Tuberculosis in East Sussex IV. A systematic examination of wild mammals other than badgers for tuberculosis

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SUMMARY

A detailed investigation of the possible role of wild mammals, other than badgers, in the maintenance of $Mycobacterium \ bovis$ in an area on the South Downs of East Sussex was carried out over 3 years. Estimates of population sizes were made where possible and minimum sample sizes were selected to be 95% certain of including at least one infected animal if the prevalence was at least 5%. Samples of wild mammals were taken from populations which had the highest potential direct or indirect contact rate with known infected badgers. *M. bovis* was not isolated from any of the 15 species of wild mammals.

It was concluded that badgers are able to maintain M. bovis in an area independently of other species, and that in the area studied other species were not a source of infection for the cattle herds.

INTRODUCTION

The possibility of the existence of reservoirs of $Mycobacterium \ bovis$ in species of free-living mammals other than the badger has been examined in two studies (Barrow & Gallagher, 1981; Little *et al.* 1982). Both studies were carried out on and around farms where infected cattle and badgers had been found. In addition free-living mammals other than badgers found dead and submitted to the Ministry of Agriculture, Fisheries and Food (MAFF) veterinary laboratories have been examined for the presence of M. bovis (Report 1983). These studies have failed to reveal any evidence of other species acting as a maintenance host for M. bovis. However, one of the recommendations made following a review of tuberculosis in cattle and badgers was a systematic examination of other wild animals for the presence of tuberculosis (Zuckerman, 1980).

This paper describes such a study over a 3-year period in an area of East Sussex

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where an infected badger population had been disclosed and would not be disturbed.

MATERIALS AND METHODS

Study area

This was the project area as described in the first paper in this series (Wilesmith *et al.* 1986*a*) and the social group and sett identities are those described in the second paper (Wilesmith *et al.* 1986*b*).

Sampling considerations

Where possible samples of animals were taken from populations which had the highest potential direct or indirect contact rate with known infected badgers. The minimum sample size for each species was selected to be 95% certain of including at least one infected animal if the prevalence of infection was at least 5%, having obtained some estimate of the population size. The approximation for the hypergeometric distribution as described by Cannon & Roe (1982) was used to determine these sample sizes.

Estimations of population sizes

Small mammals. The long-tailed field mouse (Apodemus sylvaticus), yellownecked mouse (Apodemus flavicollis), bank vole (Clethrionomys glareolus), shorttailed field vole (Microtus agrestis), harvest mouse (Micromys minutus), common shrew (Sorex araneus) and pigmy shrew (Sorex minutus) will be referred to collectively as small mammals. The numbers in the area covered by a trapping grid were estimated by Hayne's trap-out method (Hayne, 1949).

Moles. Mole (Talpa europaea) populations were estimated by trapping-out local populations in all fields where recently formed molehills were present.

Grey squirrels. Grey squirrel (Sciurus carolinensis) populations were estimated by trapping-out local populations. An attempt was also made to estimate numbers using a mark/recapture method.

Rats. Common rat (Rattus norvegicus) numbers were estimated by trapping out the population present around a group of farm buildings at a particular time.

Foxes. The overwintering adult fox (Vulpes vulpes) population was estimated by counting the number of breeding dens containing cubs at any one time and multiplying by 2 (i.e. 1 male+1 female) and an additional 12% to allow for non-breeding individuals (Page, personal communication; Lloyd, 1980). During March-May 1982 and 1983 a systematic search was made for breeding dens, and those containing cubs were identified.

Rabbits. Dawn and dusk counts were used to provide an index of rabbit (Oryctolagus cuniculus) numbers. An observer walked a predetermined route during the hour after sunrise or before sunset and, using 10×50 binoculars, counted rabbits from standard points along the route. Numbers seen were allocated to two age classes, overwintered adults or young of the year, and recorded separately for each field or other subdivision of the area scanned. Three routes were followed between April and June in 1982 and 1983. Each was counted on 4 occasions in 1982 and on 3 occasions in 1983. The maximum count of rabbits seen in each subdivision was taken as the index of numbers.

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Other species. No technique was available for estimating hedgehog (Erinaceus europaeus) numbers. No method of estimating stoat (Mustela erminea) or weasel (Mustela nivalis) numbers was used.

Sampling methods

Small mammals were trapped in Longworth traps using the methods described by Gurnell & Flowerdew (1982). The usual sampling procedure was to lay out a 100×100 m grid with single traps at 10 m spacing. This arrangement was modified where necessary as the nature of the ground dictated. The grid was centred on each main sett in turn, traps were set to catch without pre-baiting and trapping continued at each location for four nights.

In 1981, before sampling started, trapping was carried out on two setts and small mammals caught were marked by fur clipping and released. The estimated population size was used to confirm that a sufficiently large sample of the population of each species could be expected from four nights trapping. Subsequently during sampling, small mammals required as samples were killed using chloroform.

Sampling on setts was carried out in 1981 and in 1982 between September and late December. In 1983 trapping was carried out in a similar way at various locations away from badger setts in order to increase the sample of short-tailed field voles and harvest mice. These locations were mainly areas of rough grass, and the size and shape of the grid was varied to suit the location, and traps were used in pairs at 5 m spacings.

House mice (*Mus musculus*) were caught in 1983 by setting Longworth traps singly at approximately 2 m intervals within farm buildings. Traps were set to catch for four nights without pre-baiting. House mice captured were killed with chloroform.

In 1982 and 1983 Duffus and pincer spring traps at a high density (up to 20/0.1 ha) were set in all fields where molehills were observed. The area was considered trapped-out when no further moles were caught and no freshly excavated soil was seen for several days. All moles caught were dead when the traps were inspected.

The presence of grey squirrels in the woodlands on the study area was assessed by direct observations, presence of dreys and by scattering whole maize on the ground and inspecting after a few days to see whether the germ had been removed in the way characteristic of the species (Rowe, 1973).

In January and February 1982 squirrel numbers were assessed by trapping-out in woods where squirrels were present. In January and February 1984 a markrelease-recapture operation was undertaken. Squirrels were caught in singlecapture cage traps (mink traps). After a week of pre-baiting with whole maize the traps were set to catch Monday to Friday and inspected early morning and late afternoon (Rowe, 1973). Any squirrels caught were killed with chloroform. During the mark-recapture work any squirrels caught Monday to Wednesday were individually marked using aerosol stock marker in three colours and released. Those caught on Thursday or Friday were taken as samples. In addition trapping was carried out in May and June 1982 in a grain store (territory of sett D).

During the winter and spring months 1981/82 and 1982/83 farm buildings were

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periodically inspected for signs of rats. Where they were found, single-capture 'Blerdorbury' rat cage traps were placed at all points where an experienced pest operator would have placed rodenticidal baits. Traps were protected from wind and rain with available materials or heavy-duty polythene sheet, fastened open and pre-baited for a week with soaked wheat, then set to catch. Trapping was continued until no further rats were trapped and there were no further fresh signs, e.g. droppings and footprints. Holes were blocked and runs cut up to confirm that no rats remained. Rats caught were killed with chloroform.

Two methods were used to catch foxes; during January and February 1982 large cage traps baited with turkey offal were set at six locations. In January and February 1983 free-running steel snares were set in and around woodland just outside the territories of badger social groups G and H. This site was chosen because of the high level of fox activity and in order to minimize the likelihood of catching badgers. Captured foxes were killed humanely by shooting in the head with a $\cdot 22$ pistol.

Rabbits were captured by ferreting, shooting and snaring. Live rabbits were killed by dislocation of the atlanto-occipital joints. Stoats and weasels were trapped using Fenn Mark IV vermin traps set in natural or specially constructed tunnels in suitable sites around the study area (Game Conservancy, 1981). This technique will be referred to as tunnel trapping. Up to 20 traps were deployed at any one time from September 1982 to February 1983 and from July to December 1983, amounting to 1002 trap nights in 1982 and 1526 in 1983. Traps were sprung each Friday afternoon and reset on Monday mornings, and moved to different sites from time to time. Over the period traps were set in all parts of the study area except the open downland.

The area around the village was considered the most promising for catching hedgehogs, and efforts were concentrated there. Pitfall traps were constructed from round 5-gallon plastic drums, the tops of which were cut off and the drums thoroughly washed out to remove any possible taint. The drums were then sunk up to the rim in the ground at the edges of paths, fields and woods and baited with rabbit viscera. Twelve mink traps, baited with the same material, were also used in various similar sites. Trapping by both methods was carried out for 5 weeks in the autumn of 1982. Additionally, direct catching was attempted in the evening during the first 2 h after nightfall. Pasture fields, roads and tracks were scanned by spotlight and any hedgehogs seen would have been captured by hand in a hessian sack. Following the failure to capture hedgehogs by these methods a specialist in the study of hedgehogs visited the area and gave advice to the study team.

Although brown hares (*Lepus capensis*) are characteristic of downland areas their numbers have undergone a decline in recent years (Tapper & Parsons, 1984). Hares were only rarely seen in the area and no attempt was made to sample them. The sides of the two streams were regularly inspected for American mink (*Mustela vison*) footprints and during August 1982 and January 1983 cage traps baited with fish heads were set speculatively on the side of the two streams but without success. Traps were also set speculatively on the banks of a river approximately 500 m to the north of the study area.

Because few harvest mice had been caught in January 1984 a systematic search

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for nests (Harris, 1979) was made, particularly in the areas of rough grass where trapping had been carried out in 1983, and in other suitable habitats. In those woods where hazel (*Corylus avellana*) grew (mainly over sett 1) the ground was searched for hazelnuts eaten by mammals and signs of feeding by dormice (*Muscardinus avellanarius*) (Hurrell & McIntosh, 1984).

In addition to the above systematic sampling, any mammal specimens which became available from other sources were submitted for examination if they were sufficiently fresh and undamaged. These sources included road casualties and animals caught in traps set for other species. Throughout the study a watch was kept for sightings, tracks and signs of other mammal species.

Laboratory techniques

A post-mortem examination was carried out on each specimen. If there were no macroscopic lesions suspicious of tuberculosis, a pool of tissues consisting of spleen, kidneys, liver, lungs, heart and any visible lymph nodes was made. If lesions were visible, a direct smear was made, stained using the Ziehl-Neelsen technique and examined for the presence of acid-fast bacilli. A section of the lesion was taken for histological examination and the remainder of the lesion was cultured separately from the rest of the pool.

Approximately 20 ml of sterile saline was added to each pool, which was then homogenized using a Stomacher Lab-Blender 80. A 10 ml portion of the homogenate was poured into a universal bottle and 10 ml of 10% oxalic acid added, giving a final concentration of 5% oxalic acid. Each bottle was allowed to stand at room temperature for 10 min and then centrifuged at 600 g for 10 min. The supernatant was discarded, the deposit resuspended in approximately 25 ml of sterile saline and centrifuged as above. After this final wash, the deposit was resuspended in approximately 7 ml sterile saline and sterile glass beads were added to the universal bottle. After mixing on a Whirlimixer the suspension was used to inoculate modified 7H11, Stonebrink's and improved Stonebrink's slopes, which were then incubated at 37 °C for 6 weeks. Inocula from field voles (*Microtus agrestis*) were incubated for approximately 6 months in an endeavour to isolate M. microti (Wells, 1946). Inocula from the same species were pooled for guinea-pig inoculation.

RESULTS

The results of estimating small mammal population sizes and the numbers of each species caught are summarized in Table 1. No water shrews (*Neomys fodiens*) were caught. The numbers of house mice, moles, grey squirrels. rats, weasels and rabbits caught and their location are summarized in Table 2.

Fifty-eight house mice were caught in and around farm buildings. Mole activity was restricted to a few grassland fields adjoining woodland near the village and also to the flat area at the top of the chalk scarp. Twenty grey squirrels were caught, one of which was caught in a tunnel trap set for weasels and stoats. One road casualty was also submitted for examination. Insufficient squirrels were caught in the mark-recapture study for the population to be estimated by this means. In addition to the 13 weasels obtained one stoat was caught in 1983 at sett 28/1 (social group A). A total of 103 rats were caught at four locations.

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Table 1. Number of small mammals caught by species, badger social group territory and year, and estimates of population sizes

Sett/	-	198	31	198	2	1983	3	
Social	n '	^^						Total
group	Species	E*	C†	Е	С	Έ	С	caught
28/A	A. sylvaticus	15	8	—	6	10	9 + 1	24
	A. flavicollis							
	C. glareolus	—	<u> </u>		2	6 + 9	5 + 5	12
	M. agrestis		<u> </u>				3 + 1	4
	S. araneus		—		2	15	13 + 4	19
	M. minutus	<u> </u>		—		<u> </u>	2	2
1/A	A. sylvaticus	35	25					25
	A. flavicollis	19	12		_		_	12
	C. glareolus		• 5		—	-	1	6
	M. agrestis		1	_			—	1
	S. araneus	—			—	—		
0/D	S. minutus					_	1	1
2/B	A. sylvaticus	55	41	17	10		2	53
	A. flavicollis	12	8	9	6	—		14
	C. glareolus	10	4	—	—			4
	M. agrestis S. araneus			—		—		
3 and	A. sylvaticus		_			_		
4/C	A. flavicollis	_	_				_	
4/0	C. glareolus				_	5	4/2‡	6
	M. agrestis					0	$\frac{4}{2}$	$\frac{1}{2}$
	S. araneus		_	_		8	6 /7	13
	S. minutus			_		7	4	4
11 and	A. sylvaticus	24	16	13	10		_	26
29/D	A. flavicollis				_	_		
20/15	C. glareolus	17	7	<u> </u>	1		<u> </u>	8
	M. agrestis	14	9			<u> </u>	—	9
	S. araneus				4		2	6
13 and	A. sylvaticus	55	38	62	41	_		79
14/E	A. flavicollis	_	3		_			3
/	C. glareolus					_	2	2
	M. agrestis			—		1	2 2	3
	S. araneus	<u> </u>	—					
19 and	A. sylvaticus	18	10	12	9		—	19
10/F	A. flavicollis						<u> </u>	
•	C. glareolus		1		—	—		1
	M. agrestis		4			<u> </u>		4
	S. araneus						<u></u>	
5 (and	A. sylvaticus	38	25	63/47	40/36		—	101
18)/G	A. flavicollis	18	8	25/15	17/12			37
	C. glareolus	32	13		5 + 4			22
	M. agrestis	22	14		1			15
	S. araneus				2		3	5
	M. minutus		<u> </u>		<u> </u>		1	1
8/H	A. sylvaticus	30	22	43/33	32/18		2	74
	A. flavicollis			—/8	1/4			5
	C. glareolus	7	4	/32	1/21		1	27
	M. agrestis							
± **	S. araneus		—		3/1	4	4	8
* E, e	* E, estimated.							

E, estimated.

† C, caught.
‡ / Separates estimates and numbers caught at two different sites within the badger social group territory.

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Table 2. Number of animals caught by location and year

	(a) House mice caught in 1983				
Badger Social group	Sett	Number caught			
А	28/1	3			
*C	3/4	13			
\mathbf{G}	5	12			
Н	8	30			
Total		58			

(b) Moles caught in 1982 and 1983

Badger		Number caught			
Social group	Sett	1982	1983		
А	28/1	2	7		
В	2	2	1		
С	3/4	6	_		
D	11/29	7			
\mathbf{E}	13/14	2			
Н	8		18		
Total		19	26		

(c) Grey squirrels

Badger		N	umber caugl	nt
Social group	Sett	1982	1983	1984
А	28/1		3	
С	3/4	5	4*	4†
Н	8		3	2
Total		5	10	6

(d) Rats caught in 1982 and 1983

Badger		Number caught				
Social group	Sett	1982	1983			
С	3/4	15				
G	5	8	38			
Н	8	6	26^{+}_{-}			
А	28/1	4	6			
Total	·	33	70			

(e) Weasels caught in 1982–4

Badger	.,	Number caught				
Social group	Sett	1982	1983	1984		
А	28/1		2			
*C	3/4	<u> </u>	-4	1		
Е	5		3			
Н	8	3§				
Total		3ັ	9	1		

* Social group C combined with B in 1983,

+ Includes 1 road casualty,

‡ Two caught in mink trap,§ Includes 2 road casualties.

	(*)	1982			1983			1984		
Social Group	Sett		nber nted Y	Number caught		nber nted Y	Number caught	Nun coui A	nber nted Y	Number caught
А	28/1	6	4	6	15	8	4			7
В	2	11	13	0	7	7	0	—		0
С	3/4	3	3	0			0			0
D	11/29	6	6	0	2	0	2			0
\mathbf{E}	13/14	—		0			1	_		0
\mathbf{F}	19/10	4	3	0	9	5	2			0
G	5	18	23	0.	22	21	1			0
н	8	18	35	0	11	13	0			0
С	$3/4\P$	—		0			5			0
H	8 ¶	3	7	0			4			0
Total		69	94	6	66	54	19			7

Table 2. (cont).

(f) Rabbits counted and caught by location and year

|| A, adults; Y, yearling

¶ captures were nearest to these setts.

Three fox breeding dens were found in 1982 and two in 1983. A further den 150 m outside the defined boundaries of the study area was used in both years. The overwintering adult population was therefore believed to be about nine individuals for the whole study area, but as fox ranges may vary between 2.5 and 15 km² (Lloyd, 1980) it was possible to subdivide this population in terms of badger territories. Both dogs and badgers interfered with, and were caught in, the cage traps, and this method was discontinued before any foxes were caught. Eight were caught by snaring. One road casualty fox was submitted in April 1982.

Sixty-nine adult rabbits and 94 young were counted in 1982 and 66 adults and 54 young in 1983. Six rabbits were taken as samples in 1982, 19 in 1983 and 7 in 1984. Several more rabbits were caught in 1984 but were taken from the snares by foxes. The 1983 total includes one road casualty.

No hedgehog was captured by any of the methods described. Although mink footprints had been seen on one occasion in the year prior to the start of the study none was seen during the study period and no mink was captured either in cage traps or in the tunnel traps set for stoats and weasels. A ferret-like (Mustela furo) animal was seen through night viewing equipment in October 1982 in the area of sett 19. Mink traps baited with offal failed to capture the animal. The only cats in the study area were domestic pets seen close to the areas of human habitation together with three farm cats which frequented farm buildings near the centre of the area. No feral cat was seen and no cat was sampled. One road casualty bat, a pipistrelle (Pipistrellus pipistrellus), was submitted for laboratory examination.

At the start of the study, deer of all species were believed to be absent from the area, and during the period of the study no deer nor any field sign was observed. M. bovis was not isolated from any of the species examined and M. microti was not isolated from the field voles.

DISCUSSION

Two previous studies at three locations in the south-west of England have failed to provide evidence of wild animal species, other than badgers, acting as a reservoir of infection (Barrow & Gallagher, 1981; Little *et al.* 1982). The sampling methods used in these two studies were essentially intensive trapping over a relatively short period of time, and no attempt was made to estimate population sizes. The problems of such estimations have attracted the attention of ecologists and a wide range of methods have been advocated. Southern (1973) for example, has reviewed the methods used for small rodent populations and has provided some guidelines, but noted that some problems remain unsolved.

All methods of wild mammal population estimation are labour-intensive and those used in the present study were, inevitably, limited to some degree by the availability of labour and equipment. Methods of assessment involving detailed observational studies could not be considered. Where possible, priority was given to methods of assessment using trapping techniques, as these also provide samples.

Mole populations are difficult to assess by indirect means due to the subterranean habits of the species. Mead-Briggs & Woods (1973) used an index of activity to assess short-term reductions in numbers, but it is difficult to assess population density from the density of molehills alone. The method of population assessment in the present study, trapping-out of field populations, fails to estimate any reservoir population, e.g. in woodlands, but does take account of those moles most likely to come into direct or indirect content with cattle. The absence of moles from the downland area (badger territories D and E) after the first year's trapping suggests that 100% sampling was achieved, and in the absence of a nearby woodland reservoir the area remained free of moles the following year.

For much of the year, rat populations are widely dispersed in hedgerows and woods, but during the winter they move into farmyards and become concentrated (Huson & Rennison, 1981). Recent tracking studies suggest that rats may move 1 km to obtain food or to change their residence (Taylor & Quy, 1978). Thus by trapping rats in and around farm buildings the population from a wider area was sampled.

Methods of estimating fox population densities have been reviewed by Lloyd (1980). The method used was chosen because it was the most practical method with the resources available. The extent to which resident foxes foraged outside the study area, or foxes from outside the area visited the study area, is a source of inaccuracy. A second possible source of inaccuracy is that further dens may have remained undiscovered in areas of dense scrub.

No convenient method is yet available for estimating absolute numbers of rabbits over large areas of farmland. However, two direct counting methods, spotlight counting and dawn and dusk counts, give an index of rabbit numbers particularly when used to provide information on numbers of overwintering adults. The exact relationship between numbers seen and absolute numbers is not known, but probably lies between 1:2 and 1:5 for dawn and dusk counts (Tittensor, personal communication). Spotlight counts were not favoured on this site because of concern that powerful light might cause cattle to panic, consequently dawn and dusk counts were used. The attribution of rabbits to two age classes is limited by the possibility that early-born young of the year may have been indistinguishable from adults by late May.

The difficulties of estimating hedgehog populations are discussed by Morris (1983) and no convenient method of assessing numbers is available at present. The failure to catch any hedgehogs is believed to reflect a genuine absence from the study area. During 5 years regular travel to the site only one road casualty was ever seen on or near the area; this was in May 1984 2 km away from the north-west corner of the site.

The downland part of the study area is probably unsuitable for hedgehogs during much of the year because it is too well drained, and the arable areas are also likely to be unsuitable due to lack of food. The grass fields, paddocks and woodlands around the village are potentially good hedgehog habitats. However, should the species ever have become extinct in the area, e.g. as a result of keepering pressure, it may well have found difficulty in becoming re-established due to the physical obstacles presented by the downland and several deep drainage ditches which are permanently wet (Morris, personal communication). The area is known to have been intensively keepered in the past, and one farm worker questioned on the subject said that he had never seen a hedgehog in the area, although he had lived and worked there for the past 42 years.

Mink are usually found in association with streams, rivers and other bodies of water, although they may stray some distance away. Apart from two small streams mentioned, both in the territory of sett 8, and a few wet ditches in the territory of setts 28, 1, 2, 3 and 4, there was no suitable watercourse in the area. The absence of mink from the samples is therefore believed to reflect a genuine absence from the area.

The absence of yellow-necked mice from the downland setts was likely to be due to their distribution rather than a sampling failure, this species being associated with ancient woodland sites. Similarly, the variation in the distribution of field voles between the sampling sites was a reflection of the distribution of habitat types; this species is most abundant in areas of rough grass.

The literature on M. bovis infection in wild mammals has been reviewed recently (Gallagher, 1980; Little *et al.* 1982). These authors also discuss the likelihood that wild mammal species, particularly those found in areas supporting badger populations, would act as a maintenance host of M. bovis. None of the species inhabiting the present study area was considered to be capable of acting as such a host.

In the present study, it was not possible to obtain estimates of population size of the small mammals at all locations selected for sampling. Similarly, the requisite sample size was not attained at every sampling site. However, the sampling fractions of the small mammal species where most abundant and of moles, house mice, grey squirrels, rats, weasels and foxes were sufficient to have detected a prevalence of M. bovis infection of at least 5% with 95% certainty, and therefore conclude that these species were not involved in the maintenance of M. bovis in the area. Evidence of tuberculous badgers in the project area was found in each year of the study (Wilesmith *et al.* 1986*b*).

The sample of rabbits was relatively small, but the sampling fraction was sufficient to detect a prevalence of at least 15%. Experimental infection of

laboratory rabbits by inhalation has revealed a high susceptibility of some strains to M. bovis (Lurie et al. 1950). Laboratory studies have also demonstrated a rapid transmission of infection between rabbits (Francis, 1958). High prevalences of infected and tuberculous animals might therefore be expected if a wild rabbit population became infected. It is not clear whether wild rabbit strains are resistant to M. bovis or if they escape the necessary exposure. Lepper & Corner (1983) have suggested that rabbits in the wild state appear resistant to tuberculosis due to M. bovis, and despite the probable opportunity for infection from contaminated pastures there is no report of the isolation of M. bovis from wild rabbits. Cobbett (1917) suggested that wild rabbits escaped the necessary exposure at the time when pasture contamination, from infected cattle, was high. The results of the current and previous studies in Britain have provided no evidence that wild rabbits become infected with M. bovis.

The absence of M. bovis infection in wild animal species, other than badgers, indicates that these species were not a source of infection for cattle herds in the area and that badgers are able to maintain M. bovis in an area independently of any other species.

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