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Infection with *Mycobacterium microti* in Animals in France

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We describe here 35 animal cases of tuberculosis due to *Mycobacterium microti* in France (2002–2014). Recently, molecular tools that overcome the difficulty of confirming infection by this potentially zoonotic agent have revealed an increasing number of cases, suggesting that its prevalence may have been underestimated.

Mycobacterium microti belongs to the *Mycobacterium tuberculosis* complex (MTC), which also includes *M. tuberculosis*, *Mycobacterium bovis*, *Mycobacterium caprae*, *Mycobacterium africanum*, and *Mycobacterium pinnipedii*. This species was originally described as the cause of tuberculosis (TB) in wild rodents. In-

deed, field voles (*Microtus agrestis*), bank voles (*Myodes glareolus*), wood mice (*Apodemus sylvaticus*), and shrews (*Sorex araneus*), which are particularly susceptible to *M. microti* infection, constitute its natural reservoirs (1). However, an increasing number of cases have also been reported in domestic and wild mammals (2), such as cats (3, 4), pigs (5), European wild boar (*Sus scrofa*) (6), ferrets (*Mustela putorius*), badgers (*Meles meles*) (4), New World camelids (*Lama glama* and *Vicugna pacos*) (4), squirrel monkeys (*Saimiri sciureus*) (7), meerkats (*Suricata suricatta*) (8), and a dog (9). Until now, 27 cases of *M. microti* infection in both immunocompetent (10) and immunocompromised human patients (11) have been described (12), demonstrating its capacity for causing clinical illness and thus for being a potential zoonotic agent.

In the first described animal cases with *M. microti*, the infection was due to bacilli of limited genotypic diversity, as determined by spoligotyping, with the vole-type SB0118 and the llama-types SB0112 and SB0423 (13, 14) being dominant. However, Smith et al. (15) recently showed that this species is more diverse than was expected, and they described 15 new spoligotype patterns for *M. microti*. Moreover, a number of spoligotypes described in *M. microti* infection cases in humans have also been reported, although they were not submitted to the spoligotype database, confirming an even larger diversity for this mycobacterium (4, 12).

The present study, which is the first retrospective analysis of *M. microti* infection in animals in France, describes the animal *M. microti* infection cases identified by the Tuberculosis National Reference Laboratory in France from May 2002 to April 2014, with a particular focus on genotypic diversity by spoligotyping and the geographic localization of the pathogen. Here, we describe 35 cases in a large variety of domestic and wild animal species. The individuals presenting with TB-like lesions had tissues analyzed by

TABLE 1 List of *M. microti* infection cases in various hosts reported in 9 regions of France between 2002 and 2014 and the associated spoligopatterns

Yr	Host	Region	Spoligopattern	Identification method
2002	Dog	Limousin	SB2273	Bacteriology
2003	Cat	Brittany	SB0162	Molecular biology
2004	Cat	Île de France	SB0118	Bacteriology
2005	Llama	Midi-Pyrénées	SB0423	Bacteriology
2006	Cat	Languedoc-Roussillon	SB0112	Molecular biology
2007	Cat	Brittany	SB0118	Bacteriology
2007	Otter	Brittany	SB0162	Bacteriology
2007	Cat	Île de France	SB0118	Molecular biology
2008	Marmosets	Brittany	SB0118	Bacteriology
2009	Marmosets	Brittany	SB0118	Bacteriology
2009	Cat	Brittany	SB0118	Molecular biology
2010	Cat	Rhône-Alpes	SB0118	Molecular biology
2010	Cat	Midi-Pyrénées	SB0112	Molecular biology
2010	Cat	Brittany	SB2274	Molecular biology
2011	Cat	Rhône-Alpes	SB0118	Molecular biology
2011	Cat	Rhône-Alpes	SB0118	Molecular biology
2011	Dog	Midi-Pyrénées	SB0112	Bacteriology
2011	Pig	Brittany	SB2275	Molecular biology
2011	Cat	Rhône-Alpes	SB0112	Molecular biology
2012	Cat	Burgundy	SB0118	Molecular biology
2012	Cat	Limousin	SB2273 partial 1	Molecular biology
2012	Cat	Midi-Pyrénées	SB0112	Molecular biology
2013	Cat	Brittany	SB0162	Molecular biology
2013	Cat	Île de France	SB0118	Molecular biology
2013	Dog	Midi-Pyrénées	SB0112	Molecular biology
2013	Wild boar	Burgundy	SB0118	Molecular biology
2013	Cat	Brittany	SB0118	Molecular biology
2013	Wild boar	Aquitaine	SB2273 partial 2	Molecular biology
2013	Wild boar	Aquitaine	SB2273	Molecular biology
2013	Wild boar	Aquitaine	SB0118	Molecular biology
2013	Wild boar	Aquitaine	SB0118	Molecular biology
2013	Alpaca	Pays de Loire	SB0118	Molecular biology
2014	Wild boar	Aquitaine	SB2273 partial 3	Molecular biology
2014	Wild boar	Aquitaine	SB2272	Molecular biology
2014	Cat	Brittany	SB2276	Molecular biology

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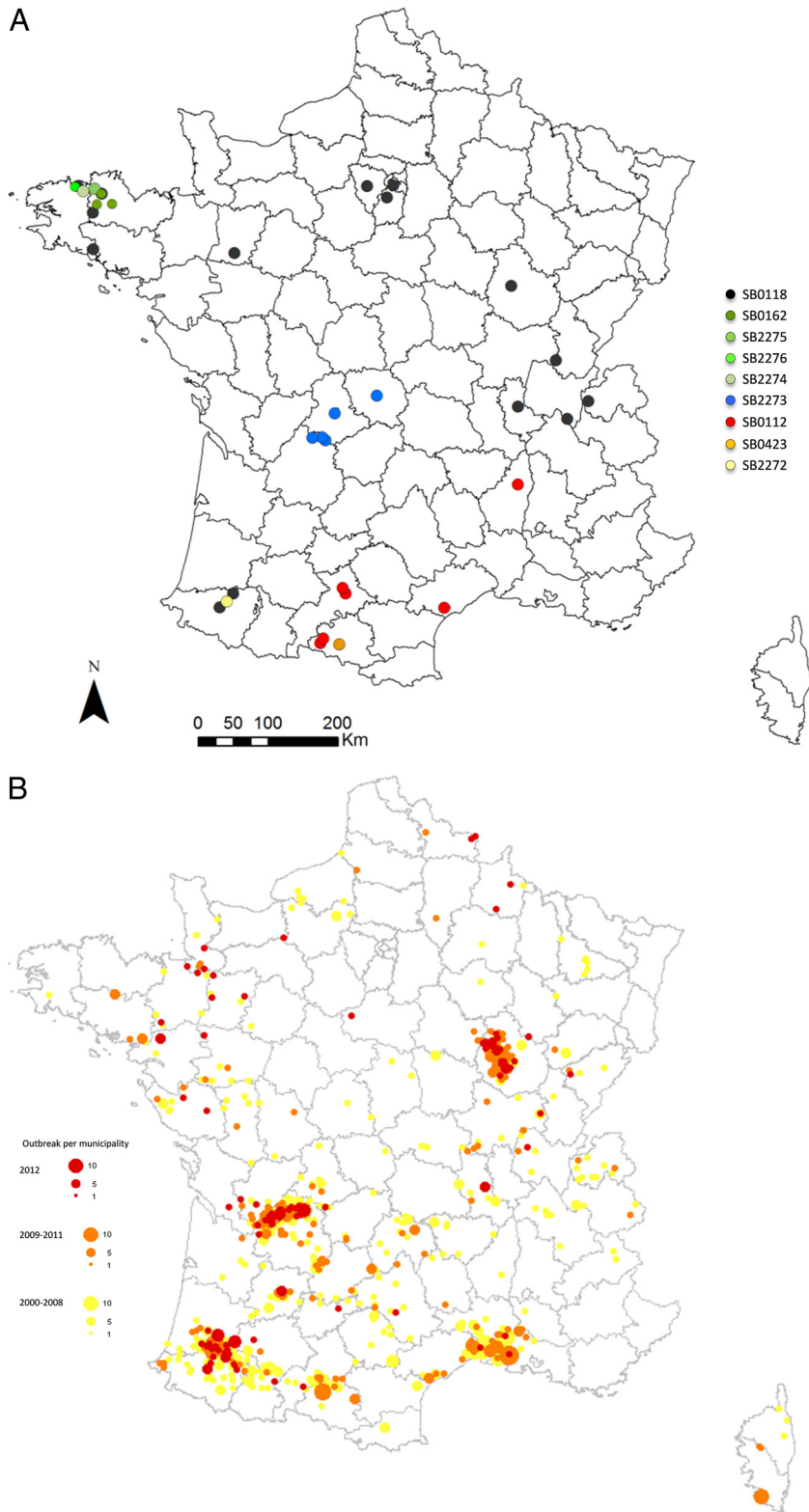


FIG 1 (A) Distribution of *M. microti* cases in France and their associated spoligopatterns. (B) Distribution by municipality of *M. bovis* outbreaks (adapted from reference 25).

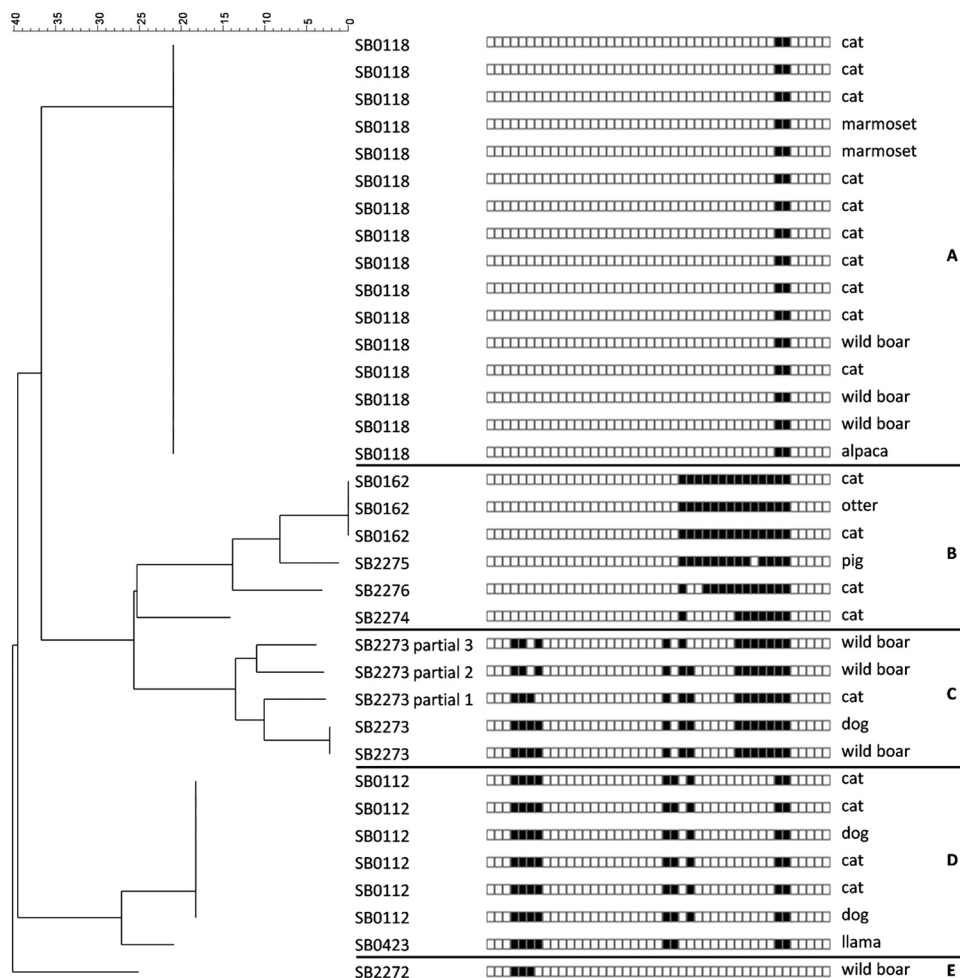


FIG 2 Neighbor-joining tree based on spoligotype patterns identified in France. The letters on the right indicate the groups A to E.

bacteriology and, from 2005, also by molecular technologies; spoligotyping was performed by Luminex either on MTC isolates or directly onto PCR-positive samples (16, 17) (Table 1). With the PCR-positive samples, in which the DNA concentration of the pathogen can be low, partial profiles were sometimes obtained. Bacteriology was positive for only eight of the cases (Table 1). The majority of the cases were in cats ($n = 19$ [54%]). The other infected animal species were wild boar ($n = 7$ [20%]), dogs ($n = 3$ [8%]), marmosets (*Callithrix jacchus*) ($n = 2$ [6%]), llamas or alpacas ($n = 2$ [6%]), a pig ($n = 1$ [3%]), and an otter (*Lutra lutra*) ($n = 1$ [3%]). The spatial distribution of the cases was obtained with the ArcGIS 9 software (Fig. 1A). As it is present in nine out of 22 French mainland regions, *M. microti* infection seems to be evenly distributed throughout the country.

The first case of *M. microti* infection described in France was identified by isolating this agent from a dog (9). Some years later, a study of six human cases of pulmonary TB due to *M. microti* was published (12). Recently, a case of feline cutaneous TB was described in Burgundy, eastern central France (18), but molecular characterization for this case was not performed. The number of cases reported in this study increased in the last few years. This increasing recognition is the result of the improved sensitivities of the diagnostic tests employed (mainly molecular typing tools),

increased awareness about the infection, and the inclusion of wildlife cases studied using molecular biology techniques since 2013. This suggests that *M. microti* is circulating both in domestic and wild populations and that the prevalence of this pathogen may have been underestimated before the introduction of molecular techniques. Indeed, until recently, wildlife TB cases were confirmed by bacteriology only, which has a very poor sensitivity for diagnosing *M. microti*, even compared to that for *M. bovis* (14, 15), which is also a quite culture-recalcitrant bacterium.

Genetic diversity. The spoligopatterns were determined for all cases in the current study and appear to form 5 groups on the neighbor-joining tree built with the BioNumerics software (Fig. 2). The authoritative names for the spoligopatterns (prefix of SB, followed by four digits) were obtained from the *M. bovis* Spoligotype Database website (<http://www.Mbovis.org>) (19). As suggested in the literature, the genetic diversity of *M. microti* seems to be greater than was previously thought (15). Until now, 28 different spoligopatterns of this MTC species (Table 2) were described in the literature, but only 24 were submitted to the *M. bovis* database. All spoligopatterns of *M. microti* are supposed to derive from a putative ancestral pattern, SB0155, also described in *M. pinnipedii* (15, 20). Spoligopatterns result from the unidirectional evolution of the direct repeat region, with accumulations of deletions

TABLE 2 Spoligopatterns of *M. microti* described in the literature and in this study

SB no. ^a	Spoligopattern	Source(s) ^a	Reference(s)
SB0155	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat, llama, alpaca, badger	15
SB1510	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Pig	15
SB0326	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Alpaca, cow	15
SB1514	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	15
SB0988 ◆◆◆◆◆◆◆◆◆◆◆◆	Cow	15
SB0162 ◆◆◆◆◆◆◆◆◆◆◆◆	Badger, cat, otter	15
SB2275 ◆◆◆◆◆◆◆◆◆◆◆◆	Pig	
SB2276 ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	
SB2274 ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	
SB2273	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Wild boar, dog, cat	
ND	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Human	12
ND	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Human	12
SB0655	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Badger	15
SB1403	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	15
SB2272	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Wild boar	
SB0112	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Alpaca, llama, pig, human, otter, cat, dog	9–11, 13–15
SB1505	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Llama	15
SB1509	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Alpaca	15
SB1511	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	15
SB0657	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	13, 15
SB0423	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat, human, llama	4, 12, 13
ND	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Human	4
SB0654	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat, llama, badger, cow	13, 15
SB1513	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Cat	15
SB0118	... ◆◆◆◆ ◆◆◆◆◆◆◆◆◆◆◆◆	Vole, ferret, llama, badger, pig, human, meerkat, squirrel monkey, cat, wild boar, alpaca, marmoset	3, 4, 7, 8, 10, 12–15
ND ◆ ◆	Human	12
SB1512 ◆ ◆	Cat	15
SB2277 ◆ ◆	Wild boar	6

^a The spoligotype and sources of French cases are indicated in bold; ND, not determined.

leading to new patterns. The most interesting case is SB2277, recently described in an Italian wild boar, for which the direct repeat (DR) region lacks the classic 43 spacers (6). Here, we describe nine different spoligopatterns of *M. microti* for only 35 cases, four of which had already been described in the past, and five of which are new patterns. SB0118 (vole type), which is one of the main spoligopatterns described in the literature (13, 14), is also the most common (16 cases) and the most widespread in France (group A). Another well-described spoligopattern (4, 13, 15), SB0112 (llama type), with six identified cases, is the second most important pattern in France. This pattern has only a one-spacer difference with SB0423, which was described in French human cases (12, 13). These close patterns form a clear group on the neighbor-joining analysis and, moreover, seem to be colocalized in the south of France (group C). SB0162, reported previously in a badger (15), is described here in three cases. This pattern forms group B, with three new spoligotypes, SB2274 to SB2276, and has the same geographical localization, i.e., Brittany, in the northwest France. A last cluster, group D, was identified on the neighbor-joining tree and is composed of cases with a new pattern, SB2273, either complete or partial. This group seems to be clearly localized in the center of France. A new spoligotype, SB2272, is described here for the first time in a wild boar. This pattern is very close to the SB1403 pattern described in a cat in Britain (15).

Epidemiology of *M. microti*. As previously demonstrated in Britain (15), this study suggests that *M. microti* is endemic in certain areas in France and absent in other areas; however, the number of cases in our study is less than that in the British study. Some authors hypothesize that in regions where *M. microti* infection is endemic in badgers or cats, it might provide some protection to cattle against *M. bovis* infection and thus explain the low incidence of bovine TB (15, 21). This protective immunity to *M. bovis* appears not to apply to the wild boar, which seem to be involved in the maintenance of *M. bovis* in some of the described regions, or for cattle, as new bovine TB outbreaks are discovered every year in these same regions. Indeed, *M. microti* and *M. bovis* infections overlap in some areas (Fig. 1A and B), especially in those regions where the wild boar was found to be infected by *M. microti*, i.e., in Aquitaine (Southwest France) and Burgundy (eastern central France). The prevalences of *M. bovis* and *M. microti* in wild boar of Aquitaine, through the surveillance of wildlife in the last 2 years, have been estimated to be 2.5% and 0.5%, respectively. A serological survey of wild boar conducted in France highlighted the distribution of animals exposed to MTC (mainly *M. bovis* and *M. microti*), which overlapped the distribution of bovine TB (bTB) outbreaks in cattle (22) and correspond to the same regions where we found wild boar infected with *M. microti*. These results do not support the hypothesis of competition between *M. microti* and *M.*

bovis and suggest that these mycobacteria do not completely exclude each other geographically in France.

Zoonotic transmission might occur from rodents to humans via spillover hosts, such as cats, South American camelids, or dogs, or via environmental contamination environments. Indeed, small rodent populations are widely distributed not only in rural habitats but also in periurban regions. Potential zoonotic infections have been suggested to involve cats (23), mice (14), and either a raccoon or raccoon dogs (24). Even if *M. microti* is considered a rare human pathogen, its potential to cause clinical illness in humans has been demonstrated. Therefore, the accurate surveillance of TB using molecular tools that overcome the particular difficulty of confirming *M. microti* by bacteriology would help characterize the epidemiology of this pathogen.

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