal communication), and as latrine activity is at a maximum at this time the rigorous faeces sampling in the project area was carried out during the spring to increase the probability of detecting *M. bovis* infection. The dynamics of *M. bovis* infection in badger populations is the subject of a prospective study (Cheeseman *et al.* 1985*b*) and infectious badgers have survived for at least 22 months. The results of faeces sampling within the project area indicated that an infectious badger had been present in each social group in at least 1 of the 4 years. The isolation of *M. bovis* from faeces samples from one social group (B) in all 4 years and from three social groups in 3 consecutive years indicates that infection is endemic in this population, but it is obviously impossible to determine whether infectious individuals had survived more than 1 year. These findings do suggest, however, that cattle in the project area were potentially at risk in each of the years 1981–84.

The rate of excretion of *M. bovis* in faeces has been shown to be variable, intermittent excretion having been observed in one individual (Report, 1983). This phenomenon and the problems associated with the isolation of *M. bovis* from faeces results in faeces examination providing a poor indication of variations in the

prevalence of infection. However, the high rate of isolation from faeces in 1982, when *M. bovis* was isolated from all social groups, was associated with an increase in the incidence of infection in the cattle herds (Wilesmith *et al.* 1986).

These investigations have illustrated the difficulties in studying the epidemiology of *M. bovis* infection in badger populations with the diagnostic tools available. They have, however, indicated that badgers in at least part of the county present a continued source of *M. bovis* for cattle.

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