Genetic Variability and Prevalence of *Bartonella henselae* in Cats in Berlin, Germany, and Analysis of Its Genetic Relatedness to a Strain from Berlin That Is Pathogenic for Humans

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Nineteen *Bartonella henselae* strains and one *Bartonella clarridgeiae* strain were isolated from blood samples of 97 pet cats and 96 stray cats from Berlin, Germany, indicating prevalence rates of 1 and 18.7%, respectively, for *B. henselae* and 0 and 1%, respectively, for *B. clarridgeiae*. Eighteen of 19 *B. henselae* isolates corresponded to 16S rRNA type II. Pulsed-field gel electrophoresis (PFGE) analysis revealed seven different PFGE types among the feline *B. henselae* strains. Interestingly, all feline isolates displayed low genetic relatedness to *B. henselae* strain Berlin-1, which is pathogenic for humans.

Bartonella henselae is the major causative agent of cat scratch disease (CSD), bacillary angiomatosis (BA), bacteremia, and other pathological manifestations (1). *Bartonella clarridgeiae* has recently been described in association with CSD (11). Domestic cats are the main natural reservoir for both species (10, 11). Previous studies have demonstrated differences in prevalence rates among cats from different geographic regions, mostly correlating with climatic conditions and the type of animal population studied (3, 8–10, 16). The first objective of this study was to determine the prevalence of *Bartonella* infection among two different cat populations in Berlin, Germany.

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Blood samples were collected between June 1999 and May 2000 from 193 cats in Berlin. Ninety-seven of these were pet cats brought to the School of Veterinary Medicine Teaching Hospital for elective procedures, and another 96 were stray cats from different neighborhoods brought to an animal asylum for neutering. One to 1.5 ml of blood was collected in a pediatric lysis-centrifugation tube (Isolator 1.5; Oxoid, Basingstoke, United Kingdom). A 0.1-ml portion was inoculated onto solid and broth culture media, including Columbia agar with 5% human blood, chocolate agar (Columbia base) with 10% sheep blood, and brucella broth supplemented with Fildes enrichment and hemin (18). Plates were incubated at 37°C in 5% CO₂ for 8 weeks. Broth cultures were plated after 1 and 3 weeks on Columbia agar, and plates were incubated for a further 8 weeks. Preliminary identification of the *Bartonella* isolates was based on phenotypic characteristics and biochemical reactions in the Rapid ID 32 A system (Bio Merieux, Marcy-l'Etoile, France). Species identification was performed

for all *Bartonella*-like isolates by partial sequencing of the 16S rRNA gene (approximately 420 bp) (2).

Twenty blood cultures revealed growth of *Bartonella* spp.; 19 isolates were identified as *B. henselae*, and 1 was identified as *B. clarridgeiae*. The Rapid ID 32A results were 000 005/7 3705 for *B. henselae*, indicating that some strains could not cleave proline. The *B. clarridgeiae* strains Ber-K185 and ATCC 51734, with the profile 000 001 3705, differed in that they hydrolyzed neither proline nor leucine-glycine. Growth characteristics of *B. clarridgeiae* strain Ber-K185 differed from those of *B. henselae* strains. Briefly, the primary isolate grew only on choc-

TABLE 1. Characteristics of the *B. henselae* isolates studied

Lane in Fig. 1A	Isolate	Clinical source	16S RNA type	PFGE type
2	ATCC 49793	HIV patient (blood)	I	A
3	Houston-1	HIV patient (blood)	I	B
4	Berlin-1	HIV patient (BA-lesion)	I	B
5	Berlin-2	Cat (CSD ^a)	II	C ₅
6	Ber-K56	Cat	II	C ₁
7	Ber-K111	Cat	II	C ₁
8	Ber-K118	Cat	II	C ₄
9	Ber-K132	Cat	II	C ₁
10	Ber-K135	Cat	II	C ₂
11	Ber-K181	Cat	II	C ₂
12	Ber-K182	Cat	II	C ₁
13	Ber-K169	Cat	II	C ₃
14	Ber-K108	Cat	II	D ₁
15	Ber-K116	Cat	II	D ₁
16	Ber-K158	Cat	II	D ₂
17	Ber-K156	Cat	II	E ₁
18	Ber-K161	Cat	II	E ₂
19	Ber-K123	Cat	II	F
20	Ber-K172	Cat	II	G
21	Ber-K186	Cat	I	F
22	Ber-K143	Cat	II	H
23	Ber-K153	Cat	II	I
24	Ber-K193	Cat	II	G

^a Blood culture isolate from the pet cat of a patient with CSD.

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FIG. 1. (A) DNA fingerprint analysis of the feline and human-pathogenic *B. henselae* isolates and the *B. elizabethae* strain ATCC 49927 by PFGE. (B) Dendrogram of the fingerprints as determined by unweighted pair group using mathematical averages. Lane 1, lambda ladder; lanes 2 to 4, human-pathogenic *B. henselae* isolates ATCC 49793 (Oklahoma), Houston-1, and Berlin-1, respectively; lanes 5 to 24, feline blood culture isolates of *B. henselae*; lane 25, *B. elizabethae*; lane 26, *Saccharomyces cerevisiae* chromosome marker.

olate agar and in supplemented brucella broth, and subcultures grew inconsistently or weakly on solid media. These findings are in accordance with previous reports on the difficulties of culturing *B. clarridgeiae* on solid culture media (7, 8). Identification of the Ber-K185 strain was confirmed by complete 16S rRNA sequencing (1,410 bp), which revealed 100% homology to published sequences for *B. clarridgeiae* (8, 11).

One *B. henselae* strain was isolated from the pet cat population, whereas 18 *B. henselae* strains and one *B. clarridgeiae* strain were grown from the stray cat population. Prevalence rates for *B. henselae* were 1% (95% confidence interval [CI]₉₅, 0.98 to 3.04%) in pet cats and 18.7% (CI₉₅, 10.94 to 26.56%) in stray cats, and those for *B. clarridgeiae* were 0 and 1% (CI₉₅, -0.99 to 3.07%), respectively. The overall prevalence of *Bar-*

tonella spp. was 1% in pet and 19.8% in stray cats. Higher rates of infection in stray or impounded cats have previously been reported for other geographic regions (6). Only one *B. clarridgeiae* strain was isolated from the 193 cats included in this study. A second isolate with high morphological similarity to the *B. clarridgeiae* type strain was lost on first subculture. Although we used additional broth cultures to achieve optimal growth conditions for *B. clarridgeiae*, we cannot exclude the possibility that some *B. clarridgeiae* isolates did not grow under these conditions. Nevertheless, the prevalence of *B. clarridgeiae* appears to be rather low among domestic cats in Berlin.

Various typing methods have been used to determine the genetic relatedness among different *B. henselae* isolates (5, 14, 15). Bergmans and coworkers have described two types differing in 3 bp as determined by partial sequencing of the 16S rRNA gene (4). Type I was the most prevalent variant (75%) in tissue samples from Dutch patients with CSD, while a minority (28%) of the Dutch cats tested were infected by this variant; therefore, the authors suggested that type I isolates may be more virulent for humans than type II variants (3). We determined the 16S RNA type by partial 16S rRNA sequencing. Eighteen of the 19 feline *B. henselae* strains revealed sequences identical to that of the BA-TF strain and therefore corresponded to 16S RNA type II (Table 1). One *B. henselae* strain showed the highest homology (99.5%) with the sequence of the type I strain *B. henselae* Houston-1 (12). These findings are in accordance with the data of Sander et al. (17), who found one type I isolate (5.9%) among 17 feline blood culture isolates in Freiburg, Germany.

Pulsed-field gel electrophoresis (PFGE) has been found to be the most powerful method for intraspecies differentiation of *Bartonella* isolates (15, 17). By using this method, we recently (2) reported that the *B. henselae* isolate Berlin-1, which was originally isolated from BA lesions of a human immunodeficiency virus (HIV)-infected patient in Berlin, is genetically indistinguishable from the *B. henselae* strain Houston-1 (12). This finding raised the question whether some strains are more likely than others to cause a particular type of pathology and whether a small number of strains account for a disproportionate amount of disease (13). Therefore, we determined the genetic relationship among the feline *B. henselae* isolates from Berlin and compared their genetic fingerprints to those obtained from the Berlin-1 strain. Because further human-derived isolates were not available from Berlin or elsewhere in Germany, the *B. henselae* strains Houston-1 (ATCC 49882) and Oklahoma (ATCC 49793) (19), which are pathogenic for humans (human pathogenic), were also included in this study. Digestion of whole chromosomal DNA with *Sma*I and PFGE were performed as previously described (2). Visual analysis of banding patterns of the feline isolates revealed seven different PFGE types differing in four or more bands from each other (Fig. 1A). These PFGE types were considered to represent genetically unrelated variants, indicating a considerable genetic heterogeneity among the feline isolates (Table 1). As previously shown (2), the human-pathogenic *B. henselae* strains Berlin-1 and Houston-1 were identical and showed high similarity (88%) to the Oklahoma strain (Fig. 1). Interestingly, none of the feline isolates revealed a banding pattern identical or similar to that obtained from the Berlin-1 isolate. Computer-assisted analysis of the fingerprints revealed that the feline

isolates displayed only 63% similarity to the human-pathogenic isolates (Fig. 1B).

Although we studied cats from a limited geographic area during a short time interval, the prevalence of the Berlin-1 strain appears to be low (<0.05%). Our findings are in line with data from the Freiburg area of Germany (17) showing that none of 17 feline *B. henselae* isolates were identical or similar to the Houston-1 strain, which is genetically indistinguishable from the Berlin-1 strain. It is therefore remarkable that the single human-pathogenic *B. henselae* isolate available from an immunocompromised patient in Germany is genetically highly related to two *B. henselae* strains isolated from a similar group of patients from another continent. To date, more human-pathogenic *B. henselae* strains are not available from Germany. Further investigations should include more human-pathogenic and feline *B. henselae* isolates from other geographic regions in order to determine whether certain strains possess a specific affinity to humans and/or immunocompromised human hosts.

Nucleotide sequence accession number. The 16S rRNA sequence of the Ber-K185 strain has been submitted to the EMBL nucleotide sequence database (accession number AJ299444).

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