**Bartonella tribocorum** sp. nov., a new *Bartonella* species isolated from the blood of wild rats

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Two *Bartonella* strains from blood of two wild rats (*Rattus norvegicus*) living in a rural environment were isolated. These strains were distinct from all previously known *Bartonella* species based on phenotypic and genotypic characteristics. This new species is distinguished by its trypsin-like activity, the absence of the ability to hydrolyse proline and tributyrin, its 16S rRNA and citrate synthase gene sequences and by whole-DNA hybridization data. This new species, for which the name *Bartonella tribocorum* sp. nov. is proposed, seems to be genetically related to *Bartonella elizabethae*, an agent isolated in a case of human endocarditis. The type strain of *Bartonella tribocorum* sp. nov. is IBS 506ᵀ (CIP 105476ᵀ).

**Keywords:** *Bartonella tribocorum* sp. nov., rat, zoonoses

**INTRODUCTION**

Since the beginning of this century, red blood cells of various animal species have been known to harbour intra-erythrocytic bacteria which were referred to as *Grahamella* (Weinman & Kreier, 1977). Most of these bacteria were not cultivated and were only detected by observation of stained blood smears. Birtles *et al.* (1995) demonstrated that species of the genus *Grahamella* should be reclassified and included in the genus *Bartonella*. Therefore, the *Bartonella* presently includes species which were formerly classified in the genera *Bartonella, Rochalimaea* and *Grahamella* (Birtles *et al.*, 1995; Brenner *et al.*, 1993). Nine *Bartonella* species are currently available from international collections: *Bartonella bacilliformis, Bartonella clarridgeiae,* *Bartonella douhiae,* *Bartonella elizabethae,* *Bartonella henselae,* *Bartonella henselea,* *Bartonella quintana,* *Bartonella taylorii* and *Bartonella vinsonii*. Two additional species have been described by Birtles *et al.* (1995), *Bartonella talpae* and *Bartonella peromysci*, but no strains or 16S rRNA sequence data are available.

Several of these *Bartonella* species have been identified as emerging pathogens for humans in the 1990s. Species of this genus have been demonstrated to be vector-borne, but can also be transmitted by animal scratches or bites. It is of importance to evaluate the presence of *Bartonella* species in animals living close to humans. It has been shown that 14% of pet cats in the Paris area and more than 50% of urban stray cats are bacteraemic with *Bartonella henselae* or *Bartonella clarridgeiae* (Gurfield *et al.*, 1997; Heller *et al.*, 1997a). Pet dogs can also be infected by *Bartonella*; *Bartonella vinsonii* subsp. *berkhoffii* was isolated from a dog suffering from endocarditis (Breitschwerdt *et al.*, 1995). We have investigated the presence of *Bartonella* in wild rats (*Rattus norvegicus*) as these animals are found in urban and rural environments. They share human habitats, especially those of people of very low socio-economic status. Contacts can be direct or indirect via haematophagous arthropods such as the rat flea (*Xenopsylla cheopis*). We report the isolation and description of a new *Bartonella* species from the blood of wild rats. This new species seems to be genetically related to *Bartonella elizabethae*, an agent isolated in a case of human endocarditis (Daly *et al.*, 1993).

**METHODS**

**Reference strains.** *Bartonella elizabethae* ATCC 49927ᵀ, *Bartonella henselae* ATCC 49882ᵀ, *Bartonella quintana* ATCC VR-358ᵀ and *Bartonella vinsonii* ATCC VR-152ᵀ

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The EMBL/GenBank accession numbers for the sequences of the 16S rRNA gene and gltA of IBS 506ᵀ are AJ003070 and AJ005494, respectively.
were purchased from the American Type Culture Collection (Rockville, MD, USA). Bartonella grahamii NCTC 12860\(^T\), Bartonella taylorii NCTC 12861\(^T\) and Bartonella dсужден NCTC 12862\(^T\) were kindly provided by R. Birtles (London).

**Animals and blood sampling.** Four wild rats (Rattus norvegicus) were trapped in February 1997 in a wetland conservation area near the Rhine river, in the eastern part of France, by a professional trapper. The rats were caught with an automatic trap and had already drowned at collection time. Blood (0.5 ml) was collected from each dead animal by intracardiac puncture and placed into a Pediatric Isolator and an automatic trap and had already drowned at collection time. Identification of the animal species was based on morphology and dentition.

**Isolation of strains.** Blood (200 μl) was streaked without prior centrifugation onto two blood agar plates made with a Columbia agar base with 5% defibrinated rabbit blood. Incubation of the plates was performed at 35 °C in a moist atmosphere containing 5% CO\(_2\). Three randomly selected control plates were incubated in parallel as a sterility control. The count (c.f.u. ml\(^{-1}\)) was determined for each blood agar plate.

**Light microscopic examination.** The blood smear was treated with May–Grünwald–Giemsa stain and examined with an oil-immersion objective at a magnification of \(\times 1000\). The cultured micro-organisms were also observed at a magnification \(\times 1000\) after enhanced Gram staining by using a counterstain of Kinyoun carbol fuchsin at a 1:20 dilution (Larson et al., 1994).

**Electron microscopy.** Bacteria grown on solid medium were prepared for electron microscopy as described by Fusseneger et al. (1996). Briefly, bacteria were resuspended in phosphate-buffered saline, spread on a water surface and absorbed to Formvar-coated nickel grids. The grids were stained in 1% uranyl acetate and air-dried. The samples were viewed under a Zeiss M109 electron microscope at 80 kV.

**Biochemical analysis.** The following biochemical tests were performed with diagnostic tablets (Rosco Diagnostica): Voges–Proskauer reaction, tributyryl hydrolysis, pyrazinamidase, proline aminopeptidase and tryptophanase-like activity. The MicroScan Rapid Anaerobe Panel (Baxter Diagnostics) was used to test the activity of preformed bacterial enzymes.

**Amplification of 16S rRNA and citrate synthase (glt\(A\)) genes.** Amplification of 16S rRNA and citrate synthase genes was performed as described by Birtles & Raoult (1996) and Heller et al. (1997a). Briefly, DNA was extracted from several (5–10) colonies. *In vitro* amplification of a 1500 bp DNA fragment was performed with two 16S rRNA gene eubacterial universal primers, P8 and P515 (Heller et al., 1997a). For gltA, the primers used for the amplification were CS140f and BHCS.1137n (Birtles & Raoult, 1996). Standard procedures to prevent sample DNA contamination were performed (Kwo & Higuchi, 1989). Negative controls were included in each experiment to check for the absence of cross-contamination between samples and between previously amplified products and field samples.

**Purification of the amplified product and DNA sequencing.** The amplified DNA fragment was purified by phenol extraction and 2-propanol precipitation (Brow, 1990). Sequencing was performed with a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing Kit with 7-deaza-dGTP (Amersham), according to the manufacturer’s instructions. The sequence was obtained with an ALF DNA Sequencer (Pharmacia), according to the manufacturer’s instructions. Sequencing of the complete 16S rRNA gene fragment was performed on coding and complementary strands with four pairs of primers (Eurogentec): P8 and P515 (5’ GTATATCCGGGCGTATGCAAC 3’), P515 (5’ GTGCCAGCAGCGCGTGAAC 3’), PC804 (5’ GACGAGGCTACGCTACCAA 3’), P784 (5’ GGATTGAGTGCCTAGGC 3’) and PC198 (5’ ACTTGGACGTGATCGTGC 3’). Partial sequencing of gltA was performed with primer pair BtchS.1137n and BtchS.781p (5’ GGGGACGCTCAGTGGG 3’). All primers were 5’-labelled with fluorescein isothiocyanate.

**Sequence analysis.** The sequences obtained were compared with similar sequences from other bacteria, including all Bartonella spp., contained in the EMBL/GenBank database. The sequences were aligned by using the CLUSTAL program ( Higgins & Sharp, 1988) with DNASTAR software.

**DNA–DNA hybridization.** Colonies were harvested from blood agar plates and DNA was extracted as described previously (Riegel et al., 1994). Hybridization between the labelled DNA and the fragmented DNA preparation was carried out at 58 °C for 16 h in 0·42 M NaCl by the nucleose-TCA method (Grimont et al., 1980; Riegel et al., 1994).

**Determination of DNA base composition.** The G + C content of DNA was determined by a capillary electrophoresis method as described previously (Riegel et al., 1995).

**Nucleotide sequence accession numbers.** The EMBL/GenBank accession numbers for the 16S rRNA gene sequences are as follows: Bartonella bacilliformis, M65249; Bartonella clarridgeiae, X8920; Bartonella ducsoniae, Z13315; Bartonella elizabethae, L01260; Bartonella grahamii, Z13319; Bartonella henselae Houston-1, M73229; Bartonella henselae BA-TF, Z11684; Bartonella quintana Fuller, N11927; Bartonella taylorii, Z13350; Bartonella vinsonii, LO1259; Bartonella vinsonii subsp. berkhoffii L35052; strain C5-rat, Z70008; strain C7-rat, Z70004; strain C1-ph, Z70006; strain C4-ph, Z70007; strain R-phy1, Z70005; strain R-phy2, Z70001; strain N40, Z70002. The EMBL/GenBank accession numbers for the gltA gene sequences, as given by Birtles & Raoult (1996), are as follows: Bartonella bacilliformis LA6.3, Z70021; Bartonella ducsoniae, Z70017; Bartonella elizabethae, Z70009; Bartonella grahamii, Z70016; Bartonella quintana Fuller, Z70014; Bartonella taylorii, Z70013; Bartonella vinsonii, Z70015; strain C1-ph, Z70022; strain C4-ph, Z70019; strain C5-rat, Z70018; strain C7-rat, Z70020; strain R-phy1, Z70010; strain R-phy2, Z70011; strain N40, Z70012. The EMBL/GenBank accession numbers for the gltA gene sequences, as given by Kosoy et al. (1997), are as follows: A1, U84372; A2, U84373; A3, U84374; B1, U84375; B2, U84376; C1, U84377; C2, U84378; D1, U84379; D2, U84380; D3, U84381; D4, U84382; D5, U84383; D6, U84384; D7, U84385.

**RESULTS**

Plates streaked with the blood of two of the four rats had to be discarded because of heavy bacterial contamination (various bacterial colonies grew within 24 h). From the other plates, small bacterial colonies
Bartonella tribocorum sp. nov. isolated from wild rats

**Fig. 1.** Negative staining of strain IBS 506T. Bar, 0.25 μm.

(100 and 150 c.f.u. ml⁻¹, respectively) appeared 10 d after inoculation. The two strains were designated IBS 500 and IBS 506T.

**Phenotypic identification**

The colonies appeared after 5 d subculture as white-cream, rough colonies with a diameter of less than 1 mm. The colonies were embedded in the agar surface, appeared compact and were difficult to emulsify. Light microscopic examination after enhanced Gram staining revealed small Gram-negative rods. Electron microscopic examination showed small bacilli without flagella but there was evidence of polar structures resembling fimbriae in both strains (Fig. 1). The two strains were catalase-negative, exhibited a negative Voges-Proskauer reaction, had negative pyrazinamide and urease activities and did not produce acid from trehalose. They did not hydrolyse bis(p-nitrophenyl) phosphate and p-nitrophenyl-β-p-glucosaminide. The strains showed amino acid arylamidase activity with leucine, methionine, lysine (alkaline as well as acid), glycine, arginine and tryptophan, but not with proline. They had glycyl-glycylarylaminidase and trypsin-like activity but no pyrrolidonylarylaminidase activity.

**16S rRNA gene sequence analysis**

Sequencing of the complete 16S rRNA gene was performed for the two strains. The two sequences obtained were identical to each other but differed from those previously determined for other Bartonella species. The species with the closest sequence homology was Bartonella elizabethae which only had six different bases (0.4%) over the whole 16S rRNA gene. The most distant sequences were those of Bartonella bacilliformis and Bartonella clarridgeiae with 29 different bases (2.1%). The main differences between the sequences of the two rat strains and the 16S rRNA gene sequences of all other known Bartonella species are presented in Table 1 and the percentage similarities are presented in Table 2. Sequencing of the 16S rRNA gene of the Bartonella elizabethae type strain was also performed. The sequence obtained was slightly different from the one in the GenBank/EMBL database. In the first 150 5' bases two gaps were filled by G residues at positions 82 and 195 (Escherichia coli numbering). Furthermore, an ambiguity was resolved, as a 'B', corresponding to G, T or C, at position 183 (E. coli numbering) was found to be a G. These three bases were identical for the two wild rat strains. Nevertheless, four of the six bases remained different between the 16S rRNA gene sequences of the wild rat strains and the type strain of Bartonella elizabethae.

**Table 1.** Major differences between the 16S rRNA genes in Bartonella

<table>
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<tr>
<th>Strain</th>
<th>Consensus sequence and position (E. coli numbering)</th>
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<td>A   T   T   A   A   C   -   T   T   G   T   C   A   A   G   C   A   A   G   C   C   T   A   C   G   C   T</td>
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<td>IBS 500T</td>
<td>G   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
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<tr>
<td>Bartonella bacilliformis</td>
<td>A   G   A   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
</tr>
<tr>
<td>Bartonella clarridgeiae</td>
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<td>Bartonella doylei</td>
<td>A   G   A   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
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<tr>
<td>Bartonella henselae</td>
<td>A   G   A   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
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<td>Bartonella henselae</td>
<td>A   G   A   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
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<td>Bartonella henselae</td>
<td>A   G   A   G   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C   C</td>
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<td>Bartonella henselae</td>
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<tr>
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<tr>
<td>Bartonella vincentii</td>
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Table 2. DNA–DNA hybridization and 16S rRNA gene similarity between *Bartonella* species and strain IBS 506T

<table>
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<tr>
<th>Strain</th>
<th>Hybridization with IBS 506T (%)</th>
<th>Hybridization with ATCC 49927T (%)</th>
<th>Similarity to 16S rRNA gene (%)</th>
<th>G + C content (mol %)</th>
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<tr>
<td>IBS 506T</td>
<td>100</td>
<td>44</td>
<td>100</td>
<td>38</td>
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<tr>
<td>IBS 500</td>
<td>94</td>
<td>46</td>
<td>100</td>
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<td><em>Bartonella bacilliformis</em></td>
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<td><em>Bartonella clarridgeae</em></td>
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<td>12</td>
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<td>100</td>
<td>99.6</td>
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<td>99.3</td>
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<td>18</td>
<td>98.9</td>
<td>41†</td>
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<td><em>Bartonella quintana</em> ATCC VR-358T</td>
<td>11</td>
<td>15</td>
<td>98.3</td>
<td>40†</td>
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<tr>
<td><em>Bartonella taylorii</em> NCTC 12861T</td>
<td>14</td>
<td>16</td>
<td>99.3</td>
<td>41†</td>
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<tr>
<td><em>Bartonella vinsonii</em> ATCC VR-152T</td>
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<tr>
<td><em>Bartonella vinsonii</em> subsp. berkshoffii</td>
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–, Not tested.
* Daly et al. (1993).
† Birtles et al. (1995).

**Citrate synthase gene sequence analysis**

A partial sequence of 332 bases in the 3' end of gltA was determined for the two strains. The sequences obtained were identical to each other but differed from all other 3' end sequences of *Bartonella* species available in the GenBank/EMBL database. The closest gltA sequences were those of the cluster described by Birtles & Raoult (1996), including *Bartonella elizabethae* (94.9% similarity) *Bartonella grahamii* (96.1%) and two strains isolated from rats in Peru, C5- rat (98.3%) and C7-rat (94.9%). All other gltA sequences available in the GenBank/EMBL database had lower similarity (< 92%).

**DNA–DNA hybridization**

DNA of strain IBS 506T and the type strain of *Bartonella elizabethae* was labelled and tested by DNA–DNA hybridization against unlabelled DNA of strains IBS 506T and IBS 500 and of the type strains of *Bartonella elizabethae*, *Bartonella doshiiae*, *Bartonella grahamii*, *Bartonella henselae*, *Bartonella quintana*, *Bartonella taylorii* and *Bartonella vinsonii*. The results are shown in Table 2 together with G + C content. DNA of strains IBS 506T and IBS 500 showed 46 and 44% relatedness, respectively, with the labelled DNA of the type strain of *Bartonella elizabethae*. Conversely, DNA of strain IBS 500 and the type strain of *Bartonella elizabethae* presented 94 and 36% relatedness, respectively, with strain IBS 506T. No DNA relatedness over 70% was found between DNA of strain IBS 506T and the type strains of the other *Bartonella* species tested, thus confirming that the two rat isolates represented a single species distinct from the other *Bartonella* species previously described.

**Blood smear examination**

No intraerythrocytic *Bartonella*-like bacterium (Weinman & Kreier, 1977) could be observed in the single blood smear from the wild rat, but we identified numerous trypanosomes, probably *Trypanosoma lewisi* which is known to infect wild rodents, especially rats (Laveran & Mesnil, 1912). However, preliminary experimental infections performed on laboratory rats by intravenous injection of a suspension of strain IBS 506T permitted the detection of intraerythrocytic bacteria in blood smears, showing the ability of these bacteria to enter the red blood cells.

**DISCUSSION**

The strains isolated from the blood of two wild rats had similar phenotypic characteristics to other *Bartonella* species. (1) The bacteria were observed within erythrocytes. (2) They grew slowly and required blood-enriched medium. (3) They appeared as small, fastidious, aerobic, oxidase-negative, Gram-negative rods. (4) They grew best on blood-enriched medium in
an atmosphere containing 5% CO₂. (5) When compared to the literature, the enzymic activities tested were similar to those of other species of the genus Bartonella (Birtles et al., 1995; Daly et al., 1993; Lawson & Collins, 1996), with the exception of proline aminopeptidase activity which was negative, as seen also for Bartonella doshiae. Proline aminopeptidase activity was negative when tested with both diagnostic tablets and the MicroScan Rapid Anaerobe Panel. However, Bartonella doshiae and the two rat strains could be differentiated, as Bartonella doshiae hydrolysed tributyrin (Birtles et al., 1995) whereas the rat strains did not. (6) The 16s rRNA gene sequence of the two rat strains was highly similar to the 16s rRNA gene sequence of all known Bartonella species. The closest sequence was that of the type strain of Bartonella elizabethae (99-6% similarity) and the most divergent sequences within the genus Bartonella were the sequences of Bartonella bacilliformis and Bartonella clarridgeiae (97-9%). Furthermore, the high level of DNA-DNA hybridization shared by the two rat strains (94%) confirmed that both belonged to the same bacterial species. The hybridization rates between the two Bartonella strains isolated from the rats and Bartonella elizabethae were between 36 and 46%, indicating that these strains and Bartonella elizabethae belonged to two different species. DNA-DNA hybridization was performed only with the type strains of the other Bartonella species having a 16s rRNA gene sequence with a similarity over 98%. Therefore, Bartonella bacilliformis and Bartonella clarridgeiae with only 97-9% similarity and also presenting a polar tuft of flagella, were not tested. Based on criteria defining a species (Stackebrandt & Goebel, 1994), we conclude that the two rat strains belong to a new species within the genus Bartonella.

For an additional characterization, 16s rRNA gene amplification and sequencing was used. This method was selected as it allows a universal characterization of tested strains, especially for unknown bacteria. The 16s rRNA gene of the two Bartonella isolates obtained from the two wild rats had exactly the same sequence which differed from sequences of all known Bartonella species. The species with the nearest 16s rRNA gene sequence was Bartonella elizabethae. Bartonella elizabethae was described in 1993 with only a single strain available, isolated from a patient with endocarditis (Daly et al., 1993). The determination of a partial sequence of gltA and its comparison to other known gltA sequences enhanced the genotypic relationship between the rat strains and Bartonella elizabethae formerly demonstrated by 16s rRNA sequencing. The closest gltA sequence corresponded to the strain C5-rat (98-3%) isolated from blood of a rat in Peru (Birtles & Raoult, 1996).

The limited sample size of this study did not allow epidemiological observations. Two different Grahamella species have previously been identified from rats, Grahamella joyeuxi by Brumpt in 1913 and Grahamella crocidurae by Schwetz and Cabu in 1930 (see Weinman & Kreier, 1977). These two Grahamella species were described only on the basis of rod-shaped structures observed in erythrocytes in blood smears stained with a Giemsa solution. Therefore, no collection type strain was available to compare our isolates to these species. Recently, Ellis et al. (1997) described the isolation of Bartonella strains from blood samples of rats (Rattus norvegicus and Rattus rattus) captured in the USA, Bolivia and Paraguay. These authors found an overall frequency of bacteremia of 16%, with rates ranging from 0 to 51%, depending on the region where the animals were trapped. Ellis et al. (1997) sequenced part of the citrate synthase gene of new Bartonella isolates obtained from rats and found high similarity with the sequence of Bartonella elizabethae, ranging from 93 to 100%. However no sequence is available in the databases. Another study was performed on wild rodents by Kosoy et al. (1997). The sequences of gltA available in the GenBank/EMBL database corresponding to isolates from the Kosoy study were compared to the sequence of strain IBS 506<sup>T</sup>: the similarity was less than 92%. This value is lower than that observed for the cluster described by Birtles & Raoult (1996), including Bartonella elizabethae, Bartonella grahamii and the two strains isolated from rats in Peru in which the similarity was more than 94-9%.

Among other reports of Bartonella isolation from wild rodents, two strains were obtained from rats in Peru (Birtles & Raoult, 1996). The 16s rRNA gene sequences of these strains were likewise highly related to the sequence of Bartonella elizabethae (99-4 and 99-2%). One of these two strains had a citrate synthase sequence identical to that of the type strain Bartonella elizabethae, suggesting that this strain could be an isolate of Bartonella elizabethae. The 16s rRNA gene sequences of these different strains are highly related and seem to constitute a cluster within the Bartonella genus. Our isolates, IBS 500 and IBS 506<sup>T</sup>, also belong to this cluster. The differences between these sequences and those of our isolates are reported in Table 1.

Birtles & Raoult (1996) concluded from their isolation of Bartonella strains from Peruvian rodents that Bartonella elizabethae infections in humans, like Bartonella henselae infections, were zoonotic. We have no indication whether the Bartonella species carried by the wild rats we tested can be transmitted to and cause any pathology in humans. Moreover, experimental infection of laboratory rats with a suspension of the type strain of Bartonella elizabethae or strain IBS 506<sup>T</sup> led to bacteremia lasting 15 d after injection, demonstrating the ability of these animals to be infected by these Bartonella species. Rats are symbiotic animals which can live in the vicinity of humans and could therefore transmit Bartonella species they harbour to humans, either directly or indirectly via ectoparasites. Such a hypothesis is supported by several studies which tested the prevalence of antibodies against Bartonella elizabethae in humans. Out of 600 patients from 45 different states of the USA suspected of having
cat scratch disease, 21% had Bartonella elizabethae antibodies (Daly et al., 1993). Among a population of blood donors in Seattle, 15% were seropositive for Bartonella elizabethae (Comer et al., 1996); in addition, one-third of 630 individuals tested in a population of intravenous drug users in Baltimore were positive for Bartonella elizabethae (Comer et al., 1996). The poor living conditions of these drug users could put them in contact with rats contaminated with a Bartonella species antigenically and/or genetically related to Bartonella elizabethae. Moreover, several human cat scratch disease cases are not serologically positive for Bartonella henselae antigens and some cases have occurred without any history of cat contact. The rate of these cases has been estimated by some authors to be as high as 60% of clinical cat scratch disease cases (Dupon et al., 1996; Yoshida et al., 1996). Many Bartonella species appear to be present in our environment (Heller et al., 1997) and should be carefully investigated. These investigations should focus on updating diagnostic tools to determine the importance of these Bartonella species in human and animal pathology.

Description of Bartonella tribocorum sp. nov.

Bartonella tribocorum [tri.bo.co’rum. L. n. gen. pl. Triboci named after the tribes mentioned by Caesar (51 BC) in his Commentarii de Bello Gallico; these tribes where living in the region near the Rhine river in the eastern part of present day France where the wild rats, from which the two strains were isolated, were trapped].

Colonies grown on blood agar appear as small, white, smooth, regular colonies (about 1 mm diam.) after 10 d. Electron microscopic examination shows small bacilli without flagella, approximately 1–2 μm long by 0.5 μm wide. Gram-negative rods, aerobic and oxidase-negative. Best growth on blood-enriched medium in a moist atmosphere containing 5% CO₂. Negative for catalase, Voges–Proskauer reaction, pyrazinamidase and urease activities and acidification of trehalose. bis(p-Nitrophenyl) phosphate and p-nitrophenyl-N-acetyl-β-D-glucosaminide not hydrolysed. Amino acid arylamidase activity with leucine, methionine, lysine (alkaline as well as acid), glycine, arginine and tryptophan, but not proline. Glycylglycylarylamidase activity. Distinguishable from other Bartonella species by its ability to hydrolyse trypsin but not proline and tributyrin, its 16S rRNA gene sequence and by whole-DNA hybridization. Type strain is IBS 506T isolated from the blood of a wild rat (Rattus norvegicus). Deposited in the Collection des Bactéries de l’Institut Pasteur as CIP 105476T.

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REFERENCES


Bartonella tribocorun sp. nov. isolated from wild rats


