

## Antigenic and Genotypic Relationships between *Bartonella henselae* Strains

The ubiquitous feline intraerythrocytic pathogen *Bartonella henselae* causes a range of important human infections, and seroprevalence in blood donors in Australia is around 5% (6). Two antigenically distinct subtypes have been described, named for the type strain (Houston-1) and the more recently recognized Marseille strain (5), which have characteristic DNA sequences in the 16S rRNA gene (rDNA) and the *gltA* (citrate synthase) gene (2, 3, 5). Culture of this fastidious, gram-negative bacterium is difficult, and serology is a primary diagnostic approach. A recent report that Houston-like strains (including Houston-1, 90-615, and SA-2) may be distinguished from serologically distinct Marseille-like strains (including URBHLLY-8, URBHLIE-9, Fizz, and CAL-1) by the faster migration of a characteristic band (ca. 29 kDa) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and immunoblotting of whole-cell lysates is therefore particularly noteworthy (7).

However, the SDS-PAGE data appear to contrast with previous publications from the same laboratory (8, 9) and

others (11). We therefore compared local human isolates with the Houston-1 and Marseille strains and also found two closely related genotypes with corresponding patterns on SDS-PAGE of whole-cell lysates (Fig. 1). All human strains appear to have either the 30-kDa band (Houston) or the 29-kDa band (Marseille), in agreement with earlier studies (10, 11). The agar-pitting colony phenotype and number of *in vitro* passages have no apparent influence (Fig. 1 and Table 1). However, a local feline strain of the type II (Marseille-like) 16S rDNA genotype has the faster-migrating (29-kDa) band and a *gltA* gene sequence typical of Houston strains, consistent with the considerable diversity known to exist in feline strains (1, 10) and illustrating a dissociation of the accepted genotype-phenotype relationships. The strains in the recent study (7) were all human isolates and were assigned to genotypes by individual sequencing of several genes, including *groEL* (12) and the 16S rDNA (3). While the 29- to 30-kDa antigen(s) may yet prove useful for serotyping of human isolates, it thus seems unlikely that reported inconsistencies in the data can be easily explained by phase variation or genotype. In addition, we have no reason to assume that the more diverse feline strains are all nonpathogenic in humans. For both of these

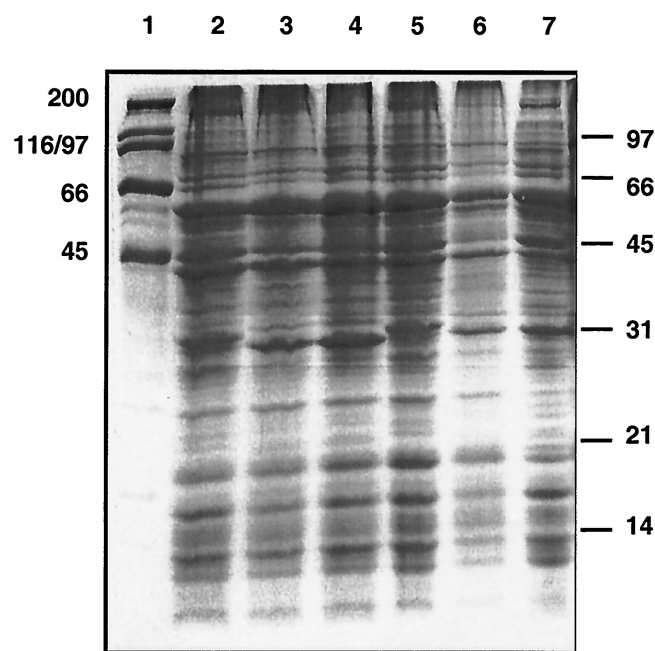


FIG. 1. Marseille and Houston types. Coomassie-stained SDS-PAGE of whole-cell lysates harvested from chocolate agar plates (grown at 35°C in 5% CO<sub>2</sub>). High-molecular-weight (lane 1 and left border) and low-molecular-weight (right border) markers are shown, with values in kilodaltons. Lanes: 2, BH5; 3, BH4; 4, URBHLLY-8 Marseille; 5, ATCC 49882 Houston-1; 6, HC35 (S4); 7, HC35 (S20).

TABLE 1. *B. henselae* isolates used in this study

Isolate	16S (Bergmans) type <sup>a</sup>	<i>gltA</i> variant <sup>a</sup>	Colonies (piliation)	Subculture (passage) no.	Source
H-1	I	H	SNP (+)	>3	ATCC 49882 (Houston-1 type strain)
49793	I	H	SNP (-)	>6	ATCC 49793
BH5	I	H	DAP (+)	2	Cat scratch disease patient (this study)
M-1	II	M	SNP (+)	>3	URBHLLY-8 (Marseille); D. Raoult (5)
BH4	II	M	SNP (+)	1	Cat scratch disease patient (this study)
HC35	II	H	DAP (+)	4	T. Gottlieb (4); feline isolate
HC35	II	H	DAP (+)	20	This study

<sup>a</sup> 16S and *gltA* types were determined by direct sequencing of PCR products as previously described (2, 3). Two variants (designated H and M) of the citrate synthase gene (*gltA*) were compared with previously published sequences (3); the BH5 *gltA* sequence was identical to that of HC35, ATCC 49793, and Houston-1; the BH4 *gltA* sequence was identical to that of URBHLLY-8 and was submitted to the EMBL database and assigned accession no. AJ439406. Colonies grew as dry-agar-pitting (DAP) or smooth, nonpitting (SNP) forms on chocolate agar (37°C; 5% CO<sub>2</sub>); piliation was determined by transmission electron microscopic examination of late-logarithmic-phase cultures grown on chocolate agar (+ = detected; - = not detected). Some strains imported or acquired from other labs had been subcultured *in vitro* prior to arrival. BH4 and BH5 were grown directly from fresh human lymph nodes in our laboratory.

reasons, we therefore urge caution in adopting this promising target as a basis for serotype distinction until the picture is clearer.

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