

Differentiation of *Bartonella* Species by a Microimmunofluorescence Assay, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis, and Western Immunoblotting

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Received 3 December 1999/Returned for modification 4 April 2000/Accepted 8 May 2000

***Bartonella* species can be differentiated by microimmunofluorescence assay, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting with murine polyclonal antisera to *Bartonella henselae*, *B. quintana*, *B. elizabethae*, and *B. bacilliformis*. A pairwise comparison on the basis of SDS-PAGE protein profiles demonstrated similarity values for proteins of different *Bartonella* species ranging from 28.6 to 86.4%. Antigenic relationships revealed by immunoblotting with murine antisera were equivalent to those of proteins observed by SDS-PAGE. A dendrogram obtained on the basis of protein bands of SDS-polyacrylamide gels showed that *Bartonella* species could be divided into three groups. *B. bacilliformis* was distinct from all other *Bartonella* species; *B. grahamii*, *B. taylorii*, *B. doshiae*, and *B. vinsonii* formed a cluster, as did *B. henselae*, *B. quintana*, *B. elizabethae*, and *B. clarridgeiae*. These relationships were consistent with those revealed by parsimony trees derived from 16S rRNA and *gltA* gene sequencing. SDS-PAGE analysis showed that 120-, 104-, 85-, 71-, 54-, 47-, 40-, 33-, 30-, and 19-kDa proteins were present in all species, with the 54-kDa protein being the most dominant. Proteins with a molecular mass of less than 54 kDa allow the differentiation of species and are a possible target for future species-specific antibodies and antigens.**

The genus *Bartonella* presently contains 14 species (3, 12, 16, 17, 24): *Bartonella bacilliformis*, *B. henselae*, *B. quintana*, *B. vinsonii*, *B. elizabethae*, *B. talpae*, *B. peromysci*, *B. grahamii*, *B. taylorii*, *B. doshiae*, *B. clarridgeiae*, *B. tribocorum*, *B. alsatica*, and *B. koehlerae*. A number of studies of the genetic relationship among *Bartonella* species have been carried out, including determinations of guanine-plus-cytosine contents (3, 6) and whole-genome DNA-DNA hybridization analyses (9, 33). These studies have demonstrated significant levels of similarity among the *Bartonella* species. Comparison of 16S rRNA gene sequences also indicated a high degree of similarity, ranging from 97.9 to 99.6%, within the genus *Bartonella* (3). A comparison of citrate synthase gene (*gltA*) sequences of different *Bartonella* species has shown the levels of similarity between sequences to be from 83.8 to 93.5% (4). *Bartonella* species may, however, be differentiated by restriction fragment length polymorphism PCR of the 16S rRNA gene with a combination of the restriction endonucleases *DdeI* and *MnII* (5) and of the citrate synthase gene (18). Few phenotypic characteristics (8, 10, 14, 26, 32) have been reported for the identification of *Bartonella* species. In this report, we describe the protein and antigenic characteristics of *Bartonella* species as determined by comparative microimmunofluorescence assay (MIF), sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE), and Western immunoblotting.

MATERIALS AND METHODS

***Bartonella* strains.** The sources of all strains used in this study are presented in Table 1. *Bartonella* isolates were cultivated on blood agar (BioMérieux, Marcy l'Etoile, France) at 37°C in a 5% carbon dioxide incubator with the exception of *B. bacilliformis*, for which cultivation was carried out at 32°C. After between 5

and 7 days of culture, bacteria were harvested and suspended in deionized water for use in SDS-PAGE or in phosphate-buffered saline (PBS) for use in the MIF.

Antiserum production. Murine polyclonal antisera directed against *B. henselae*, *B. quintana*, *B. bacilliformis*, *B. elizabethae*, *B. taylorii*, and *B. vinsonii* were produced in BALB/c mice. The mice were immunized intraperitoneally three times, at 7-day intervals, with 200 µg of *Bartonella* proteins. Sera were obtained from the mice 1 week after the final immunization. Antibody titers of all sera collected were determined by MIF.

MIF. Antigens were deposited on 24-well microscope slides with the nib of a pen. The antigens were fixed in methanol for 10 min at room temperature. Murine antisera to *Bartonella* species were diluted 1:2 to 1:8,192 in PBS containing 3% milk powder and added to the wells, and the slides were incubated in a humidified chamber at 37°C for 30 min. The slides were then washed twice in PBS (5 min each) and rinsed with distilled water. After being air dried at 37°C, the slides were incubated at 37°C for 30 min with fluorescein (dechlorotriazinyl aminofluorescein)-conjugated goat anti-mouse immunoglobulin G (IgG) plus IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa.) diluted 1:100 in PBS containing 0.2% Evans blue (BioMérieux). The slides were washed as described above, mounted with Fluoprep (BioMérieux), and then read on a Zeiss epifluorescence microscope (Axioskop 20; Carl Zeiss Göttingen, Germany) at 400× magnification.

SDS-PAGE and Western immunoblotting. SDS-PAGE was performed by a modification of the method described by Laemmli (22), using a 12% polyacrylamide separating gel and a 5% stacking gel. Aliquots (10 µl) of antigens preparations, previously adjusted to contain 4 mg of protein/ml, were suspended in an equal volume of sample buffer (0.0625 M Tris hydrochloride [pH 8.0], 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) and heated for 5 min at 100°C. The dissolved antigens were separated in an electrophoretic cell (Mini Protein II; Bio-Rad, Richmond, Calif.) at a constant current (8 to 10 mA per gel) for 3 to 4 h in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS). Prestained SDS-PAGE standards (Low-Range; Bio-Rad) were used on each gel as a reference.

The separated antigens were transferred to 0.45-µm-pore-size nitrocellulose membranes (Hybond-C; Amersham, Little Chalfont, United Kingdom) in an electrophoretic transfer cell (Mini Trans-Blot; Bio-Rad) with transfer buffer (2.5 mM Tris base, 192 mM glycine, 20% methanol) at 50 V and 4°C for 1 h. After transfer was completed, the nitrocellulose membranes were incubated overnight in PBS with 5% nonfat dry milk to block nonspecific binding sites, washed three times with PBS, and air dried. The membranes were then incubated at room temperature for 1 h with murine antisera diluted 1:100 in PBS containing 3% nonfat dry milk and washed as described above. After being incubated at room temperature for 1 h with peroxidase-conjugated F(ab')₂ fragment goat anti-mouse IgG (heavy and light chains; AffiniPure; Jackson ImmunoResearch) diluted 1:500 in PBS containing 3% nonfat dry milk, the blots were washed in PBS

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TABLE 1. Studied strains

Species	Strain ^a	Source ^b
<i>B. bacilliformis</i>	Monzon 812	Bartonellosis patient, Peru
<i>B. henselae</i>	Houston-1 ^T (ATCC 49882)	Septicemia
	URBHLLY8	CSD patient, Marseille, France
	URBHLLY9	IE patient, Marseille, France
	CAL-1	Septicemia patient, United States
	Cat 6	South African cat
	Fizz	Swiss cat
	King	USA
	SA2	CSD patient, United States
<i>B. quintana</i>	Fuller ^T (ATCC VR-358)	TF patient
<i>B. elizabethae</i>	F9251 ^T (ATCC 49927)	IE patient
<i>B. grahamii</i>	V2 ^T (NCTC 12860)	Blood of <i>C. glareolus</i>
<i>B. taylorii</i>	M6 ^T (NCTC 12861)	Blood of <i>Apodemus</i> spp.
<i>B. doshiae</i>	R18 ^T (NCTC 12862)	Blood of <i>M. agrestis</i>
<i>B. clarridgeiae</i>	URBCMHC26	French cat
<i>B. vinsonii</i>	Baker ^T (ATCC VR-152)	Spleen of <i>M. pennsylvanicus</i>

^a T, reference strain.

^b Abbreviations: BA, bacillary angiomatosis; CSD, cat scratch disease; IE, infective endocarditis; LY, lymphadenopathy; TF, trench fever.

and bound peroxidase enzyme was detected with 4-chloro-1-naphthol as the substrate.

Calculation of protein molecular sizes. A standard curve was generated by using molecular mass standards on each Western blot so that the sizes of the *Bartonella* proteins could be determined.

Numerical taxonomic analysis. A score of 1 was given for each band in the SDS-PAGE profile or Western blot profile. These scores were used to construct a dendrogram from the matrix by the unweighted-pair group method with an arithmetic mean (UPGMA), available in the PC-TAXAN software package (Sea Grant College, University of Maryland, College Park), according to the manufacturer's instructions. The Jaccard coefficients (S_j) derived from matrix analysis were used as measures of similarity of antigenicity among different *Bartonella* species. Parsimony trees inferred from alignment of *Bartonella* 16S rRNA and *gluA* gene sequences obtained from GenBank were compared with the obtained dendrograms.

RESULTS

While MIF titers of the polyclonal mouse antisera were highest against the homologous antigens (Table 2), cross-reactive antibodies against the other *Bartonella* species could generally be detected. The highest titers of cross-reacting antibodies were in antisera to *B. henselae* when tested against *B. quintana*.

SDS-PAGE analysis of *Bartonella* species showed distinct protein profiles (Fig. 1), with differences being evident mainly among proteins with molecular masses smaller than 54 kDa. Although minor differences between *B. grahamii* and *B. taylorii* were detected, their SDS-PAGE profiles were almost identical. *B. bacilliformis* was clearly distinct from all other *Bartonella*

species. Different strains belonging to the same species had identical protein profiles, with the exception of the two *B. henselae* serotypes, which showed only minor differences (data not shown). SDS-PAGE of *Bartonella* species antigens showed that several proteins, including those of 120, 104, 85, 71, 54, 47, 40, 33, 30, and 19 kDa, were common to all (Fig. 1; Table 3). The 54-kDa protein band gave the most prominent staining reaction in each species (Fig. 1; Table 3), and dominant and species-specific proteins of less than 54 kDa were also observed (see Fig. 4).

Western blots revealed unique profiles for each species (Fig. 2A to D), with the exception of antisera to *B. grahamii* and *B. vinsonii*, which could not differentiate *Bartonella* species (Fig. 2E and F). Although there were visible differences among *B. henselae* serotypes and between *B. grahamii* and *B. taylorii*, these organisms generally had very similar profiles (Fig. 2A to D). Species-specific antigens were clearly identified in a low-molecular-mass range. Each polyclonal murine antiserum reacted with the 120-, 104-, and 54-kDa antigen bands of each *Bartonella* species (Fig. 2; Table 3), and in addition, antisera to *B. henselae* and *B. quintana* (Fig. 2; Table 3) reacted with a 19-kDa protein in each species. Homologous Western blots identified several antigens, and the most immunoreactive bands were detected with anti-*B. bacilliformis* serum. Eleven of these bands had estimated molecular sizes of 120, 104, 71, 54, 47, 41, 37, 33, 30, 27, and 25 kDa and were highly reactive. The

TABLE 2. MIF titers of *Bartonella* antigens, obtained with murine antisera

Species no.	Species ^a	MIF titer determined using antiserum to species:					
		1	2	4	5	6	9
1	<i>B. bacilliformis</i>	4,096	16	16	4	128	
2	<i>B. henselae</i> H	128	4,096	64	32	16	16
3	<i>B. henselae</i> M	128	2,048	32	32	16	16
4	<i>B. quintana</i>	128	512	4,096	8	64	4
5	<i>B. elizabethae</i>	128	64	16	1,024	32	
6	<i>B. grahamii</i>	64	16	16	4	1,024	
7	<i>B. taylorii</i>	128	16	16	8	512	
8	<i>B. doshiae</i>	128	16	16	8	64	32
9	<i>B. vinsonii</i>	128	16	16	16	64	1,024
10	<i>B. clarridgeiae</i>	128	16	16	8	32	16

^a *B. henselae* H and *B. henselae* M are *B. henselae* subspecies *houstonae* and *B. henselae* *massiliae*, respectively.

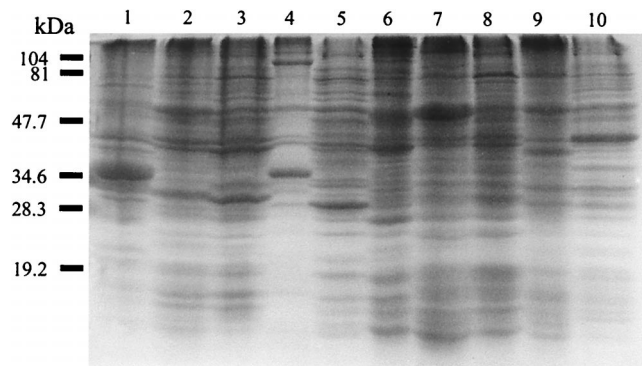


FIG. 1. Coomassie brilliant blue staining profiles of *Bartonella* strains after SDS-PAGE. Lanes: 1, *B. bacilliformis*; 2, *B. henselae* serotype Houston; 3, *B. henselae* serotype Marseille; 4, *B. clarridgeiae*; 5, *B. quintana*; 6, *B. elizabethae*; 7, *B. grahamii*; 8, *B. taylorii*; 9, *B. doshiae*; 10, *B. vinsonii*. The positions of molecular mass markers of 104, 81, 47.7, 34.6, 28.3, and 19.2 kDa are noted on the left.

less-reactive antisera were mostly against *B. grahamii* and *B. vinsonii* (Fig. 2E and F). Species-specific immunoreactive bands of 41, 23, 21, 32, and 26 kDa were prominently observed for *B. bacilliformis*, *B. henselae* subtype Houston, *B. henselae* subtype Marseille, *B. quintana*, and *B. elizabethae*, respectively (Fig. 2; Table 4).

A pairwise comparison of SDS-PAGE protein profiles demonstrated protein similarity values ranging from 28.6% (for *B. clarridgeiae* and *B. doshiae*) to 86.4% (for *B. grahamii* and *B. taylorii*). The protein similarity value for *B. henselae* strains Houston and Marseille was 76.3%. However, the similarity between different strains within each serotype was 100% (Table 5). Antigenic relationships identified by Western blotting with murine antiserum to *B. henselae* were similar to those found in protein profiles obtained by SDS-PAGE (Table 5).

If an *S_i* similarity value of 50.1% was used as the cutoff for the dendrogram obtained from SDS-PAGE, the *Bartonella* species could be divided into three groups (Fig. 3). *B. bacilliformis* was the single member of the first group and was most distant; *B. grahamii*, *B. taylorii*, *B. doshiae*, and *B. vinsonii* formed a second cluster, while *B. henselae*, *B. quintana*, *B. elizabethae*, and *B. clarridgeiae* formed a third (Fig. 3). The dendrogram based on immunoreactive bands observed with murine antiserum to *B. henselae* also identified these three groups (Fig. 4).

As for comparison of these dendrograms with phylogenetic trees, SDS-PAGE and Western blot findings were consistent with the analysis of the 16S rRNA and *gltA* gene data (100% bootstrap) (Fig. 3 and 4) obtained by parsimony analysis, showing the relatively deeply rooted divergence of *B. bacilliformis* from other *Bartonella* species. However, the two other groups were not identified, and none of the significant bootstrap values was found in a phylogenetic tree inferred from the 16S rRNA gene sequence data. However, three significant nodes were determined from the *gltA*-derived data. Two groups were also found with the Western blot data based on UPGMA; one contained *B. taylorii* and *B. vinsonii* (90% bootstrap value) (Fig. 4), and the second included *B. henselae* and *B. quintana* (94% bootstrap value) (Fig. 4). The third node, clustering *B. elizabethae* and *B. grahamii* (100% bootstrap) (Fig. 4), was not found when Western blot data were used.

DISCUSSION

In this study, nine *Bartonella* species were clearly differentiated by their distinct SDS-PAGE protein profiles, with bands

of molecular mass less than 54 kDa being responsible for most of the observed differences. Western blotting with antisera to *B. quintana*, *B. henselae*, *B. bacilliformis*, and *B. elizabethae* also enabled the differentiation of all species and subspecies of *Bartonella* studied. These results support the earlier suggestion that phenotypic criteria may be used to characterize *Bartonella* spp. (26). They also show that SDS-PAGE and Western blotting are effective methods for phenotypic identification of the *Bartonella* group to the species level, in accord with the observed specific reactivity between *B. quintana* and *B. henselae*. Moreover, the identification of genus- and species-specific bands may allow the identification of genus- and species-specific polyclonal and monoclonal antibodies. The species-specific antigen could be the source of species-specific serology.

Data obtained in SDS-PAGE and Western blot analyses enabled us to determine that there are three groups within the species *Bartonella*. The first contains only *B. bacilliformis*, the etiological agent of Carrion's disease, whose distribution is limited to the inter-Andean valleys of South America. The trees derived from the similarity matrix based on our SDS-PAGE and Western blotting results indicate that *B. bacilliformis* is the most distant of the *Bartonella* species, which is consistent with the results of previous DNA hybridization studies (9, 33) and with data obtained in a series of studies of both 16S rRNA and *gltA* gene sequences (4). The second cluster contains *B. henselae*, *B. quintana*, *B. elizabethae*, and *B. clarridgeiae*. *B. henselae*, a new species first described in 1992 (29), contains two serotypes and two genotypes (2, 11, 25, 30, 31), and we believe that they are subspecies (Z. Liang, V. Roux, B. La Scola, and D. Raoult, unpublished data). The dendrogram confirms that *B. henselae* is very closely related to *B. quintana*. These species can cause a number of clinical manifestations, some of which, such as bacillary angiomatosis and endocarditis, are identical (7, 15, 19, 21, 27, 28). Both exhibit a high degree of serological cross-reactivity (1, 13, 23). DNA-DNA hybridization experiments (9, 33) and comparisons of the sequences of the 16S rRNA and *gltA* genes revealed a high level of genetic homology (3, 4). *B. clarridgeiae* was isolated from an American cat in 1996 (24), and it has subsequently been suggested that this organism may be one of the etiological agents of cat scratch disease (20). The dendrogram produced in the present study demonstrates that this species is very closely related to *B. henselae*. To date, only a single isolate of *B. elizabethae* has been obtained from a human patient with endocarditis (9).

TABLE 3. *Bartonella* genus-specific antigens detected by SDS-PAGE and Western blotting with antibodies against various *Bartonella* species

Protein size (kDa)	SDS-PAGE	Results of immunoblot with murine serum to ^a :					
		bac	hen	qui	eli	gra	vin
120	+	++	+	++	++	+	+
104	+	+	++	++	++	++	+
85	+	+	++	+	+	+	-
71	+	+	+	-	+++	-	-
54	++++	++++	++++	+++	++++	++++	+++
47	+	+++	+	++	++	-	+++
40	+	+++	++	-	+	+	-
33	+	+	+	-	-	-	-
30	+	+++	+	+++	++	-	-
19	+	-	++	+++	-	-	-

^a Antigens were *B. bacilliformis* (bac), *B. henselae* (hen), *B. quintana* (qui), *B. elizabethae* (eli), *B. grahamii* (gra), and *B. vinsonii* (vin). -, no band evident; +, weak band; ++, moderate-intensity band; +++, intense band; +++++, very intense band.

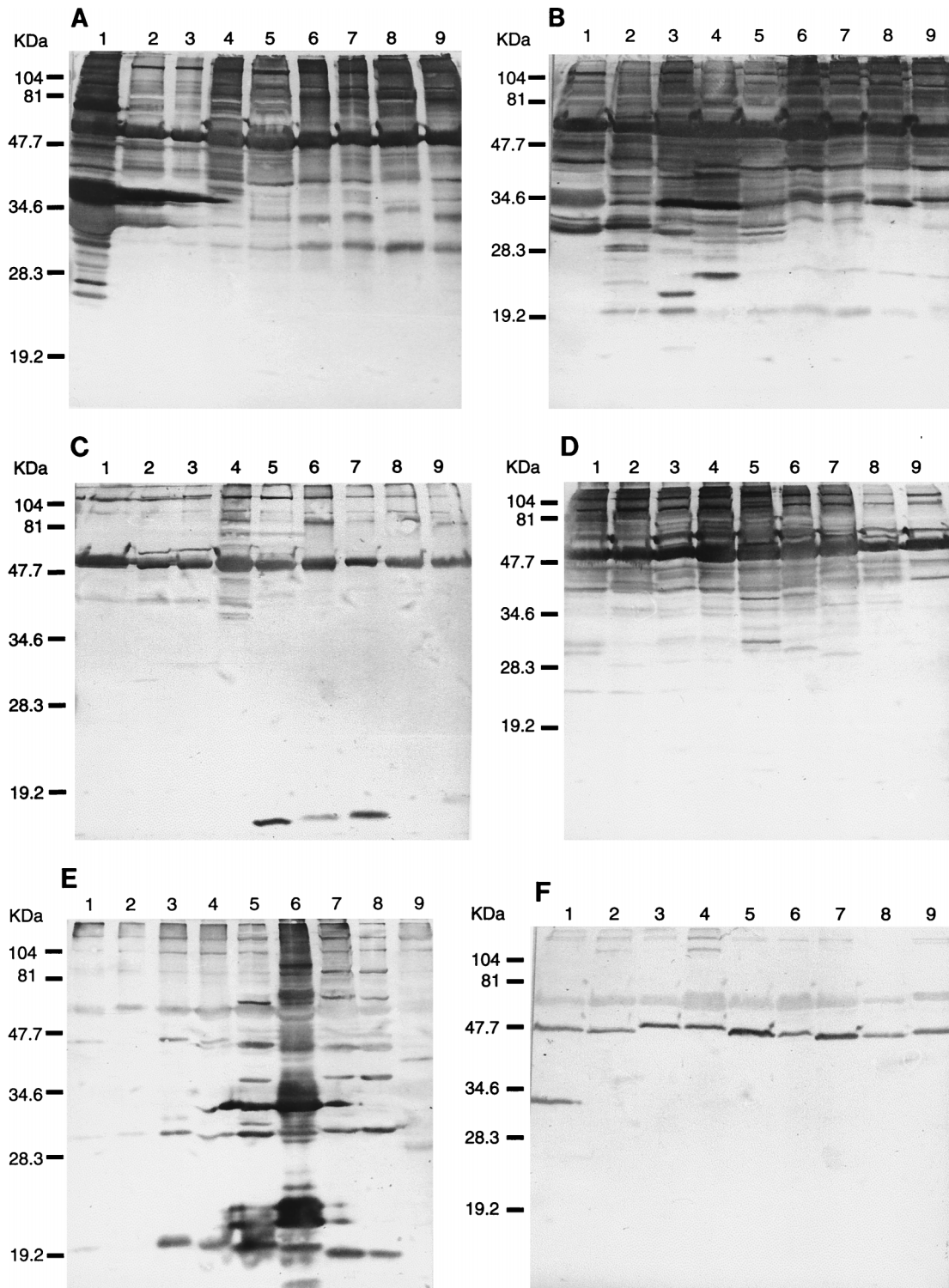


FIG. 2. Immunoblotting of *Bartonella* antigens with murine antisera to *B. bacilliformis* (A), *B. henselae* (B), *B. grahamii* (C), *B. elizabethae* (D), *B. quintana* (E), and *B. vinsonii* (F). (A to C) Lanes: 1, *B. bacilliformis*; 2, *B. henselae* Houston; 3, *B. henselae* Marseille; 4, *B. quintana*; 5, *B. elizabethae*; 6, *B. grahamii*; 7, *B. taylorii*; 8, *B. doshiae*; 9, *B. vinsonii*. (D to F) Lanes: 1, *B. vinsonii*; 2, *B. doshiae*; 3, *B. taylorii*; 4, *B. grahamii*; 5, *B. elizabethae*; 6, *B. quintana*; 7, *B. henselae* Marseille; 8, *B. henselae* Houston; 9, *B. bacilliformis*. The positions of molecular mass standards are noted on the left of each panel.

This isolate clustered differently in our study and was found to be closely related to *B. quintana* and *B. henselae*; 16S rRNA and *gltA* gene sequence analysis showed it to cluster with *B. grahamii*.

The third cluster contains *B. grahamii*, *B. taylorii*, *B. doshiae*, and *B. vinsonii*. These organisms were isolated from rodents; *B. vinsonii* was also isolated from dogs.

A previous investigation (4) has shown that analysis of the

TABLE 4. Dominant and species-specific proteins of less than 54 kDa in *Bartonella* species

Method used	Protein (kDa)	Results for ^a :												
		<i>B. bacilliformis</i>	<i>B. henselae</i>	Houston	<i>B. henselae</i>	Marseille	<i>B. quintana</i>	<i>B. elizabethae</i>	<i>B. garharnii</i>	<i>B. taylorii</i>	<i>B. doshiae</i>	<i>B. vinsonii</i>	<i>B. clarridgeiae</i>	
SDS-PAGE	41			+++	+++									
	40.5						++						+++	+++
	40	+++		+++										
	37	+++												
	34	+++	+++											
	32													+++
	31													
	30	+++			+++									+
	29	+++												
	27													
26														
23														
Immunoblotting with antiserum to <i>B. henselae</i>			+++											
41														
38														
35														
34		+++												
31		+++												
30		++												
29		++												
28		+++												
24		+++												
23				++										
21														
19				++										
45														
Immunoblotting with antiserum to <i>B. quintana</i>														
44														
41		++												
37														
34														
32														
31														
30														
29														
28		++												
23														

^a +, weak; ++, moderate; +++, intense; +++++, very intense; NT, not tested.

TABLE 5. Antigenic S_j similarity among *Bartonella* isolate pairs as revealed by SDS-PAGE and immunoblotting with antiserum to *B. henselae*

Isolate no.	<i>Bartonella</i> isolate	Antigenic similarity (S_j ; %) among <i>Bartonella</i> isolate ^a :															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	<i>B. bacilliformis</i>		56.0	56.0	56.0	48.1	48.1	48.1	48.1	48.1	NT	60.9	50.0	22.2	25.9	30.4	33.3
2	<i>B. henselae</i>	34.7	100	100	100	75.0	75.0	75.0	75.0	75.0	NT	64.3	60.7	23.5	26.5	30.0	32.1
3	<i>B. henselae</i>	34.7	100	100	100	75.0	75.0	75.0	75.0	75.0	NT	64.3	60.7	23.5	26.5	30.0	32.1
4	<i>B. henselae</i>	34.7	100	100	100	75.0	75.0	75.0	75.0	75.0	NT	64.3	60.7	23.5	26.5	30.0	32.1
5	<i>B. henselae</i>	45.7	76.3	76.3	76.3	100	100	100	100	100	NT	56.7	48.4	26.5	25.7	29.0	26.7
6	<i>B. henselae</i>	45.7	76.3	76.3	76.3	100	100	100	100	100	NT	56.7	48.4	26.5	25.7	29.0	26.7
7	<i>B. henselae</i>	45.7	76.3	76.3	76.3	100	100	100	100	100	NT	56.7	48.4	26.5	25.7	29.0	26.7
8	<i>B. henselae</i>	45.7	76.3	76.3	76.3	100	100	100	100	100	NT	56.7	48.4	26.5	25.7	29.0	26.7
9	<i>B. henselae</i>	45.7	76.3	76.3	76.3	100	100	100	100	100	NT	56.7	48.4	26.5	25.7	29.0	26.7
10	<i>B. clarridgeiae</i>	40.0	61.5	61.5	61.5	64.1	64.1	64.1	64.1	64.1	NT	NT	NT	NT	NT	NT	NT
11	<i>B. quintana</i>	43.5	50.0	50.0	50.0	59.5	59.5	59.5	59.5	59.5	61.5	65.4	33.3	36.7	42.3	45.8	
12	<i>B. elizabethae</i>	42.9	48.9	48.9	48.9	54.3	54.3	54.3	54.3	54.3	55.8	59.1	34.5	37.9	38.5	47.8	
13	<i>B. grahamii</i>	35.9	32.5	32.5	32.5	31.7	31.7	31.7	31.7	31.7	38.9	39.5	39.0	94.7	65.0	63.2	
14	<i>B. taylorii</i>	42.1	31.7	31.7	31.7	31.0	31.0	31.0	31.0	31.0	37.8	38.5	41.5	86.4	61.9	68.4	
15	<i>B. doshiae</i>	37.1	33.3	33.3	33.3	32.4	32.4	32.4	32.4	32.4	28.6	33.3	30.0	52.2	50.0	64.7	
16	<i>B. vinsonii</i>	36.8	33.3	33.3	33.3	32.5	32.5	32.5	32.5	32.5	40.0	44.4	36.6	50.0	48.1	54.5	

^a The values on the upper right are the antigenic similarities of *Bartonella* spp. as determined by SDS-PAGE, and the values on the lower left are the antigenic similarities of *Bartonella* spp. as determined by immunoblotting with antiserum to *B. henselae*.

^b NT, not tested.

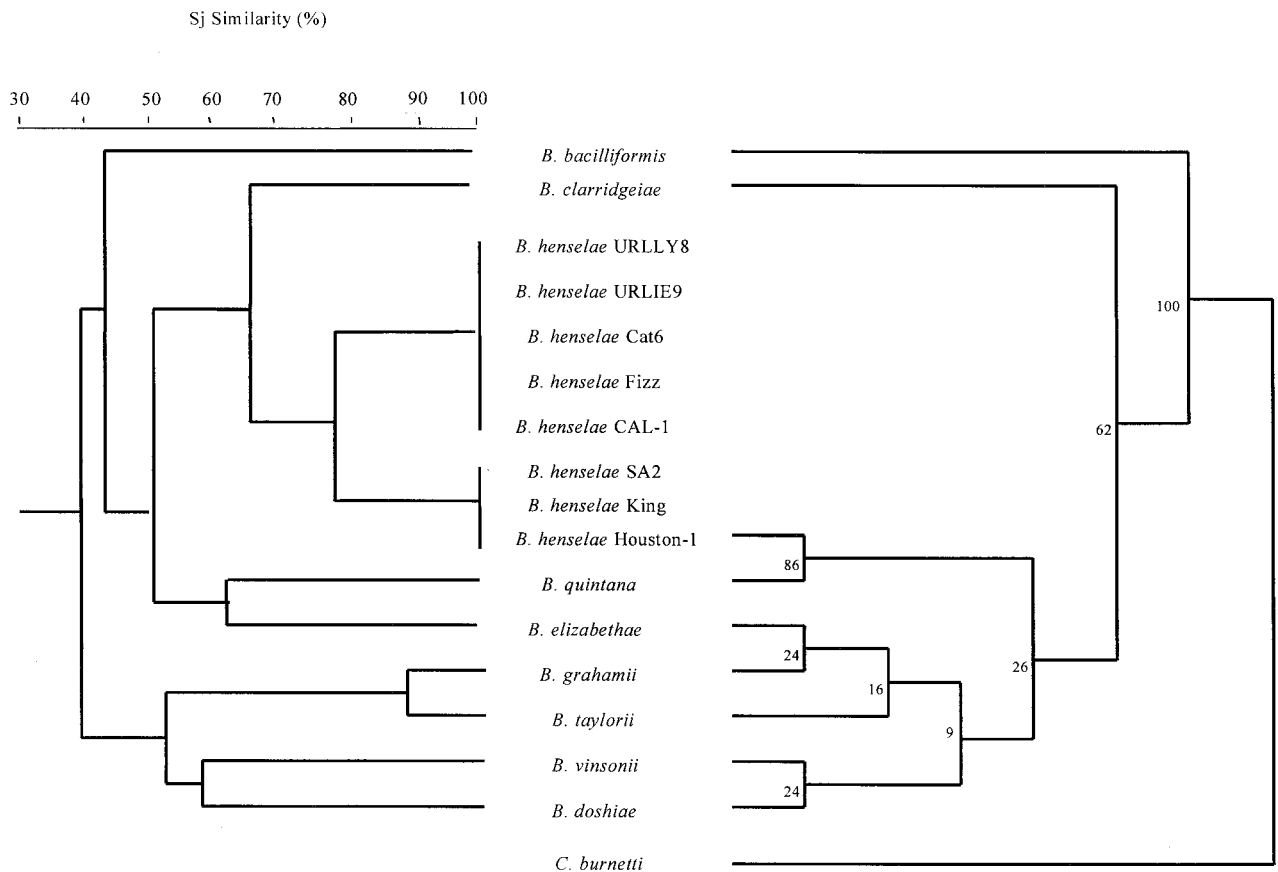


FIG. 3. Dendrogram of *Bartonella* strains inferring S_j similarities obtained by UPGMA on the basis of the molecular masses of different protein bands on SDS-PAGE (left), and parsimony tree of *Bartonella* strains and *Coxiella burnetii* derived from 16S rRNA gene comparison (right). The numbers at the nodes are the proportions of 100 bootstrap resamplings that support the topology shown. This analysis provided tree topology only, and the lengths of both the vertical lines and the horizontal lines are not significant. The GenBank accession numbers for the 16S rRNA gene sequences included are as follows: *B. bacilliformis*, Z70003; *B. henselae*, Z11684; *B. quintana*, M11927; *B. clarridgeiae*, X97822; *B. elizabethae*, L01260; *B. grahamii*, Z31349; *B. taylorii*, Z31350; *B. doshiae*, Z31351; *B. vinsonii*, Z31352; and *C. burnetii*, D89792.

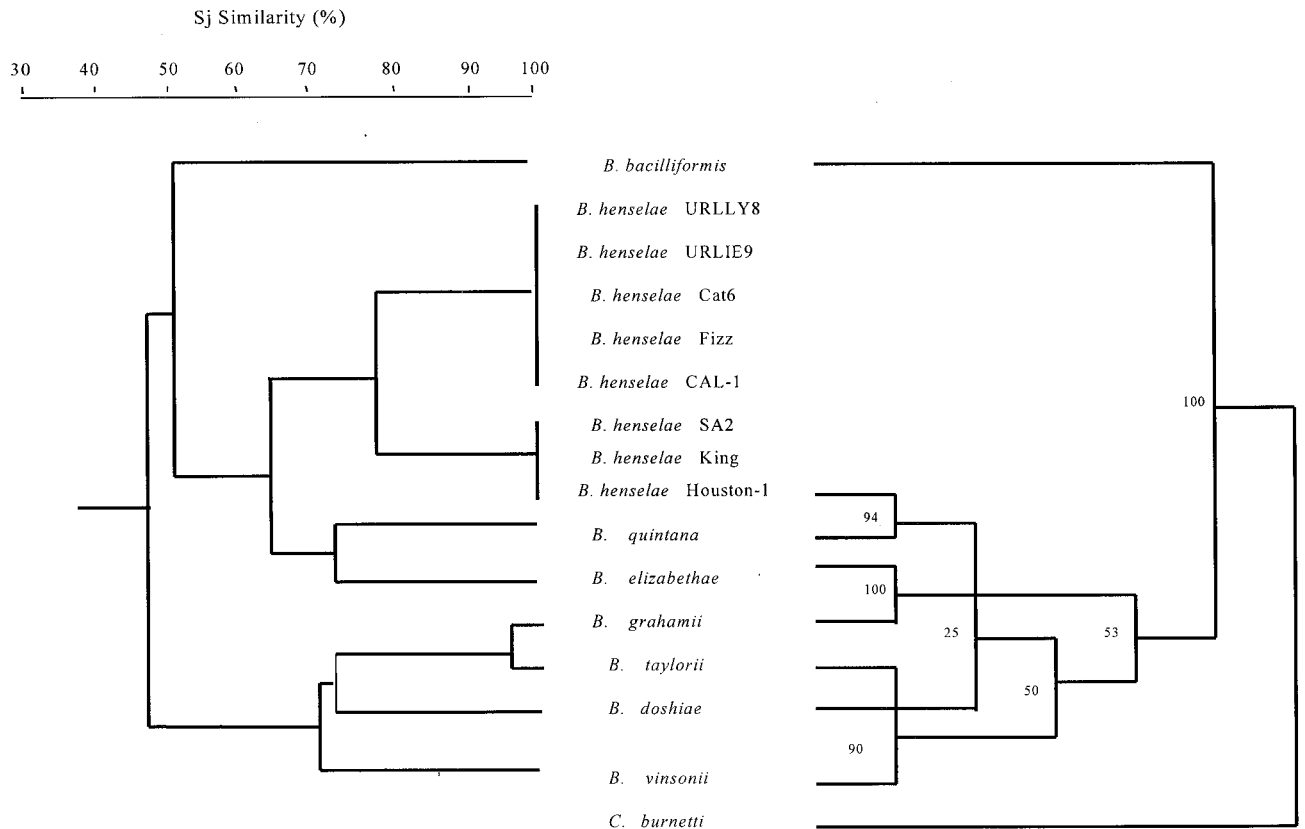


FIG. 4. Dendrogram of *Bartonella* strains representative of *Coxiella* inferring S_j similarities obtained by UPGMA on the basis of immunoblotting with murine antiserum to *B. henselae* (left), and parsimony tree of *Bartonella* strains and *Coxiella burnetii* derived from *gltA* gene comparison (right). The support for each branch, as determined from bootstrap samples, is indicated by the value at the node. This analysis provided tree topology only, and lengths of both the vertical lines and the horizontal lines are not significant. The GenBank accession numbers for *gltA* gene sequences included are as follows: *B. bacilliformis*, Z70021; *B. henselae*, L38987; *B. quintana*, Z70014; *B. elizabethae*, Z70009; *B. grahamii*, Z70016; *B. taylorii*, Z70013; *B. doshiae*, Z70017; *B. vinsonii*, Z70015; and *C. burnetii*, M36338.

gltA gene is more suitable for phylogenetic studies of *Bartonella* species than is analysis of the 16S rRNA gene. The results of our study support this finding, as the taxonomic relationships among the *Bartonella* species we inferred were generally consistent with those derived from phylogenetic analysis of the *gltA* gene data.

ACKNOWLEDGMENTS

We are grateful to Hervé Tissot-Dupont and Veronique Roux for making the dendrogram and to P. Kelly for reviewing the manuscript.

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