Genomic Variation of *Bartonella henselae* Strains Detected in Lymph Nodes of Patients with Cat Scratch Disease

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*Bartonella henselae* is the primary agent of cat scratch disease (CSD). In order to study the genetic variation of *B. henselae* and the correlation of the various genotypes with epidemiological and clinical findings, two seminested, groEL- and *pap31*-based PCR assays were carried out with specimens from 273 patients. Amplicons were sequenced to determine the genotype of the causative *Bartonella* species. Compared to our reference intergenic spacer region-based PCR, the groEL- and *pap31*-based assays were 1.7 and 1.9 times more sensitive, respectively. All 107 positive patients were infected with *B. henselae*; neither *Bartonella claridgeiae* nor other species were detected. Based on the groEL and *pap31* sequences, *B. henselae* amplicons were classified into two genogroups, Marseille and Houston-1, and into four variants, Marseille, CAL-1, Houston-1, and a new variant, ZF-1. Patients infected with either one or the other genogroup did not exhibit different epidemiological or clinical characteristics. Our study highlights the genotypic heterogeneity of *B. henselae* in patients with CSD.

Cat scratch disease (CSD) is most frequently reported in children and young adults, in whom it usually presents as a benign, self-limited lymphadenopathy in lymph nodes draining the site of a cat scratch or bite (13). The first presumed agent of CSD was isolated in 1988 by English and colleagues and was named *Afipia felis* (11). In 1993, Dolan and colleagues isolated *Bartonella henselae* from the lymph nodes of patients with CSD for the first time (18). Since then, many data from epidemiological, serological, and molecular biology-based studies (2, 7, 17) have demonstrated the role of *B. henselae* rather than *A. felis* as the main causative agent of CSD. *B. henselae* exhibits an important heterogeneity; two serotypes, Houston-1 and Marseille, have been described (19), and these correspond to two genotypes based on 16S rRNA sequences, genotypes I and II, respectively (8). To date, the respective pathogenicity spectra of serotypes Marseille and Houston-1 have not been established. In addition, two other *Bartonella* species, *Bartonella quintana* and *Bartonella claridgeiae*, have been suspected as alternative agents of CSD (4, 20, 34, 47, 58).

Serological analysis by immunofluorescence or enzyme-linked immunosorbent assay is a useful tool for the diagnosis of *B. henselae* infections (5, 15, 46). However, the specificity of serological analysis has been questioned due to the cross-reactivity between *B. henselae* and other species including *B. quintana*, *Coxiella burnetii* (21, 36, 55), and *Chlamydia* species (41). Culture has not proved to be very useful for the diagnosis of CSD because of the fastidious nature of *Bartonella* species (37, 42, 43). PCR-based detection of *Bartonella* species coupled with nucleotide sequencing has also been used for the diagnosis of CSD. In our laboratory, we have been using primers derived from *gltA* (31), *rpoB* (50), and the 16S-23S intergenic spacer region (ITS) (51) for the detection of *Bartonella* DNA from human specimens. Unfortunately, none of these genes was adequate for the subtyping of *B. henselae* variants. Moreover, conventional PCR may be impaired by the inactivation of many DNA polymerases by immunoglobulins in patients’ lymph nodes (1).

Recently, we demonstrated that sequences from the groEL and *pap31* genes are suitable for the identification and subtyping of *B. henselae* genotypes, respectively (unpublished data).

The purpose of our work was to increase the sensitivity of the PCR-based detection of *Bartonella* DNA from lymph node biopsy specimens by using primers that allow the amplification of all known *Bartonella* species pathogenic for humans and, thus, the involvement of *Bartonella* species in CSD. We designed two seminested PCR assays, one that amplifies the groEL gene, which results in specific sequence signatures for each of the seven currently known *Bartonella* species pathogenic for humans, and another that amplifies the *pap31* gene, for which sequences present a signature region allowing the differentiation of *B. henselae* variants. We tested 289 lymph node biopsy specimens from 273 patients with a clinical diagnosis of CSD. We compared these results to those obtained by the ITS-based PCR. We also compared the epidemiological and clinical aspects of CSD according to the infecting *B. henselae* genogroup.

**MATERIALS AND METHODS**

**Study design.** Patients were clinically suspected of having CSD on the basis of findings such as the presence of chronic lymphadenopathy without a specific diagnosis and contacts with cats (22). *Bartonella* infection was diagnosed on the basis of the results of direct identification of a *Bartonella* sp. by PCR from lymph node tissue. Samples were collected from January 1996 to January 2001. Two hundred eighty-nine lymph node biopsy specimens or aspirates from 273 patients clinically suspected of having CSD were sent to the Unité des Rickettsies to be tested for the presence of *Bartonella* spp. A standardized questionnaire was completed for each CSD patient. Items completed on the questionnaire included whether the patient had had contact with cats or cat fleas; the presence of fever, cat scratches or bites, or fleabites; the presence of a cutaneous lesion at the inoculation site; the previous administration of antibiotic therapy; and the outcome within 1 month following the diagnosis of CSD.

**Molecular biology-based methods.** (i) DNA extraction and PCR amplification. Total genomic DNA was extracted from samples with a QIAamp Tissue kit (QIAGEN, Hilden, Germany), as described by the manufacturer. A total of 10
to 25 mg of tissue or 200 µl of aspirate was used. Samples were handled under sterile conditions to avoid the risk of cross-contamination. A total of 125 µl of elution buffer was used to resuspend the DNA. Genomic DNAs were stored at 4°C until their use as templates in PCR assays.

The primers used for amplification and sequencing are presented in Table 1. Primers were selected by using Primer3 software (52) and were purchased from Eurobio (Les Ulis, France). Primers positions were numbered relative to the ITS of B. henselae strain Houston-1 (GenBank accession number L35101), the sequence of the groEL gene of B. bacilliformis (GenBank accession number Z15160), and the sequence of the pap31 gene of B. henselae strain Houston-1 (GenBank accession number AF001274). Arrows indicate the directions of the primers (→, forward; ←, reverse).

A PCR protocol for amplification of the Bartonella ITS DNA fragment was carried out with these specimens as described previously (51). Seminested PCR protocols for amplification of the Bartonella groEL and pap31 genes with three primers at the same time (primers HSPps1, HSPps2, and HSPps4, respectively) were applied to the clinical samples.

FIG. 1. Multiple-sequence alignment of partial groEL sequences of B. henselae Houston-1 (BhHo), B. henselae Marseille (BhMa), B. quintana (Bqui), B. vinsonii subsp. berkhoffii (Bvbe), B. elizabethae (Beli), B. claridgeiae (bla), B. grahamii (bgra), and B. bacilliformis (bbac) (a) and partial pap31 sequences of seven B. henselae variants (b).
RESULTS

Molecular biology-based assay results. Of the 289 lymph node specimens tested, amplicons were obtained from 114 samples from 108 patients by combining the results from the three PCR assays.

ITS amplification and sequencing. Amplicons were obtained from 57 specimens (19.7%) by 56 patients by the ITS-based PCR. 49 of which were also groEL positive and 56 of which were also paps31 positive (Fig. 2). One specimen was amplified only by the ITS-based PCR. None of the negative controls were positive. The sequences derived from these PCR products were 100% identical to the B. henselae sequence for all 57 specimens. Therefore, these sequences did not allow us to distinguish between B. henselae genotypes.

groEL seminested PCR and sequence variation. PCR amplification of part of the groEL gene resulted in two bands. The PCR products generated consisted of 231- and 269-bp fragments, corresponding to the amplifications obtained by PCR.

FIG. 2. Comparison of results of the PCR assays carried out with ITS-, groEL-, and paps31-derived primers with lymph node specimens from 273 patients. The numbers represent the numbers of specimens.

### Table 2. Bacterial strains and sequences used in this study

<table>
<thead>
<tr>
<th>Species (strain)</th>
<th>Source</th>
<th>Collection no.</th>
<th>Reference</th>
<th>GenBank accession no.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartonella henselae (Houston-1)</td>
<td>ATCC 49882</td>
<td>48</td>
<td>AF001274</td>
<td>9</td>
<td>AF014829</td>
</tr>
<tr>
<td>Bartonella henselae (URLLY 8)</td>
<td>CIP 104756</td>
<td>19</td>
<td>AF308169</td>
<td>UD</td>
<td>AF304019</td>
</tr>
<tr>
<td>Bartonella henselae (CAL-1)</td>
<td>CDC, septicemia</td>
<td>19</td>
<td>AF308166</td>
<td>UD</td>
<td>AF304020</td>
</tr>
<tr>
<td>Bartonella henselae (Fizz)</td>
<td>CDC</td>
<td>19</td>
<td>AF308167</td>
<td>UD</td>
<td>AF304022</td>
</tr>
<tr>
<td>Bartonella henselae (SA2)</td>
<td>CDC</td>
<td>18</td>
<td>AF306165</td>
<td>UD</td>
<td>AF304023</td>
</tr>
<tr>
<td>Bartonella henselae (ZF-1)</td>
<td>CDC</td>
<td>18</td>
<td>AF308168</td>
<td>UD</td>
<td>AF304021</td>
</tr>
<tr>
<td>Bartonella bacilliformis</td>
<td>ZI5160</td>
<td>UD</td>
<td>AF014831</td>
<td>39</td>
<td>AF014833</td>
</tr>
<tr>
<td>Bartonella claridgeiae</td>
<td>ZI5160</td>
<td>UD</td>
<td>AF014831</td>
<td>39</td>
<td>AF014833</td>
</tr>
<tr>
<td>Bartonella elizabethae</td>
<td>ZI5160</td>
<td>UD</td>
<td>AF014834</td>
<td>39</td>
<td>AF014839</td>
</tr>
<tr>
<td>Bartonella quintana</td>
<td>ZI5160</td>
<td>UD</td>
<td>AF014836</td>
<td>39</td>
<td>AF014839</td>
</tr>
</tbody>
</table>

*Abbreviations: ATCC, American Type Culture Collection, Manassas, Va.; CIP, Collection de l’Institut Pasteur; CDC, Centers for Disease Control and Prevention, Atlanta, Ga.; UD, unpublished data.*
with the HSPps4-HSPps2 and HSPps1-HSPps2 primer pairs, respectively. None of the negative controls were positive. Amplification of the groEL sequence resulted in the identification of *B. henselae* from 104 (36%) specimens from 98 patients, all of which were also *pap31* positive and 49 of which were also ITS positive (Fig. 2). Only *B. henselae* could be detected. Additionally, sequences from this amplified region also allowed us to subtype *B. henselae* amplicons into two clusters according to the sequence variations seen at position 1348 (red in Fig. 1a). Samples from two of the six patients were positive; the amplicons from both samples had the same sequence. Of the sequences from the 98 patients, 60 (61.2%) exhibited 100% sequence identity with the sequence of *B. henselae* genotype Marseille and 38 (38.8%) exhibited 100% sequence identity with the sequence of *B. henselae* genotype Houston-1.

**pap31 seminested PCR, sequence variation, and phylogeny.** PCR amplification of part of the *pap31* gene resulted in two bands. The PCR products generated consisted of 209- and 275-bp fragments, corresponding to the amplification products obtained by PCR with the PA Pen1-PA Pen2 and PA Pen1-PA Pen2 primer pairs, respectively. None of the negative controls were positive. The sequences deduced by the *pap31*-based PCR identified only *B. henselae* DNA in 113 specimens from 107 patients. Samples from two of the six patients were positive; the amplicons from both samples had the same sequence. Among the 107 patients, 64 (59.8%) were infected with the Marseille genogroup and 43 (48.2%) were infected with the Houston-1 genogroup. Within these two main genogroups, four different *pap31* sequences were identified: the CAL-1 genotype in 63 patients (58.8%), the Marseille genotype in 1 patient (0.9%), the Houston-1 genotype in 3 patients (2.8%), and a previously undescribed *pap31* genotype, which we called the ZF-1 genotype, in 40 patients (38.1%). The CAL-1, Marseille, Houston-1, and ZF-1 genotypes were differentiated on the basis of specific *pap31* signatures at 22 positions (Fig. 1b). For one patient, only the ITS-based PCR was positive, but an amplicon could not be obtained by either the *pap31*-based PCR or the groEL-based PCR; therefore, the precise genotype could not be determined.

Phylogenetic analysis by the maximum parsimony and neighbor-joining methods provided trees with similar organizations when *pap31* sequences were used (Fig. 3). *B. henselae* isolates clustered into two genogroups. The first, which we called Marseille, contained *B. henselae* strains Fizz, CAL-1, and Marseille. The second group, Houston-1, included *B. henselae* strains SA-2, 90-615, Houston-1, and ZF-1. The two clusters were statistically supported by elevated bootstrap values (100%) by both methods. Elevated bootstrap values were also obtained within both clusters.

**Comparative sensitivities of the three PCR assays.** All three PCR assays were conducted under the same conditions and so were subjected to the same effects of inhibitory elements. Overall, 114 lymph node specimens were positive by at least one of the three tests (Fig. 2). When compared to each other, the sensitivity of the ITS assay was 0.50, that of the groEL assay was 0.91, and that of the *pap31* assay was 0.99.

**Patients’ characteristics.** The mean ± standard deviation age of the 108 patients with proven *B. henselae* lymphadenopathy was 25.6 ± 22.7 years (range, 4 to 82 years). Forty-three patients (39.8%) were younger than 20 years, including 23 (21.3%) who were younger than 10 years. Sixty-eight patients (63.0%) were male. All patients had contact with cats, but only 82 (75.9%) reported scratches, 34 (31.5%) reported bites, and 20 (18.5%) reported flea bites. None was immunocompromised. Forty-nine (45.3%) patients developed a lesion at the inoculation site, and 35 (32.4%) had a temperature of >38.5°C. The infected lymph nodes were the axillary lymph nodes in 58 patients (53.7%), the inguinal lymph nodes in 33 patients (30.5%), the cervical lymph nodes in 13 patients (12.0%), and the popliteal lymph nodes in 3 patients (2.8%). None of the patients developed any visceral complication. Compared to the 64 patients with CSD caused by *B. henselae* genogroup Marseille, the 43 patients with CSD caused by *B. henselae* genogroup Houston-1 were not significantly different epidemiologically or clinically (Table 3). We also compared patients within each group infected with one or the other genotype but observed no significant differences.

**DISCUSSION**

PCR-based detection methods have been widely used for the diagnosis of CSD (Table 4). We compared the DNA detection efficacy of one conventional, ITS-based PCR assay, previously considered in our laboratory as the best tool for the detection of *B. henselae*.
of Bartonella DNA from clinical specimens, and those of two seminested, groEL- and pap31-based PCR assays. All three tests were carried out with 289 lymph node samples from 273 patients with a clinical diagnosis of CSD, which represents the largest reported series of lymph node samples from patients with a diagnosis of CSD tested by PCR (Table 5).

The importance of the groEL gene as a sensitive and valuable tool for the phylogenetic analysis (39, 61) and identification (27) of bacterial species has recently been highlighted. We selected primers that allowed the amplification and identification, following sequencing, of all seven Bartonella species currently recognized to be pathogenic for humans (Fig. 1a). We

<table>
<thead>
<tr>
<th>Variable</th>
<th>B. henselae genogroup Marseille</th>
<th>B. henselae genogroup Houston-1</th>
<th>P value</th>
<th>B. henselae genogroup Marseille</th>
<th>B. henselae genogroup Houston-1</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>64</td>
<td>43</td>
<td></td>
<td>63</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>1.78</td>
<td>1.52</td>
<td>0.70</td>
<td>1.74</td>
<td>1.0</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean ± SD age</td>
<td>24.2 ± 23.4</td>
<td>25.9 ± 23.3</td>
<td>0.71</td>
<td>24.6 ± 23.7</td>
<td>11.0*</td>
<td>NA*</td>
</tr>
<tr>
<td>No. of patients with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cat scratches</td>
<td>52</td>
<td>30</td>
<td>0.17</td>
<td>51</td>
<td>1</td>
<td>0.70</td>
</tr>
<tr>
<td>Cat bites</td>
<td>21</td>
<td>13</td>
<td>0.78</td>
<td>21</td>
<td>0</td>
<td>0.67</td>
</tr>
<tr>
<td>Cat flea bites</td>
<td>13</td>
<td>7</td>
<td>0.60</td>
<td>13</td>
<td>0</td>
<td>0.79</td>
</tr>
<tr>
<td>Inoculation lesion</td>
<td>29</td>
<td>20</td>
<td>0.90</td>
<td>28</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>Fever</td>
<td>19</td>
<td>16</td>
<td>0.41</td>
<td>19</td>
<td>0</td>
<td>0.74</td>
</tr>
<tr>
<td>Usual affected lymph node location (axillary + inguinal)</td>
<td>55</td>
<td>36</td>
<td>0.75</td>
<td>54</td>
<td>1</td>
<td>0.86</td>
</tr>
<tr>
<td>Unusual affected lymph node location (cervical + popliteal)</td>
<td>9</td>
<td>7</td>
<td>0.75</td>
<td>9</td>
<td>0</td>
<td>0.86</td>
</tr>
<tr>
<td>Axillary lymph nodes affected</td>
<td>35</td>
<td>23</td>
<td>0.90</td>
<td>34</td>
<td>1</td>
<td>0.54</td>
</tr>
<tr>
<td>Inguinal lymph nodes affected</td>
<td>20</td>
<td>13</td>
<td>0.91</td>
<td>20</td>
<td>0</td>
<td>0.68</td>
</tr>
<tr>
<td>Cervical lymph nodes affected</td>
<td>8</td>
<td>5</td>
<td>0.89</td>
<td>8</td>
<td>0</td>
<td>0.87</td>
</tr>
<tr>
<td>Popliteal lymph nodes affected</td>
<td>1</td>
<td>2</td>
<td>0.35</td>
<td>1</td>
<td>0</td>
<td>0.98</td>
</tr>
</tbody>
</table>

a Standard deviation not applicable.
b NA, not applicable.
also used pap31-derived primers. The pap31 gene encodes a major protein associated with a phage isolated from \textit{B. henselae} and is probably implicated in its pathogenesis (9). Previously, a major protein associated with a phage isolated from \textit{B. henselae} strains into two clusters, \textit{B. henselae} Houston-1 and \textit{B. henselae} Marseille, on the basis of 22 nucleotide differences (Fig. 1b) (unpublished data). To the best of our knowledge, our study is the first to use a seminested PCR assay. Compared to the conventional, ITS-based PCR assay used in our laboratory for the diagnosis of CSD, our seminested assays with the \textit{groEL} and the pap31 genes increased the numbers of positive samples by 82 and 98\%, respectively. Therefore, while our new seminested detection method exhibits a specificity of 100\%, it was nearly twice as sensitive as the conventional, ITS-based PCR for the detection of \textit{B. henselae} DNA in specimens from patients with CSD. When the assays were compared to each other, the seminested, pap31-based assay was 8\% more sensitive than the seminested, \textit{groEL}-based assay. The sensitivity of the PCR assay may be influenced by the fact that the sequences of the pap31- and \textit{groEL}-derived primers have been chosen from conserved regions of DNA and the fact that the sequences of the primers used for ITS amplification have been chosen from a variable region of DNA. However, one ITS-positive amplicon was not detected by the two seminested PCR assays; this may be due to either the presence of a different \textit{B. henselae} genotype not amplified by any of two detection assays or DNA damage because of long-term storage of the sample. Compared to the largest (7) of the previously published studies of \textit{B. henselae} DNA detection (Table 5), the proportions of positive samples by our PCR assays (i.e., 36.0 and 39.1\% by the \textit{groEL}- and pap31-based assays, respectively) may seem low, but in the previous study, PCR was performed with specimens from patients with a diagnosis of CSD that had already been confirmed by a skin test, whereas in our series, patients were only clinically suspected of having CSD.

In contrast to ITS sequences, which were discriminant only to the species level, the \textit{groEL} sequences classified positive patients as being infected with \textit{B. henselae} genogroup Houston-1 (38.8\%) or genogroup Marseille (61.2\%); and pap31 sequences allowed a similar but more precise subtyping of \textit{B. henselae} variants, as four \textit{B. henselae} genotypes were identified. When \textit{groEL} sequences were used, 60 patients were found to be infected with \textit{B. henselae} genogroup Marseille and 38 were found to be infected with genogroup Houston-1. When pap31 sequences were used, 1 patient exhibited a genotype Marseille infection, 63 patients exhibited genotype CAL-1 infections, 40 patients exhibited genotype ZF-1 (a previously undescribed genotype) infections, and 3 exhibited genotype Houston-1 infections (Fig. 1b). The great heterogeneity among \textit{B. henselae} variants confirms the interest in the pap31 gene for the subtyping of this bacterium.

\textit{B. henselae} has been described as the main agent of CSD, but two additional \textit{Bartonella} species, \textit{B. quintana} and \textit{B. claridgeiae}, have also been proposed as possible agents of the disease. The role of \textit{B. quintana} as an agent of CSD appears to be limited, as it has been isolated only from lymph node biopsy specimens of two immunocompromised adults in contact with cats and/or cat fleas (20, 47) and was recently reported in a 10-month-old girl with a chronic lymphadenopathy (4). However, although both patients presented with \textit{B. quintana}-induced lymphadenopathies, their epidemiological and clinical characteristics were not the same as those of patients with typical CSD. Recently, we isolated both \textit{B. quintana} and \textit{M. tuberculosis} from a human immunodeficiency virus-positive patient who had no contact with cats and who presented with lymphadenitis (unpublished data). Moreover, \textit{B. quintana} is the agent of rare cases of chronic lymphadenopathy (47). However, as observed in patients with bacillary angiomatosis, \textit{B. quintana} is less likely than \textit{B. henselae} to cause adenopathies, and when \textit{B. quintana} was present, the infection did not proceed to suppuration (33). Therefore, the role of \textit{B. quintana} in CSD and other lymphadenopathies should be further investigated.

The role of \textit{B. claridgeiae} as an agent of CSD has been suspected on the basis of the fact that cats are the reservoir of both \textit{B. claridgeiae} and \textit{B. henselae} (30, 32, 34, 40, 50) and that coinfection with both species has been reported (14, 28). As \textit{B. claridgeiae} represents up to 36\% of \textit{Bartonella} isolates from cats (6, 30), one would expect humans to be frequently exposed to this bacterium. The demonstration of the pathogenic role of \textit{B. claridgeiae} for humans is currently based on indirect evidence obtained either by PCR-restriction fragment length polymorphism analysis of an isolate from the cat of a patient with CSD (34) or by serology for a patient with CSD (38). Recently, Sander et al. (58) detected antiflagellin (anti-FlaA) antibodies to \textit{B. claridgeiae} in 3.9\% of 724 patients suffering from lymphadenopathy. However, among the previously published studies, only two reported the use of PCR primers able to detect \textit{B. claridgeiae} (16, 51). In our study, and despite the use of \textit{groEL}-derived primers that allowed the amplification of \textit{B. claridgeiae}, we did not find this bacterium among 98 positive patients. Moreover, in the study of Sander et al. (58), no bacterial agent was reported in cultures of samples from serologically positive patients, and the only PCR carried out with a sample from a \textit{B. claridgeiae} anti-FlaA-positive patient detected and identified \textit{B. henselae} but not \textit{B. claridgeiae}. None
of the other diagnostic studies conducted so far could detect \textit{B. claridgeae} from patients with CSD either by culture or by PCR (2, 7, 8, 29, 49, 54, 57). Therefore, if \textit{B. claridgeae} is pathogenic for humans, it is probably much less virulent than \textit{B. henselae}.

Two main pathogenic \textit{B. henselae} variants, Houston-1 and Marseille, have been identified in both animals and humans, and both of these variants are involved in CSD (8, 19, 24, 28, 53, 54, 56, 57). Sander et al. (54) reported on the detection of three \textit{B. henselae} variants in human lymph node specimens by PCR: Houston-1 in 59% of patients, Marseille in 23% of patients, and a third variant in 18% of patients. In our study, we identified two \textit{B. henselae} genogroups using the \textit{groEL} sequences and four genotypes using the \textit{pap31} sequences; among the 107 patients infected with the Marseille genogroup, 0.9% were infected with the Marseille genotype and 58.9% were infected with the CAL-1 genotype, whereas among the patients infected with the Houston-1 genogroup, 2.8% of the patients were infected with the Houston-1 genotype and 37.4% of the patients were infected with the ZF-1 genotype. This is the first description of the ZF-1 genotype, which represented the major genotype among isolates within the Houston-1 genogroup in our study. Therefore, the genotypic variability of \textit{B. henselae} is greater than that observed previously and may explain the absence of cross-protection between both variants in cats (28). Other investigators have studied the geographic distributions of variants Houston-1 and Marseille detected in human lymph node specimens (Table 4). In Germany and The Netherlands, the Houston-1 variant is more frequent than the Marseille variant (8, 54), whereas in France (16) and Switzerland (10), the Marseille variant is the most common. As cats are the source of both \textit{B. henselae} variants, it may be speculated that the prevalence in cats reflects that in humans. However, when trying to correlate the distribution of \textit{B. henselae} variants in humans and cats (Table 4), discrepancies were noted in the literature. In Switzerland, both cat and human \textit{B. henselae} isolates are mainly the Marseille variant (10) (Table 4). In The Netherlands and Germany, the majority of \textit{B. henselae} isolates from cats are the Marseille variant, whereas isolates from humans are mostly identified as \textit{B. henselae} Houston-1 (6, 8, 54, 57) (Table 4). In France, the opposite results have been reported: humans are mainly infected with \textit{B. henselae} Marseille, whereas most cats are infected with the Houston-1 variant (16, 28) (Table 4).

On the basis of the distributions of the \textit{B. henselae} variants observed in humans and cats in Germany, Sander et al. (54) have proposed that \textit{B. henselae} Houston-1 may be more pathogenic for humans than the Marseille variant. As described above, this assertion is not supported by the results of Swiss and French studies (Table 4). Moreover, in our study, when we compared the epidemiological and clinical characteristics of patients infected with either one or the other of the two main \textit{B. henselae} genogroups, Marseille and Houston-1, we found that our data were in accordance with the findings usually observed for patients with CSD (62). However, we observed no statistically supported differences between the two genogroups and, within each genogroup, between genotypes; these results do not favor differences in the pathogenicities of the two genotypes. We suggest that further epidemiological studies with \textit{B. henselae} variants should take the four genotypes described above into consideration and should investigate whether these various genotypes are correlated with specific serotypes, human pathologies, or distributions in cats.

In this study we have reported on a seminested PCR-based detection and identification method which allowed us to increase the sensitivity of detection and identification of causative agents of CSD directly with lymph node specimens by use of the \textit{groEL} and \textit{pap31} genes. Although our seminested, \textit{pap31}-based PCR was the most sensitive detection tool, it failed to detect \textit{B. henselae} in a patient found to be positive by the ITS-based PCR. Therefore, we recommend the use of a combination of both the ITS- and the \textit{pap31}-based PCR methods for the diagnosis of CSD from lymph node specimens. By the \textit{pap31} gene-based PCR, the \textit{B. henselae} organisms that caused CSD were classified into two genogroups, Marseille and Houston-1, and four genotypes, mainly CAL-1 and ZF-1, but also Marseille and Houston-1. The \textit{pap31} gene should be considered a valuable tool for the subtyping of \textit{B. henselae}.

Further studies with patients with CSD may clarify whether the various pathogenic genotypes of \textit{B. henselae} have specific geographic distributions and/or virulences and may identify additional variants.

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REFERENCES


