***Bartonella birtlesii* sp. nov., isolated from small mammals (*Apodemus* spp.)**

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Three strains isolated from *Apodemus* spp. were similar to *Bartonella* species on the basis of phenotypic characteristics. Furthermore, genotypic analysis based on sequence analysis of the 16S rRNA and *gltA* genes and on DNA–DNA hybridization showed that the three isolates represented a distinct and new species of *Bartonella*. The name *Bartonella birtlesii* is proposed for the new species. The type strain of *B. birtlesii* sp. nov. is IBS 325^T (= CIP 106294^T = CCUG 44360^T).

Keywords: *Bartonella birtlesii*, rodents, taxonomy, citrate synthase, 16S rDNA gene

INTRODUCTION

Prior to 1993, *Bartonella bacilliformis* was the only member of the *Bartonella* genus. Now, after only a few years of research and taxonomic study, this genus includes 12 approved species available in the international bacterial collections (Brenner *et al.*, 1993). For two species [*B. bacilliformis* (Weinman, 1968) and *Bartonella quintana* (Vinson & Fuller, 1961)] the human is the natural host, for three others [*Bartonella henselae* (Regnery *et al.*, 1992; Welch *et al.*, 1992), *Bartonella clarridgeiae* (Lawson & Collins, 1996) and *Bartonella koehlerae* (Droz *et al.*, 1999)] it is the cat (*Felis domesticus*) and for one other (*Bartonella alsatica*; Heller *et al.*, 1999) it is the rabbit (*Oryctolagus cuniculus*). For five *Bartonella* species, small mammals are the hosts: the rat (*Rattus rattus*) is the host for *Bartonella tribocorum* (Heller *et al.*, 1998), *Microtus*

pennsylvanicus is the host for *Bartonella vinsonii* (Baker, 1946), *Apodemus* spp. are the hosts for *Bartonella taylorii* (Birtles *et al.*, 1995), *Clethrionomys glareolus* is the host for *Bartonella grahamii* (Birtles *et al.*, 1995) and *Microtus agrestis* is the host for *Bartonella doshiae* (Birtles *et al.*, 1995). Furthermore, *B. taylorii*, *B. grahamii* and *B. doshiae* are able to infect numerous species of small woodland mammals and there is no narrow animal host spectrum of infection. The 12th approved species is *Bartonella elizabethae* (Daly *et al.*, 1993), for which only a single isolate, originating from a human case of endocarditis, was described. The 16S rRNA gene sequence of this species is close to that of the rat *Bartonella* sp., *B. tribocorum* (Heller *et al.*, 1998) and to that of other isolates not yet validly described, but all were isolated from small wild mammals (Birtles & Raoult, 1996; Kosoy *et al.*, 1997). In addition to these 12 species, two others have been described in *Peromyscus* spp. [*Bartonella peromysci* (Birtles *et al.*, 1995; Ristic & Kreier, 1984)] and in the mole [*Bartonella talpae* (Birtles *et al.*, 1995; Ristic & Kreier, 1984)], but type strains for these species are no longer extant. Moreover, *B. vinsonii* (Baker, 1946;

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequence and the partial 3'-end gene sequence of strain IBS 325^T (= CIP 106294^T = CCUG 44360^T) are AF204274 and AF204272, respectively.

Breitschwerdt *et al.*, 1995), now includes three subspecies, one of which, *B. vinsonii* subsp. *arupensis*, was isolated from humans and mice (*Peromyscus leucopus*) and is therefore presumptively considered as pathogenic for humans (Welch *et al.*, 1999; Hofmeister *et al.*, 1998).

Thus, several *Bartonella* species have been isolated from numerous small mammals. For one species that is potentially pathogenic for humans (*B. elizabethae*) small woodland mammals are suspected as a reservoir; *B. vinsonii* subsp. *arupensis* (Welch *et al.*, 1999), however, was isolated from mice and humans. *B. grahamii* DNA was detected from the anterior chamber fluid of the eye in an HIV-negative woman who presented with bilateral eye inflammatory disease. This detection was performed by using PCR amplification and sequence analysis of the 16S rRNA gene (Kerkhoff *et al.*, 1999). Thus, the members of the bacterial genus *Bartonella*, particularly species isolated from small woodland mammals, appear to be a group of emerging human pathogens. Better knowledge of the bacteria harboured by wild small mammals is therefore necessary.

The aim of this work was to characterize a new *Bartonella* group, members of which were isolated in France and the UK from the blood of small mammals belonging to the genus *Apodemus*.

METHODS

Animals, blood sampling and culture conditions. Small mammals were caught in February 1997 by a professional trapper in a wetland conservation area (at point kilometre PK 275, from the Bodensee in Germany), near the River Rhine, in Gerstheim, France. Identification of the small mammals was based on morphology and dentition. Animals were anaesthetized by diethylether inhalation and then 0.2 ml blood was collected from each animal by intracardiac puncture. Blood was transferred to a paediatric lysis-centrifugation tube (Wampole Laboratories). After centrifugation, the pellet was plated onto Columbia-base agar plates containing 5% fresh, defibrinated rabbit blood. The plates were incubated at 35 °C in a moist atmosphere containing 5% CO₂ for 4 weeks.

Bacterial strains. Two isolates obtained from *Apodemus* spp. and named IBS 325^T and IBS 358 were examined. (We were unable to identify the *Apodemus* spp. precisely, because the animals captured were too young.) In addition, strain N40, kindly provided by R. Birtles (Bristol, UK) and isolated in the UK from *Apodemus sylvaticus*, was included in the study.

Reference strains used for DNA-DNA hybridization. *B. elizabethae* ATCC 49927^T, *B. henselae* ATCC 49882^T and *B. quintana* ATCC VR-358^T were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). *B. alsatica* CIP 105477^T and *B. tribocorum* CIP 105476^T were kindly provided by R. Heller (Strasbourg, France). *B. koehlerae* ATCC 700693^T was kindly given by B. B. Chomel (Davis, CA, USA). *B. doshiae* NCTC 12862^T, *B. grahamii* NCTC 12860^T, *B. taylorii* NCTC 12861^T and *B. vinsonii* ATCC VR-152^T were kindly provided by R. Birtles (Bristol, UK). *B. clarridgeiae* ATCC 51734^T was kindly given by J. Clarridge (Houston, TX, USA).

Electron microscopy. Bacteria were grown on solid medium and submitted to electron microscopy as described previously (Fussenecker *et al.*, 1996). Briefly, bacteria were suspended in PBS (0.145 M NaCl, 0.15 M sodium phosphate), spread onto a water surface, absorbed onto Formvar-coated nickel grids, stained in 1% (w/v) uranyl acetate and then air-dried. Samples were examined with a JEOL 100CX2 electron microscope.

Biochemical analysis. The following biochemical assays were performed with diagnostic tablets (Rosco Diagnostica): the Voges-Proskauer reaction, tributyrine hydrolysis and pyrazinamidase, proline aminopeptidase and trypsin-like activities. Pre-formed bacterial enzyme activity was tested using the MicroScan Rapid Anaerobe Panel (Dade International) according to the manufacturer's instructions and as described previously (Welch *et al.*, 1993; Birtles *et al.*, 1995).

Amplification of 16S rRNA and citrate synthase genes. DNA was extracted from bacterial suspensions via the Chelex extraction technique (De Lamballerie, 1992). An approximately 1500 bp fragment of the 16S rRNA gene was amplified from the extracted DNA by using the eubacterial universal primers specific for the 16S rRNA gene, i.e. primer P8 (5'-AGAGATTTGATCCTGGCTCAG-3') and primer Pc1544 (5'-AAGGAGGTGATCCAGCCGCA-3') (Heller *et al.*, 1998). PCR amplification of the citrate synthase gene was performed, as described by Birtles & Raoult (1996), with two primers, i.e. primer CS.140f (5'-TTACTTATGATCCKGGYTTTA-3', where K represents equimolar amounts of G and T and Y represents equimolar amounts of C and T) and primer BhCS.1137n (5'-AATGCAAAAAGAACAGTAAACA-3'). Standard procedures for preventing sample DNA cross-contamination were undertaken (Kwok & Higuchi, 1989). Each set of reactions also included negative controls to confirm the absence of cross-contamination between samples and previously amplified DNA or field samples. The presence of the desired amplicons was controlled by electrophoresis on a 1.5% agarose gel, followed by ethidium bromide staining and visualization on a UV transilluminator.

Purification of the amplicons and DNA sequencing. The amplified fragments were purified via phenol extraction and 2-propanol precipitation (Brow, 1990). Sequencing of the complete 16S rRNA gene was performed on the coding and complementary strands by using the following four primer pairs 5'-labelled with FITC (Eurogentec): P8 and Pc535 (5'-GTATTACCGCGGCTGCTGGCAC-3'); P515 (5'-GTGC-CAGCAGCCGCGGTAACAC-3') and Pc804 (5'-GACTA-CCAGGGTATCTAATCC-3'); P784 (5'-GGATTAGATACCCTGGTAGTC-3') and Pc1198 (5'-ACTTGACGTTATCCCCACCTTCC-3'); and P1174 (5'-GAGGAAGGTGGGGATGACGTC-3') and Pc1544.

Partial sequencing of the 3'-end of the citrate synthase gene (*gltA*) was performed on coding and complementary strands by using primer pairs BhCS.1137n and BhCS.781p (5'-GGGGACCAGCTCATGGTGG-3'). All primers were 5'-labelled with fluorescein isothiocyanate.

The sequencing reaction was performed by using a Thermo Sequenase Fluorescent Labelled Primer Cycle Sequencing kit with 7-deaza-dGTP (Amersham), according to the manufacturer's instructions. The sequences were obtained with an ALF DNA Sequencer (Pharmacia Biotech) used according to the manufacturer's instructions.

Sequence analysis. The DNA sequences obtained were

compared with those from other *Bartonella* spp. and other bacteria, contained in the EMBL/GenBank database. The sequences were aligned by using the CLUSTAL method (Higgins & Sharp, 1988) with DNASTAR software.

Nucleotide sequence accession numbers. The EMBL/GenBank accession numbers for the 16S rRNA sequences used for the sequence comparison given are as follows: *B. alsatica*, AJ002139; *B. bacilliformis*, M65249; *B. clarridgeiae*, X8920; *B. doshiae*, Z31351; *B. elizabethae*, L01260; *B. grahamii*, Z31349; *B. henselae* Houston-1, M73229; *B. henselae* BA-TF, Z11684; *B. koehlerae*, AF76237; *B. quintana* Fuller, M11927; *B. taylorii*, Z31350; *B. tribocorum*, AJ003070; *B. vinsonii*, L01259; *B. vinsonii* subsp. *berkhoffii*, L35052; *B. vinsonii* subsp. *arupensis*, U71322; *B. vinsonii* Baker, Z31352; *Escherichia coli*, Z46753; *Afipia felis*, M65248; *Brucella abortus*, AF091354; *Agrobacterium tumefaciens*, M11223; *Rickettsia prowazekii*, M21789; *Rickettsia rickettsii*, M21293; *Bartonella* strain C7-rat, Z70004; *Bartonella* strain C5-rat, Z0008; *Bartonella* strain C4-phy, Z70007; *Bartonella* strain C1-phy, Z70006; *Bartonella* strain R-phy2, Z70001; *Bartonella* strain R-phy1, Z70005; and *Bartonella* strain N40, Z7002.

The EMBL/GenBank accession numbers for the citrate synthase gene sequences used for sequence comparisons, as given by Birtles & Raoult (1996), are as follows: *B. bacilliformis* LA6.3, Z70021; *B. clarridgeiae*, U84386; *B. doshiae*, Z70017; *B. elizabethae*, Z70009; *B. grahamii*, Z70016; *Bartonella henselae*, L38987; *B. quintana* Fuller, Z70014; *B. taylorii*, Z70013; *B. tribocorum*, AJ005494; *B. vinsonii*, Z70015; *B. vinsonii* subsp. *berkhoffii*, U28075; *Bartonella* strain C7-rat, Z70020; *Bartonella* strain C5-rat, Z70018; *Bartonella* strain C4-phy, Z70019; *Bartonella* strain C1-phy, Z70022; *Bartonella* strain R-phy2, Z70011; *Bartonella* strain R-phy1, Z70010; and *Bartonella* strain N40, Z70012.

The EMBL/GenBank accession numbers for the citrate synthase gene sequences of *Bartonella* spp., as given by Kosoy *et al.* (1997), are as follows: strain A1, U84372; strain A2, U84373; strain A3, U84374; strain B1, U84375; strain B2, U84376; strain C1, U84377; strain C2, U84378; strain D1, U84379; strain D2, U84380; strain D3, U84381; strain D4, U84382; strain D5, U84383; strain D6, U84384; strain D7, U84385.

The EMBL/GenBank accession number for the citrate synthase gene sequences of *B. vinsonii* subsp. *arupensis* (Welch *et al.*, 1999) and *B. alsatica* (Heller *et al.*, 1999) are U77057 and AF204273, respectively.

DNA–DNA hybridization. DNA extraction and purification were performed as described previously (Riegel *et al.*, 1994). DNA hybridizations between the labelled DNA and the unlabelled fragmented DNA were performed at 58 °C for 16 h in 0.42 M NaCl by using the nuclease trichloroacetic acid method as described earlier (Grimont *et al.*, 1980; Riegel *et al.*, 1994).

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

RESULTS

Among the 28 *Bartonella* strains isolated from the 34 small mammals, two (IBS 325^T and IBS 358) were

isolated from two *Apodemus* spp. and had exactly the same 16S rRNA gene and *gltA* sequences (Table 1). These sequences were identical to those of strain N40, which was also isolated from an *Apodemus* species. The 16S rRNA gene sequences of 26 other strains isolated from the other wild mammals were different and so these strains were not included in this study.

Phenotypic characteristics

The three strains produced homogeneous, rough, round, grey–white colonies, 0.6–1.0 mm in diameter, that were slightly adherent to the surface of the culture medium. Bacterial suspension in PBS was not homogeneous because of bacterial aggregation. Gram-staining revealed short, slender, straight or slightly curved Gram-negative bacilli. Electron microscopy examination with negative staining showed small bacilli that lacked flagella. They were catalase and oxidase-negative, gave a negative Voges–Proskauer reaction, had no urease activity and did not produce acid from trehalose. They hydrolysed bis-*p*-nitrophenyl phosphate but not *N*-acetyl β -D-glucosaminide. They had amino acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. The strains had trypsin-like activity and glycylglycylarylamidase activity but no pyrrolidonylarylamidase activity. These data were identical for the three strains tested; only the results obtained with strain IBS 325^T are given in Table 2.

16S rRNA gene sequence analysis

The length of the 16S rRNA gene sequenced was 1394 bp (all located within the 16S rRNA gene). We sequenced the amplicon obtained with the eubacterial universal primers P8 and Pc1544.

Strains IBS 325^T, IBS 358 and *Bartonella* isolate N40 had an identical 16S rRNA gene sequence, which differed from that of the 12 other *Bartonella* species contained in the EMBL/GenBank database. The percentage similarities are presented in Table 1.

The species with the closest 16S rRNA gene sequence was *B. taylorii* (99.3% similarity). The most distant 16S rRNA gene sequences within the genus *Bartonella* were those of *B. clarridgeiae* and *B. bacilliformis* (97.0 and 97.2% similarity, respectively).

When bacterial genera close to *Bartonella* were studied, the 16S rRNA gene sequence of strain IBS 325^T was found to be 93.8% similar to that of *B. abortus* and 91.4% similar to that of *A. tumefaciens*.

Citrate synthase gene sequence analysis

The partial sequence of 320 bp in the 3'-end of the citrate synthase gene was identical for the three strains, but differed from the corresponding sequences of all

Table 1. DNA–DNA hybridization rate and 16S rRNA gene and *gltA* similarity between the *Bartonella* species and the strain IBS 325^T

—, Not tested.

<i>Bartonella</i> strain	Mean hybridization (%) with strain IBS 325 ^T of (trial 1 and trial 2)*	Hybridization with strain IBS 325 ^T (%)	Similarity (%)		Actual base differences (16S rRNA gene)	G + C content (mol %)
			320 bp of 3' end (<i>gltA</i> gene)	1394 bp of 16S rRNA gene		
Strain IBS 325 ^T	(100/100)	100	100	100	0	38
Strain IBS 358	(92/96)	94	100	100	0	38
Strain N40	(94/95)	95	100	100	0	38†
<i>B. alsatica</i> CIP 105477 ^T	(28/27)	27	92.5	98.8	8	37‡
<i>B. bacilliformis</i> ATCC 35685 ^T	—	—	84.5	97.2	19	—
<i>B. clarridgeiae</i> ATCC 51734 ^T	—	—	85.6	97.0	25	—
<i>B. doshiae</i> NCTC 12862 ^T	(27/32)	28	88.4	98.5	7	41§
<i>B. elizabethae</i> ATCC 49927 ^T	(17/21)	18	87.7	97.9	11	41
<i>B. grahamii</i> NCTC 12860 ^T	(22/24)	22	89.6	98.8	6	40§
<i>B. henselae</i> ATCC 49882 ^T	(21/20)	21	90.6	97.5	15	41
<i>B. koehlerae</i> ATCC 700693 ^T	(24/27)	25	90.9	98.3	10	—
<i>B. quintana</i> ATCC VR-358 ^T	(28/31)	29	87.4	98.1	14	40
<i>B. taylorii</i> NCTC 12861 ^T	(23/27)	25	90.6	99.3	3	41§
<i>B. tribocorum</i> CIP 105476 ^T	(22/23)	23	90.6	98.5	8	38¶
<i>B. vinsonii</i> subsp. <i>vinsonii</i> ATCC VR-152 ^T	(27/33)	30	90.9	98.8	6	41
<i>B. vinsonii</i> subsp. <i>arupensis</i> ATCC 700727 ^T	—	—	90.8	98.8	6	41#
Strain C1-phy	—	—	87.1	99.0	5	—
Strain C4-phy	—	—	87.7	98.8	6	—
Strain R-phy1	—	—	88.4	99.1	4	—
Strain R-phy2	—	—	87.7	99.0	5	—
Strain A1	—	—	89.3	—	—	—
Strain A2	—	—	88.4	—	—	—
Strain A3	—	—	88.4	—	—	—
Strain B1	—	—	91.2	—	—	—
Strain B2	—	—	91.8	—	—	—
Strain D7	—	—	90.6	—	—	—

* Mean of data from trial 1/trial 2.

† Birtles & Raoult (1996).

‡ Heller *et al.* (1999).§ Birtles *et al.* (1995).|| Daly *et al.* (1993).¶ Heller *et al.* (1998).# Welch *et al.* (1999).

type strains of *Bartonella* species available in the EMBL/GenBank database.

The closest *gltA* sequences were those of *B. alsatica* (92.5 % similarity) isolated from wild rabbits (Heller *et al.*, 1999) and *Bartonella* strain B2 (91.8 %) isolated from a small rodent (Kosoy *et al.*, 1997). All of the results are presented in Table 1. All other 3'-end citrate

synthase gene sequences available in the EMBL/GenBank database shared less than 91.2 % similarity.

DNA–DNA hybridizations

DNA of the strain IBS 325^T was labelled and hybridized with unlabelled DNA of isolate N40, isolate IBS 358, isolate 325 and of the type strains of *B. alsatica*,

Table 2. Differential phenotypic characteristics of the isolate IBS 325^T when compared with those of type strains of the *Bartonella* species tested

Taxa (with references): 1, IBS 325^T; 2, *B. alsatica*⁷; 3, *B. bacilliformis*^{1,2,8}; 4, *B. clarridgeiae*²; 5, *B. doshiae*¹; 6, *B. elizabethae*^{3,9}; 7, *B. grahamii*¹; 8, *B. henselae*^{3,4,8}; 9, *B. koehlerae*⁵; 10, *B. quintana*^{3,4,8}; 11, *B. taylorii*¹; 12, *B. tribocorum*⁶; 13, *B. vinsonii*^{3,4,8}.
References: 1, Birtles *et al.* (1995); 2, Clarridge *et al.* (1995); 3, Daly *et al.* (1993); 4, Drancourt & Raoult (1993); 5, Droz *et al.* (1999); 6, Heller *et al.* (1998); 7, Heller *et al.* (1999); 8, Welch *et al.* (1993); 9, Welch *et al.* (1999). All species were positive for hydrolysis of arginine, glycine and leucine. The characteristic of Gram-staining was omitted. +, Positive; –, negative; NT, not tested; v, variable; w, weakly positive.

Characteristic	Taxa:												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Oxidase	–	–	–	–	–	–	–	–	–	v	–	–	v
Catalase	–	–	+	–	–	–	–	–	–	–	–	–	–
Haemolysis	–	–	–	–	–	+	–	–	–	–	–	–	–
Motility	–	–	+	+	–	–	–	–	–	–	–	–	–
Urea	–	–	–	–	NT	–	NT	–	NT	–	NT	–	–
Voges–Proskauer	–	–	NT	NT	+	NT	+	–	–	+	+	–	–
Glycylglycine	+	+	NT	NT	+	NT	+	NT	+	NT	+	+	+
Lysine (alkaline)	+	+	NT	NT	+	+	+	+	+	+	+	+	v
Lysine (acidic)	+	+	+	NT	+	w	+	+	–	–	+	+	v
Methionine	+	+	NT	+	+	NT	+	+	+	NT	+	+	NT
Proline	+	+	–	+	–	+	+	+	–	+	+	–	v
Tryptophan	+	+	NT	+	+	+	+	+	+	+	+	+	+

B. doshiae, *B. elizabethae*, *B. grahamii*, *B. henselae*, *B. koehlerae*, *B. quintana*, *B. taylorii*, *B. tribocorum* and *B. vinsonii*. IBS 325^T showed less than 30% relatedness with all type strains of *Bartonella* species tested, but showed more than 94% relatedness with DNA from IBS 358 and DNA from N40.

DISCUSSION

Strains IBS 325^T, IBS 358 and *Bartonella* isolate N40 exhibited phenotypic characteristics similar to those of other *Bartonella* species: they grew slowly (10 d for primary isolation and 6 d for subcultures), required CO₂ and produced small colonies on Columbia–blood agar plates. Furthermore, they were negative for oxidase, catalase and the Voges–Proskauer reaction. They did not hydrolyse trehalose. They had amino acid arylamidase activity with arginine, lysine, glycine, leucine, methionine, proline and tryptophan and had a trypsin-like activity.

Additional data were obtained from genetic analysis. The three isolates had exactly the same sequence for the 16S rRNA gene. All of them belonged to the *Bartonella* genus, since this sequence showed 97.0–99.3% similarity to those of other *Bartonella* species; this is within the range of similarity found between the type strains of other *Bartonella* species (97.5–99.3%; Birtles & Raoult, 1996). The closest 16S rRNA gene sequences were those of *B. taylorii*, *B. vinsonii* and *B. vinsonii* subsp. *arupensis* and those of strains C1-phy, C4-phy, R1-phy and R2-phy (Birtles & Raoult, 1996),

all of which had been isolated from small woodland mammals (Fig. 1).

Furthermore, the three isolates IBS 325^T, IBS 358 and N40 showed a unique 3'-end citrate synthase sequence. The level of sequence similarity between IBS 325^T and the previously described *Bartonella* species was in the range 84.5–92.5%. This range was similar to those previously described for the type strains of *Bartonella* species [83.8–93.2% (Birtles & Raoult, 1996) and 88.7–96.4% (Kosoy *et al.*, 1997)]. These data, together with the 16S rRNA gene sequence (Table 1), the culture characteristics (colonial morphology, duration of bacterial growth, microscopic appearance of the bacteria after Gram-staining) and the isolation of the bacteria from the blood of *Apodemus* spp., all support the classification of these three isolates within the genus *Bartonella*.

On the basis of DNA–DNA hybridization, the three isolates IBS 325^T, IBS 358 and N40 belong to the same species, since they showed more than 94.0% hybridization to each other. Furthermore, the DNA of strains N40 and IBS 325^T showed 95 and 97% hybridization, respectively, with the labelled DNA of strain IBS 358 (data not shown). Conversely, the DNA of strains IBS 358 and N40 showed 94 and 95% relatedness, respectively, with the labelled DNA of strain IBS 325^T. Moreover, all of the type strains of *Bartonella* species tested showed DNA relatedness to strain IBS 325^T of less than 30% (Table 1). These data indicated that the three isolates belong to a single *Bartonella* species distinct from all those previously described, according

Fig. 1. Phylogenetic tree derived from a 1394 bp fragment of the 16S gene for the *Bartonella* strains and other representatives of the *Eubacteria*. This phylogenetic tree was constructed using the CLUSTAL method with DNASTAR software. The lengths of the horizontal lines are significant. The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree measures the distance between sequences. Units indicate the number of substitution events.

to previously established criteria (Stackebrandt & Goebel, 1994).

In spite of the fact that the three isolates of the new *Bartonella* species were isolated from wild *Apodemus* spp., this animal genus should not be regarded as a specific host, since we used IBS 325^T to inoculate outbred and inbred laboratory mice and found that they developed long-term bacteraemia (unpublished data). The ability of this new species to infect humans should be assessed, since other rodent-associated *Bartonella* spp. associated with human diseases have been described, namely *B. grahamii* (Kerkhoff *et al.*, 1999) and *B. vinsonii* subsp. *arupensis* (Hofmeister *et al.*, 1998; Welch *et al.*, 1999).

To detect possible cases of human infection by *Bartonella birtlesii*, serological and genetic tools specific for *B. birtlesii* should be established.

Description of *Bartonella birtlesii* sp. nov.

Bartonella birtlesii (birt.les'i.i. M.L. gen. n. *birtlesii* of Richard J. Birtles, whose studies have contributed to an improved understanding of the taxonomy of the *Bartonella* genus).

Colonies grown on blood agar appeared homogeneous, rough, round and grey-white. Electron microscopic examination showed small bacilli without flagella. Primary culture of strain *B. birtlesii* was obtained after 10 d incubation at 35 °C in a moist atmosphere containing 5% CO₂. Subculture on agar was obtained after 6 d culture in the same conditions. The three strains tested were all oxidase-negative and catalase-negative, had negative Voges-Proskauer reactions, showed no urease activity and were unable to hy-

drolyse trehalose or *N*-acetyl β -D-glucosaminide. They all hydrolysed bis-*p*-nitrophenyl phosphate and had amino acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. The new species showed a trypsin-like activity and a glycylglycylarylamidase activity but no pyrrolidonyl-arylamidase activity. The new species was distinguished from the other *Bartonella* species by its 16S rRNA gene sequence and by whole-DNA hybridization. The type strain is IBS 325^T (isolated from the blood of an *Apodemus* sp.) and is deposited in the Collection de l'Institut Pasteur, Paris, France (CIP 106294^T = CCUG 44360^T).

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