

## ***Bartonella bovis* Bermond *et al.* sp. nov. and *Bartonella capreoli* sp. nov., isolated from European ruminants**

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**Two novel species of *Bartonella* isolated from European ruminants are described. *Bartonella capreoli* sp. nov. was isolated from the blood of roe-deer (*Capreolus capreolus*) captured in Chizé, France. The type strain is IB5 193<sup>T</sup> (= CIP 106691<sup>T</sup> = CCUG 43827<sup>T</sup>). It is distinct from another European ruminant isolate that originated from a cow from a French herd of 430 dairy cattle. The latter isolate belongs to a novel species named *Bartonella bovis* Bermond *et al.* sp. nov. The type strain is strain 91-4<sup>T</sup> (= CIP 106692<sup>T</sup> = CCUG 43828<sup>T</sup>). The two bacteria appeared as small, fastidious, aerobic, oxidase-negative, Gram-negative rods. Their biochemical properties were similar to those of members of the genus *Bartonella*. The sequences of the 16S rRNA and citrate synthase genes obtained from the two type strains were highly related to sequences of the different *Bartonella* species. Hybridization values when testing type strains of recognized *Bartonella* species, obtained with the nuclease/trichloroacetic acid method, support the creation of two novel species.**

**Keywords:** *Bartonella capreoli*, *Bartonella bovis*, ruminants, citrate synthase gene, 16S rDNA

### **INTRODUCTION**

The bacteria belonging to the genus *Bartonella* are parasites of the red blood cells of wild and domestic mammals. These bacteria have been recovered, often in large numbers, from the blood of humans [*Bartonella bacilliformis* (Weinman, 1968), *Bartonella quintana* (Vinson & Fuller, 1961)], small woodland mammals [*Bartonella vinsonii* subsp. *vinsonii* (Baker, 1946), likely *Bartonella elizabethae* (Daly *et al.*, 1993), *Bartonella doshiae*, *Bartonella taylorii* and *Bartonella grahamii* (Birtles *et al.*, 1995), *B. vinsonii* subsp. *arupensis* (Welch *et al.*, 1999; Hofmeister *et al.*, 1998), *Bartonella birtlesii* (Bermond *et al.*, 2000) and other unnamed species], rats [*Bartonella tribocorum* (Heller *et al.*, 1998)], rabbits [*Bartonella alsatica* (Heller *et al.*, 1999a)], domestic and free-ranging cats [*Bartonella henselae* (Regnery *et al.*, 1992; Welch *et al.*, 1992; Bass

*et al.*, 1997), *Bartonella clarridgeiae* (Lawson & Collins, 1996), *Bartonella koehlerae* (Droz *et al.*, 1999)], roe-deer [*Bartonella schoenbuchensis* (Dehio *et al.*, 2001)] and canids [*B. vinsonii* subsp. *berkhoffii* (Breitschwerdt *et al.*, 1995)].

The bacteria are mainly transmitted by haematophagous arthropods, such as sandflies (mainly *Lutzomyia verrucarum*) for *B. bacilliformis*, body lice (*Pediculus humanus* var. *corporis*) for *B. quintana* and fleas (*Ctenocephalides felis*) for *B. henselae*. It is assumed that all *Bartonella* species are vector-borne (Breitschwerdt & Kordick, 2000). This assumption is based on the fact that blood is the natural habitat of all known *Bartonella* species and that, in wild ruminants, *Bartonella* strains have been isolated from roe-deer and DNA of *Bartonella* spp. has been detected in ticks collected on roe-deer in The Netherlands (Schouls *et al.*, 1999; Bergmans, 1996). Many *Bartonella* species have been shown to be zoonotic. Identification of possible human infections requires specific diagnostic tools, such as serological testing or *in vitro* gene amplification. When investigating the distribution of

The GenBank/EMBL accession numbers for the 16S rRNA gene and partial 3'-end *gltA* sequences of strains IB5 193<sup>T</sup> and 91-4<sup>T</sup> are respectively AF293389 and AF293391 (16S rRNA) and AF293392 and AF293394 (*gltA*).

*Bartonella* species present in domestic and wild animals, we isolated *Bartonella* strains from cattle (*Bos taurus*) and roe-deer (*Capreolus capreolus*) (Chomel *et al.*, 1999; Heller *et al.*, 1999b). These bacteria could be potential zoonotic pathogens, as demonstrated recently for *B. vinsonii* subsp. *berkhoffii*, as humans could be exposed to blood during slaughter and evisceration of roe-deer and cattle. In this publication, we describe two novel *Bartonella* species from ruminants.

## METHODS

**Animals, blood sampling and culture conditions.** In January 1996, 69 wild roe-deer were captured by a professional trapper in Chizé forest conservation hunting area (western France). In June 1998, blood samples were collected from 36 cows belonging to a herd of 430 dairy cattle located in Bissy, 50 km south of Paris, France. Blood samples (2 ml) from roe-deer were collected into lysis-centrifugation tubes (Wampole Laboratory). Blood samples (4 ml) from domestic cattle were collected into plastic 4 ml EDTA tubes (Becton Dickinson). These tubes were frozen at  $-80^{\circ}\text{C}$  and stored until tested. The cattle blood samples were thawed before plating. All blood samples were centrifuged at 1800 *g* for 60 min. After centrifugation, the pellets were plated onto Columbia base agar plates (Difco) containing 5% fresh defibrinated rabbit blood. The plates were incubated at  $35^{\circ}\text{C}$  in a moist 5%  $\text{CO}_2$  atmosphere for 4 weeks.

**Bacterial strains.** Two isolates obtained from two roe-deer, IBS 193<sup>T</sup> and IBS 206, and one isolate obtained from a cow, 91-4<sup>T</sup>, were selected for species identification. Two additional *Bartonella* isolates were included in the study: (i) isolate *Bartonella* sp. FC7049UT or '*Bartonella weissii*', kindly provided by Russell L. Regnery (Atlanta, GA, USA) and isolated in the USA from a domestic cat (Regnery *et al.*, 2000), and (ii) *Bartonella schoenbuchensis* DSM 13525<sup>T</sup>, isolated from a German roe-deer in Autumn 1999 (Dehio *et al.*, 2001).

**Reference strains used for DNA-DNA hybridization.** *B. bacilliformis* ATCC 35685<sup>T</sup>, *B. elizabethae* ATCC 49927<sup>T</sup>, *B. henselae* ATCC 49882<sup>T</sup> and *B. quintana* ATCC VR-358<sup>T</sup> were purchased from the ATCC (Manassas, VA, USA). *B. alsatica* CIP 105477<sup>T</sup>, *B. tribocorum* CIP 105476<sup>T</sup>, *B. schoenbuchensis* DSM 13525<sup>T</sup> and *B. birtlesii* CIP 106294<sup>T</sup> were described previously in our laboratories. *B. koehlerae* ATCC 700693<sup>T</sup> was kindly given by J. E. Koehler (San Francisco, CA, USA). *B. doshiae* NCTC 12862<sup>T</sup>, *B. grahamii* NCTC 12860<sup>T</sup>, *B. taylorii* NCTC 12861<sup>T</sup> and *B. vinsonii* subsp. *vinsonii* ATCC VR-152<sup>T</sup> were kindly provided by R. Birtles (Bristol, UK). *B. clarridgeiae* ATCC 51734<sup>T</sup> 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 (Fusseneger *et al.*, 1996). Briefly, bacteria were suspended in PBS (0.145 M NaCl, 0.15 M sodium phosphate), spread on a water surface and adsorbed to Formvar-coated nickel grids, stained with 1% (w/v) uranyl acetate and air-dried. Samples were examined with a Philips transmission electron microscope (roe-deer isolate) or a JEOL 100CX2 electron microscope (bovine isolate).

**Biochemical analysis.** The following biochemical assays were performed with diagnostic tablets (Rosco Diagnostica): Voges-Proskauer reaction, tributyrin hydrolysis, pyrazinamidase, proline aminopeptidase and trypsin-like activity.

Preformed bacterial enzyme activity was tested using the MicroScan Rapid Anaerobe Panel (Dade International) according to the manufacturer's instructions and as described previously (Birtles *et al.*, 1995; Welch *et al.*, 1993).

**Amplification of 16S rRNA and citrate synthase genes.** DNA was extracted from bacterial suspensions via the Chelex extraction technique (de Lamballerie *et al.*, 1992). An approximately 1400 bp fragment of the 16S rRNA gene was amplified from the extracted DNA by using eubacterial universal primers specific for the 16S rRNA gene: P8 (5'-AGAGTTTGATCCTGGCTCAG-3') and Pc1544 (5'-AAGGAGGTGATCCAGCCGCA-3') (Heller *et al.*, 1998). PCR amplification of a 1000 bp fragment of the citrate synthase gene was performed as described by Birtles & Raoult (1996) with two primers, CS.140f (5'-TTACTTATG-ATCCKGGYTTTA-3', where K is an equimolar amount of G and T and Y is an equimolar amount of C and T) and BhCS.1137n (5'-AATGCAAAAAGAACAGTAA-ACA-3'). Standard procedures were undertaken to prevent sample DNA cross-contamination (Kwok & Higuchi, 1989). Each set of reactions also included negative controls to evaluate the absence of cross-contamination between samples and previously amplified DNA or field samples. The presence and size of the desired amplicons were determined 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 by CHROMA SPIN columns (Clontech). Sequencing of the complete 16S rRNA gene was performed on the coding and complementary strands by using four primer pairs, 5'-labelled with fluorescein isothiocyanate (Eurogentec): P8 and Pc535 (5'-GTAT-TACCGCGGCTGCTGGCAC-3'), P515 (5'-GTGCCAG-CAGCCGCGGTAACAC-3') and Pc804 (5'-GACTACC-AGGGTATCTAATCC-3'), P784 (5'-GGATTAGATAC-CCTGGTAGTC-3') and Pc1198 (5'-ACTTGACGTTATC-CCCACCTTC-3') and P1174 (5'-GAGGAAGGTGGG-GATGACGTC-3') and Pc1544.

Sequencing of 310 nt from the 3' end of the citrate synthase gene (*gltA*) was performed on the coding and complementary strands by using primers BhCS.1137n and BhCS.781p (5'-GGGGACCAGCTCATGGTGG-3'), 5'-labelled with fluorescein isothiocyanate.

Sequencing reactions were performed by using a Sequi-Therm EXCEL II Long-Read DNA Sequencing kit-ALF (Epicentre Technologies), according to the manufacturer's instructions. The sequences were obtained with an ALF DNA Sequencer (Pharmacia Biotech), according to the manufacturer's instructions.

**Analysis of sequence data and construction of a phylogenetic tree.** The DNA sequences obtained were compared with sequences from other *Bartonella* species and other bacteria contained in the EMBL/GenBank database. The sequences were aligned by using the CLUSTAL method (Higgins & Sharp, 1988) with DNASTar software. Partial citrate synthase gene sequences were then aligned with each other and also with other partial citrate synthase genes of recognized *Bartonella* species by using the CLUSTAL X multisequence alignment program (Thompson *et al.*, 1997).

A total of 100 bootstrap samples for alignment was produced by using the program SEQBOOT from PHYLIP version 3.5 (Felsenstein, 1989) and a phylogenetic tree was inferred from each bootstrap sample by using parsimony (DNAPARS pro-

gram in PHYLIP). The resulting tree was combined to yield a consensus tree (CONSENSE program in PHYLIP). A matrix of evolutionary distances was also derived from each bootstrap alignment by using the DNADIST program in PHYLIP. A tree was inferred from the matrices by using the FITCH program in PHYLIP. The resulting tree was visualized to yield a consensus tree (CONSENSE program in PHYLIP).

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

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

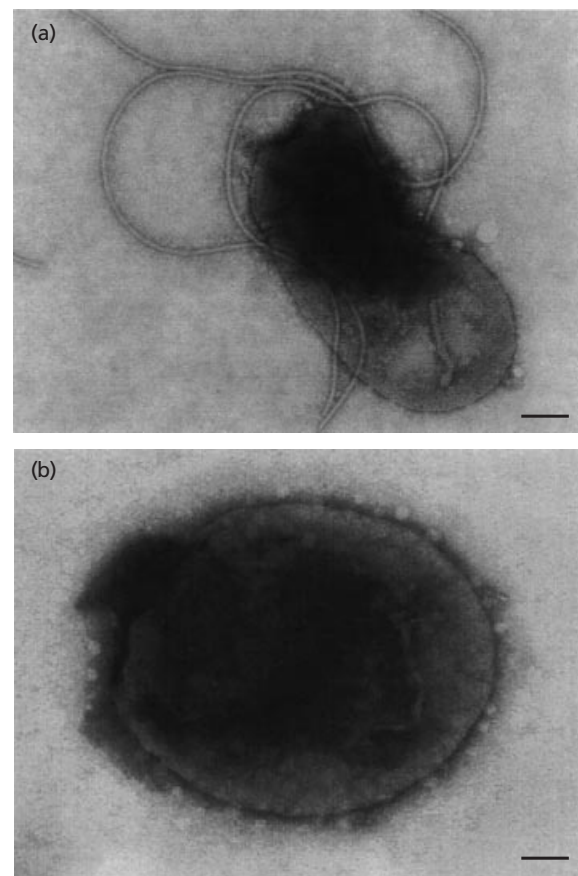
**Nucleotide sequence accession numbers.** The EMBL/GenBank accession numbers for the 16S rRNA sequences used for sequence comparison were: *B. alsatica*, AJ002139; *B. bacilliformis*, M65249; *B. birtlesii*, AF204274; *B. claridgeae*, X89208; *B. doshae*, Z31351; *B. elizabethae*, L01260; *B. grahamii*, Z31349; *B. henselae* Houston-1<sup>T</sup>, M73229; *B. henselae* BA-TF, Z11684; *B. koehlerae*, AF76237; *B. quintana* Fuller<sup>T</sup>, M11927; *B. taylorii*, Z31350; *B. tribocorum*, AJ003070; *B. vinsonii* subsp. *vinsonii*, L01259; *B. vinsonii* subsp. *berkhoffii*, L35052; *B. vinsonii* subsp. *arupensis*, U71322; *Escherichia coli*, Z83204; *Brucella abortus*, AF091354; *Agrobacterium tumefaciens*, M11223; *Bartonella* strain C7-rat, Z70004; *Bartonella* strain C5-rat, Z70008; *Bartonella* strain C4-phy, Z70007; *Bartonella* strain C1-phy, Z70006; *Bartonella* strain R-phy2, Z70001; *Bartonella* strain R-phy1, Z70005; '*B. weissii*', AF199502; *B. schoenbuchensis*, AJ278187. The EMBL/GenBank accession numbers for the citrate synthase gene sequences used for the sequence comparison are given in Fig. 2.

## RESULTS AND DISCUSSION

### Phenotypic characteristics

The two roe-deer isolates, IBS 193<sup>T</sup> and IBS 206, produced smooth colonies, 0.5–1.2 mm in diameter, with short, slender Gram-negative rods. Electron microscope examination with negative staining showed small bacilli with multiple unipolar flagella (Fig. 1a). The two roe-deer isolates tested were oxidase- and catalase-negative, had a negative Voges–Proskauer reaction and no urease activity and were able to hydrolyse trehalose, *N*-acetyl  $\beta$ -D-glucosaminide and bis-*p*-nitrophenyl phosphate. The two isolates had amino-acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. The two isolates had trypsin-like activity and glycyglycylarylamidase activity, but no pyrrolidonylarylamidase activity.

The bovine isolate, 91-4<sup>T</sup>, produced small colonies, 0.3–1.1 mm in diameter, with straight and slender Gram-negative bacilli. Electron microscope examination of a subculture of bovine isolate 91-4<sup>T</sup> with negative staining showed small bacilli without flagella



**Fig. 1.** Electron micrographs of negatively stained cells of *B. capreoli* IBS 193<sup>T</sup> (a) and *B. bovis* 91-4<sup>T</sup> (b). Bars, 0.2  $\mu$ m.

(Fig. 1b). It was oxidase- and catalase-negative, had a negative Voges–Proskauer reaction and no urease activity and was able to hydrolyse trehalose, *N*-acetyl  $\beta$ -D-glucosaminide and bis-*p*-nitrophenyl phosphate. It had amino-acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. This isolate had trypsin-like activity and glycyglycylarylamidase activity, but no pyrrolidonylarylamidase activity.

### Genotypic characteristics

Genotypic characteristics of all *Bartonella* type strains and of several additional isolates (16S rRNA gene and 3'-end citrate synthase gene similarities and/or DNA–DNA hybridization and total DNA G + C content) are presented in Table 1.

### 16S rRNA gene sequence analysis

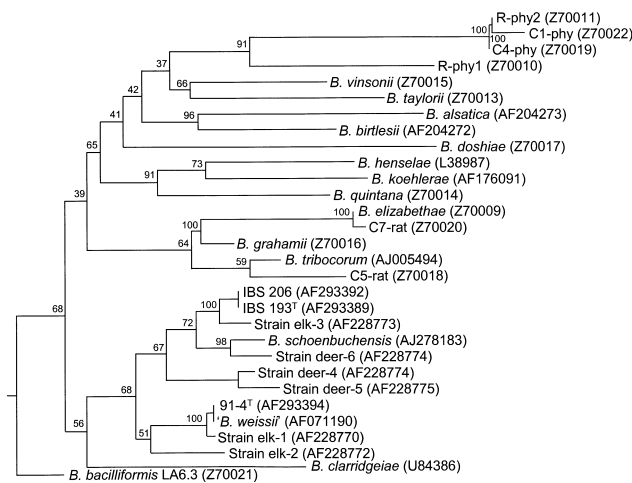
The roe-deer isolates IBS 193<sup>T</sup> and IBS 206 had identical 16S rRNA gene sequences, which differed slightly from the sequences of the bovine isolate 91-4<sup>T</sup> and the 15 *Bartonella* type strains. This 16S rRNA

**Table 1.** DNA–DNA hybridization and 16S rRNA and *gltA* gene similarity between strains IBS 193<sup>T</sup> and 91-4<sup>T</sup> and *Bartonella* speciesSequences compared contained 1390 bp (16S rRNA) and 310 bp (*gltA* 3' end).

Strain	G + C content (mol %)*	Sequence similarity (%) to:				DNA–DNA hybridization (%) with:	
		Strain IBS 193 <sup>T</sup>		Strain 91-4 <sup>T</sup>		Strain IBS 193 <sup>T</sup>	Strain 91-4 <sup>T</sup>
		16S rRNA gene	<i>gltA</i> 3' end	16S rRNA gene	<i>gltA</i> 3' end		
IBS 193 <sup>T</sup>	38	100	100	99.2	93.8	100	45
IBS 206	38	100	100	99.2	93.8	90	45
91-4 <sup>T</sup>	38	99.2	93.8	100	100	48	100
<i>B. alsatica</i> CIP 105477 <sup>T</sup>	37 <sup>e</sup>	98.5	87.1	97.8	85.9	25	22
<i>B. bacilliformis</i> ATCC 35685 <sup>T</sup>	NT	97.8	90.4	97.8	77.7	19	21
<i>B. birtlesii</i> CIP 106294 <sup>T</sup>	38 <sup>c</sup>	98.2	85.2	97.4	89.2	24	19
<i>B. clarridgeiae</i> ATCC 51734 <sup>T</sup>	NT	97.9	88.5	97.6	90.2	26	23
<i>B. doshiae</i> NCTC 12862 <sup>T</sup>	41 <sup>b</sup>	98.9	86.6	98.1	87.5	24	27
<i>B. elizabethae</i> ATCC 49927 <sup>T</sup>	41 <sup>a</sup>	98.0	87.6	97.8	87.2	18	25
<i>B. grahamii</i> NCTC 12860 <sup>T</sup>	40 <sup>b</sup>	98.2	90.0	97.5	90.5	25	24
<i>B. henselae</i> ATCC 49882 <sup>T</sup>	41 <sup>a</sup>	98.8	85.2	98.2	89.2	30	33
<i>B. koehlerae</i> ATCC 700693 <sup>T</sup>	NT	99.4	86.6	98.8	88.2	40	39
<i>B. quintana</i> ATCC VR-358 <sup>T</sup>	40 <sup>a</sup>	98.2	85.6	97.5	87.9	38	27
<i>B. taylorii</i> NCTC 12861 <sup>T</sup>	41 <sup>b</sup>	97.7	88.5	97.0	87.2	20	17
<i>B. tribocorum</i> CIP 105476 <sup>T</sup>	38 <sup>d</sup>	98.0	88.0	97.1	89.1	28	19
<i>B. vinsonii</i> subsp. <i>vinsonii</i> ATCC VR-152 <sup>T</sup>	41 <sup>a</sup>	98.7	88.5	97.8	87.5	33	23
<i>B. vinsonii</i> subsp. <i>arupensis</i> ATCC 700727 <sup>T</sup>	41 <sup>f</sup>	98.5	85.6	97.8	88.6	NT	NT
' <i>B. weissii</i> '	NT	99.2	93.8	100	100	48	80
<i>B. schoenbuchensis</i> DSM 13525 <sup>T</sup>	NT	99.4	98.1	99.8	94.1	53	45

\* Data not obtained in this study were taken from: *a*, Daly *et al.* (1993); *b*, Birtles *et al.* (1995); *c*, Bermond *et al.* (2000); *d*, Heller *et al.* (1998); *e*, Heller *et al.* (1999a); *f*, Welch *et al.* (1999).

NT, Not tested.



**Fig. 2.** Phylogenetic analysis of members of the genus *Bartonella* derived from sequence comparison of 310-bp fragments from the 3' end of *gltA*. The support for each branch, as determined from 100 bootstrap samples, is indicated above each node. This analysis provided the tree topology only, and the lengths of vertical and horizontal lines are not significant. Accession numbers are given in parentheses.

gene sequence was also different from that of all other isolates in the EMBL/GenBank database. The levels of similarity are presented in Table 1. For isolate IBS 193<sup>T</sup>, the most distant 16S rRNA gene sequence was *B. taylorii*, with 97.7% similarity. The species with the closest 16S rRNA gene sequences were *B. koehlerae* and *B. schoenbuchensis* (Dehio *et al.*, 2001), both with 99.4% similarity, whereas the bovine isolate 91-4<sup>T</sup> and '*B. weissii*' had 99.2% similarity (Table 1).

The bovine isolate 91-4<sup>T</sup> and the isolate '*B. weissii*' had identical 16S rRNA gene sequences that differed from the 16S rRNA gene sequences of other *Bartonella* species contained in the EMBL/GenBank database. The isolates with the closest 16S rRNA gene sequences were *B. koehlerae* and *B. schoenbuchensis*, with 98.8 and 99.8% similarity. Furthermore, the bovine isolate 91-4<sup>T</sup> had 99.2% similarity to the roe-deer isolate IBS 193<sup>T</sup> (Table 1). The most distant 16S rRNA gene sequences within the genus *Bartonella* were those of *B. tribocorum* and *B. taylorii*, with 97.1 and 97.0% similarity (Table 1).

The 16S rRNA gene sequences of the roe-deer isolate IBS 193<sup>T</sup> and the bovine isolate 91-4<sup>T</sup> were respectively 94.1 and 93.9% similar to that of *Brucella abortus* and 92.0 and 91.8% similar to that of *Agrobacterium tumefaciens* DSM 30105 (data not shown).

### Citrate synthase gene sequence analysis

The roe-deer isolates IBS 193<sup>T</sup> and IBS 206 had identical 3'-end *gltA* sequences, which differed from the corresponding sequences of the bovine isolate 91-4<sup>T</sup> and the 15 other type strains of *Bartonella* (Fig. 2). This 3'-end *gltA* gene sequence was also

different from that of all other isolates contained in the EMBL/GenBank database. The levels of similarity are presented in Table 1. The most distant *gltA* gene sequences within the genus *Bartonella* were those of *B. birtlesii* and *B. henselae*, each with 85.2% similarity. The species with the closest *gltA* gene sequences were *B. bacilliformis* and *B. schoenbuchensis*, with 90.4 and 98.1% similarity. Furthermore, the sequence of strain IBS 193<sup>T</sup> had 93.8% similarity to that of isolate 91-4<sup>T</sup>, isolated from a French cow, and 93.8% to that of '*B. weissii*', isolated from a domestic North American cat (Table 1).

The bovine isolate 91-4<sup>T</sup> and the cat isolate '*B. weissii*' showed a unique and identical 310 bp sequence of the 3' end of *gltA*, which differed from all corresponding *gltA* gene sequences of other *Bartonella* species (Table 1). The species with the most distant *gltA* gene sequence was *B. bacilliformis*, with 77.7% similarity. The species with the closest *gltA* gene sequence were *B. grahamii* and *B. schoenbuchensis*, with 90.5 and 94.1% similarity. Furthermore, bovine isolate 91-4<sup>T</sup> had 93.8% similarity to the French roe-deer isolate IBS 193<sup>T</sup> (Table 1).

### DNA-DNA hybridization

DNA of the isolates IBS 193<sup>T</sup>, 91-4<sup>T</sup> and '*B. weissii*' was labelled and hybridized against unlabelled DNA of roe-deer isolates IBS 206 and IBS 193<sup>T</sup>, bovine isolate 91-4<sup>T</sup>, North American cat isolate '*B. weissii*' and 14 *Bartonella* type strains. The results are presented in Table 1.

Roe-deer isolate IBS 193<sup>T</sup> showed 90, 53 and 48% hybridization, respectively, with roe-deer isolate IBS 206, the type strain of *B. schoenbuchensis* and bovine isolate 91-4<sup>T</sup> and less than 40% hybridization with all other type strains of *Bartonella* species tested (Table 1).

Bovine isolate 91-4<sup>T</sup> exhibited 80, 45 and 45% hybridization, respectively, with the cat isolate '*B. weissii*', *B. schoenbuchensis* and roe-deer isolate IBS 193<sup>T</sup>. It had less than 39% hybridization with all other type strains of *Bartonella* species tested (Table 1).

DNA of isolates IBS 193<sup>T</sup>, IBS 206 and 91-4<sup>T</sup> respectively presented 42, 44 and 82% hybridization with labelled DNA of the strain '*B. weissii*' (data not shown).

### Discussion of the taxonomic position of the novel isolates

Isolates IBS 193<sup>T</sup>, IBS 206 and 91-4<sup>T</sup> exhibited phenotypic characters that were characteristic of the genus *Bartonella*: they were all isolated from blood, they grew slowly (10 d for primary isolation and 6 d for subcultures), required CO<sub>2</sub> and produced small colonies on Columbia blood-agar plates.

Biochemical profiles cannot be used routinely for differentiation of *Bartonella* species because of their

inert biochemical nature. By measuring preformed enzymes (Rapid Anaerobe Panel), the tested isolates were found to be biochemically inert except for the production of peptidases, characteristic of *Bartonella* profiles (10077640).

The 16S rRNA gene sequences of isolates 91-4<sup>T</sup>, IBS 193<sup>T</sup> and IBS 206 were also highly related to the sequences of members of *Bartonella*, exhibiting 97–99.4 % similarity. These results indicate that isolates IBS 193<sup>T</sup>, IBS 206 and 91-4<sup>T</sup> belong to the genus *Bartonella*, since the similarity of 16S rRNA gene sequences between type strains within the genus *Bartonella* was previously described to be in the range 97.5–99.3 % (Birtles & Raoult, 1996). The genetic and phenotypic data provide sufficient evidence to classify these three isolates in the genus *Bartonella*.

Additional data were obtained from genetic analysis. Firstly, the strains isolated from blood of roe-deer, IBS 193<sup>T</sup> and IBS 206, had a unique and identical 16S rRNA gene sequence. Secondly, bovine isolate 91-4<sup>T</sup> had exactly the same 16S rRNA gene sequence as the cat isolate '*B. weissii*'.

On the basis of DNA–DNA hybridization, two novel species were identified, one from roe-deer and one from cattle. The two roe-deer isolates, IBS 193<sup>T</sup> and IBS 206, belonged to the same species, since they had more than 90 % hybridization with each other. Strains of the novel species are different from the type strain of *B. schoenbuchensis*, as hybridization with the latter was only 53 %. Similarly, the French roe-deer isolate IBS 193<sup>T</sup> does not belong to the same species as the bovine isolate 91-4<sup>T</sup>, since the hybridization between them was 48 %. These results indicate that strain IBS 193<sup>T</sup> belongs to a novel *Bartonella* species distinct from all previously described species and also distinct from *B. schoenbuchensis*, according to previously established criteria (Stackebrandt & Goebel, 1994).

The bovine isolate 91-4<sup>T</sup> showed 45 % hybridization with the roe-deer isolates IBS 193<sup>T</sup> and *B. schoenbuchensis*, indicating that this isolate belonged to a species different from the known roe-deer isolates. However, isolate 91-4<sup>T</sup> had 80 % DNA relatedness to the cat isolate '*B. weissii*'. These data indicated that the bovine isolate 91-4<sup>T</sup> and the cat isolate '*B. weissii*' are closely related and belong to the same species, which is distinct from all those previously described and also distinct from the species isolated from roe-deer, according to previously established criteria (Stackebrandt & Goebel, 1994). '*B. weissii*' has been isolated from a few cats in the USA. However, isolate 91-4<sup>T</sup> DNA has been detected in more than 35 % of 36 French dairy cattle. It could be possible that '*B. weissii*' resulted from a transfer from a bovine to a feline population. In support of this hypothesis, Chang *et al.* (2000) obtained bovine isolates from American herds, closely related to French isolate 91-4<sup>T</sup>, with a high prevalence of bacteraemia (49 %).

The first report of the infection of cattle with a

bacterium called '*Bartonella bovis*' was made by Donatien & Lestoquard (1934), on the basis of microscope examination of a stained blood smear. Similar descriptions were presented later from American cattle (Lotze & Yiengst, 1942) and British cattle (Brocklesby, 1970); the bacteria observed were called '*Haemobartonella bovis*' by the latter two groups of authors. The presence of '*H. bovis*' in American or British cattle was associated with the isolation of strains of *Theileria* sp. or *Anaplasma* sp., two well-known agents of tick-borne infections.

The first report of cultivation of *Bartonella* species from free-ranging wild ruminants was made in Europe from the blood of French roe-deer (Heller *et al.*, 1999b). Other isolates were later obtained from German roe-deer (Dehio *et al.*, 2001) and also from wild and domestic ruminants from North America (Chomel *et al.*, 1999; Chang *et al.*, 2000) and domestic cattle in France (H.-J. Boulouis and others, unpublished data). *Bartonella* species are therefore frequently encountered in the blood of ruminants and can be transmitted to humans or various other animal species. *Bartonella* DNA was also detected in more than 70 % of ticks collected from roe-deer in The Netherlands (Schouls *et al.*, 1999; Bergmans, 1996).

#### Description of *Bartonella capreoli* sp. nov.

*Bartonella capreoli* (ca.pre.o'li. L. gen. n. *capreoli* of the roe-deer).

Colonies grown on blood agar appear homogeneous, round and grey–white. Electron microscope examination shows small bacilli with multiple unipolar flagella. Primary culture obtained after 10 d of incubation at 35 °C in a moist atmosphere containing 5 % CO<sub>2</sub>. Subculture on agar obtained after 6 d under the same conditions. Gram-negative, oxidase- and catalase-negative, negative for Voges–Proskauer reaction, no urease activity and able to hydrolyse trehalose, *N*-acetyl β-D-glucosaminide and bis-*p*-nitrophenyl phosphate. Shows amino-acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. Has trypsin-like activity and glycyglycylarylamidase activity, but no pyrrolidonylarylamidase activity. Distinguished from the other *Bartonella* species by 16S rRNA and *gltA* gene sequences and by genomic DNA hybridization.

The type strain, IBS 193<sup>T</sup> (= CIP 106691<sup>T</sup> = CCUG 43827<sup>T</sup>), was isolated from the blood of a roe-deer (*Capreolus capreolus*). Its 16S rRNA gene sequence and partial 3'-end *gltA* gene sequence have been deposited in EMBL/GenBank under accession numbers AF293389 and AF293392.

#### Description of *Bartonella bovis* Bermond *et al.* non Donatien and Lestoquard 1934 sp. nov.

*Bartonella bovis* (bo'vis. L. gen. n. *bovis* of the ox).

This species is named *Bartonella bovis* Bermond *et al.* 2002 non Donatien and Lestoquard 1934 sp. nov. to distinguish it from the organism described as '*Bartonella bovis*' by Donatien & Lestoquard (1934). The latter name did not appear in the *Approved Lists of Bacterial Names* (Skerman *et al.*, 1980).

Primary culture obtained after 10 d of incubation at 35 °C in a moist atmosphere containing 5% CO<sub>2</sub>. Subculture on agar obtained after 4 d of culture under the same conditions. Colonies grown on 5% rabbit blood agar appear homogeneous, round and grey-white. Electron microscope examination shows small bacilli without flagella. Gram-negative, oxidase- and catalase-negative, with a negative Voges-Proskauer reaction and no urease activity. Able to hydrolyse trehalose, *N*-acetyl  $\beta$ -D-glucosaminide and bis-*p*-nitrophenyl phosphate. Exhibits amino-acid arylamidase activity with the following amino acids: arginine, lysine (alkaline as well as acid), glycine, leucine, methionine, proline and tryptophan. Has trypsin-like activity and glycyglycylarylamidase activity, but no pyrrolidonylarylamidase activity. Distinguished from the other *Bartonella* species by 16S rRNA gene and *gltA* gene sequences and by genomic DNA hybridization.

The type strain is strain 91-4<sup>T</sup> (= CIP 106692<sup>T</sup> = CCUG 43828<sup>T</sup>), isolated from the blood of a domestic cow. Its 16S rRNA gene sequence and partial 3'-end *gltA* gene sequence have been deposited in EMBL/GenBank under accession numbers AF293391 and AF293394.

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