

Bovine tuberculosis in domestic and wild mammals in an area of Dorset. I. Tuberculosis in cattle

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SUMMARY

A major outbreak of tuberculosis occurred in cattle on a farm in Dorset between 1970 and 1976. Six hundred and twenty-six cattle were slaughtered either because they reacted to the tuberculin test or had been exposed to infection. No source of infection was found until 1974 when badgers infected with *Mycobacterium bovis* were first discovered.

An analysis of the tuberculin test records of this herd and the six surrounding herds indicated that tuberculosis had been a sporadic problem since the early 1960's. Two peaks of infection occurred in the most severely affected herd in 1970 and 1974 when 29·8% and 27·3% of animals, respectively, reacted to the tuberculin test. These figures are exceptionally high. During the last 20 years there have been two periods when all the herds in the area had synchronous outbreaks consistent with a common source.

Analysis indicated that cattle were at greatest risk in April and May and suggest that there was re-exposure to infection at this time each year. In addition the cattle were apparently exposed to *M. bovis*, at sufficiently high levels for transmission to occur, for only a relatively short period of time.

INTRODUCTION

Tuberculosis in badgers due to *Mycobacterium bovis* was first identified in Great Britain in Gloucestershire in 1971 (Muirhead, Gallagher & Burn, 1974). Since then the role that this mammal may play in disseminating infection in cattle has been investigated in various parts of the South West of England. This paper and the two following papers (Little *et al.*, 1982*a*, 1982*b*) describes the disease in cattle, badgers and other domestic and wild mammals in an area of Southern Dorset where infected badgers were first discovered in 1974.

A major outbreak of tuberculosis occurred in cattle on a farm (the central farm) in the area between 1970 and 1977. Six hundred and twenty-six cattle were slaughtered either because they reacted to the tuberculin test or because they had

been in close contact with reactor cattle. The relatively isolated situation of the farms in the area, the disclosure of infection in badgers in 1974 (Reports, 1976, 1977, 1979), the accumulating evidence of widespread infection amongst badgers with the absence of any other source of infection and the severity of the outbreak of disease in cattle were factors which led to a decision to remove the entire badger population within a defined area – the control area – in order to eliminate the infection.

The control area of 1200 hectares is shown in Fig. 3, and contains seven farms.

The central farm consists of four holdings run as a single unit and the eastern part of about 80 hectares is owned by the farm, while the main western part of about 324 hectares is rented from the Ministry of Defence. The northern boundary of the farm is a range of hills running east and west, and the southern boundary is the sea. The boundary fields on the eastern side of the farm are arable and provide a barrier from cattle on neighbouring farms while the western boundary is a military restricted area. Thus the central farm is well isolated and contact with cattle on surrounding properties is not a problem.

A range of hills runs across the central farm forming a vale to the north containing the two dairies and the coastal fields to the south.

The two dairies operate as separate units for milking purposes but draw on a common pool of replacements. The milking cows graze the fields adjacent to the dairies. The dry cows are moved to the south-eastern coastal fields where they mix with in-calf heifers, but usually the cows return to the same dairy after calving. A small beef suckler herd is run on the hills to the west. Heifers are allocated to one or other of the dairy herds unless substandard, in which case they may be retained in the suckler herd.

Calves born in the dairies receive colostrum and milk from the dam for several days and are then housed for 6–9 months depending on the season. Due to a respiratory disease problem in 1974 at the main dairy farm all the rearing was done at the other dairy farm, but normally the calves do not mix until they are turned out to grass on the northern hills and fields. The older heifers and steers are moved on to the ridge of hills in the centre of the farm.

The other six farms contain cattle, five of these have common boundaries with the central farm. The remaining farm (C) rents grazing land within the control area.

The vegetation types of the study area are broadly related to the underlying geology. The calcareous grasslands are situated on the chalk or limestone escarpments and much has remained unimproved, i.e. not ploughed or reseeded. *Brachypodium pinnatum* (Tor grass) is present in all areas and is dominant in many. Where *Brachypodium* is very dense, the floristic diversity is diminished.

The main areas of species-rich grassland occurring in the study area are the edges of cliff, though enclaves of this type also occur on the south-facing slopes and locally on part of the northern ridge. The vegetation height is lower, and the number of grass and herb species is greatly increased including such plants as thyme, salad burnett, flax, burnett saxifrage, stemless thistle and sedges, as well as several orchid species.

Parts of the northern ridge are capped with flint gravels and Greensand and

Reading Beds outcrop on the slopes. This produces a more acid soil, and a characteristic chalk–heath type of vegetation. It comprises species such as gorse, bracken, woodsage, betony, foxglove and heather.

The major part of the valley is Wealden Beds. The grassland to the west remains unimproved. These grasslands are of a neutral type, varying with soil depth and past management. The fields at the western extremity show the clear remains of ridge and furrow systems, and are grass-dominated (*Festuca/Agrostis* type). Areas of *Juncus* occur where drainage is impeded.

Elsewhere, the composition of the grassland sward appears to be dominated by *Brachypodium* with some herbs and scrub development.

The north-facing wood is predominantly ash with some oak, white-beam, maple, yew etc., most of which has been coppiced in the past. The ground flora is characteristic of a calcareous woodland, dominated by bluebells, dog's mercury and wild garlic, with a few locally rare species, e.g. nettle-leaved bellflower. The other large wood in the south-west of the area is also a mixed deciduous woodland with areas predominantly of elm suffering from Dutch Elm Disease.

The hedgerows and gwyles (wooded glens) mostly survive intact, though Dutch Elm Disease has badly affected them in some areas. The predominant species remaining are ash, sycamore, blackthorn and hawthorn, with old man's beard, rose and brambles.

This paper describes in epidemiological terms, the extensive outbreaks of tuberculosis which occurred amongst cattle on the central farm and the less severe outbreaks which occurred in the other cattle herds. The data are drawn primarily from the records of tuberculin tests carried out by the State Veterinary Service from 1955 to 1979.

CENTRAL FARM

Materials and methods

The herd is divided into separate units on the basis of management and age. In this analysis the animals in these units were considered to form one herd as there was considerable movement of animals, especially young stock, between units.

The frequency with which tuberculin tests have been carried out are shown in Table 1. The herd was tested in every year except 1958. From 1955 to 1969 and in 1978 and 1979 the herd was tested once per year and from 1970 to 1977 on two or more occasions in each year. All cattle present were tested at each herd test. Weybridge avian purified protein derivative (PPD) and human PPD were used in the tuberculin test from 1955 to January 1975. In the herd test carried out in May 1975 and subsequently, Weybridge bovine PPD was used in place of human PPD. The tuberculin test charts completed at the time of these tests, indicating the age, sex, breed and result of the tuberculin test, were used to calculate reactor incidence rates. Reactors were categorized on the presence (visible lesion, VL) or absence (no visible lesion, NVL) of lesions indicative of tuberculosis on post-mortem examination.

The annual incidences of total (VL and NVL) reactors were calculated as shown

Table 1. *Frequency of herd tests 1955–1979*

Year	Month of herd test	Year	Month of herd test
1955	August	1973	January
1956	October		*November/December
1957	November	1974	*February/March
1959	March		May
1960	May		August
1961	May		November
1962	August	1975	January
1963	November		May
1964	March		July
1965	*June/November		October
1966	*August/October		*December/January
1967	*June/November	1976	March
1968	*July/December		June
1969	*October/December		September
1970	*October/November	1977	May
	*December/January		August
1971	March	1978	January
	June	1979	February
	*September/October		
1972	January		
	October		

* Herd tested in two parts.

Table 2. *Derivations of annual and 4 week reactor incidence rates*

$$1. \text{ Annual incidence (total reactors \%)} = \frac{\text{total no. of reactors}}{\text{animal years at risk}} \times 100$$

where:

$$\text{animal years at risk} = \frac{\text{total no. of animal tests performed at herd tests during the year}}{\text{No. of herd tests performed in the year}}$$

$$2. \text{ Four-week total reactor incidence (\%)*} = \frac{\text{No. reactors at test 2}}{\text{animal days at risk/28}} \times 100$$

where:

$$\text{animal days at risk} = \frac{1}{2}(\text{No. animals present at test 1} - \text{no. reactors at test 1} + \text{no. animals present at test 2}) \times \text{days between tests 1 and 2}$$

* E.g. between test 1 and test 2.

in Table 2. Annual VL reactor incidence rates were calculated in a similar way as were age-specific annual, total and VL, reactor incidence rates. It was not possible to determine field or building specific attack rates as the large number of animals and the within-farm movements prohibited the record keeping necessary for such calculations.

In order to examine seasonal variations in incidence, four-week incidence rates for each inter-test period from December 1970/January 1971 to August 1977 were

Table 3. Annual incidence rates of total and visible lesioned reactors, 1955–1979

Year	Animal years at risk	Total reactors	VL reactors	Annual incidence	
				Total reactors (%)	V.L. reactors (%)
1955	173	0	0	0	0
1956	235	0	0	0	0
1957	230	0	0	0	0
1959	276	0	0	0	0
1960	312	0	0	0	0
1961	237	0	0	0	0
1962	226	0	0	0	0
1963	286	14	6	4.9	2.1
1964	216	1	0	0.5	0
1965	483	0	0	0	0
1966	431	1	1	0.2	0.2
1967	389	0	0	0	0
1968	377	0	0	0	0
1969	429	0	0	0	0
1970	450	134	66	29.8	14.7
1971	377	13	9	3.5	2.4
1972	426	6	0	1.4	0
1973	550	45	18	8.2	3.3
1974	505	138	33	27.3	6.5
1975	479	61	32	12.7	6.7
1976	539	6	0	1.1	0
1977	524	6	0	1.1	0
1978	549	0	0	0	0
1979	511	0	0	0	0

V.L. = visible lesion.

calculated as shown in Table 2. Where the whole herd was not tested on the same day the mid-point between the two part-herd tests was used to determine days between tests.

Results

Annual reactor incidence

The annual incidence of reactors varied considerably over this 25-year period (Table 3 and Fig. 1).

Reactors were first disclosed in 1963 when the incidence of total reactors was 4.9%. The herd remained free of reactor animals until 1970, other than one reactor found in 1964 and one other in 1966.

From 1970 to 1979 the herd experienced two relatively short-lived epidemics of infection. The first peak occurred in 1970 when the incidence of total (VL and NVL) reactors was 29.8%. In the following years, 1971 and 1972, the incidence of reactors declined to 3.5% and 1.4% respectively. No VL reactors were found in 1972. Reactor incidence in 1973 increased to 8.2% and in 1974 the second peak occurred when the total reactor incidence was 27.3%.

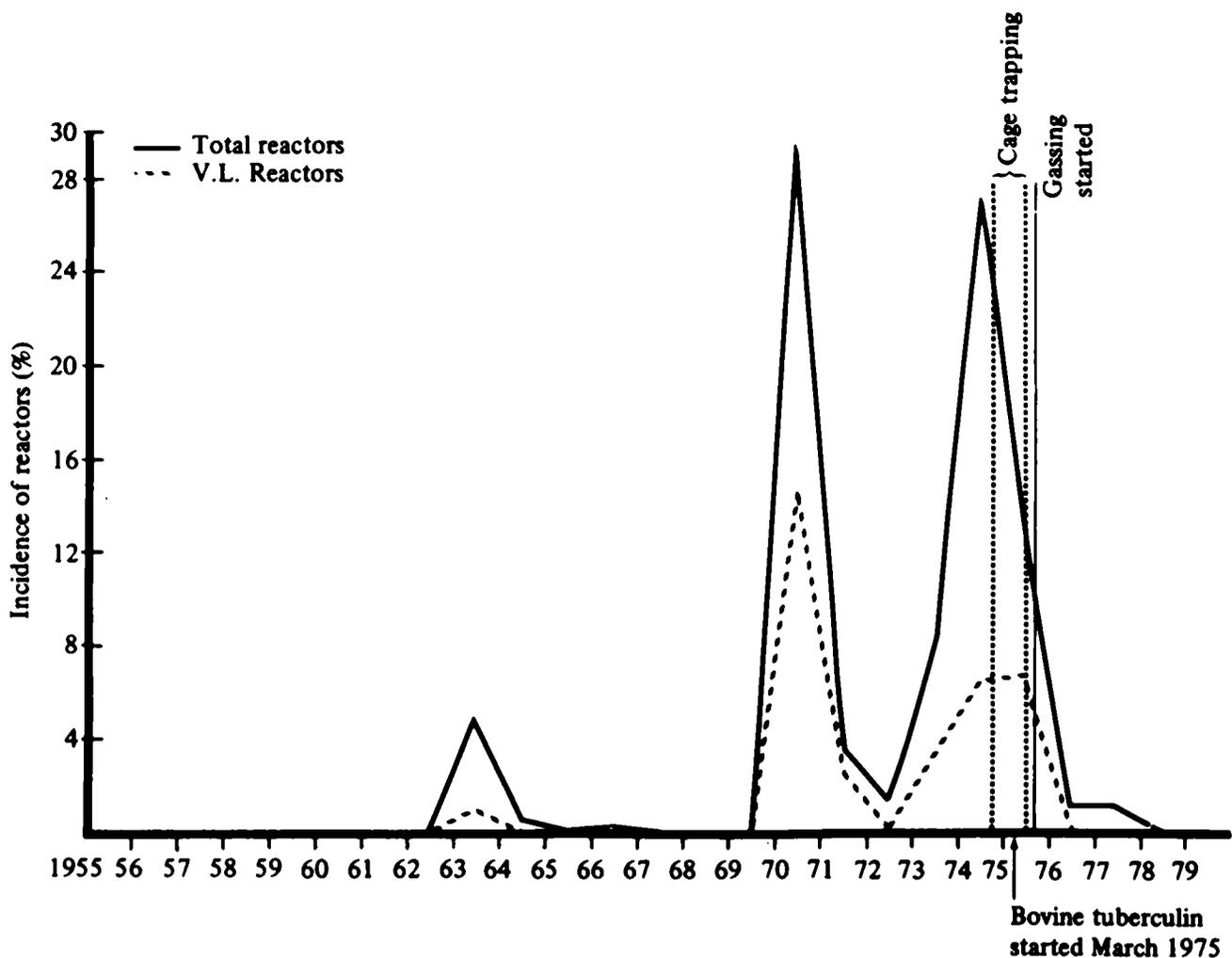


Fig. 1. Annual reactor incidence percentage.

From 1975 to 1977 the incidence of reactors declined and in 1978 the herd was again free of reactors. VL reactors have not occurred in the herd since 1975.

Incidence of reactors in the inter-test periods (1971–1977)

In the three inter-test periods in 1971, the peak incidence occurred from March to May (Fig. 2). During the last three months of 1971 no animals developed sensitivity to the tuberculin test. The herd was tested only twice in 1972, but again no animals became reactors during the last quarter of the year.

In the three months, December 1973 to February 1974, the incidence of reactors increased fivefold compared to that in the preceding inter-test period (January to November 1973). In the following 2½ months (March to mid-May 1974) the incidence of reactors showed a slight reduction, but in the subsequent six weeks (mid-May to end of June) a dramatic increase in incidence occurred.

The incidence of reactors gradually declined in the three inter-test periods from July 1974 to mid-May 1975, but a marked increase in incidence occurred in the period mid-May to mid-July 1975. The incidence declined in the following two inter-test periods (mid-July to mid-September and mid-September to the end of December 1975) and in the first three months of 1976 no reactors occurred. In the ten weeks from April to the beginning of June 1976 the incidence was again relatively high after which the incidence remained at a constant low level.

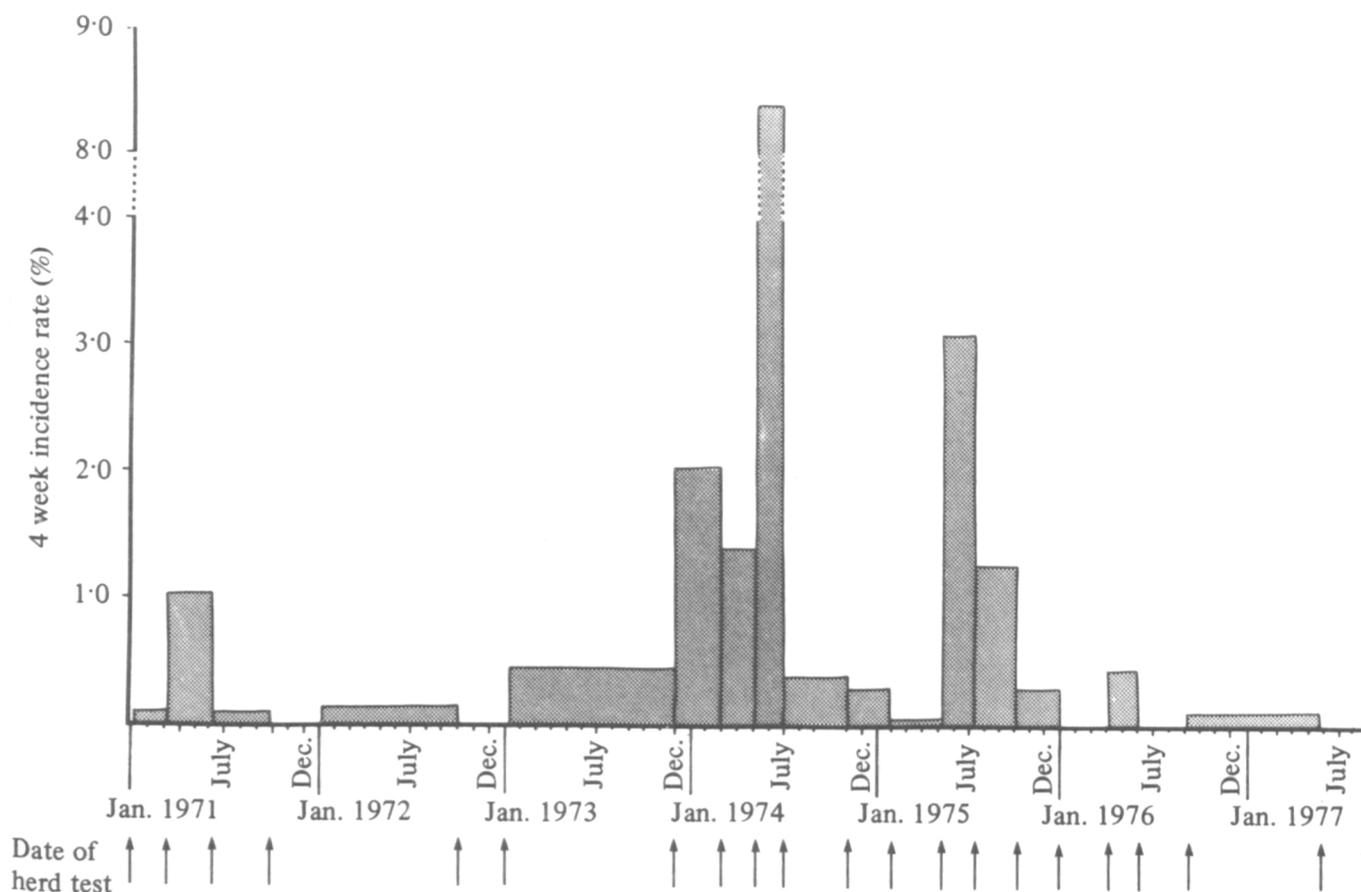


Fig. 2. 4 week percentage incidence of VL and NVL reactors in the inter-test periods 1971-7.

Table 4. Mean age of herd and of T.B. reactors

Year	Mean age of herd (months)	Mean age of reactors (months)
1963	21.56	18.0
1964	22.19	18.0
1966	33.54	90.0
1970	35.46	37.73
1971	33.41	24.86
1972	42.83	86.0
1973	34.57	43.6
1974	34.95	31.38
1975	38.29	32.48
1976	34.0	63.82
1977	32.40	57.27

Age-specific incidence rates

There was no consistent association between age and risk of infection as determined by age-specific annual incidence rates, other than cattle less than six months of age experiencing very low or zero incidence rates (Appendix 1).

In six of the 11 years in which reactors occurred the mean age of the reactors was greater than that of the whole herd (Table 4). In these 11 years cattle more

Table 5. *Relative age-specific incidence of reactors in years in which reactors found in herd*

Age group	Relative incidence reactors (%)
< 6 months	0.242
6 months - 2½ years	11.14
2½ - 4½ years	8.4
4½ - 8½ years	7.3
> 8½ years	13.1

Table 6. *Sites of lesions in 126 VL reactors disclosed from 1970 to 1974*

Site	Number
Lung	5
Lymph nodes	
Retropharyngeal/Submaxillary	50
Mediastinal/bronchial	87
Mesenteric	9
Precrural/prescapular	4

than 8½ years of age experienced the highest incidence (Table 5). The incidence in cattle 6 months to 2½ years of age was greater than that in cattle between 2½ years and 4½ years old and that of cattle between 4½ and 8½ years old.

Post-mortem findings in reactors

The proportion of reactors with visible lesions varied from year to year and in the two peak incidence years, this proportion was greater in 1970 (49.2%) than in 1974 (23.9%) (Table 3).

The distribution of lesions in the 126 VL reactors disclosed from 1970 to 1974 is shown in Table 6. Lesions in the lymph nodes of the respiratory system predominated; lesions in mesenteric lymph nodes were detected in only nine animals. This distribution of lesions was similar in each of these years.

TUBERCULOSIS IN CATTLE HERDS ON OTHER FARMS WITHIN THE CONTROL AREA

Materials and methods

The location of these farms in relation to the central farm is shown in Fig. 3. The cattle herds have all been in existence since 1955.

The annual incidence of reactors (VL and NVL) to the tuberculin test was calculated as described for the Central Farm from 1955. However, results of tuberculin tests in farms A, B and F are only available from 1961, 1960 and 1957, respectively.

Results

The herds have been subjected, in general to annual tuberculin tests or at more frequent intervals when reactors were disclosed. The annual incidences of reactors

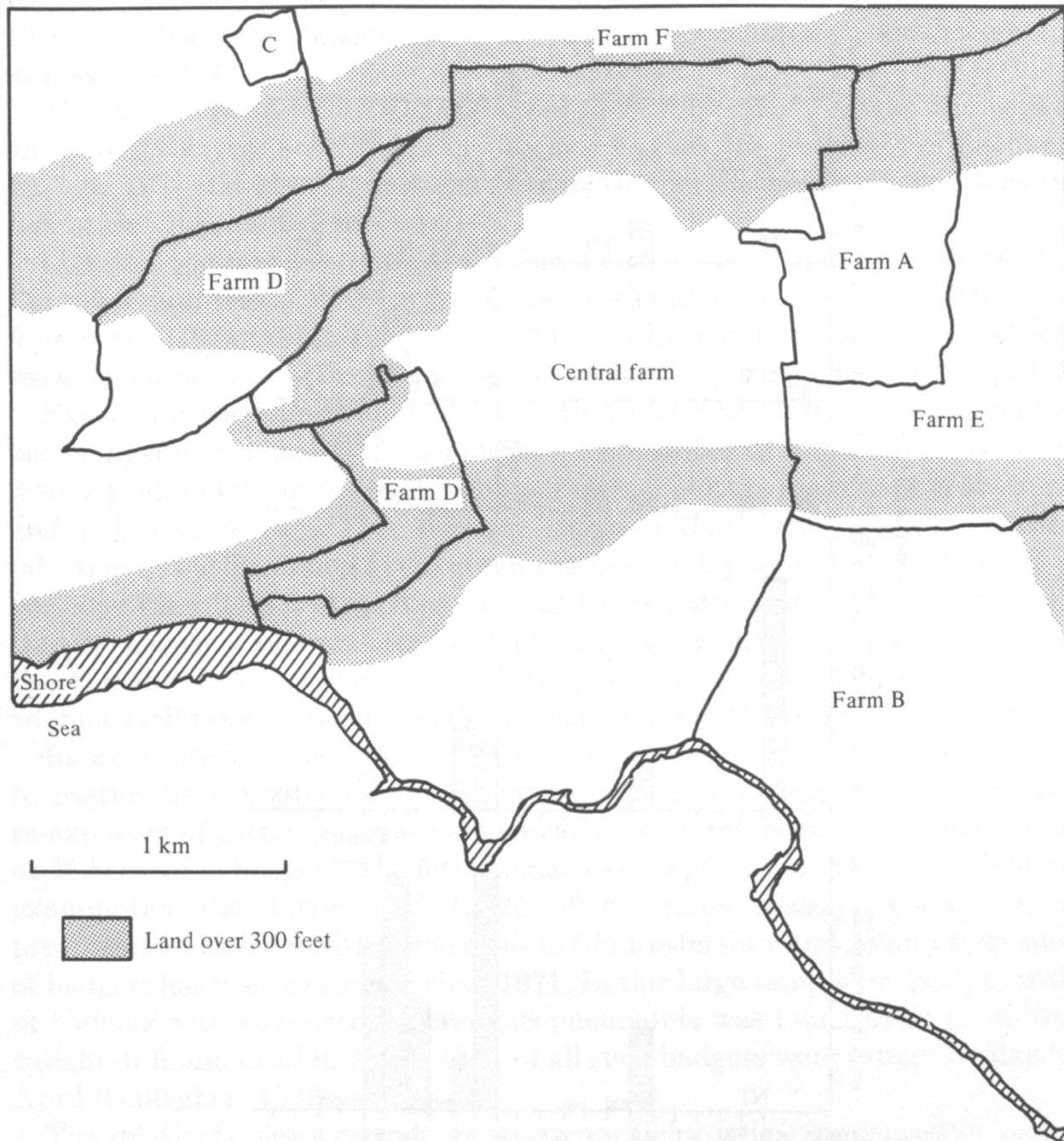


Fig. 3. Location of the seven farms with cattle in the control area.

are shown in Fig. 4. All herds have experienced outbreaks of infection over the last 25 years for which the origin of infection was not identified until infected badgers were disclosed in the area in 1974.

These outbreaks show certain common features. Infection rates have been low, only small numbers of cattle being infected in each outbreak. The outbreaks have been short-lived, infection persisting for less than two years and classically sporadic in their occurrence. Also, these herds have become infected simultaneously at least twice during the last 25 years. Four herds became infected in 1963. Similarly, infection reappeared in three herds in 1968/9 and in Farm F in 1970. The incidence of herd infection was in general higher at this time than in the previous outbreak in 1963.

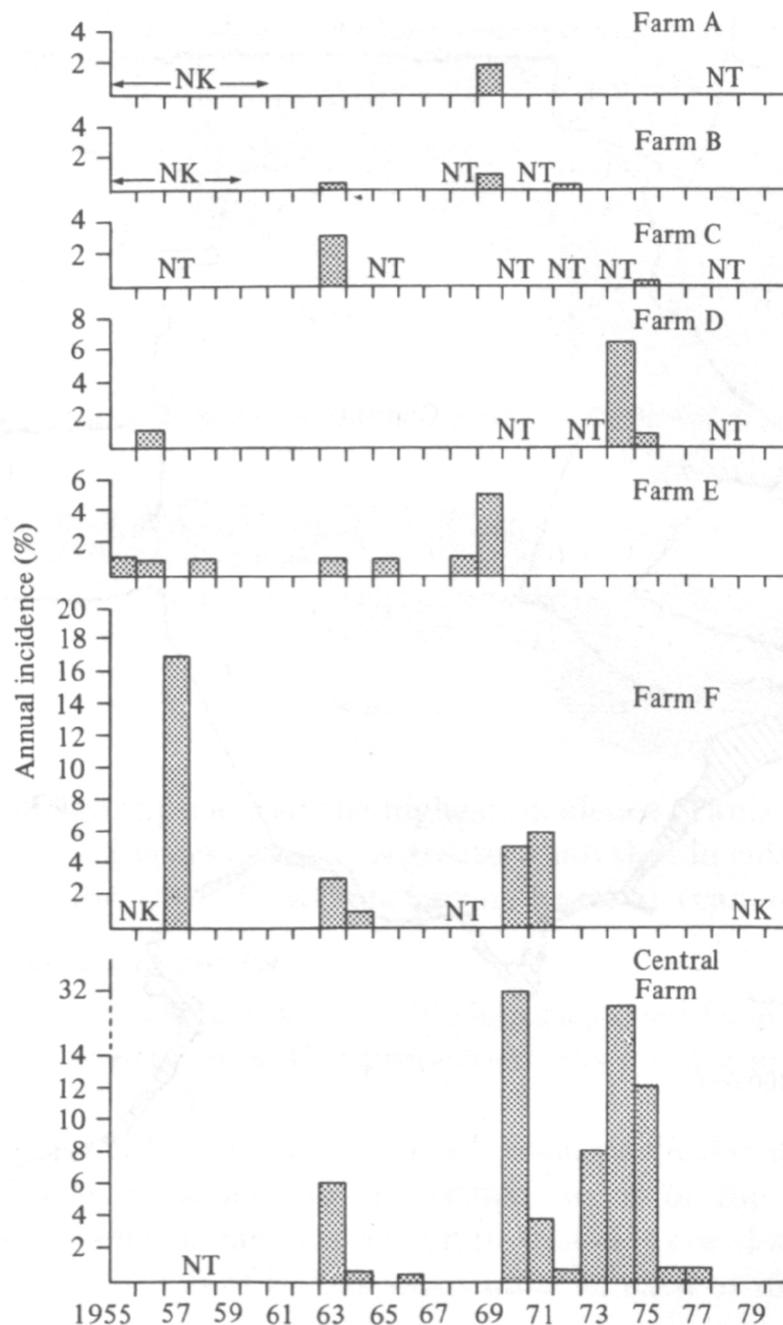


Fig. 4. Annual incidence of TB reactors (%) in the seven herds within the control area 1955–79. NT, herd not tested in that year; NK, records not available of tuberculin test.

The epidemic suffered by the central farm from 1973 to 1975 was not experienced by five of the neighbouring herds, but in Farm D infection reappeared in 1974 when the incidence of reactors was 6.6% and a further two reactors were disclosed in 1975. One reactor was also disclosed in Farm C in 1975.

Discussion

Central Farm

Since 1970 the herd has been tested at sufficiently frequent intervals to conclude that the variations in the annual incidence of reactors are real. Exposure of cattle

to infection was therefore not constant from year to year, although in 1970 and 1974 the cattle were probably exposed to high levels of infection which were similar in magnitude (Fig. 1).

Although herd tests in 1972 and 1973 were only carried out at the beginning and end of the year and in 1977 in May and August, the frequency of testing from 1971 to 1977 did allow a detailed examination of the seasonal variations in the risk of cattle acquiring infection.

In cattle the median time from infection to the development of sensitivity to the tuberculin test is of the order of four weeks (Francis, 1947). Therefore, in the four years (1971, 1974, 1975 and 1976) in which it is possible to examine for seasonal variations cattle were at greatest risk of acquiring infection in April/May.

Except for the winter of 1973/74 cattle were at a relatively low or no risk of becoming infected during the winter months (October to March), the risk declining from a peak in late spring/early summer throughout the remainder of the summer and following winter. This strongly suggests that cattle were re-exposed to infection at the beginning of the grazing season each year and for a relatively short period of time. This is in marked contrast to the pattern of infection in herds when the prevalence of tuberculosis was high, prior to the national eradication scheme. Cattle were then at greatest risk during the winter housing period when close contact facilitated cattle-to-cattle transmission via the respiratory route.

Re-exposure to infection in each year is not entirely coincident with a re-exposure to pasture as young stock are out-wintered. The peak risk of infection and the re-exposure of cattle are however coincident with the peak in potential exposure of *M. bovis* from badgers. Too few badgers were captured in this area for laboratory examination (see Little *et al.* 1982a) to determine seasonal variations in the prevalence of badgers with lesions, but in Gloucestershire and Avon a large number of badgers has been examined since 1971. In this large sample the peak prevalence of badgers with advanced tuberculous pneumonia was found to occur in badgers caught or found dead in April; 43% of all such badgers were caught in March and April (Gallagher, 1982).

The relatively short period of exposure which cattle experienced in each year is consistent with the poor survival, up to four weeks, of *M. bovis* on pasture during spring and summer (Maddock, 1933; Report, 1979) and the low prevalence and small number of tuberculous badgers detected from May to December in the Gloucestershire study (Gallagher, 1982).

The relatively high incidence of infection during the winter of 1973/74 was anomalous. It is possible that cattle-to-cattle transmission was facilitated during this time as the density of stock was at a maximum (see Appendix 2). In the absence of a herd test during the summer of 1973 cattle-to-cattle transmission could have commenced during the latter half of the year. However, the efficacy of the tuberculin test in curtailing cattle-to-cattle transmission during this time and during the other periods of higher levels of herd infection is evident.

It was not possible to calculate field specific attack rates but the between-year variation in the age-specific annual incidences (Appendix 1) indicate that exposure was not at the same level throughout the farm in any given year as the herd is

subdivided on the basis of age and management. Similarly, the high incidence in the young stock (6 months to 2½ years) in 1970 and 1974, compared to that in older cattle probably reflects a difference in exposure rather than susceptibility or predisposition associated with age. During their first six months of life cattle were undoubtedly infrequently exposed to infection.

The between-year variation in the proportion of reactors with visible lesions merely reflects the frequency of testing. This proportion was lower in 1974 than in 1970 as testing during 1974 and in the previous three years had been more frequent than that pre-1970 (Table 1). The high proportion (92·9%) of reactor animals with lesions in the lymph nodes associated with the respiratory tract or lungs is similar to that found in other countries. Francis (1971) noted that the post-mortem findings in some 56000 infected cattle from various countries showed that the thoracic cavity contained lesions of tuberculosis ten times more frequently than the abdominal cavity and therefore concluded that the route of infection in 90% of cases was aerogenous. These post-mortem results were accumulated from countries where the prevalence of tuberculosis was still relatively high and cattle-to-cattle transmission predominated. A proportion of the infected cattle on the central farm may have contracted *M. bovis* by contact with excreting cattle, but the distribution of lesions suggests that infection in cattle as a result of exposure to badgers and their excretions also takes place predominantly via the respiratory route. This is consistent with the known insusceptibility of cattle to infection by ingestion relative to the number of organisms required to infect cattle via the respiratory route (Francis, 1958).

Tuberculosis in all herds

The sporadic occurrence of *M. bovis* infection in these herds implies that infection has not been endemic in the cattle in this area during the last 25 years. The synchronous occurrence of outbreaks of herd infection suggest a common source of infection.

Interestingly, although six of the seven herds became infected within a four year period (1968–1971) it seems that a 'wave' of infection moved across the area from east to west during this time. The three herds (on Farms A, B and E) in the eastern sector of the area became infected in 1969 and the most western herd (Farm D) not until 1974.

The simultaneous exposure in 1963 and later 'wave' of infection cannot be explained by contact of cattle between herds or by inter-herd purchases and movements of cattle. Similarly, investigations of other potential sources of infection, apart from badgers, proved negative.

The sporadic occurrence of herd infection and the simultaneous infection of herds, resulting in temporal variations in the incidence of infection, have also been observed in areas in Gloucestershire where badgers have been the only possible source of infection (Wilesmith and Muirhead, unpublished findings).

Such variations in cattle infection are probably indicative of the temporal variations in the prevalence of infection in badgers resulting from fluctuations in population density. Cyclical variations are known to occur in some small mammals

(Elton, 1942; Finerty, 1980), but sufficiently long term studies on the dynamics of badger populations have not been carried out.

The central farm indicates the risk for cattle which can occur when there is a high level of exposure to badgers suffering a major epidemic of tuberculosis.

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REFERENCES

- ELTON, C. (1942). *Voles, Mice and Lemmings*. Oxford University Press, Oxford.
- FINERTY, J. P. (1980). *Population Ecology of Cycles in Small Mammals*. New Haven and London: Yale University Press.
- FRANCIS, J. (1947). *Bovine Tuberculosis*, p. 135. London: Staple Press.
- FRANCIS, J. (1958). *Tuberculosis in Animals and Man*, p. 357. London: Cassell.
- FRANCIS, J. (1971). Susceptibility to tuberculosis and the route of infection. *Australian Veterinary Journal* **47**, 414.
- GALLAGHER, J. (1982). *Natural History of Spontaneous Tuberculosis in Wild Badgers in Gloucestershire* London University. (In preparation.)
- LITTLE, T. W. A., SWAN, C., THOMPSON, H. V. and WILESMITH, J. W. (1982*a*). Bovine tuberculosis in domestic and wild mammals in an area of Dorset. II. The badger population its ecology and tuberculosis status. *Journal of Hygiene* **89**, 211–224.
- LITTLE, T. W. A., SWAN, C., THOMPSON, H. V. and WILESMITH, J. W. (1982*b*). Bovine tuberculosis in domestic and wild mammals in an area of Dorset. III. The prevalence of tuberculosis in mammals other than badgers and cattle. *Journal of Hygiene* **89**, 225–234.
- MADDOCK, E. C. G. (1933). Studies on the survival time of the bovine tubercle bacillus in soil, soil and dung, in dung on grass, with experiments on the preliminary treatment of infected organic matter and the cultivation of the organism. *Journal of Hygiene* **33**, 103–117.
- MUIRHEAD, R. H., GALLAGHER, J. and BURN, K. J. (1974). Tuberculosis in wild badgers in Gloucestershire: epidemiology. *The Veterinary Record* **95**, 552–555.
- REPORT (1976). *Bovine Tuberculosis in Badgers*. Ministry of Agriculture, Fisheries and Food, London.
- REPORT (1977). *Bovine Tuberculosis in Badgers. Second Report*. Ministry of Agriculture, Fisheries and Food. London.
- REPORT (1979). *Bovine Tuberculosis in Badgers. Third Report*. Ministry of Agriculture, Fisheries and Food, London.

Appendix 1. Age-specific annual TB reactor incidence (%) 1955-1979

	Annual incidence TB reactors (%)											All Ages	
	< 6 months	6 months to 2½ years	2½-4½ years	4½-6½ years	6½-8½ years	8½-10½ years	10½-12½ years	12½-14½ years					
1955	—	0	0	—	—	—	—	—	—	—	—	—	0
1956	—	(0/124)	(0/49)	—	—	—	—	—	—	—	—	—	(0/173)
1957	0	(0/115)	(0/120)	—	—	—	—	—	—	—	—	—	(0/235)
1959	0	(0/131)	(0/86)	0	—	—	—	—	—	—	—	—	(0/230)
1960	0	(0/147)	(0/124)	(0/3)	—	—	—	—	—	—	—	—	(0/276)
1961	0	(0/217)	(0/74)	(0/5)	0	—	—	—	—	—	—	—	(0/312)
1962	0	(0/132)	(0/88)	(0/10)	(0/1)	—	—	—	—	—	—	—	(0/237)
1963	0	(0/189)	(0/34)	(0/1)	—	—	—	—	—	—	—	—	(0/226)
1964	0	(0/12)	(14/229)	(0/5)	—	—	—	—	—	—	—	—	4.9
1965	0	(0/4.5)	(1/184)	(0/9)	0	—	—	—	—	—	—	—	(14/286)
1966	0	(0/83)	(0/257)	(0/62)	(0/2)	0	—	—	—	—	—	—	0.46
	0	(0/51)	(0/231.5)	(0/32)	(0/30)	0	0	0	0	0	0	0	(1/216)
	0		0	0	2.35	0	0	0	0	0	0	0	0
			0	(0/61)	(1/42.5)	(0/8)	(0/3.5)	(0/1.5)					(0/483)
			(0/61)	(0/32)	(0/8)	(0/3.5)	(0/1.5)						0.23
			(0/61)	(0/32)	(0/8)	(0/3.5)	(0/1.5)						(1/431)

1967	0 (0/51)	0 (0/165)	0 (0/74)	0 (0/55)	0 (0/31)	0 (0/9)	0 (0/3)	0 (0/1)	0 (0/389)
1968	0 (0/31)	0 (0/157)	0 (0/107)	0 (0/30)	0 (0/31)	0 (0/16)	0 (0/4)	0 (0/1)	0 (0/377)
1969	0 (0/86)	0 (0/157)	0 (0/45)	0 (0/38)	0 (0/45)	0 (0/30)	0 (0/17)	0 (0/11)	0 (0/429)
1970	1·19 (1/84)	40·59 (69/170)	40·23 (35/87)	25·42 (15/59)	28·13 (9/32)	25·0 (3/12)	20·0 (1/5)	100·0 (1/1)	29·77 (134/450)
1971	0 (0/58)	5·21 (9/172·7)	3·57 (2/56)	1·86 (1/53·7)	0 (0/31·2)	18·52 (1/5·4)	—	—	3·44 (13/377)
1972	0 (0/67·5)	0 (0/54·5)	0 (0/83·5)	3·95 (3/76)	2·82 (1/35·5)	36·36 (2/5·5)	0 (0/3)	—	1·41 (6/426)
1973	0 (0/120)	9·84 (18/183)	11·43 (12/105)	11·77 (10/85)	8·33 (4/48)	13·33 (1/7·5)	0 (0/0·5)	0	8·18 (45/550)
1974	0·82 (1/122·8)	56·41 (102/180·8)	19·05 (10/52·5)	16·56 (13/78·5)	11·86 (7/59)	42·55 (5/11·8)	—	—	27·13 (138/505)
1975	0 (0/54)	20·78 (46/221·4)	2·14 (1/46·8)	10·07 (9/89·4)	7·09 (4/56·4)	10·2 (1/9·8)	0 (0/0·4)	0 (0/0·4)	12·73 (61/479)
1976	0 (0/98·7)	0·89 (2/224)	0 (0/83·7)	2·7 (2/74)	2·17 (1/46)	8·33 (1/12)	0 (0/0·7)	—	1·11 (6/539)
1977	0 (0/75·5)	0 (0/251)	3·16 (3/95)	3·67 (2/54·5)	0 (0/35·5)	8·7 (1/11·5)	0 (0/1)	—	1·15 (6/524)
1978	0 (0/46)	0 (0/275)	0 (0/114)	0 (0/42)	0 (0/48)	0 (0/17)	0 (0/7)	—	0 (0/549)
1979	0 (0/34)	0 (0/250)	0 (0/101)	0 (0/71)	0 (0/30)	0 (0/22)	0 (0/3)	—	0 (0/511)

* Figures in parentheses refer to number of reactors disclosed in the year/animal years at risk.

Appendix 2. *Four-week percentage incidence of VL and NVL reactors in the inter-test periods 1971–1977*

	Days between tests	No. of animals at risk	No. of new reactors	4 week incidence of reactors (%)*
10.i.71–16.iii.71	65	352	1	0·12
16.iii.71–14.vi.71	90	363	12	1·03
14.vi.71–3.x.71	111	386·5	1	0·07
3.x.71–7.i.72	96	391	0	0
7.i.72–13.x.72	280	425·5	6	0·14
13.x.72–12.i.73	91	484·5	0	0
12.i.73–1.xii.73	323	549·5	45	0·41
1.xii.73–1.iii.74	90	561·5	37	2·05
1.iii.74–17.v.74	77	534	20	1·36
17.v.74–3.vii.74	47	508·6	72	8·43
3.vii.74–12.xi.74	132	424	7	0·35
12.xi.74–27.i.75	76	436	3	0·25
27.i.75–12.v.75	105	469	1	0·06
12.v.75–14.vii.75	63	490·5	34	3·08
14.vii.75–13.x.75	91	465	19	1·26
13.x.75–2.i.76	81	465·5	4	0·30
2.i.76–30.iii.76	88	498	0	0
30.iii.76–7.vi.76	69	534	6	0·46
7.vi.76–7.ix.76	92	498·5	0	0
7.ix.76–21.v.77	256	531·5	6	0·12
21.v.77–2.viii.77	73	519·5	0	0

* VL and NVL reactors.