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Isolation of *Brucella microti* from Mandibular Lymph Nodes of Red Foxes, *Vulpes vulpes*, in Lower Austria

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Abstract

From the mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) hunted in the region of Gmünd, Lower Austria, two gram-negative, oxidase- and urease-positive, coccoid rod-shaped bacteria (strains 257 and 284) were isolated. Cells were fast growing, nonmotile, and agglutinated with monospecific anti-*Brucella* (M) serum. Both strains were biochemically identified as *Ochrobactrum anthropi* by using the API 20NE test. However, sequencing of the 16S rRNA and *recA* genes clearly identified strains 257 and 284 as *Brucella* spp. Further molecular analysis by *omp2a/b* gene sequencing, multilocus sequence typing and multilocus variable number tandem repeats analysis revealed *Brucella microti,* a recently described *Brucella* species that has originally been isolated from diseased common voles (*Microtus arvalis*) in South Moravia, Czech Republic in 2000. Our findings demonstrate that *B. microti* is prevalent in a larger geographic area covering the region of South Moravia and parts of Lower Austria. Foxes could have become infected by ingestion of infected common voles.

Key Words: *Brucella microti*; Red foxes; Austria; South Moravia; Wide geographic distribution

Introduction

Recently, we reported the isolation of an atypical *Brucella* isolate from the common vole *Microtus arvalis* in the region of South Moravia, Czech Republic (Hubálek et al. 2007). This isolate was later described as a novel *Brucella* species, termed *B. microti* (Scholz et al. 2008a). Unlike the other *Brucella* species, *B. microti* is characterized by nonfastidious, rapid growth on standard media and its biochemical profile is similar to that of *Ochrobactrum* (*O.*), a facultatively pathogenic bacterial genus that is genetically and phylogenetically closely related to *Brucella* (Scholz et al. 2008b).

Here we report the isolation of *B. microti* (strains 257 and 284) in the year 2007 from the mandibular lymph nodes of red foxes in the region of Gmünd in Lower Austria, located approximately 130 km southwest of the region where *B. microti* was isolated for the first time in 2000.

Methods

The foxes were hunted in two different hunting grounds 20 km apart during a rabies screening program. Besides screening for rabies, animals are routinely screened for *Francisella tularensis*, the causative agent of tularemia, and the presence of *Brucella*, in particular *B. suis* biovar 2, which is known to have a reservoir in wild animals like hares and wild boars (Hubálek et al. 1993, Al Dahouk et al. 2005).

Amplification and sequencing of the *omp2a/b* genes was carried out as described previously (Cloeckaert et al. 1995, Cloeckaert et al. 2001, Scholz et al. 2008a).

Results

Strains 257 and 284 agglutinated with monospecific anti-*Brucella* (M) serum but the rapid and nonfastidious growth on standard media (colonies of 2–3 mm diameter within 24

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h) was unusual for *Brucella*. As previously described for *B. microti* isolated from the common voles (Hubálek et al. 2007, Scholz et al. 2008a), strains 257 and 284 were biochemically identified as "*O. anthropi*" by using the API 20NE system. Molecular analysis by 16S rRNA and *recA* gene sequencing (Scholz et al. 2008b), however, clearly identified both isolates as *Brucella* spp. with 100% sequence identity to the other *Brucella* species in both genes.

Amplification and sequencing of the *omp2a/b* genes showed that both strains exhibited the characteristic combination of *omp2a* and *omp2b* as described for *B. microti* and some *B. suis* biovars (Scholz et al. 2008a). Sequence identities of *omp2a* of both strains were 100% to *B. microti* strains CCM 4915^T and CCM 4916, and 99% to *B. suis* biovar (bv) 5 (strain NCTC 11996), respectively (not shown). The *omp2b* genes of strains 257 (AM943809) and 284 (AM943810) again were identical to each other but exhibited one nucleotide difference when compared to *B. microti* strains CCM 4915^T and CCM 4916, isolated from the common vole and two nucleotides difference to *B. suis* bv 5, strain NCTC 11996.

Further molecular characterization by multilocus variable number tandem repeats analysis cluster analysis using eight-minisatellite (panel 1) and eight-microsatellite (panel 2A/B) variable number tandem repeats markers (Le Flèche et al.

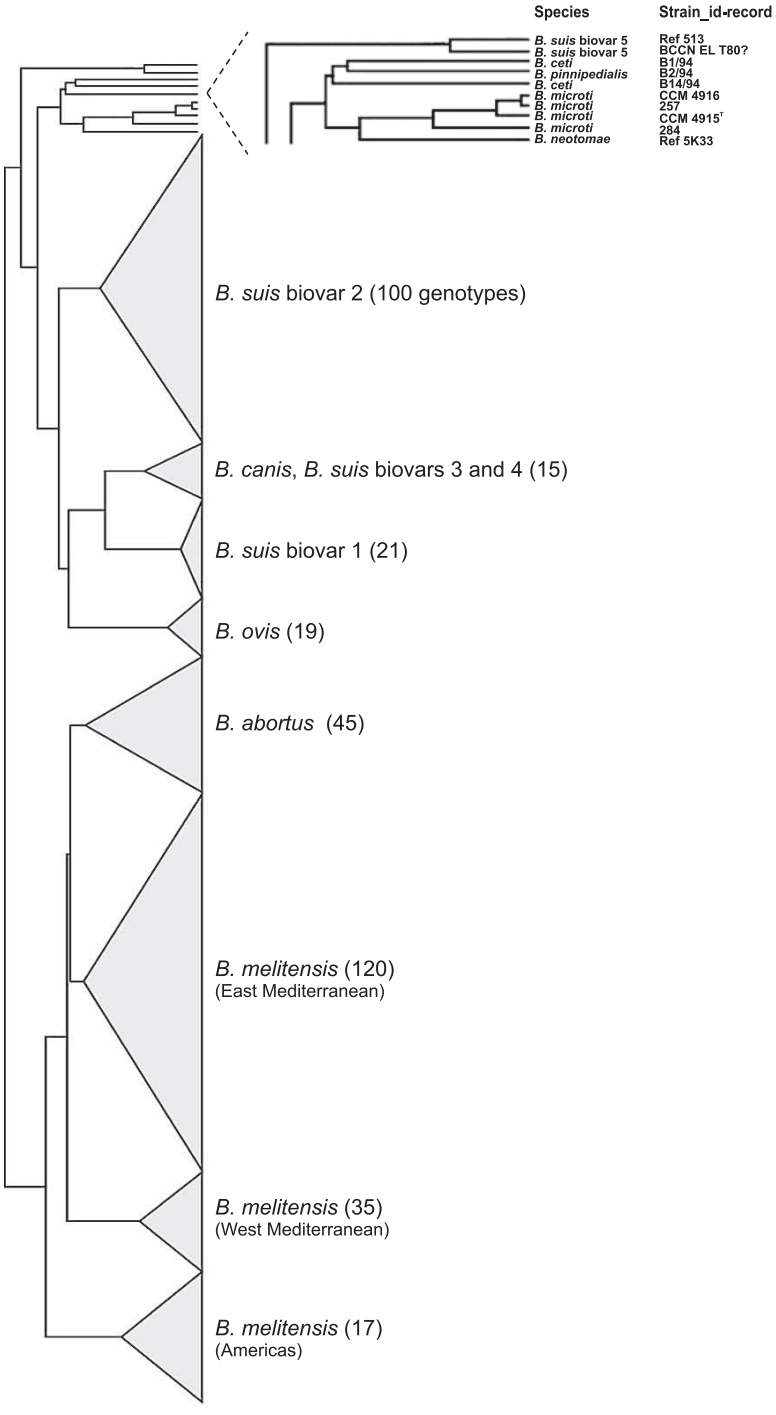

FIG. 1. Condensed dendrogram (unrooted) of clustered multilocus variable number tandem repeats analysis-16 genotypes obtained with 530 Brucella isolates corresponding to 386 genotypes (corresponding to the *Brucella* 2007 database, http://mlva.u-psud.fr/).

2006, Al Dahouk et al. 2007, Scholz et al. 2008a) also placed strains 257 and 284 together with *B. microti* strains CCM 4915^T and CCM 4916 (Fig. 1). Strain 257 is very closely related to strain CCM 4916, with identical panel 1 and panel 2A genotypes and three differences in panel 2B (bruce07, bruce09, and bruce30). Strain 284 is slightly more distinct, with a new panel 1 and panel 2A genotypes.

B. microti was also confirmed by multilocus sequence typing analysis using nine different loci as described by Whatmore et al. 2007 (not shown). In summary, the results from phenotypic and molecular analyses of strains 257 and 284 clearly demonstrate that both isolates belong to the recently described species *B. microti*.

Discussion

B. microti was first isolated in the year 2000 in South Moravia, Czech Republic from systemically infected common voles (Hubálek et al. 2007) and now has been isolated in 2007 from foxes in Lower Austria more than 100 km distant. Common voles with typical lesions such as edematous hind extremities were also reported in Central Moravia (E. Tkadlec, Z. Hubálek, unpublished). This demonstrates that *B. microti* occurs over a larger geographic range than initially thought, covering at least South Moravia and parts of Lower Austria. The isolation of *B. microti* 7 years after its primary isolation further demonstrates that *B. microti* persists in this region and suggests that the foxes might have been infected by ingestion of *Brucella*-infected common voles.

Currently, a larger screening program for the detection of *Brucella* in foxes is being carried out in Austria in order to gain a deeper insight in the occurrence and distribution of *B. microti* in these animals. One major question that still needs to be answered is the natural reservoir of *B. microti*. Recently, we could demonstrate the direct isolation of *B. microti* from soil samples collected at the same site 7 years after primary isolation of this novel species from common voles and also after a 6-month storage of the same samples at 4°C (Scholz et al. 2008). This indicates long-term survival of *B. microti* in the environment; thus soil might function as a reservoir of infection. However, it cannot be excluded that other vectors, such as nematodes, might represent the primary habitat of *B. microti*.

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