Experimental Transmission of *Bartonella henselae* by the Cat Flea


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*Bartonella henselae* is an emerging bacterial pathogen, causing cat scratch disease and bacillary angiomatosi.

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trigulated, and the pellet was spread onto heart infusion agar containing 5% fresh rabbit blood. The plates were incubated at 36°C in 5% CO₂ for 3 weeks. Identification of isolates as *B. henselae* was confirmed by PCR-restriction fragment length polymorphism analysis with two different restriction endonucleases, TaqI and *Hha*I, to digest the single product amplified with the primers described previously (22) for negative and positive PCR controls. Digestion of the *B. henselae* amplicon with TaqI produces three bands, and digestion with *Hha*I results in two bands, as described previously (20).

**Experimental transmission of *B. henselae* to SPF cats via fleas removed from bacteremic cattery cats.** The 47 cattery cats were primarily stray cats that were kept indoors in a private home after being adopted. Each cat was clinically examined, and 2.5 ml of blood was drawn for culture and serology. Serological testing by indirect immunofluorescence-antibody (IFA) testing was performed as described previously (6). The blood collections were performed five times over 12 months, in April 1994, June 1994, September 1994, March 1995, and April 1995. The microbiological and serological data from the three 1994 blood collections were reported along with data for a larger group of 205 cats from a study of the relationship between feline *B. henselae* bacteremia and the presence of antibodies (6). Fleas were collected from cattery cats at each of the visits described above with the exception of the first visit and were used either for experimental flea-borne transmission experiments or for PCR detection of *B. henselae* DNA.

Prior to experimental infection or inoculation, all SPF kittens were tested twice by blood culture and IFA testing to ascertain that they had not been exposed to *B. henselae*. The SPF kittens were isolated in a confined, arthropod-free environment and were raised for 6 months according to the regulations of the University of California, Davis. Two 5-month-old SPF kittens were infested with fleas collected from several cattery cats known to be highly bacteremic (>10⁴ CFU/ml) during the collection period. The kittens were tested 1 and 2 weeks earlier. Two pools of 14 fleas and the other of seven fleas, were collected from these cats and were deposited on the bodies of each of the two SPF kittens. SPF kittens experimentally infected with fleas were examined once a week, and blood was collected for culture, serology, and complete blood count (CBC).

After these two SPF kittens became infected with flea-borne *B. henselae*, the flea transmission experiment was repeated 10 months later with three new SPF kittens. *B. henselae*-negative, 3- to 5-month-old SPF kittens that also were isolated in a confined environment were used because we found early in the study that we could not predict either which cattery cats were bacteremic or which fleas contained *B. henselae* at a given time. A total of 63 fleas (five from each of the SPF kittens, and the fourth pool of 14 fleas was frozen at -70°C immediately for subsequent PCR detection of *B. henselae* DNA. The kittens were examined clinically, and blood was collected every week for culture, serology, and CBC.

**Evaluation for direct, arthropod-independent transmission of *B. henselae* from kitten to kitten.** To identify the direct transmission of *B. henselae* among cats during activities such as playing, scratching, biting, and communal eating in an arthropod-free environment, two 4-month-old *B. henselae*-negative SPF kittens were used. Eighteen of the 47 fleas were randomly assigned to each kitten. Fleas were infested in individual cages and the simultaneously obtained blood culture to permit us to make a direct association between the presence of *B. henselae* in individual fleas and the simultaneously obtained blood culture and serology results for the same cat.

**Statistical analysis.** Descriptive data for the cattery cats were analyzed by using Epi-Info, version 6.0 (7). Frequency distributions were obtained, and chi-square tests of homogeneity for 2-by-2 contingency tables were used to examine the statistical significance of any associations. Fisher’s exact test was used to examine the association between the presence and level of *B. henselae* bacteremia and antibody titers in cats and the presence of PCR-positive fleas. S-PLUS statistical software (S-PLUS for Windows, version 3.2, 1994; StaSci, a division of MathSoft, Inc., Seattle, Wash.) was used to evaluate the relationship between levels of bacteremia or antibody titers and the number of fleas that were PCR positive for *B. henselae* DNA.

**RESULTS AND CASE REPORT**

**Experimental transmission of *B. henselae* to SPF cats via fleas removed from bacteremic cattery cats.** Eighty-five percent (40 of 47) of the cattery cats were ≥1 year old, and their ages ranged from 3 months to 18 years (mean, 4.4 years; standard error, 0.56 years). Overall, 89% (42 of 47) of the cats were bacteremic at the time of at least one of the different blood collections (range, 67 to 100%) (Table 1). PCR-restriction fragment length polymorphism analysis of all isolates produced bands characteristic of *B. henselae* (digestion of the amplicon with TaqI produced three bands, whereas digestion with *Hha*I resulted in two bands) (20). The proportion of cats with high-grade bacteremia (>10⁴ CFU/ml of blood) during the 12 months ranged from 25 to 56%. Blood was collected from 17 cats at least three times over the 1-year period; 9 of the 17 (53%) cats were bacteremic with *B. henselae* at all blood collections, and 2 of the 17 cats were never bacteremic. All 47 cattery cats were serologically positive, with titers ranging from 1:64 to 1:2,048 (mode and median, 1:256).

Bacteremia was first detected in four of the SPF kittens at 2 weeks and in the fifth kitten at 6 weeks after the experimental flea infestation. The level of bacteremia rose rapidly to very high levels (10⁴ to 10⁶ CFU/ml) for all kittens. Antibodies were detected by IFA testing 4 to 5 weeks after experimental infestation. In the first experiment, both kittens became culture negative after 11 to 12 weeks of bacteremia, but one of these kittens had three relapses of bacteremia during the 1-year observation period, despite the persistence of *B. henselae* antibodies (data not shown). The infection and seroconversion of the three kittens in the second experiment are represented in Fig. 1; the bacteremia and elevated antibody titers persisted during the entire observation period for these kittens. No kitten showed clinical or CBC abnormalities.

**Evaluation for direct, arthropod-independent transmission of *B. henselae* from kitten to kitten.** All five SPF kittens experimentally infected by the intradermal route became bacteremic 2 weeks after inoculation and developed bacteremia for a minimum of 15 weeks (>10⁴ CFU/ml of blood; data not shown). None presented clinical signs of infection or abnormal CBC values. Despite fight and play among the five infected kittens after inoculation, no kittens for 21 weeks, neither of the two control kittens became infected or seroconverted.

**Detection of *B. henselae* DNA in fleas from bacteremic cattery cats.** All cattery cats were heavily infested with fleas (five or more detectable fleas per cat), identified as the cat flea, *C. vol. 34, 1996 TRANSMISSION OF *B. HENSELAE* BY THE CAT FLEA 1953
A 25-year-old male veterinary student was scratched by a cattery cat during the first blood drawing. He subsequently developed CSD, including a small inoculation vesicle, fever, and lymphadenopathy (epitrochlear and axillary). Blood was drawn for *B. henselae* culture and serology while he was symptomatic, 7 weeks after the scratch. A banked serum sample collected 2 weeks before the scratch and a sample collected 10 weeks after the scratch also were tested. He denied receiving any other cat scratches or flea bites. *B. henselae* was not recovered from the blood culture, but seroconversion (titer, 1:128) was demonstrated.

**TABLE 1.** *B. henselae* bacteremia and detection of *B. henselae* DNA in fleas from cattery cats in a private home

<table>
<thead>
<tr>
<th>Date of blood collection</th>
<th>No. of cats whose blood was cultured</th>
<th>No. (%) of cats with:</th>
<th>No. of cats from which blood was drawn for culture and individual fleas were removed</th>
<th>PCR detection of <em>B. henselae</em> DNA in fleas (no. of fleas positive/no. of fleas tested [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. henselae</em> bacteremia</td>
<td>Bacteremia of $&gt;10^4$ CFU/ml</td>
<td>Individual fleas</td>
</tr>
<tr>
<td>April 1994</td>
<td>21</td>
<td>14 (67)</td>
<td>5 (36)</td>
<td>ND*</td>
</tr>
<tr>
<td>June 1994</td>
<td>12</td>
<td>9 (75)</td>
<td>5 (56)</td>
<td>ND</td>
</tr>
<tr>
<td>September 1994</td>
<td>34</td>
<td>26 (76)</td>
<td>10 (38)</td>
<td>13</td>
</tr>
<tr>
<td>March 1995</td>
<td>31</td>
<td>24 (77)</td>
<td>6 (25)</td>
<td>25</td>
</tr>
<tr>
<td>April 1995</td>
<td>7</td>
<td>7 (100)</td>
<td>3 (43)</td>
<td>7</td>
</tr>
</tbody>
</table>

* Fleas collected at the same time or closely proximate times as the pooled fleas used for experimental infestations.

* ND, not done.

**FIG. 1.** Mean *B. henselae* bacteremia and antibody titers of SPF kittens infected by experimental cat flea infestation. Fleas combed from cattery cats with *B. henselae* bacteremia were placed on the bodies of three SPF kittens under experimentally controlled, arthropod-free conditions at time zero. Bacteremia developed in the SPF kittens within 2 weeks of infestation with infected fleas. The median *B. henselae* antibody titer (by IFA testing) and bacteremia level (measured in CFU per milliliter of blood) are shown for the experimentally infested kittens. Bars represent standard deviations.

**DISCUSSION**

*B. henselae* represents an emerging human pathogen causing a diverse spectrum of infections including prolonged, relapsing, and even fatal manifestations. A large reservoir for *B. henselae* exists among the 57 million pet cats residing in approximately one-third of the homes in the United States (28). The factors contributing to the creation and maintenance of this large reservoir are unknown, yet their identification is essential for developing strategies to prevent human and feline infection. In two separate experiments, we established that the cat flea is a competent vector in the transmission of *B. henselae* to cats. The infected kittens had no detectable clinical disease, and *B. henselae* antibodies detected by the IFA test did not appear to protect against a relapse of bacteremia. In contrast to flea-borne transmission, direct cat-to-cat transmission among kittens could not be demonstrated in the absence of fleas.

The flea-infested, naturally *B. henselae*-infected cattery cats, living indoors in a private home, provided an excellent population for studying the relationship between feline bacteremia and the presence of *B. henselae* organisms in fleas over time and for providing fleas naturally infected with *B. henselae* for experimental infestations. Of the 47 cats, 89% were bacteremic with *B. henselae* at one or more of five samplings over a 1-year period, with 67 to 100% being bacteremic at any single sampling time. The cattery cats were heavily infested with fleas, and one-third of the 132 fleas tested individually contained *B. henselae* DNA, although there was no direct association between the presence or level of bacteremia in the cats and the presence of *B. henselae*-positive fleas carried by these cats. Millions of *B. henselae* bacilli can be present in 1 ml of feline blood, and the female cat flea consumes an average of 13.6 μl of blood per day (9). Thus, the potential for ingestion of thousands of *B. henselae* bacilli is substantial. Whether propagative
transmission occurs has not yet been determined, but the cat flea is obligately hematophagous and B. henselae has a requirement for hemin (24) that is satisfied by the constant ingestion of blood by the flea, making multiplication of B. henselae organisms in the flea gut feasible.

Fleas have been established as vectors in the transmission of other human pathogens, e.g., Yersinia pestis, Rickettsia typhi, Francisella tularensis, and the endoparasite Dipylidium caninum. In addition to B. henselae, another bacterium, Rickettsia felis, was recently isolated from the cat flea (12). The vertebrate host becomes infected with these organisms via fleas through different routes. Infection with D. canum occurs after ingestion of fleas by children or cats (11). For plague, the number, and the endoparasite transmission has not yet been determined, but the cat flea B. henselae is likely infected via fleas carrier. The cat flea can pass the organism into the host when the flea is regurgitated into the host via a bite or a scratch. Flea saliva contains various enzymes, including proteases, lipases, and peptidases, which aid in the digestion of blood and facilitate the transmission of pathogens. The pathogen is ingested by the flea, which in turn is ingested by the host when the flea bites or scratches the host.

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REFERENCES


