

Mycobacterium microti: More Diverse than Previously Thought[∇]

N. H. Smith,^{1,2*} T. Crawshaw,³ J. Parry,¹ and R. J. Birtles⁴

VLA Weybridge, New Haw, Surrey KT15 3NB, United Kingdom¹; Centre for the Study of Evolution, University of Sussex, Brighton BN1 9QL, United Kingdom²; VLA Starcross, Staplake Mount, Starcross, Exeter EX6 8PE, United Kingdom³; and Disease Ecology Group, Faculty of Veterinary Science, University of Liverpool, CH64 7TE, United Kingdom⁴

Received 30 March 2009/Returned for modification 22 May 2009/Accepted 8 June 2009

Mycobacterium microti is a member of the *Mycobacterium tuberculosis* complex of bacteria. This species was originally identified as a pathogen of small rodents and shrews and was associated with limited diversity and a much reduced spoligotype pattern. More recently, specific deletions of chromosomal DNA have been shown to define this group of organisms, which can be identified by the absence of chromosomal region RD1^{mic}. We describe here the molecular characteristics of 141 strains of the *Mycobacterium tuberculosis* complex isolated in Great Britain over a 14-year period. All strains have characteristic loss of some spoligotype spacers and characteristic alleles at the ETR-E and ETR-F variable-number tandem-repeat (VNTR) loci, and a sample of these strains was deleted for regions RD7, RD9, and RD1^{mic} but intact for regions RD4 and RD12. We therefore identified these strains as *M. microti* and show that they have much more diverse spoligotype patterns and VNTR types than previously thought. The most common source of these strains was domestic cats, and we show that the molecular types of *M. microti* are geographically localized in the same way that molecular types of *Mycobacterium bovis* are geographically localized in cattle in the United Kingdom. We describe the pathology of *M. microti* infection in cats and suggest that the feline disease is a spillover from a disease maintained in an unknown wild mammal, probably field voles. The location of the cats with *M. microti* infection suggests that they do not overlap geographically with the strains of *Mycobacterium bovis* in Great Britain.

In 1946, Wells described a form of tuberculosis found in over 20% of 4,309 voles (*Microtus agrestis*) collected between 1936 and 1942 from many sites dispersed throughout Great Britain (43). In the same study, over 10% of 223 bank voles (*Clethrionomys glareolus*, now known as *Myodes glareolus* [18]), over 2% of 175 wood mice (*Apodemus sylvaticus*), and 1.5% of 550 shrews (*Sorex araneus*) were also found to be infected with tuberculosis (43). In all cases, acid-fast bacteria were demonstrated in a stained smear or in culture. For voles with only a single observed site of infection, the lymph glands draining the intestinal tract were most commonly infected; the lungs were a primary site in 11% of animals. The author also describes a second type of disease pathology, an infiltration of the subcutaneous tissue with a white gritty substance full of acid-fast bacteria; in these animals, the skin frequently ulcerates over these masses, and the lungs, liver, and spleen are heavily infected. Transmission of organisms from both wild-caught infected voles and experimentally infected voles kept together with laboratory-bred animals was demonstrated. Infected voles can live for as long as 52 weeks, and the author comments on how extensive tuberculosis can be without producing obvious symptoms. Although we cannot be certain that all the organisms described in the 1946 manuscript are *M. microti*, although some strains were shown to have distinctive *M. microti* spoligotype patterns and deletion profiles in later studies (1, 5, 25), the distinctive cellular morphology when viewed microscopically supports this identification. The author mentions that growth is very slow and variable upon primary isolation

and is substantially faster after repeated subculture and also reports on a number of studies using live strains of the vole bacillus as a vaccine in guinea pigs, cattle, and humans and suggests that the vole bacillus does not cause progressive disease in these animals unless given in large doses (43). The organisms described by Wells were later designated *Mycobacterium microti* (42).

In the 1950s, live strains of *M. microti* were administered in large-scale trials as an antituberculosis vaccine in Czechoslovakia, the United Kingdom, and northern Rhodesia (19, 31, 36). Although both attenuated and nonattenuated vole bacillus vaccines proved both safe and effective, vaccination with *M. microti* was found to be no more effective than the commonly used BCG—an attenuated strain of *Mycobacterium bovis*. However, in 1998, *M. microti* infections were identified as causing pathology in both immunocompetent and immunocompromised humans from The Netherlands (41) and have subsequently been identified in humans from Germany (30), Switzerland (5), England, and Scotland (summarized in reference 45). Recently, evidence of multihost clustering of *M. microti* infection of the same genotype, in an alpaca and an immunocompromised human from Scotland, has been described (C. McGoldrick, A.-L. Seagar, I. F. Laurenson, N. H. Smith, W. Stewart, K. Kerr, and J. Douglas, submitted for publication).

In the late 1990s, molecular methods were developed to identify strains of the *Mycobacterium tuberculosis* complex, and in particular, spoligotyping was shown to be a good technique for simultaneous strain discrimination and identification (24). Spoligotyping identifies polymorphism in the presence of spacer units in the direct repeat (DR) region in strains of the *M. tuberculosis* complex (24, 38). The DR is composed of multiple, virtually identical, 36-bp regions interspersed with unique DNA spacer sequences of similar sizes (direct variant repeat, or

* Corresponding author. Mailing address: VLA Weybridge, New Haw, Surrey KT15 3NB, United Kingdom. Phone: (44) 1273 873502. Fax: (44) 1932 347046. E-mail: Noel@Sussex.ac.uk.

[∇] Published ahead of print on 17 June 2009.

DVR, units). Spacer sequences are unique to the DR region and copies are not located elsewhere in the chromosome (39). The DR region may contain over 60 DVR units; however, 43 of the spacer units were selected and are used in the standard application of spoligotyping to strains of the *M. tuberculosis* complex (16, 24). The DR region is polymorphic because of the loss (deletion) of single or multiple spacers, and each spoligotype pattern from strains other than *M. tuberculosis* is given an identifier, such as SB0118, by www.Mbovis.org. More recently variable-number tandem-repeat (VNTR) typing, a form of mini-satellite typing, has been shown to be an effective method for genotyping strains of the *M. tuberculosis* complex (34).

In 1998, Kremer et al. described a series of spoligotype patterns associated with isolates from animals and a human from the United Kingdom (25). The traditional *M. microti* spoligotype pattern (SB0118) with only two spacers hybridizing was identified; however, more extensive spoligotype patterns were found in strains isolated from cats in southern England (up to eight spacers hybridizing; spoligotype patterns SB0112 and SB0657); strains with these more extensive patterns were referred to as “llama” strains because a strain of this type had previously been isolated from a llama in a Belgian zoo (40).

The distribution of vole tuberculosis in Great Britain was revisited 60 years after Wells by Cavanagh et al. (5), who trapped small rodents in Northumberland (Keilder Forest) and Cheshire, United Kingdom. In the part of the Kielder Forest study that included dissection, the prevalence of characteristic tuberculous lesions was recorded as being as high as 21%. Overall, the authors described characteristic skin lesions in 2% of 4,852 field voles collected in Northumberland between 1998 and 2000 and, once again, described the characteristic spoligotype pattern as hybridization to spacers 37 and 38 only. Using advanced molecular techniques (IS6110 restriction fragment length polymorphism), the authors were able to demonstrate that modern isolates were distinct from Wells' isolates of 1946 but that all *microti* strains isolated in Great Britain were more closely related to each other than to strains of *M. microti* that had been isolated from a variety of animals in Belgium and The Netherlands. The hyrax (dassie) strain mentioned in this report, and in others, is not a strain of *M. microti* and is more closely allied to strains of *Mycobacterium africanum* subtype 1 (clade 2) (20, 35).

The phylogenetic position of *M. microti* within the *M. tuberculosis* complex was identified by Brosch et al. (2), who used a series of phylogenetically informative deletions and single nucleotide polymorphisms to position these strains within the lineage leading from *M. tuberculosis* to *M. bovis*. Strains of *M. microti* were found to be closely allied to strains of *Mycobacterium pinnipedii* and to lack chromosomal regions of difference RD7 to -10 but, unlike strains of *M. bovis*, were intact at regions RD4, -12, and -13. Later, the loss of certain spoligotype spacers was shown to be informative within the phylogenetic framework established by Brosch et al., and it was suggested that the different clades of the *M. tuberculosis* complex might better be referred to as ecotypes rather than species (35). Using the ecotype approach strains of *M. microti* would be considered a vole-adapted ecotype of *M. tuberculosis* while the very closely related *M. pinnipedii* could be considered an ecotype adapted to marine mammals (35). The use of molecular markers and spoligotype patterns to identify different spe-

cies of the *M. tuberculosis* complex was later confirmed in an extensive study of many strains by Huard et al. (20), who also identified a number of *M. microti*-specific single nucleotide polymorphisms within the RD13 locus and the 16S rRNA gene.

Using bacterial artificial chromosomes, Brodin et al. identified 10 regions of difference (deletions) between a strain of *M. microti* and strains of *M. bovis* and *M. tuberculosis* (1). Surveying for the presence of these deletions in a collection of nine *M. microti* strains, they showed that several deletions, including RD1^{mic}, were characteristic of *M. microti* strains. They also showed that the deletion of a region called MiD1 was responsible for the reduced spoligotype pattern seen in strains showing hybridization to only two spoligotype spacers. In other strains of *M. microti* with more extensive spoligotype patterns, the MiD1 region was either only partially deleted or intact.

Apart from small mammals, strains of *M. microti* have most frequently been isolated from domestic cats in Great Britain but have also been found in skin test reactor cattle and domestic pigs with tuberculous lesions at slaughter (21, 37). Gunn-Moore et al. describe 19 cats from Great Britain in which the signalment, pathology, and geographical location of the cases are remarkably similar to those of our findings below (17). The authors speculate that the cats were infected when hunting prey such as small rodents. Later, six of the strains described by Gunn-Moore et al., the majority of them isolated from cats in Kent and Sussex, were identified as having *M. microti*-like spoligotype patterns of the same type as the one we report from this area below (25).

We describe the molecular characteristics of 141 strains of the *M. tuberculosis* complex isolated primarily from cats and identified as *M. microti* by deletion typing and spoligotyping. We show that both the spoligotype pattern diversity and the genotypic diversity of *M. microti* strains isolated in Great Britain are greater than previously thought and that, like *M. bovis* strains from cattle in Great Britain, genotypes of *M. microti* are geographically localized. We argue that *M. microti* infection in cats is probably a spillover from a disease prevalent in wildlife such as the field vole, and we suggest that areas with *M. bovis* infection in cats and *M. microti* infection in cats are geographically distinct.

MATERIALS AND METHODS

Deletion assays. The status of the RD1^{mic} region was assessed by PCR with two sets of primers using heat-killed lysed material from each strain. One PCR targeted the flanking regions of RD1^{mic} (RD1mic Fl Fw, 5'-GCAGTGCAAAGGTGCAGATA, and RD1mic Fl Rv, 5'-GATTGAGACACTTGCCACGA), and a second used a set of primers hybridizing with the internal region of RD1^{mic} (RD1mic Int Fw, 5'-TCCGTACCTTCCGACTATC, and RD1mic Int Rv, 5'-CGGGAAGGTGTTATCCTC). The products were identified by agarose gel electrophoresis; strains with RD1^{mic} intact will generate a product of 642 bp with the internal primer and flanking primer, and strains with RD1^{mic} deleted will generate a product of ~360 bp with the flanking primers only. The statuses of all other RD deletions were assayed in a similar fashion according to the method of Brosch et al. (2).

Spoligotyping and VNTR typing. Strains were spoligotyped according to the method of Kamerbeek et al. (24). VNTR typing targeted the six loci originally described by Frothingham and Meeker-O'Connell (13) according to the protocol described by Cadmus et al. (4). The ETR-F locus contains two types of tandem repeats of different lengths (79 bp and 55 bp). We displayed the repeat number of the 79-bp repeats followed by the repeat number of the 55-bp repeats separated by a period.

TABLE 1. Hosts and frequencies of isolation of spoligotypes^a

Spoligotype pattern		No. of strains from indicated host											Total
International	VLA	Cat	Alpaca	Badger	Llama	Cow	Pig	Ferret	Vole	Human	Giant otter	Pony	
SB0112	19	65	3		2	2	3			1	1	1	78
SB0118	34	19	1	1			1	1	1				24
SB0654	32	4		2	2	1							9
SB0155	31	4	1	1	2								8
SB0326	18		5			1							6
SB0657	3	3											3
SB0655	52			2									2
SB0162	64			1									1
SB1403	95	1											1
SB0988	109					1							1
SB1505	110				1								1
SB1513	111	1											1
SB1507	112	1											1
SB1514	113	1											1
SB1509	115		1										1
SB1510	116						1						1
SB1511	117	1											1
SB1512	118	1											1
Totals	1,369	101	11	7	7	5	5	1	1	1	1	1	141

^a International spoligotype name assigned by www.Mbovis.org. *M. microti* isolates from humans in Great Britain are underrepresented here because those strains are analyzed by the Health Protection Agency (HPA) instead of VLA.

Spatial localization of cats. The distribution of cats in nonurban and urban settlement areas was determined by cat location (Ordnance Survey coordinates translated from the postcode of cat domicile) in relation to 2001 settlement data (owned by Communities and Local Government), which covers England and Wales and corresponds to the 2001 census data. The settlement data were extracted from the Ordnance Survey base maps, as at 1 April 2001. Cats located within areas of population designated >10,000 were considered urban. All cats located outside of these areas were designated nonurban. Maps were drawn using ArcView GIS, Environmental Systems Research Institute, Inc.

Samples for culture. The spoligotype database of the Veterinary Laboratories Agency (VLA) Weybridge was examined for samples showing unusual (nonbovine) spoligotype patterns for the period from 1994 to October 2008. The treatment and culture of tissue are described by Jahans and Worth (22) with the exception that, in this study, nonbovine samples are cultured for up to 14 weeks. Samples from species other than those of cattle and deer were submitted to VLA in an ad hoc manner from private veterinary surgeons prior to 2006. However, the Tuberculosis (England) Order came into force in February 2006 (and similar orders came into force in Wales and Scotland). The orders introduced a legal obligation to report suspicions of tuberculosis in carcasses of any domestic mammal to the Divisional Veterinary Manager. The Department of Environment, Food, and Rural Affairs (Defra) funds a free culture service for mycobacteria to support this obligation (12). Notification of suspicion of tuberculosis in a living domestic animal (other than deer or cattle) is not an obligation under the orders; however, where there is a reasonable suspicion of tuberculosis, the submission of samples is encouraged, and the free mycobacterial culture service is extended to these cases (27). Additionally, the isolation of *M. bovis* strains from animal tissues, secretions, excretions, or carcasses is notifiable to VLA Weybridge. Feline *M. bovis* and *M. microti* infections have very similar clinical and pathological manifestations and can be differentiated only by mycobacterial culture of tissues and molecular typing. These legislative changes, the increasing awareness of *M. bovis* infections in species other than cattle, and the provision of free bacteriological tests for animal tuberculosis at VLA have contributed to the marked rise in the number of nonbovine submissions examined for tuberculosis in recent years. However, over 50% of all feline tuberculosis submissions to VLA fail to yield any mycobacterial isolates whatsoever after the usual extended culture period for nonbovine tissues.

RESULTS

Identification of *M. microti* strains. Over a 14-year period through 2008, 18 unusual spoligotype patterns were identified in 141 cultures of primarily nonbovine isolates at VLA Wey-

bridge, United Kingdom (Table 1). These strains had been identified as members of the *M. tuberculosis* complex by growth characteristics on selective media (22). Strains of this type were most frequently isolated from cats and had distinct genotypes (spoligotype plus VNTR type), compared to the great majority of the 28,500 strains from cattle that had been genotyped during the same period. A sample of the spoligotype patterns from these isolates is shown in Fig. 1. In addition to the loss of spoligotype spacers 3, 9, 16, and 39 to 43, which are missing in all strains of *M. microti*, *M. pinnipedii*, *M. bovis* subsp. *caprae*, and *M. bovis* (34, 35), these strains all had spoligotype patterns with spacers 1 to 3 and spacers 8 to 22 deleted. Among these spoligotype patterns are those previously reported for strains of *M. microti* (SB0118, SB0265, SB0112, SB0657 [25]) and *Mycobacterium pinnipedii* (SB0155 [7]).

Deletion typing was carried out on a sample of these strains to determine their phylogenetic statuses (Table 2). Table 2 shows that all strains sampled with these unusual spoligotype patterns were deleted for RD1^{mic} and that the chromosomal status of the other regions surveyed is compatible with these strains being members of *M. microti*. In particular, these strains were intact at the RD4 and RD12 loci, which distinguishes them from strains of *M. bovis* and *M. bovis* subsp. *caprae*, and were deleted for RD1^{mic}, which distinguishes them from strains of *M. pinnipedii*.

The VNTR profiles for these strains at the ETR-E and -F loci were distinct from those for *M. bovis* isolates from cattle. We assayed the ETR-F locus for 112 of these strains; the locus was virtually monomorphic for a "2.2" allele ($n = 110$ strains; two strains had a "2.1" allele). For the ETR-E locus, assayed in 110 strains, a "1" allele was found in 81 strains, and 29 strains had a "2" allele. If we exclude the five strains from cattle that are part of this study (Table 1), these VNTR results for the ETR-E and -F loci are in contrast to those of over 28,500 strains from cattle in Great Britain, with typical *M. bovis*

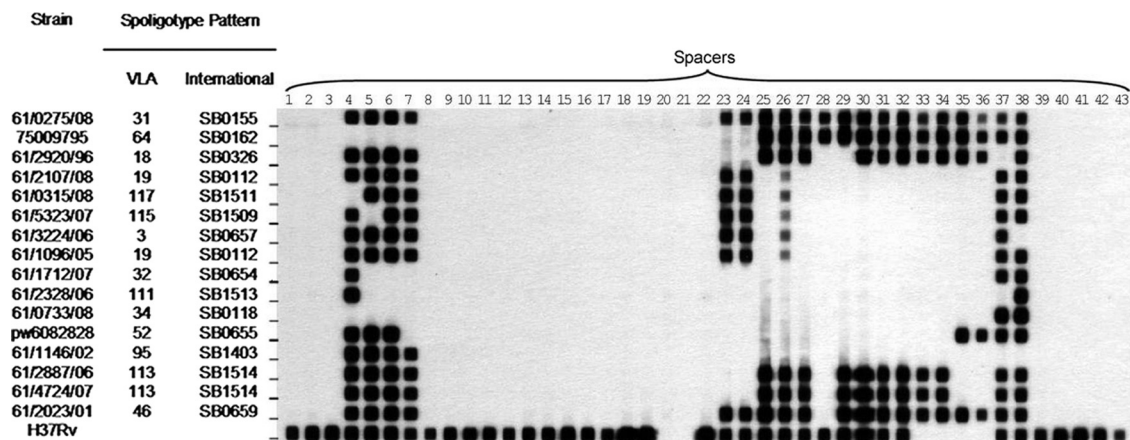


FIG. 1. Sample of *M. microti* spoligotype patterns identified between 1994 and October 2008. VLA names for the spoligotype pattern, the international names for spoligotype patterns assigned by www.Mbovis.org, and the spoligotype patterns are shown. Control strains of *M. pinnipedii* (SB0659, [7]) and *M. tuberculosis* (H37Rv) are included. We also isolated strains with spoligotype patterns SB0988, SB1505, SB1507, SB1510, and SB1512.

spoligotype patterns, for which a full VNTR profile has been obtained. A “2.2” allele was not present at the ETR-F locus of cattle samples, and only one strain from cattle had a “1” allele at the ETR-E locus; furthermore, less than 7.5% of cattle

isolates have a “2” allele at the ETR-E locus. These VNTR results for ETR-E and -F loci in *M. bovis* from cattle in Great Britain are similar in kind to the data from over 250 isolates of *M. bovis* isolated from cattle in Nigeria, Cameroon, Mali, and

TABLE 2. Chromosomal status of RD deletions for a sample of strains^a

Strain	Spoligotype pattern		Chromosomal status of deletions							Species	Host
	International	VLA	RD4	RD7	RD9	RD12	RD13	RD1 ^{mic}	MiD4		
61/0422/06	SB0657	3	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0284/05	SB0657	3	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/2917/96	SB0236	18	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Alpaca
61/0339/08	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/4814/07	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/2316/05	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0477/08	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/4535/07	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/4253/07	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/3883/02	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/1856/05	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0172/08	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/0517/08	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/0688/01	SB0112	19	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Cat
CR6509/94	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
CAR 5514	SB0112	19	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
CAR 9541/94	SB0155	31	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0275/08	SB0155	31	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61-1216-99	SB0155	31	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Llama
61-1216-99	SB0155	31	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Llama
61/2138/06	SB0654	32	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0547/05	SB0654	32	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/3438/00	SB0654	32	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Llama
71002795	SB0654	32	Intact			Intact	Intact	Deleted		<i>M. microti</i>	Badger
61/5510/03	SB0118	34	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/0496/08	SB0118	34	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Vole
61/3312/06	SB0118	34	Intact	Deleted	Deleted	Intact		Deleted	Deleted	<i>M. microti</i>	Cat
61/2785/00	SB0118	34	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Ferret
75009795	SB0162	64			Deleted		Intact	Deleted		<i>M. microti</i>	Badger
61/5323/07	SB1509	115	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Alpaca
61/4724/07	SB1514	113	Intact	Deleted	Deleted	Intact		Deleted		<i>M. microti</i>	Cat
61/2023/01	SB0659	46	Intact	Deleted	Deleted	Intact		Intact	Deleted	<i>M. pinnipedii</i>	Llama
61/1967/01	SB0659	46	Intact	Deleted	Deleted	Intact		Intact	Deleted	<i>M. pinnipedii</i>	Tapir
AF/2122	SB0140	9	Deleted	Deleted	Deleted	Deleted	Deleted	Intact	Intact	<i>M. bovis</i>	Cow
H37Rv			Intact	Intact	Intact	Intact	Intact	Intact	Intact	<i>M. tuberculosis</i>	Human

^a Two strains of *M. pinnipedii* (SB0659) as well as control strains of *M. tuberculosis* and *M. bovis* are also shown.

TABLE 3. Allelic diversity at each VNTR locus for samples from cattle, one selected from each farm (unique CPH), or for the full data set (all isolates), compared with allelic diversity at these loci for the 99 *M. microti* strains^a

Isolate	Sample size	Allelic diversity at:					
		ETR-A	ETR-B	ETR-C	ETR-D	ETR-E	ETR-F
Cattle sample (unique CPH)	9,456	0.44	0.14	0.24	0.47	0.16	0.15
Cattle (all isolates)	28,526	0.45	0.14	0.22	0.47	0.14	0.13
<i>M. microti</i> -like	99	0.60	0.36	0.01	0.64	0.39	0.03

^a Allelic diversity is calculated by the method of Selander et al. (33). CPH, County Parish Holding unique identifier.

Chad (29) and 240 strains of *M. bovis* from France with spoligotype pattern SB0140 (unpublished data). Interestingly, a sample of four *M. microti* strains isolated in The Netherlands and one from Belgium, although differing from Great Britain strains at other loci, shared the same alleles at the ETR-E and -F loci with British strains.

We concluded, based on the distinct deletion profile and the similarities in spoligotype pattern and VNTR type, that these 141 strains are all closely related and represent strains of *M. microti*.

Diversity of *M. microti* strains. Although a true measure of the diversity of *M. microti* is difficult to measure because of sampling bias, we have compared the spoligotype diversity in these 141 strains to the diversity of the *M. bovis* strains isolated in cattle. The allelic diversity (the probability of identifying different types in two isolates chosen at random [33]) of the 141 *M. microti*-like strains based on spoligotype pattern was 0.65 and can be compared with previously published estimates of 0.79, 0.69, and 0.55 for *M. bovis* strains from populations of cattle in Great Britain, the Republic of Ireland, and Northern Ireland, respectively (34). For the 99 *M. microti* isolates with both spoligotype and six-locus VNTR data, the allelic diversity was 0.83, which is comparable with a diversity of 0.90 for a sample of 9,456 strains isolated from cattle in Great Britain during the same period (one isolate randomly selected from each farm that had a bovine tuberculosis breakdown).

We also calculated the allelic diversity for each of the six ETR loci used in VNTR typing. In (Table 3), the allelic diversity for each VNTR locus in the *M. microti* isolates is compared with the same data for a sample of 9,456 strains of *M. bovis* isolated from cattle (described above) and also compared with all *M. bovis* isolates from cattle with full spoligotype and VNTR type. The allelic diversities at each locus are similar for the two cattle samples. However, comparing the allelic diversity between the *M. microti* samples and the cattle samples shows some surprising differences. The ETR-A, -B, -D, and -E loci in *M. microti* strains all exceed the equivalent allelic diversity in cattle strains, whereas the allelic diversity of the ETR-F locus and, especially, the ETR-C locus is close to zero and much lower in the *M. microti* strains compared to that in the *M. bovis* strains.

Geographical localization of genotypes. We noticed a geographical clustering of genotypes in the *M. microti* strains. For example, all strains with spoligotype SB0112 from the Kent, Sussex, and Surrey area were of two closely related VNTR types ($n = 25$), whereas the strains showing the other four VNTR types of this spoligotype pattern were each clustered separately elsewhere in the country ($n = 23$). The host location was available for 82 strains of *M. microti* and included 48

strains of spoligotype SB0112 (VLA type 19), which could be further subdivided into six different VNTR types called 19:a to 19:f. The geographical localization of the five common VNTR types of spoligotype SB0112 (19:a to 19:e) and the four spoligotypes for which we had more than one isolate of each type is shown in Fig. 2A ($n = 64$). For comparison, the geographical localization of the eight common genotypes of the *M. bovis* strains that were isolated from cats at VLA Weybridge is also shown ($n = 35$) (Fig. 2B).

Pathology of *M. microti*. Details of the pathology of *M. microti* strains isolated from cats were available for 61 isolates. The majority of these infected cats were domestic house cats, the mean age of the cats was 8.3 years (range, 3 to 16 years), and 26 were male and 24 female (the sex was not stated for 11 cats). Thirty-one cases of lesions of the skin and superficial lymph nodes of the head were reported, as well as two cases of gingivitis. Eight cases involved the superficial lymph nodes or skin of the limbs and tail; three of these were located at the tail base. An additional six cases involved superficial lymph nodes or skin of the neck and torso. In the remaining 14 cases the lesions indicated more generalized disease with lesions involving a combination of lung, liver, spleen, and mesenteric lymph nodes sometimes with concurrent skin and lymph node lesions.

Where data were available, the pathology seen in the *M. microti*-infected alpacas ($n = 6$), llama, pony, and giant otter all involved generalized disease. Four of the seven badgers with *M. microti* infection had visible lesions, two had no visible lesions, and data were unavailable in one case. The five bovine samples were from cattle that were skin test reactors, and two had lesions visible at postmortem examination. The cattle from which *M. microti* strains were isolated were identified following a short interval skin test (after a confirmed *M. bovis* breakdown; $n = 2$), a routine herd test, a premovement test, and a check test and were geographically dispersed.

Growth of strains. Isolates for culture at VLA Weybridge are routinely put onto solid Stonebrinks, Lowenstein-Jensen (base, with glycerol or pyruvate), and Middlebrook 7H11 (22) media, and the growth characteristics of 36 isolates of *M. microti*, of various spoligotypes, were monitored. The average time to observed growth was 8.5 weeks, although this varied between 3 weeks ($n = 2$) and 12 weeks ($n = 4$). Culture of nonbovine samples is routinely abandoned between 12 and 14 weeks. In general, growth, compared with that of the more commonly isolated *M. bovis*, was recorded as minimal, although in four isolates it was recorded as profuse. The type of medium on which growth occurred was highly variable; 14 isolates grew on Stonebrinks medium only, nine on Middlebrook 7H11 only, and the other isolates grew on two, three, or four medium types. There were no easily discernible relation-

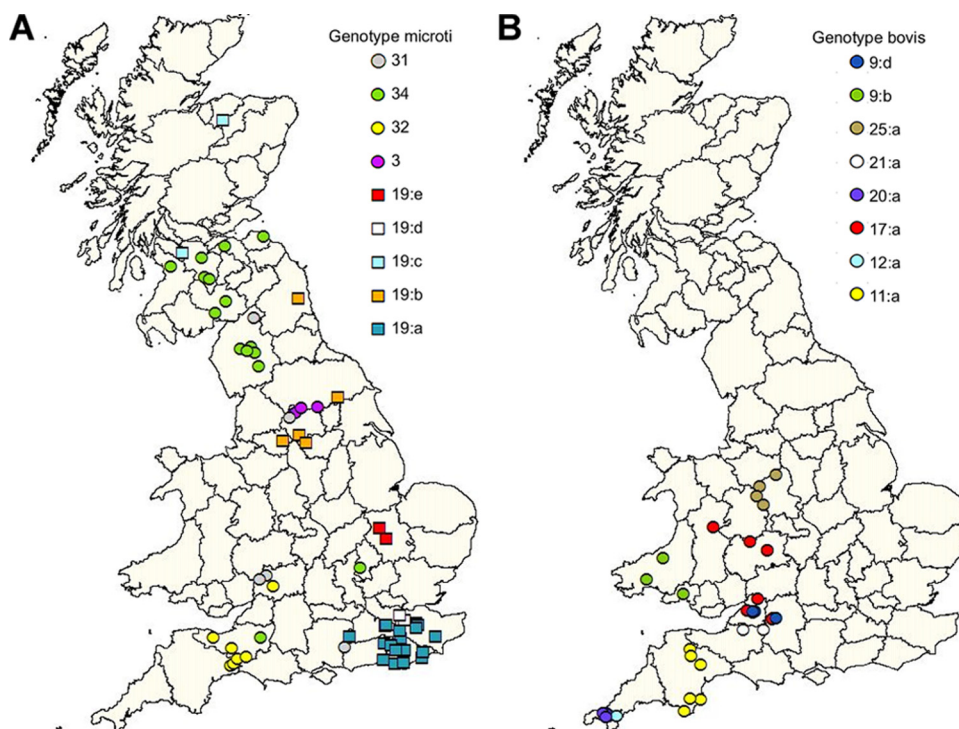


FIG. 2. Geographical localization of the common *M. microti* genotypes of all hosts and the common *M. bovis* genotypes of cats. (A) Locations of the common genotypes (more than a single isolate) of *M. microti*. Four spoligotypes are shown as well as strains with spoligotype SB0112 (VLA type 19) which have been divided into genotypes (19:a to 19:e), using a combination of spoligotype and VNTR type. (B) Locations of *M. bovis* genotypes (spoligotype plus VNTR type), with more than a single isolate, from cats.

ships between the spoligotype patterns of strains and their growth characteristics.

DISCUSSION

We have described 141 strains of the *M. tuberculosis* complex isolated at VLA Weybridge between 1994 and 2008. All strains had spoligotype patterns deleted for spacers 1 to 3 and 8 to 22 and were generally isolated from nonbovine sources—most frequently, domestic cats. The 18 spoligotype patterns of these strains include those traditionally associated with *M. microti* (SB0118) as well as the “llama” type of *M. microti* (SB0112) (1, 8, 25, 30). A sample of these strains was shown to be deleted for phylogenetically informative regions RD7 and RD9 and MiD4 but intact for RD4 and RD12; this deletion profile is distinct from those of strains of *M. bovis* subsp. *caprae* and *M. bovis* and places them in the *M. pinnipedii*-*M. microti* clade of the *M. tuberculosis* complex (2, 20, 35). We show that a sample of these strains was also deleted for chromosomal region RD1^{mic}, which has previously been shown to be intact in all other members of the *M. tuberculosis* complex, including *M. pinnipedii*, and the deletion of which has been previously identified as an *M. microti*-specific deletion (1).

In the absence of recombination, the deletion of a nonrepetitive region of the chromosome cannot be repaired and will mark all the descendants of the cell in which the deletion occurred (29, 34). The group of strains described here, which includes traditional *M. microti* strains, are linked to a recent common ancestor by the deletion RD1^{mic} and have other char-

acteristics in common, including variable/slow growth, similar losses of spoligotype spacers, and isolation from British domestic cats. The close phylogenetic relationship of these strains is also marked by similarities in the VNTR profile, in particular at the ETR-E and ETR-F loci. We therefore suggest that these strains should be referred to as *M. microti* or, perhaps, the vole-adapted ecotype of *M. tuberculosis* (35).

The most complete spoligotype pattern identified here in *M. microti* strains (SB0155) has previously been identified in strains of *M. pinnipedii* from Argentina, Uruguay, and Australia which were isolated, primarily, from several species of fur seals in the Southern hemisphere (7). However, in contrast to strains of *M. pinnipedii*, the British strains with this spoligotype pattern are deleted for RD1^{mic} and were isolated from cats (four strains), camelids (three strains), and a badger, rather than marine mammals (Table 1). This is the first report, to our knowledge, of two species of the *M. tuberculosis* complex sharing the same spoligotype pattern and highlights the dangers of using spoligotype pattern alone to assign a strain to a species. The sharing of a spoligotype pattern reemphasizes the close phylogenetic relationship between *M. pinnipedii* and *M. microti*, marked by the sharing of an MiD4 deletion as well as a distinct deletion profile for RD4, -7, -8, -9, -10, -12, and -13 (1, 2, 20, 27, 28, 35). It is reasonable to assume that the most recent common ancestor of these two species had the spoligotype pattern SB0155, as well as an MiD4 deletion, and that this spoligotype pattern is identical by descent in strains from these two species; *M. microti* strains have region RD1^{mic} deletion during this descent from the most recent common ancestor of

the two species (see reference 14 for an alternative explanation for the presence of MiD4 in these two species). However, convergence of spoligotype pattern in *M. pinnipedii* and *M. microti* cannot be ruled out. All spoligotype patterns for *M. microti* strains reported here can be developed from this putative ancestral spoligotype pattern (SB0155) by loss of spoligotype spacers. The host adaptation of the most recent common ancestor of these two species is unknown; it could be either pinnipeds, rodents, or an as-yet-unidentified host.

For the strains of *M. microti* described here, the growth rate on first isolation in culture and the best medium to use were highly variable between isolates and were not useful characteristics of *M. microti* strains. Some strains grew rapidly on a single medium, while others grew very slowly on several medium types. Problems with the growth of strains of this type have frequently been reported before; in one case, it was reported that growth on solid medium took 6 months (15). In general, most strains of *M. microti* would not be detected in the 6 weeks of culture routinely allowed for the growth of *M. bovis* samples from cattle in Great Britain.

There is no evidence in the data in Table 1 to suggest a difference in host specificity for the different spoligotypes of *M. microti* strains. The commonest and second most common spoligotypes found in cats are also the commonest and second most common types found in other mammals. The five spoligotype SB0326 strains found in alpacas were all isolated from a single herd breakdown in 1996 (Table 1). We also show that *M. microti* infections are capable of causing visible lesions in a variety of mammals, including cattle and badgers, as has been shown previously for pigs (37). However, the frequency of generalized disease in mammals caused by *M. microti* cannot be determined from these data; the decision to culture material from animals other than cattle is frequently determined by the observation of visible lesions. The genotypic diversity of *M. microti* strains in Great Britain is not dissimilar to the genotypic diversity found in *M. bovis* strains isolated from cattle, suggesting that the population of *M. microti* strains is not as restricted as previously thought. For the last 3 years, the ratio of *M. microti* strains from cats to *M. bovis* strains from cats isolated at VLA Weybridge has been fairly constant at 1.5 to 1, also suggesting that *M. microti* is not uncommon in Great Britain.

We find it unlikely that cats, or any of the mammals (other than voles) in Table 1, are the maintenance host for *M. microti* in Great Britain. The incidence rate of *M. microti* infection in cats is very low, and most are associated with extra-urban locations (see below); we assume that the density of the domestic cat population is much higher in urban areas and that a cat-maintained disease would also be at much higher frequency in urban areas. Furthermore, it seems unlikely that the geographical localization of genotypes of *M. microti* (Fig. 2) would be maintained if cats were the maintenance host, as the significant movement of cats with their owners would tend to confuse the distribution. Although infection could be maintained by cats and spread by bite wounding, the gross pathology of disease in the cats implies that in the majority of cases, introduction of infection is via the skin, as 45 of the 61 cases involved the skin or superficial lymph nodes. This is in clear contrast to *M. bovis* infection in cattle, badgers, and camelids in Great Britain. However, if cat-to-cat bite transmission were

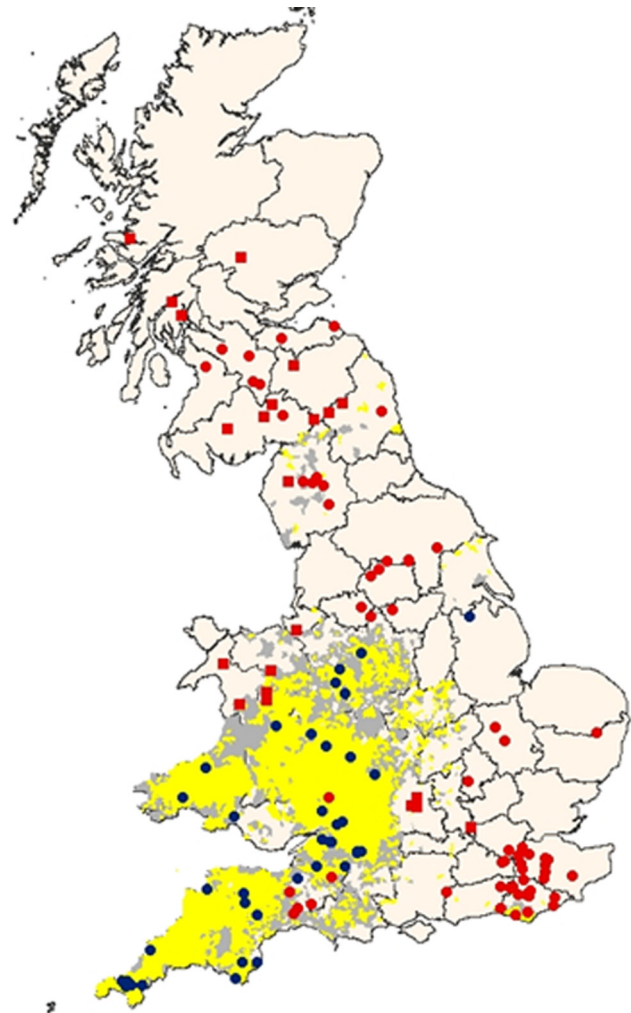


FIG. 3. Location of cats with *M. bovis* infection (black circles) ($n = 43$), cats with *M. microti* infection (red circles) ($n = 67$), captured voles with *M. microti* infection (red squares) (5, 43), and 1- (yellow) and 2-year (gray) testing parishes for bovine tuberculosis in cattle (last quarter of 2008). Two cats, one with *M. microti* infection and one with *M. bovis* infection, are located in unexpected areas, possibly as the result of their owners relocating.

involved, we would expect a greater proportion of the lesions on the tail base, as this is a frequent site for bite wounding by cats.

The distribution of gross lesions on the head in cats is consistent with injuries sustained while hunting, and some cats catch prodigious numbers of birds, mammals, and reptiles (44). We therefore suggest that cats and the mammals other than voles listed in Table 1 are acting as a spillover host for disease present in a wildlife maintenance host; the geographical localization of *M. microti* genotypes would be preserved by the distribution of infection in the wildlife maintenance host. The most likely maintenance host for *M. microti* in Great Britain is the field vole (*Microtus agrestis*), which has been reported as having a high prevalence of the disease in several studies (3, 5, 6, 43).

In Fig. 3, we have plotted the locations of all cats with *M. microti* and *M. bovis* infections, reports of tuberculosis in voles

(5, 43), and 1- and 2-year testing parishes for bovine tuberculosis in cattle. The location of cats with *M. bovis* infection is significantly correlated with 1-year testing parishes for cattle, and the genotypes of *M. bovis* strains found in cats match those found in local cattle, suggesting a common source of infection and that cats are a spillover host (sentinel) for *M. bovis* (27). In the same way, we argue that cats are also a sentinel species for the presence of *M. microti* in local voles. It could be suggested that the two samples of cats infected with different types of tuberculosis were from two different populations; for example, cats with *M. microti* infection could be “city” cats and those infected with *M. bovis* could be “country” cats. Two preliminary analyses were carried out to determine if there were major differences between the samples of cats. First, we used the location of cats to determine if each cat was from an urban or nonurban area. For both cats with *M. microti* infection and cats with *M. bovis* infection, the ratio of cats located in urban areas to cats in nonurban areas was the same: 0.43 (13/30 for cats with *M. bovis* infection and 20/47 for cats with *M. microti* infection). In a further analysis, we estimated the distance from each infected cat to the nearest herd of cattle. The distances for both groups of cats were very similar (an average of 870 meters for cats with *M. microti* infection and 794 meters for cats with *M. bovis* infection). These analyses give us confidence that the two samples of cats are not from dissimilar environments, and the high proportion of cats with *M. microti* and *M. bovis* infections in nonurban areas reinforces the association of these diseases with the countryside rather than the city. However, the results of all these analyses on cat location must be interpreted carefully, as a number of confounding factors and biases, including specific veterinary practices being more aware of *M. microti* infection or feline tuberculosis infections in general, can easily be suggested.

The distribution of *M. microti* strains found in cats and the geographic localization of genotypes suggest that *M. microti* is endemic to certain areas of Great Britain and absent, or at low frequency, in other areas in a manner similar to the distribution of *M. bovis* strains from cattle in Great Britain (34). What is surprising is that those areas to which bovine tuberculosis in cattle is endemic do not seem to overlap with those areas in which cats or voles are infected with *M. microti* (Fig. 3). In support of this observation, we found that cattle herds closest to the location of cats with *M. bovis* infection were tested for bovine tuberculosis more frequently (average testing interval, 1.3 years) than were cattle closest to cats with *M. microti* infection (average testing interval, 3.7 years). Cattle herds in Great Britain are tested for bovine tuberculosis at a variable frequency ranging from 1 to 4 years, depending on the historical incidence of herd breakdowns in a locality (parish). Cats infected with *M. microti* are apparently associated with areas of low tuberculosis incidence, whereas feline *M. bovis* infections are associated with areas of high bovine tuberculosis incidence.

How can *M. microti* in voles and *M. bovis* in cattle be mutually exclusive? The simplest explanation for the absence of cats with *M. microti* infection in high-incidence areas of bovine tuberculosis in cattle is that *M. microti* is not present in the local wildlife although other explanations are possible. Bovine tuberculosis is known to be pathogenic for field voles (9, 23); however, major surveys of small mammals in high-incidence

areas of bovine tuberculosis have not identified a high frequency of *M. bovis* infection (9, 10, 26).

The absence of *M. bovis* infection in cattle in areas where *M. microti* infection is common may have a more interesting explanation. Badgers are involved in the maintenance of *M. bovis* in large tracts of Great Britain (11), and one possible explanation for the geographical separation of *M. microti* and *M. bovis* is that infection with *M. microti* in badgers provides some protection from *M. bovis* infection. We argue that if cats are a spillover host for *M. microti* that is prevalent in voles and if they are infected while hunting small mammals, then it is reasonable to suggest that, in these areas, badgers, large omnivores, may also be a spillover host for *M. microti* infection. If badgers are infected with *M. microti*, it is not unreasonable to suggest that they have some protective immunity from *M. bovis* infection, and this may explain, in some part, the low incidence of bovine tuberculosis in local cattle in those areas where *M. microti* is apparently endemic. The protective effect of one species of the *M. tuberculosis* complex against infection with another species has been shown with both *M. microti* (referenced above) and *M. bovis* BCG (32) for *M. tuberculosis* in humans, and in cattle, the protective effect of *M. tuberculosis* against *M. bovis* was discussed by Emil von Behring in his Nobel Prize acceptance lecture (http://nobelprize.org/nobel_prizes/medicine/laureates/1901/behring-lecture.html). We find it plausible that in areas where *M. microti* is at high frequency in wildlife, strains of *M. bovis* have been unable to establish themselves in the local badger population because of cross-immunity with *M. microti*. It is interesting to note that throughout Ireland, where there are no native voles (46) and presumably no *M. microti*, bovine tuberculosis is prevalent in both cattle and badgers.

ACKNOWLEDGMENTS

We thank K. Gover, M. Okker, P. Upton, and J. Dale for their excellent technical assistance.

This work was funded by the Department of Environment, Food and Rural Affairs, United Kingdom (project code SB4020).

REFERENCES

- Brodin, P., K. Eiglmeier, M. Marmiesse, A. Billault, T. Garnier, S. Niemann, S. T. Cole, and R. Brosch. 2002. Bacterial artificial chromosome-based comparative genomic analysis identifies *Mycobacterium microti* as a natural ESAT-6 deletion mutant. *Infect. Immun.* **70**:5568–5578.
- Brosch, R., S. V. Gordon, M. Marmiesse, P. Brodin, C. Buchrieser, K. Eiglmeier, T. Garnier, C. Gutierrez, G. Hewinson, K. Kremer, L. M. Parsons, A. S. Pym, S. Samper, D. van Soolingen, and S. T. Cole. 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci. USA* **99**:3684–3689.
- Burthe, S., M. Bennett, A. Kipar, X. Lambin, A. Smith, S. Telfer, and M. Begon. 2008. Tuberculosis (*Mycobacterium microti*) in wild field vole populations. *Parasitology* **135**:309–317.
- Cadmus, S., S. Palmer, M. Okker, J. Dale, K. Gover, N. Smith, K. Jahans, R. G. Hewinson, and S. V. Gordon. 2006. Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. *J. Clin. Microbiol.* **44**:29–34.
- Cavanagh, R., M. Begon, M. Bennett, T. Ergon, I. M. Graham, P. E. De Haas, C. A. Hart, M. Koedam, K. Kremer, X. Lambin, P. Roholl, and D. van Soolingen. 2002. *Mycobacterium microti* infection (vole tuberculosis) in wild rodent populations. *J. Clin. Microbiol.* **40**:3281–3285.
- Chitty, D. 1954. Tuberculosis among wild voles: with a discussion of other pathological conditions among certain mammals and birds. *Ecology* **35**:227–237.
- Cousins, D. V., R. Bastida, A. Cataldi, V. Quse, S. Redrobe, S. Dow, P. Dugnan, A. Murray, C. Dupont, N. Ahmed, D. M. Collins, W. R. Butler, D. Dawson, D. Rodr guez, J. Loureiro, M. I. Romano, A. Alito, M. Zumarraga, and A. Bernardelli. 2003. Tuberculosis in seals caused by a novel member of the *Mycobacterium tuberculosis* complex: *Mycobacterium pinnipedii* sp. nov. *Int. J. Syst. Evol. Microbiol.* **53**:1305–1314.

8. Deforges, L., H. J. Boulouis, J. L. Thibaud, L. Boulouha, W. Sougakoff, S. Blot, G. Hewinson, C. Truffot-Pernot, and N. Haddad. 2004. First isolation of *Mycobacterium microti* (llama-type) from a dog. *Vet. Microbiol.* **103**:249–253.
9. Delahay, R. J., A. N. De Leeuw, A. M. Barlow, R. S. Clifton-Hadley, and C. L. Cheeseman. 2002. The status of *Mycobacterium bovis* infection in UK wild mammals: a review. *Vet. J.* **164**:90–105.
10. Delahay, R. J., G. C. Smith, A. M. Barlow, N. Walker, A. Harris, R. S. Clifton-Hadley, and C. L. Cheeseman. 2007. Bovine tuberculosis infection in wild mammals in the south-west region of England: a survey of prevalence and a semi-quantitative assessment of the relative risks to cattle. *Vet. J.* **173**:287–301.
11. Donnelly, C. A., G. Wei, W. T. Johnston, D. R. Cox, R. Woodroffe, F. J. Bourne, C. L. Cheeseman, R. S. Clifton-Hadley, G. Gettinby, P. Gilks, H. E. Jenkins, A. M. Le Fevre, J. P. McNerney, and W. I. Morrison. 2007. Impacts of widespread badger culling on cattle tuberculosis: concluding analyses from a large-scale field trial. *Int. J. Infect. Dis.* **11**:300–308.
12. Edwards, T. 2006. New Tuberculosis (England) Order. *Vet. Rec.* **158**:246.
13. Frothingham, R., and W. A. Meeker-O'Connell. 1998. Genetic diversity in the *Mycobacterium tuberculosis* complex based on variable numbers of tandem DNA repeats. *Microbiology* **144**:1189–1196.
14. Garcia-Pelayo, M. C., K. C. Caimi, J. K. Inwald, J. Hinds, F. Bigi, M. I. Romano, D. van Soolingen, R. G. Hewinson, A. Cataldi, and S. V. Gordon. 2004. Microarray analysis of *Mycobacterium microti* reveals deletion of genes encoding PE-PPE proteins and ESAT-6 family antigens. *Tuberculosis (Edinburgh)* **84**:159–166.
15. Geiss, H. K., R. Feldhues, S. Niemann, O. Nolte, and R. Rieker. 2005. Landouzy septicemia (sepsis tuberculosa acutissima) due to *Mycobacterium microti* in an immunocompetent man. *Infection* **33**:393–396.
16. Groenen, P. M., A. E. Bunschoten, D. van Soolingen, and J. D. van Embden. 1993. Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*; application for strain differentiation by a novel typing method. *Mol. Microbiol.* **10**:1057–1065.
17. Gunn-Moore, D. A., P. A. Jenkins, and V. M. Lucke. 1996. Feline tuberculosis: a literature review and discussion of 19 cases caused by an unusual mycobacterial variant. *Vet. Rec.* **138**:53–58.
18. Harris, S., and D. W. Yalden. 2008. Mammals of the British Isles: handbook, 4th ed. Mammal Society, Southampton, United Kingdom.
19. Hart, P. D., and I. Sutherland. 1977. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early adult life. *Br. Med. J.* **2**:293–295.
20. Huard, R. C., M. Fabre, P. de Haas, L. C. Lazzarini, D. van Soolingen, D. Cousins, and J. L. Ho. 2006. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. *J. Bacteriol.* **188**:4271–4287.
21. Jahans, K., S. Palmer, J. Inwald, J. Brown, and S. Abayakoon. 2004. Isolation of *Mycobacterium microti* from a male Charolais-Hereford cross. *Vet. Rec.* **155**:373–374.
22. Jahans, K., and D. Worth. 2006. The laboratory diagnosis of bovine tuberculosis. *Gov. Vet. J.* **16**:53–57.
23. Jespersen, A. 1976. Multiplication of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in *Microtus agrestis* (field vole). *Acta Pathol. Microbiol. Scand. [B]*. **84**:57–60.
24. Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J. Clin. Microbiol.* **35**:907–914.
25. Kremer, K., D. van Soolingen, J. van Embden, S. Hughes, J. Inwald, and G. Hewinson. 1998. *Mycobacterium microti*: more widespread than previously thought. *J. Clin. Microbiol.* **36**:2793–2794.
26. Mathews, F., D. W. Macdonald, G. M. Taylor, M. Gelling, R. A. Norman, P. E. Honess, R. Foster, C. M. Gower, S. Varley, A. Harris, S. Palmer, G. Hewinson, and J. P. Webster. 2006. Bovine tuberculosis (*Mycobacterium bovis*) in British farmland wildlife: the importance to agriculture. *Proc. Biol. Sci.* **273**:357–365.
27. Monies, B., R. de la Rúa, and K. Jahans. 2006. Bovine tuberculosis in cats. *Vet. Rec.* **158**:490–491.
28. Mostowy, S., D. Cousins, J. Brinkman, A. Aranaz, and M. A. Behr. 2002. Genomic deletions suggest a phylogeny for the *Mycobacterium tuberculosis* complex. *J. Infect. Dis.* **186**:74–80.
29. Müller, B., M. Hilty, S. Berg, M. C. Garcia-Pelayo, J. Dale, M. L. Boschirolì, S. Cadmus, B. Nare, R. Ngandolo, S. Godreuil, C. Diguimbaye-Djaibe, R. Kazwala, B. Bonfoh, B. M. Njanpop-Lafourcade, N. Sahraoui, D. Guetarni, A. Aseffa, M. H. Mekonnen, V. Rasolofoa Razanamparany, H. Ramarokoto, B. Djonje, J. Oloya, A. Machado, C. Mucavele, E. Skjerve, F. Portaels, L. Rigouts, A. Michel, A. Muller, G. Kallenius, P. D. van Helden, R. G. Hewinson, J. Zinsstag, S. V. Gordon, and N. H. Smith. 2009. African 1, an epidemiologically important clonal complex of *Mycobacterium bovis* dominant in Mali, Nigeria, Cameroon, and Chad. *J. Bacteriol.* **191**:1951–1960.
30. Niemann, S., E. Richter, H. Dalügge-Tamm, H. Schlesinger, D. Graupner, B. Königstein, G. Gurath, U. Greiner, and S. Risch-Gerdes. 2000. Two cases of *Mycobacterium microti* derived tuberculosis in HIV-negative immunocompetent patients. *Emerg. Infect. Dis.* **6**:539–542.
31. Paul, R. 1961. The effects of vole bacillus vaccination of African mine workers in the northern Rhodesian copper mines. *Br. J. Ind. Med.* **18**:148.
32. Ritz, N., W. A. Hanekom, R. Robins-Browne, W. J. Britton, and N. Curtis. 2008. Influence of BCG vaccine strain on the immune response and protection against tuberculosis. *FEMS Microbiol. Rev.* **32**:821–841.
33. Selander, R. K., D. A. Caugant, H. Ochman, J. M. Musser, M. N. Gilmour, and T. S. Whittam. 1986. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Appl. Environ. Microbiol.* **51**:873–884.
34. Smith, N. H., S. V. Gordon, R. de la Rúa-Domenech, R. S. Clifton-Hadley, and R. G. Hewinson. 2006. Bottlenecks and broomsticks: the molecular evolution of *Mycobacterium bovis*. *Nat. Rev. Microbiol.* **4**:670–681.
35. Smith, N. H., K. Kremer, J. Inwald, J. Dale, J. R. Driscoll, S. V. Gordon, D. van Soolingen, R. G. Hewinson, and J. M. Smith. 2006. Ecotypes of the *Mycobacterium tuberculosis* complex. *J. Theor. Biol.* **239**:220–225.
36. Sula, L., and I. Radkovsky. 1976. Protective effects of *M. microti* vaccine against tuberculosis. *J. Hyg. Epidemiol. Microbiol. Immunol.* **20**:1–6.
37. Taylor, C., K. Jahans, S. Palmer, M. Okker, J. Brown, and K. Steer. 2006. *Mycobacterium microti* isolated from two pigs. *Vet. Rec.* **159**:59–60.
38. van der Zanden, A. G., A. H. Hoentjen, F. G. Heilmann, E. F. Weltevreden, L. M. Schouls, and J. D. van Embden. 1998. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* complex in paraffin wax embedded tissues and in stained microscopic preparations. *Mol. Pathol.* **51**:209–214.
39. van Embden, J. D., T. van Gorkom, K. Kremer, R. Jansen, B. A. van Der Zeijst, and L. M. Schouls. 2000. Genetic variation and evolutionary origin of the direct repeat locus of *Mycobacterium tuberculosis* complex bacteria. *J. Bacteriol.* **182**:2393–2401.
40. van Soolingen, D. 1998. Utility of molecular epidemiology of tuberculosis. *Eur. Respir. J.* **11**:795–797.
41. van Soolingen, D., A. G. van der Zanden, P. E. de Haas, G. T. Noordhoek, A. Kiers, N. A. Foudraine, F. Portaels, A. H. Kolk, K. Kremer, and J. D. van Embden. 1998. Diagnosis of *Mycobacterium microti* infections among humans by using novel genetic markers. *J. Clin. Microbiol.* **36**:1840–1845.
42. Wayne, L. G., and G. P. Kubica. 1986. *The Mycobacteria*. The Williams and Wilkins Co., Baltimore, MD.
43. Wells, A. Q. 1946. The murine type of tubercle bacillus (the vole acid-fast bacillus). Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom.
44. Woods, M., R. A. McDonald, and S. Harris. 2003. Predation of wildlife by domestic cats *Felis catus* in Great Britain. *Mammal Rev.* **33**:174–188.
45. Xavier Emmanuel, F., A. L. Seagar, C. Doig, A. Rayner, P. Claxton, and I. Laurenson. 2007. Human and animal infections with *Mycobacterium microti*, Scotland. *Emerg. Infect. Dis.* **13**:1924–1927.
46. Yalden, D. 1999. *The history of British mammals*. T & AD Poyser Natural History, London, United Kingdom.