Molecular Epidemiology of *Bartonella henselae* Infection in Human Immunodeficiency Virus–Infected Patients and Their Cat Contacts, Using Pulsed-Field Gel Electrophoresis and Genotyping

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*Bartonella henselae* causes severe disease in immunocompromised individuals. *B. henselae* was isolated from 12 human immunodeficiency virus (HIV)–infected individuals with bacillary angiomatosis and/or peliosis hepatitis and from their 15 cat contacts. Specific associations between the 2 *B. henselae* genotypes, individual pulsed-field gel electrophoresis (PFGE) patterns, and different clinical syndromes and pathogenicity were investigated. The role of cat contacts as the source of human infection was also examined. Three of the 4 patients with *B. henselae* genotype I infection, but none of the 8 patients with genotype II infection, had hepatosplenic vascular proliferative lesions (*P* = .018). Four of 5 human-cat pairs had closely-related PFGE patterns and concordant results by 16S rDNA typing, which strongly suggests that human infection was caused by the cat contact. These results corroborate the major role of cats in the transmission of *B. henselae* to humans and suggest that *B. henselae* genotypes may induce different pathological features in HIV-infected patients.

*Bartonella* species are gram-negative, extremely fastidious bacteria that cause both cat scratch disease (CSD) and bacillary angiomatosis (BA) [1–4]; they also are emerging pathogens that cause potentially fatal opportunistic infection in persons with AIDS. In 1990, a new bacterium was identified in tissue and blood samples from immunocompromised patients with BA or bacillary peliosis hepatitis that was closely related to *Bartonella quintana* [1, 2]. This organism was subsequently named *Bartonella henselae* [5, 6]. Serological and molecular studies further showed that *B. henselae* is the etiologic agent of both BA and CSD [3, 7]. *B. quintana* is also an etiologic agent of BA [4] and, in addition, causes trench fever [8, 9]. The angiogenic lesions of BA are most often recognized in cutaneous or subcutaneous tissues and can be clinically indistinguishable from Kaposi sarcoma [10]. Unlike BA lesions caused by *B. quintana*, which are observed in subcutaneous or deep soft tissues and bone, vascular proliferative lesions caused by *B. henselae* are more likely to involve lymph nodes (BA) and parenchymal organs, such as liver and spleen (peliosis hepatitis) [11].

Epidemiological studies identified the major risk factors for acquiring contemporary *Bartonella* infections. *B. quintana* infection is associated with exposure to the human body louse, *Pediculus humanus* [11, 12], and homelessness [11–13], whereas the major risk factor associated with *B. henselae* infection is cat exposure, especially cat scratches [11, 14–16]. As many as 40% of cats in a geographic region can be bacteremic [16, 17] for a prolonged period of time [18, 19]. As the result of these studies, risks associated with cat ownership were identified, and guidelines were established [20] that enable human immunodeficiency virus (HIV)–infected and other immunocompromised persons to continue to enjoy the psychological benefits [21] of their pet cats.

Since the initial studies were done of the molecular epidemiology of BA infections in patients with AIDS, molecular techniques have been developed that have an improved ability to discriminate among *B. henselae* isolates, leading to the iden-
Identification of 2 different genotypes of *B. henselae* [22, 23]. In addition, multiple variants within these 2 genotypes of *B. henselae* have been identified by pulsed-field gel electrophoresis (PFGE) [24–26]; the diversity among *B. henselae* strains appears to be much greater than that among *B. quintana* strains [27]. In Western Europe, domestic cats are more frequently infected with *B. henselae* genotype II than genotype I [23, 26, 28, 29]. However, most human cases of CSD in Germany and The Netherlands are caused by *B. henselae* genotype I [23, 30]. It has been suggested that pathogenicity differences between *B. henselae* genotypes I and II may explain the higher frequency of human cases of CSD caused by genotype I [23, 30], and there are no studies to date that evaluate potential differences between pathogenicity or clinical manifestations of the different *B. henselae* strains in patients with BA.

In the present study, we used genotyping with polymerase chain reaction (PCR) restriction fragment–length polymorphism (RFLP) analysis of the 16S rRNA gene (16S rDNA) and PFGE pattern analysis of *Smal*-digested genomic DNA, to determine whether the clinical manifestations of *B. henselae* infection in HIV-infected patients are associated with a specific *B. henselae* genotype or PFGE pattern and whether genotyping and PFGE pattern analysis of source-cat *B. henselae* isolates show concordance with those of the corresponding *B. henselae*–infected patient with BA.

**Materials and Methods**

*B. henselae* isolates. *B. henselae* was isolated from 12 HIV-infected patients, as well as from 15 of their cat contacts from 7 different households, using methods described elsewhere [4]. We also isolated *B. henselae* from a flea collected from 1 of the 15 cats [16]. All human and feline strains used for these studies were passed <3 times before PCR or PFGE analysis. The bacteria used for PCR and PFGE were obtained from the same colony.

We numbered the patient (Pt)–cat pairs according to the order in which their *B. henselae* isolates were recovered. Isolates were available from 4 households for both human and cat contacts: Pt 3 and Cat 3A, Pt 4 and Cat 4A, Pt 5 and Cat 5A, and Pt 6 and Cat 6A. The fifth household included 1 human isolate (Pt 7) and isolates from 7 cat contacts (Cats 7A–7G) [11]. The last 2 pairs (Cat 1A and Cat 1B, and Cat 2A and Cat 2B) were from cats in 2 households where the owner had BA, but from whom no human isolate was available. The isolate Flea–Cat 1A was from a flea collected from Cat 1A [16]. All the patients and their cats resided in the San Francisco Bay Area except Pt 14, who resided in Atlanta, and Pt 4 and Cat 4A, who lived in Oregon.

**Molecular Identification of *B. henselae* by PCR-RFLP.** To ensure that DNA for molecular analysis represented a clonal population, a single colony was picked for each isolate and serially passed once or twice on heart infusion agar containing 5% rabbit blood until a sufficient amount of bacteria was obtained. Cultures were incubated in 5% CO₂ at 35°C. All isolates were identified as *B. henselae* by use of multiple methods, including sequencing of a 241-bp fragment amplified from the 16S rDNA [1, 4] and PCR-RFLP of the citrate synthase gene (*gltA*) [31, 32] and 16S rRNA [33]. These methods allow for discrimination between *B. henselae* and other *Bartonella* species. *B. henselae* subtyping (genotype I or II) was performed using PCR-RFLP of the 16S rDNA, as described elsewhere [34].

For PCR-RFLP analysis, ~2 cm³ of confluent *B. henselae* growth was harvested and resuspended in 100 µL of sterile water; the bacterial suspension then was heated at 100°C for 15 min and centrifuged at 15,000 g for 10 min at 4°C. A 1:10 dilution of the supernatant was used as DNA template for PCR amplification. An ~400-bp fragment of the *gltA* gene [32] and a 1500-bp fragment of the 16S rDNA [33] were amplified and verified by gel electrophoresis. The amplified product of the *gltA* gene was digested with *TaqI* (Promega) and *Hhal* (New England Biolabs) restriction endonucleases. The amplified product of the 16S rDNA was digested with *DdeI* (Boehringer Mannheim) restriction endonuclease to identify the *B. henselae* genotypes, designated as genotypes I and II [34]. Banding patterns were compared with reference strains of the 2 *Bartonella* species most commonly isolated from domestic cats, *B. henselae* (feline strain U-4; University of California, Davis; [18]) and *B. claridgeiae* (ATCC 51734).

**PFGE.** For PFGE, a single colony of each *B. henselae* isolate was subcultured to confluence (in a total of 1 or 2 passages) on 5% rabbit agar at 35°C for 5–7 days in a 5% CO₂ atmosphere. The bacteria grown on the agar plates were scraped off, suspended in sterile saline, and washed twice by centrifugation at 15,000 g for 5 min at 4°C. The turbidity of the suspension was adjusted to McFarland standard 6 [35], and 0.5 mL of this suspension was mixed gently but thoroughly with an equal volume of 2% ultrapure low-melting-point agarose (Gibco BRL Life Technologies). The mixture was solidified in plug molds at 4°C, and the agarose plugs were transferred into lysozyme solution (10 mM Tris [pH 7.2], 50 mM NaCl, 0.2% sodium deoxycholate, 0.5% sodium lauroyl sarcosine, and 1 mg/mL lysozyme) and incubated at 37°C overnight.

The plugs were rinsed with sterile water and incubated in protease K solution (100 mM EDTA [pH 8.0], 0.2% sodium deoxycholate, 1% sodium lauroyl sarcosine, and 1 mg/mL protease K) at 50°C overnight. The protease K incubation was repeated a second time. The plugs then were washed 4 times in 10 mL of washing buffer (50 mM EDTA and 20 mM Tris [pH 8.0]) for 1 h at room temperature with gentle agitation. Protease K was inactivated by the addition of 1 mM phenyl-methyl-sulfonyl-fluoride solution during the second wash. The plugs were stored in wash buffer at 4°C before endonuclease digestion. Before digestion, the plugs were transferred to 1.5-mL sterile microtubes with 0.1× wash buffer at 4°C overnight and then equilibrated in 1× endonuclease-specific reaction buffer for 1 h. *SmaI* restriction endonuclease (New England Biolabs) was used for the analysis of whole *Bartonella* species genomic DNA [24, 35] by digesting bacterial DNA in reaction buffer at 28°C overnight. After digestion, plugs were equilibrated in 0.5× TBE buffer (45 mM Tris-borate and 1 mM EDTA [pH 8.0]) for 30 min. The chromosomal restriction fragments were separated by PFGE in a CHEF-DRIII system (Bio-Rad) using a 1.5% pulsed-field certified agarose gel (Bio-Rad) in 0.5× TBE buffer. The electrophoresis was equilibrated at 14°C for 26 h at a constant voltage of 5.7 V/cm for the *SmaI*-digested plugs. Separation of the digested...
genomic DNA in plugs was achieved with pulse times of 3–10 s. After electrophoresis, the gel was stained and photographed. Lambda ladder pulsed-field gel marker (48.5–970 kbp; Bio-Rad) was used for molecular weight standards. *B. henselae* Houston-1 (ATCC 49882) was always included as a positive control for assurance of the consistent performance of the digestion and electrophoresis conditions.

**Analysis of PFGE profiles.** The PFGE profiles were analyzed by cluster analysis with Molecular Analyst Software (Fingerprinting, version 1.12; Bio-Rad). The images were processed, and then Dice coefficients ($S_d$) of band-based similarity were calculated as $S_d = 2N_{ab}/(N_a + N_b)$, where $N_{ab}$ is the number of bands common for A and B, $N_a$ is the total number of bands in A, and $N_b$ is the total number of bands in B. The Dice coefficient was chosen to give more weight to matching bands. Dendrograms based on results of the matrix of similarity values were created with the unweighted-pair group method by using average linkages clustering.

**Statistical analysis.** Significant statistical association ($P < .05$) between a specific genotype and the presence of specific lesions in the human patients was determined by Fisher’s exact test with EpiInfo software (version 6.04; CDC).

**Results**

All 28 isolates had the same profile by PCR-RFLP analysis of the *gltA* gene, using *TaqI* and *HhaI* endonucleases, and were identified as *B. henselae* relative to the profiles of the reference *Bartonella* species strains. The results were further confirmed by sequence analysis of a 241-bp fragment amplified from the 16S rDNA. The human and feline isolates had 2 different profiles, designated as genotypes I and II, on the basis of the PCR-RFLP analysis of the 16S rDNA by using *DdeI* endonuclease digestion. Eight (66.7%) of the 12 human isolates and 12 (80%) of the 15 feline isolates belonged to genotype II. The flea isolate was *B. henselae* genotype I. Concordant genotyping results of *B. henselae* isolates from human-cat pairs were demonstrated in 4 of 5 investigated households (3 patients and their cat contacts had genotype II infection, and 1 patient and his cat contact had genotype I infection). The discordant pair included isolates from Pt 3 (*B. henselae* genotype I) and Cat 3A (*B. henselae* genotype II); this patient stated that he avoided any contact with Cat 3A and that he had been scratched by a cat in another household. Isolate Pt 7 was identified as *B. henselae* genotype II. This patient was caring for and feeding 7 neighborhood cats on a daily basis. All 7 of these cats were also infected with *B. henselae* genotype II. *B. henselae* infection was documented by PCR but not by culture in 2 human patients with BA, each of whom had contact with 2 cats in their household (Cats 1A and 1B and Cats 2A and 2B). One of the 2 cats within each household was infected with *B. henselae* genotype I, and 1 was infected with genotype II. No information about acquisition and source is known about the discordant cats in these households, but Cats 2A and 2B were from different breeds (Siamese and tabby) and were likely acquired at different times. They also

![Figure 1](image-url)
might have been exposed to different genotypes of *B. henselae* before arrival in the household. Of interest, the *B. henselae* Flea–Cat 1A strain isolated from the flea collected on Cat 1A belonged to genotype I and had the identical genotype and PFGE pattern as the isolate from this cat.

PFGE analysis of genomic DNA from each isolate demonstrated that the cat-human pairs from the same household with concordant results by 16S rDNA PCR-RFLP analysis also had closely-related PFGE profiles (figure 1). The PFGE patterns of the Pt 7 and Cat 7A–7G isolates constituted a cluster. Of interest, only 2 main PFGE profiles were observed in these 7 cats, with isolates from Cats 7A, 7E, 7F, and 7G showing an almost indistinguishable profile and isolates from Cats 7B, 7C, and 7D revealing a second profile. Using the cluster analysis software, the PFGE profile of DNA from the human isolate Pt 7 clustered in the center between the 2 feline profile groups. Similarly, the PFGE profile for the human isolate Pt 5 clustered with the profile of the isolate from his cat contact, Cat 5A, and the human isolate Pt 6 clustered with that of his cat, Cat 6A. The final related pair, human isolate Pt 4 and the isolate from his cat, Cat 4A, were both genotype II and grouped closely, although not directly adjacent, to each other. Figure 2 shows the PFGE profiles of *B. henselae* isolates from the 12 humans and demonstrates 2 predominant clusters. There was no apparent clustering of isolate PFGE profiles according to temporal order of isolation (e.g., Pt 1 to Pt 7) or geographic origin; isolates from Atlanta (Pt 14) and Oregon (Pt 4) were each closely related to a different isolate from San Francisco (Pt 10 and Pt 6, respectively). We found the PFGE technique to be reproducible; PFGE pattern analysis of 2 single-colony picks from the same individual were always identical (data not shown). No significant relationship between either of the 2 16S rDNA genotypes and the different PFGE profiles was observed. The association between genotypes and the type of vascular proliferative lesion (BA or peliosis) in each patient is summarized in table 1. Two patients were bacteremic with *B. henselae* (1 with genotype I and 1 with genotype II) but did not develop BA lesions. Among those patients with *B. henselae* lesions, skin and lymph node lesions were the most common (7/12 [58%] for each). None of the patients with *B. henselae* had bone lesions. One patient infected with a genotype I strain (Pt 3), had multiple lesions of the skin, lymph node, liver, and spleen, and another patient, also infected with a genotype I strain (Pt 8), had skin and liver lesions. Of note, 3 of the 4 patients infected with *B. henselae* genotype I, but none of the 8 patients infected with *B. henselae* genotype II, had hepatic and/or splenic vascular proliferative lesions (P = .018). All 4 patients infected with *B. henselae* genotype I but only 3 of the 8 patients infected with *B. henselae* genotype II had any hepatosplenic disease (P = .07).

**Discussion**

*B. henselae* is a gram-negative pathogen transmitted from cats to humans, resulting in serious, even life-threatening illness in immunocompromised patients. An earlier study identified different tissue tropisms for *B. henselae*, compared with those for *B. quintana*, in patients with AIDS and BA [11]. *B. henselae* was associated uniquely with hepatosplenic disease and lymphadenopathy but not with osseous disease. Cutaneous BA lesions were caused by both *B. quintana* and *B. henselae*. For the present study, we further characterized *B. henselae* isolates with genotyping and PFGE pattern analysis to determine additional characteristics of *B. henselae* infection in HIV-infected patients and their cat contacts.

We first determined the genotype of each of the patient and cat contact isolates, using PCR-RFLP analysis of DdeI-digested
Furthermore, the prevalence of the 2 genotypes varies in cats from different geographic regions [30, 33, 34]. It has been hypothesized that there might be a difference in pathogenicity of the 2 different genotypes during human infection [30, 36]. It is a unique association of the 2 different B. henselae genotypes during human infection [30, 36].

Although the present study is limited by a small sample size, the data indicate that patients with B. henselae genotype I infection were more likely to have vascular proliferative lesions of the liver and/or spleen (3/4 patients) than were patients with genotype II infection. The fourth patient with genotype I infection did not have peliosis hepatitis documented but did have hepatosplenicomegaly, as did another patient reported in the literature (1 of the original patients from whom B. henselae genotype I was isolated [6]). In contrast, only 1 (Pt 11) of the 8 patients in our study with B. henselae genotype II infection had focal hepatic lesions; however, these were not the vascular proliferative lesions of peliosis hepatitis but, instead, were demonstrated to be granulomatous hepatitis. Two additional patients with B. henselae genotype II had hepatosplenicomegaly, and the remaining patients with genotype II (5/8) had no hepatosplenic abnormalities. The predominant location of BA lesions in patients with genotype II infection was in skin and/or lymph nodes (7/8). No osseous BA lesions were found with B. henselae infection in these 12 patients, which is consistent with what was observed elsewhere [11]. Further studies will be necessary to determine the genotype of other B. henselae isolates from patients with hepatosplenic disease, to determine whether there is a unique association of the B. henselae genotype I with focal vascular proliferative lesions of the liver and spleen.

The concordance of 16S rDNA PCR-RFLP results and the clustering of PFGE patterns from both molecular analyses support the hypothesis that all patients but 1 among our patient-cat pairs acquired their infection from their domestic cat contacts. PFGE profile analysis revealed that there was considerable diversity among B. henselae isolates from both humans and cats (figure 1). In some pairs (e.g., Pt 5 and Cat 5A), the individual PFGE profiles were blindly paired by the clustering program but revealed that 1 or several genomic fragments of the human isolate differed in size from the cat-source isolate. Although the cat was the only possible source for the human infection for the Pt 5–Cat 5A pair, the change in profile in this and other paired strains may be the result of genomic rearrangement during antigenic or phase variation; such a mechanism would not be unusual for an organism that can persist in the bloodstream of animals or humans for >1 year. It would be interesting to look for evidence of this virulence strategy in serial isolates from the same mammalian host.

One patient was infected with a B. henselae strain that had a genotype and PFGE pattern discordant with the isolate from the cat in his home. The source of infection in this patient was likely to have been another cat, because, on the questionnaire completed by this patient before molecular typing of his isolate, he stated that he avoided the cat owned by his roommate and that he had been scratched by a different cat several months earlier. However, feline coinfection of B. henselae genotypes I and II can occur [33, 36]; therefore, in a household with discordant molecular typing patterns for human and cat isolates,
the patient might have acquired the infection from a cat that was actually coinfected with genotypes I and II but from which our cultures only identified genotype II. Nevertheless, the known exposure to another cat seems to be a more likely explanation in this case.

Two-thirds of the immunocompromised patients in our study were infected by *B. henselae* genotype II, as were 80% of their cat contacts, suggesting that *B. henselae* genotype II may be the more common genotype in cats and humans in northern California. In contrast, previous observations from patients with CSD in The Netherlands and Germany revealed genotype I to be more common [23, 30], although the most recent data from French patients with CSD [40] identified, by PCR, *B. henselae* genotype II infection in the majority (61.2%) of patient lymph nodes. As with other bacterial pathogens, the distribution of different *B. henselae* strains may be highly regional, and it will be important to confirm these results in a larger sample of domestic cats and human patients with *B. henselae* infection from the same geographic region.

It is evident from the results of the present study that both genotype I and genotype II can cause debilitating disease in immunocompromised patients. Prevention of transmission of *B. henselae* of either genotype is extremely important for these patients, to enable them to keep their companion animals. Cats appear to be the principal vector transmitting *B. henselae* to humans, via scratches; in one large study, most patients with CSD had a scratch present at the time of diagnosis [41]. In turn, the major vector that transmits *B. henselae* among cats is the cat flea [16, 42, 43], and, although it has been speculated that humans could acquire *B. henselae* infection from flea bites [44], there is currently no clinical evidence for this. In the present study, we demonstrated that 2 cats from the same household were infected with different *B. henselae* genotypes and that the genotype and PFGE profile of the *B. henselae* flea isolate was identical to the isolate from the cat from which it was removed. Unfortunately, we were unable to identify the genotype of the *B. henselae* causing infection in the cats’ owner, so we cannot identify the source for the human infection.

Regardless of whether direct flea-to-human transmission can be documented, it is evident that the flea plays a significant, indirect role in human infection and that flea eradication is an important strategy for interrupting the transmission cycle among cats, thus decreasing the risk of human acquisition of *B. henselae* from bacteremic cats [20, 45]. In addition, young (<1 year old) and stray or impounded cats were more likely to be *B. henselae* bacteremic [17, 28], and infected cats were bacteremic from several months to a year [19]. Finally, kittens were more likely to interact with and scratch humans; therefore, it is recommended that, if immunocompromised patients wish to acquire a cat, they should choose a cat >1 year old, avoid rough play with their pets, and, if scratched, should wash the wound immediately [20, 45]. These measures should help to minimize exposure to *B. henselae* and permit immunocompromised patients to keep and enjoy their feline companions.

References


