Epidemiol. Infect. (1993), **111**, 9–19

Mycobacterium bovis in the European badger (*Meles meles*): epidemiological findings in tuberculous badgers from a naturally infected population

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(*Accepted 30 March 1993*)

SUMMARY

This study investigates the course of tuberculosis in a naturally infected badger population, its impact on the population and the risk of spread to other species in the light of capture data and post-mortem findings from 47 tuberculous badgers, stratified by age group and sex, accrued since 1975. The findings are compared with those for 260 badgers from the same population in whom no evidence of infection was detected. Detailed estimates of seasonal variations in bodyweight for uninfected male and female cub, yearling and adult badgers are presented and compared to the weights at post-mortem examination of the tuberculous badgers, in whom poor condition and weight loss were the principal presenting signs. Lesions were seen especially in the lungs and associated lymph nodes, and in the kidneys. Organisms were detected intermittently in faeces, urine, sputum and discharging bite wounds. Infected animals could survive for nearly 2 years and produce cubs successfully.

INTRODUCTION

Since *Mycobacterium bovis* was first isolated from the European badger in Switzerland [1], the endemicity of infection in the badger population of Great Britain has been described [2, 3] and its impact on the national cattle herd discussed at length [4, 5].

As part of the Ministry of Agriculture, Fisheries and Food (MAFF) research programme into the epidemiology of tuberculosis in badgers, a study was initiated in 1975 in an area of the Cotswold escarpment in Gloucestershire. The objective initially was to gain a better understanding of the ecology and behaviour of badgers. A second objective, which commenced in earnest in 1981, was to study tuberculosis and its effects in a naturally infected badger population. Some of the findings of this study, which is still in progress, have been reported previously [3, 6–9].

The natural history of the species presents the researcher with many practical difficulties, not least the high rate of loss to follow up. This too often results in an animal's age, date and cause of death, and disease processes which would have been revealed at post-mortem examination remaining unknown. During the 17

years of the study, only 47 badgers have been found at post-mortem examination to have visible evidence of tuberculosis. They represent a unique group of animals in the field of tuberculosis research in badgers, since 25 of them had one or more pre-mortem capture records. This paper describes the 47 animals from capture data during their lifetimes, where available, and from the post-mortem findings at their deaths or humane killing to help elucidate the course of the disease in infected badgers, the impact of the disease on the study population, and the implications for transmission to other species.

MATERIALS AND METHODS

The study area in Gloucestershire, covering an area in excess of 8 km^2, has been described in detail previously [6, 9].

Individual social group territories were delineated by bait marking each spring. The boundaries remained stable, provided that the population remained undisturbed [9, 10]. Badgers within these groups were cage-trapped, marked by tattoo and ear-tags, weighed (kg), condition scored (1 to 4, 'poor' to 'very good') and clinically sampled, under ketamine anaesthesia, every 3 months as previously described [8, 9, 11, 12]. Evidence of bite wounds was noted. At each capture a sample of blood was taken for serological examination for *M. bovis* using a monoclonal antibody-based, indirect ELISA developed at the Central Veterinary Laboratory. Where possible, samples of urine and faeces, a tracheal aspirate or laryngeal swab and swabs or needle biopsies of bite wounds and abscesses were also taken for bacterial culture of *M. bovis*. Animals known from a previous capture to be infected were radio-tagged to facilitate recovery of the carcase at death.

Badgers which were found dead on or, if previously captured, near the study area or killed *in extremis* for humane reasons with intracardiac pentobarbitone sodium, were ascribed a cause of death where possible and subjected to detailed post-mortem examination. Visible lesions (VL) of tuberculosis were recorded and samples taken for histopathological examination. Tissue samples from all lymph nodes and major internal organs were taken for bacteriological culture [13].

Animals were aged at first capture as cubs (less than 1 year), yearlings (between 1 and 2 years) or adults (2 years or older) using a birth date of 1 January of the year of capture, 1 January of the year before, or 1 January of 2 years before, respectively. Only animals caught as cubs or yearlings were considered to be of known age.

A record was prepared for each capture for each animal of known age caught during the period of the project until it showed evidence of *M. bovis* infection (either a positive culture result from clinical samples or at post-mortem examination or seroconversion to the ELISA). Each record consisted of the weight, year and age at capture, the sex of the animal and the social group in which it was captured. There were 1658 cub, 1132 yearling and 1568 adult records. These records were used to calculate the mean monthly body weights and 95% confidence intervals for male and female cub, yearling and adult sections of the badger population at the study site.

Table 1. *Primary cause of death in tuberculous and non-tuberculous badgers*

	Tuberculous			Non-tuberculous			
Cause of death	Female	Male	Totals	Female	Male	Unknown	Totals
Tuberculosis	10	9	19	0	0	0	0
Starvation	0	2	2	6	8	1	15
Humane killing	1	2	3	0	0	0	0
Iatrogenic	1	1	2	2	3	0	5
Killed	8	6	14	39	31	0	70
Road accident	0	3	3	80	69	0	149
Unspecified	1	3	4	13	8	0	21
Totals	21	26	47	140	119	1	260

RESULTS

Forty-seven badgers were found at post-mortem examination to have visible evidence of tuberculosis with subsequent confirmation by bacterial isolation or, in one case, by histopathological examination only. This compares with a total of 260 animals where visible evidence of tuberculosis was not found at post-mortem examination and *M. bovis* was not isolated. Thirteen of the tuberculous badgers and 52 of the negative badgers had evidence of bite wounds during their lives ($P = 0{\cdot}247$ by Fisher's exact test).

The primary cause of death for each badger in the two groups is summarized in Table 1. Nineteen of the 47 tuberculous animals were considered to have died from tuberculosis, as determined by the extent of pathology and the absence of any other cause. It is probable that tuberculosis also contributed to the deaths of those dying from starvation (2), humane killing (3) and iatrogenic causation (2). Those noted as 'killed' (14) were mostly part of MAFF badger control operations (9). There was no significant difference in the sex or age distribution between the two groups of animals, although the distribution by primary cause was very different.

The condition scores at death were noted for 44 of the tuberculous badgers; 27 badgers were in poor condition, 18 with tuberculosis as the primary cause of death. Their distribution by primary cause of death is summarized in Table 2 and may be compared with that in 224 'negative' badgers.

The mean monthly weights and 95% confidence intervals for male and female cubs, yearlings and adult animals are summarized in Figs. 1, 2a and b. Although both social group and year of study were possible variables of significance, the overriding variables determining body weight as shown by simple linear regression were age, sex and time of year. The body weights of the 47 tuberculous badgers at post-mortem examination have been plotted against the mean monthly figures. Five of six cubs, the 4 yearlings and 22 of 37 adults weighed less than the lower 95% confidence limit at death as did 18 of the 19 animals with tuberculosis as the primary cause of death.

It was possible to determine the reproductive status during the year of death for 8 of the 14 adult females. Three had not lactated, four had lactated and one was pregnant at the time of death. The primary cause of death of seven of these animals was tuberculosis.

Table 2. *Distribution of condition scores in tuberculous and non-tuberculous badgers according to the primary cause of death*

	Condition				
Cause of death	Poor	Fair	Good	V. good	Totals
Tuberculous badgers					
Tuberculosis	18	1	0	0	19
Starvation	2	0	0	0	2
Humane killing	2	0	0	1	3
Iatrogenic	1	1	0	0	2
Killed	1	0	11	0	12
Road accident	1	0	0	1	2
Unspecified	2	1	1	0	4
Totals	27	3	12	2	44
Non-tuberculous badgers					
Tuberculosis	0	0	0	0	0
Starvation	13	1	0	0	14
Humane killing	0	0	0	0	0
Iatrogenic	1	2	1	0	4
Killed	3	7	57	1	68
Road accident	15	21	77	6	119
Unspecified	5	5	8	1	19
Totals	37	36	143	8	224

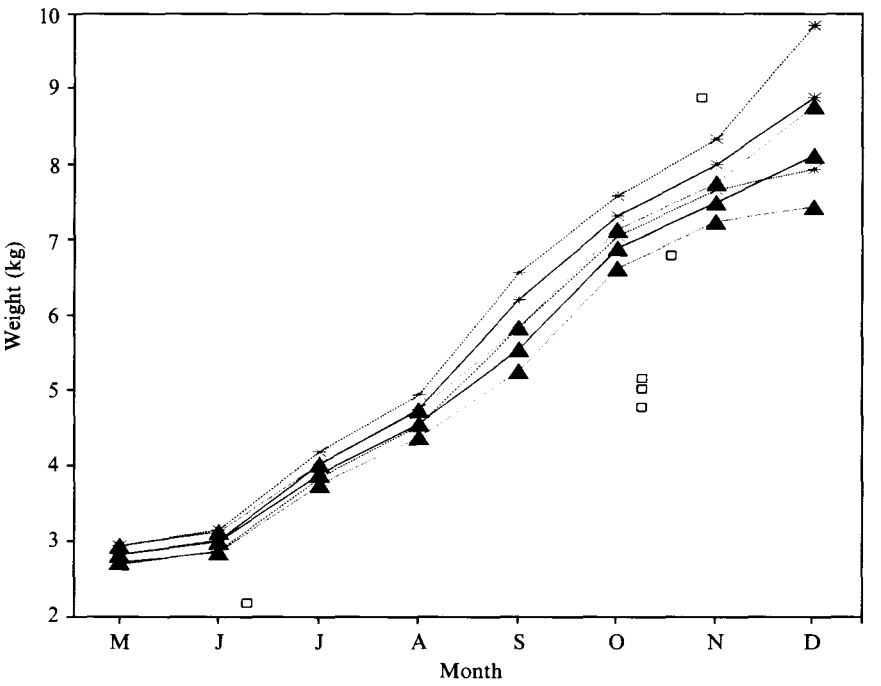

Fig. 1. Mean monthly weights (solid lines) with 95% confidence intervals (dotted lines) of uninfected cubs, and weights of tuberculous cubs at post-mortem. ▲, female; *, male, □, cubs at post-mortem examination.

https://doi.org/10.1017/S0950268800056624 Published online by Cambridge University Press

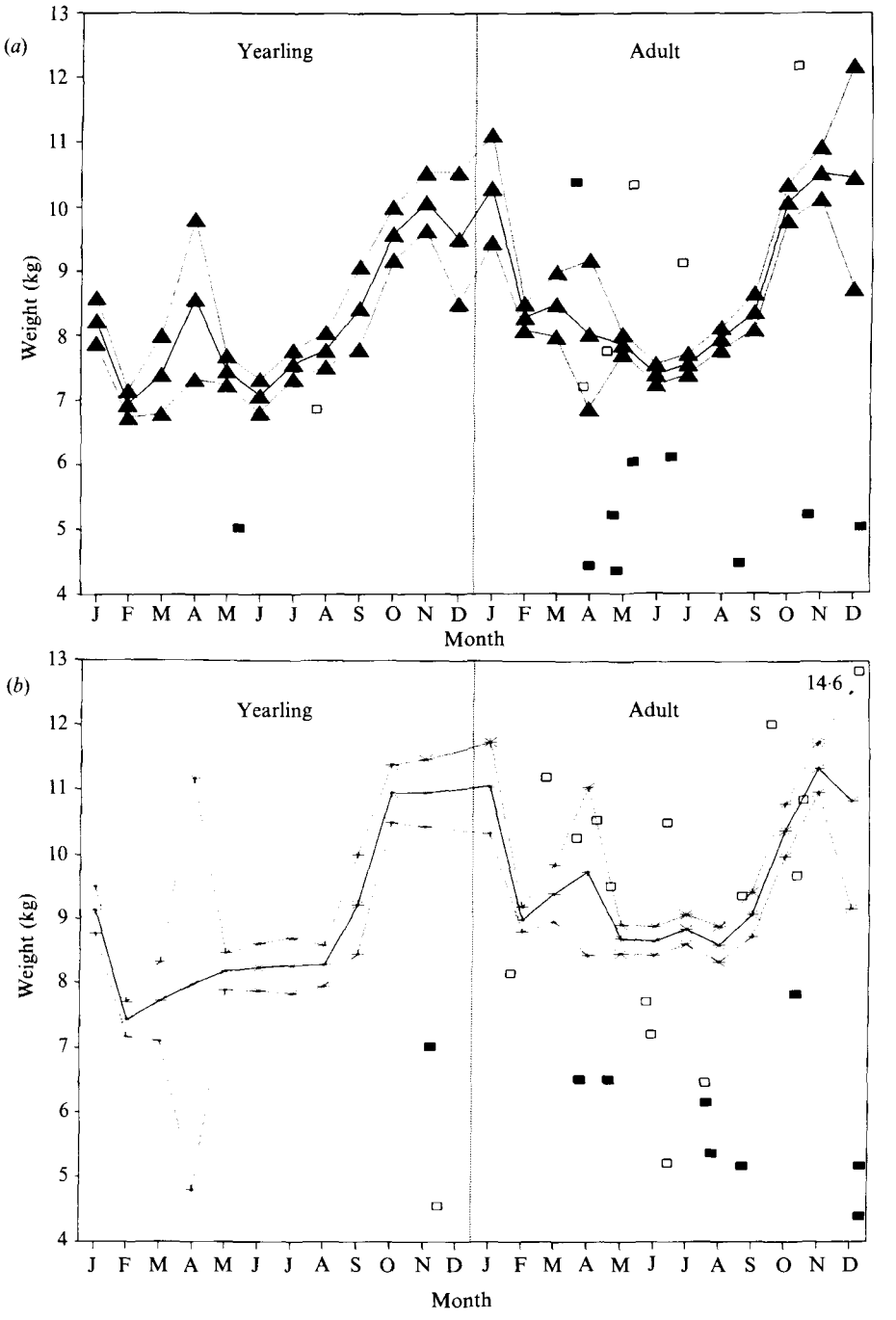

Fig. 2. Mean monthly weights (solid lines) with 95% confidence intervals (dotted lines) of uninfected yearlings and adults, and weights of tuberculous yearlings and adults at post-mortem. (*a*) Females. (*b*) Males. □, death not due to tuberculosis; ■, death due to tuberculosis.

https://doi.org/10.1017/S0950268800056624 Published online by Cambridge University Press

Table 3. *Clinical samples from which* M. bovis *was cultured for 11 tuberculous badgers*

Badger identity	Sex	First +ve sample*	Later +ve samples*	Interval (days) from first +ve sample to death
B7	Male	B	B/F/T	434
B22	Female	A	F/T	566
F30	Male	B/F/T/U	None	21
G20	Female	A	A/F/U/biopsy	649
G30	Male	B/L	None	22
G31	Female	F	A/F/L/U	611
K12	Male	B	None	7
L5	Male	F/T/U	None	190
O4	Male	A	None	219
P11	Female	B	None	99
P12	Female	T	A/F/L/T	709

*A, abscess; B, bite wound; F, faeces; L, laryngeal swab; T, tracheal aspirate; U, urine.

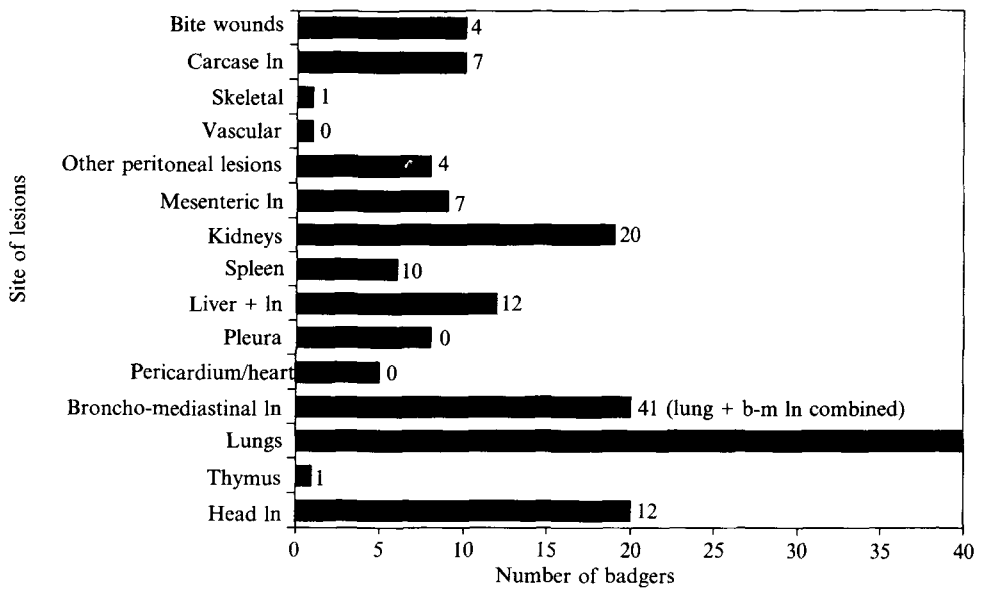

Fig. 3. Lesion distribution and number of *M. bovis* isolations from samples at post-mortem examination in 47 tuberculous badgers. ln, lymph nodes. Number of *M. bovis* culture +ve samples at head of bars.

Twenty-three of the 47 tuberculous badgers had been captured a total of 130 times and had clinical samples taken on a total of 117 times prior to their death. Positive culture results were obtained from 11 of the 23 animals. Six of these were captured and sampled after the initial positive results on a further 21 occasions. Four of these 6 animals were negative at subsequent captures on 5 of these 21 occasions. The mean time from first positive sample to death for these animals was 360 days (95% confidence interval 320–400 days). The maximum period between initial diagnosis and death was 709 days. The types of sample resulting in the initial diagnosis for each case and subsequent positive samples are summarized in

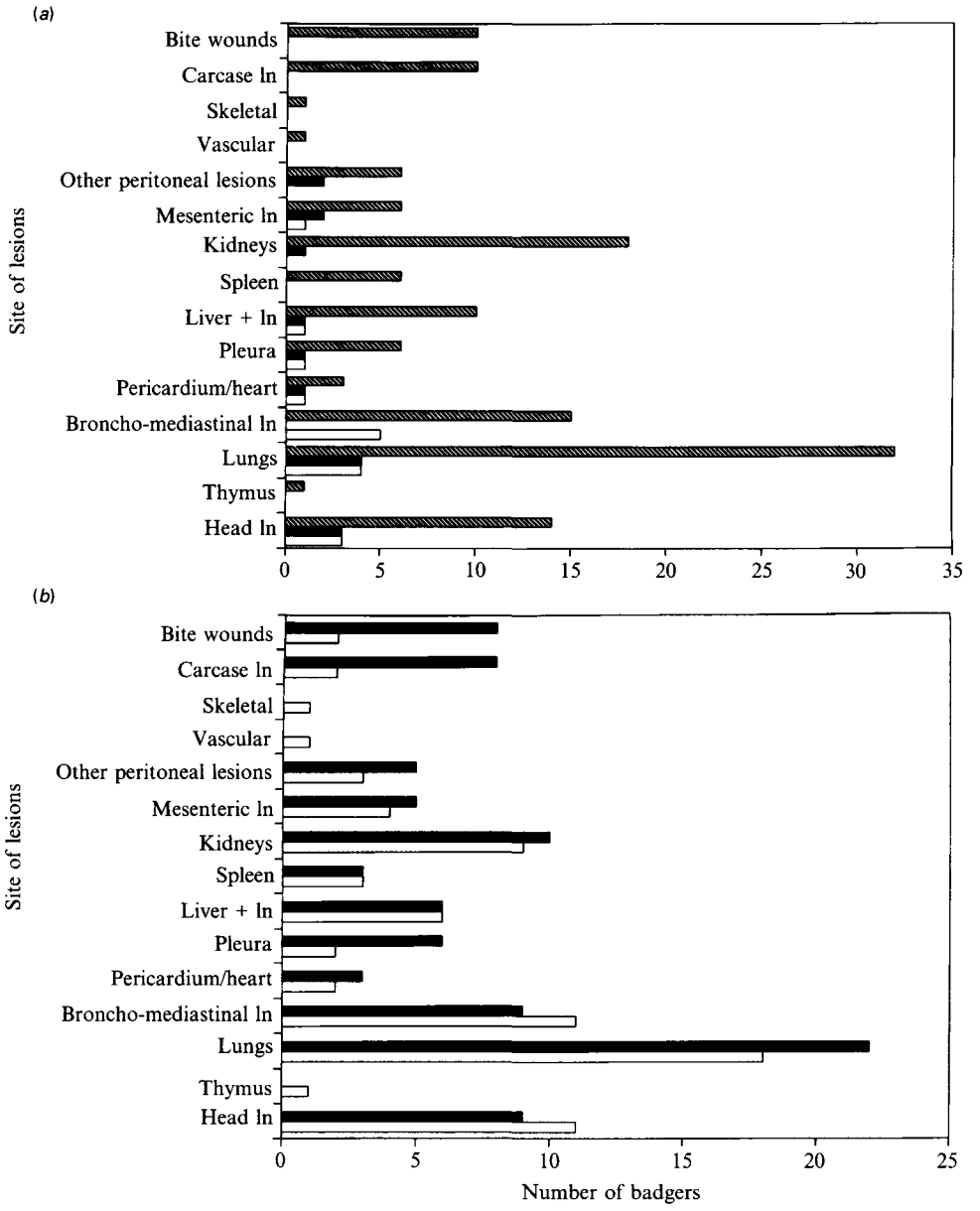

Fig. 4. Lesion distribution at post-mortem examination of 47 tuberculous badgers. (*a*) Age distribution: ▨, adults; ■, yearlings; □, cubs. (*b*) Sex distribution: ■, male; □, female. ln, lymph nodes.

Table 3. In eight cases the initial culture was from bite wounds (5) or abscesses (3). The mean survival of those with infected bite wounds was 117 days, 95% CI −180 to +341 days, compared with 491 days, 95% CI +253 to +729 days, in those first diagnosed from other samples.

The number of animals at post-mortem examination with lesions by site of lesion, together with the total number of *M. bovis* culture positive samples for each site are summarized in Fig. 3. Changes in the lymph nodes associated with the

https://doi.org/10.1017/S0950268800056624 Published online by Cambridge University Press

head (20), in the lungs (40) and associated lymph nodes (20) and in the kidneys (19) accounted for over half of the lesions seen. The distribution of the lesions by age group and by sex are summarized in Figs. 4a and b. Bite wounds and carcase lymph node lesions were only found in adults and principally in male animals. All except one affected kidneys were also in adults.

DISCUSSION

The marked seasonal variation in body weight of adult badgers has been previously noted from limited studies in both free-living [8, 14, 15] and captive animals [13, 14] with the lowest body weights being found in midsummer and the highest from November to December. The extensive body weight data from the current study have allowed a far more precise estimation of this variation both by sex and age group. Cub weights increased progressively up to the end of their first year with sexual differentiation being apparent from as early as September (Fig. 1). The seasonal pattern of weight change was established in the yearlings, with a sharp decrease in weight in both sexes after the New Year, a prolonged plateau phase over most of the summer being more marked in males, and a sharp increase of weight from September to November (Figs. 2a and b). Despite these well-established seasonal trends, the main presenting signs in the tuberculous badgers were poor condition and emaciation. Mechanisms for this weight loss involving a macrophage-secreted factor, tumour necrosis factor or cachectin, have previously been described in other species [16].

In contrast, animals from the tuberculous group which had 'killed' as their primary cause of death were generally in good condition and within or above the 95% confidence limits for the monthly mean weight (Table 2, Figs. 1, 2a and b) despite a positive diagnosis of tuberculosis being made in some instances many months previously. In this study, 5 of 11 animals diagnosed as infected during their lifetimes survived for more than a year after initial diagnosis and the potential threat from environmental contamination to other badgers and cattle over such a period cannot be ignored.

All except 3 of the 47 badgers had visible lesions of tuberculosis in the lungs and/or broncho-mediastinal lymph nodes, varying from miliary tubercles to more extensive caseous abscesses. This would tend to support the contention that the initial route of infection for *M. bovis* in badgers is mainly respiratory, as it is in most other mammalian species [17]. Whatever the initial route of infection, the lungs and associated lymph nodes were apparently the predilection sites for multiplication of *M. bovis*. Subsequent excretion of the organism could be detected in tracheal aspirates or laryngeal swabs and faeces samples (Table 3). Other internal organs, such as the liver, spleen and especially the kidney, were also apparently affected by secondary haematogenous spread from lung lesions or bite wounds. Shedding of *M. bovis* in badger urine is potentially of particular importance in the epidemiology of cattle tuberculosis [18], especially when the large numbers of organisms found in infected badger urine are considered [19].

Five of the 11 animals known to be infected prior to death had infected bite wounds at first diagnosis. These badgers tended not to survive as long as those diagnosed from other samples. This may imply a more rapidly disseminated form

of tuberculosis, perhaps due to haematogenous spread, following inoculation of the organism, as is seen in experimentally inoculated badgers [13]. These findings support the results of a previous study based on post-mortem examinations, in which it was considered that infection originating from bite wounds accounted for 14·1% of tuberculous badgers [20] and that the post-mortem appearance in these animals suggested that a particularly severe and acute form of tuberculosis resulted. However, it may be argued that intracellular tubercle bacilli may preferentially locate at sites of traumatic wounds or the lymph nodes draining them, the host macrophages being attracted there as part of the immune response. Infection at the site of a bite wound may simply be an expression of an ongoing haematogenous dissemination of organisms from another site. It would not be surprising for animals with such lesions to have a reduced survival. Bite wound prevalence was not significantly greater in the tuberculous than the negative group in this study.

The survival of infected badgers for long periods suggests that there is a control mechanism which, in many cases, hinders spread of the organism within and from the primary lesion. Indeed, the negative sampling occasions after a positive diagnosis was made suggest that shedding organisms may be an intermittent event in badgers as in cattle [21]. The evidence presented suggests that badgers can cope remarkably well with the chronic infection, to the extent that cubs may be produced and a successful lactation occur. At some point this mechanism appears to fail and massive multiplication of the organism occurs [13, 22]. The trigger for this is unknown, although a range of 'stress' factors, such as food shortage, may be postulated. It is interesting that tuberculosis has apparently not been a major cause of death in this endemically infected badger population. Only 5·2% of all badgers autopsied during the study have had tuberculosis as the primary cause of death, although 22·9% have bacteriological evidence of infection. These figures also ignore the biases associated with the sample of the population represented by those autopsied, such as the effect of radiotagging known-infected animals [23] on the recovery rate of carcases. Nevertheless, the impact of infection on cubs dying before they are sampled may be severe, as the estimated cub pre-capture mortality rates have in some years, especially those at the peak of the epidemic cycle, exceeded 50% [24].

The information presented here is understandably incomplete given the species studied and the chronic loss to follow up, where only 363 animals have been autopsied out of a total of nearly 1400 individuals so far captured and of those more than 150 have no previous capture record before being found dead. However, the body of data is unique, relating as it does to the course of tuberculosis in a group of naturally infected badgers of known lesion status at post-mortem examination. The data suggest that infected badgers, subsequently shown to have gross tuberculous pathology, may survive for long periods, produce cubs, contaminate their environment intermittently and show a marked decline in body weight before they die. The impact of the disease on the yearling and adult population appears limited but may be significant in young cubs. The principal sites of pathology were the lungs and associated lymph nodes, and the kidneys. The positive clinical samples suggested that contamination may occur from faeces, urine, sputum or discharging bite wounds.

ACKNOWLEDGEMENTS

We would like to thank the farmers, landowners and members of the public who have given access to their property and allowed this project to be undertaken. The diligence and dedication of Dr C. L. Cheeseman and his staff of the Central Science Laboratory in making this study possible and the skill and efficiency of the many other scientific staff who have been involved in the collection and laboratory screening of samples and in the post-mortem examination of badger carcases are gratefully acknowledged. The project is funded by the Ministry of Agriculture, Fisheries and Food.

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