Prevalence of Bartonella Species in Domestic Cats in The Netherlands

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Cats have been shown to provide the only known reservoir of Bartonella henselae, the causative agent of cat scratch disease. To determine the prevalence of Bartonella bacteremia and antibodies in Dutch cats, blood samples from 113 cats from shelters (sheltered cats), 50 pet cats, and 25 specific-pathogen-free (SPF) cats were analyzed. Culture and subsequent PCR-restriction fragment length polymorphism (RFLP) analysis of the 16S-23S rRNA intergenic region and 16S rRNA gene PCR-hybridization assays revealed a prevalence of Bartonella bacteremia in 22% of the sheltered cats and showed no bacteremia in the SPF cats. Three spacer RFLP types were found: types A, B, and G, with type B being predominant over types A and G. An important finding was the existence of mixtures of different Bartonella species. Bartonella DNA was detected in 7 of 27 DNA extracts from fleas combed from the sheltered cats (26%). Seropositivity was 50% for sheltered cats and 56% for pet cats, as determined by a B. henselae enzyme-linked immunoassay.

Recent evidence has linked Bartonella henselae to various infectious diseases of humans. B. henselae can cause cat scratch disease (CSD) (1, 5, 13, 26, 33), bacillary angiomatosis (BA) (18, 27), bacteremia (28), relapsing fever (24, 28), and endocarditis (15).

Exposure to cats has proven to be an important risk factor in the human acquisition of B. henselae infections. Thirty-eight percent of pet cats not associated with humans with disease and 81% of cats associated with humans with CSD were found to have Bartonella-specific immunofluorescent antibodies (33). Microbiologic evidence for the assumption that the cat serves as a reservoir for B. henselae was obtained when the organism was isolated from the blood of a cat that was not linked to a human with disease (25). Kocher et al. (19) isolated B. henselae from cutaneous lesions of three of four BA patients and from the blood of all seven asymptomatic pet cats with which the four BA patients had prolonged contact. Forty-one percent of pet or impounded cats in the greater San Francisco Bay region had asymptomatic B. henselae bacteremia (19). Several fleas combed from these cats also contained B. henselae, as shown by culture and PCR (19). Kordick et al. (20) found 89% of cats associated with CSD patients to be bacteremic with Bartonella species, while only 28% of the cats from veterinary students were bacteremic. Thirteen of 24 bacteremic cats, respectively, remained culture positive during the ensuing 12-month period.

Several reports on serologic testing of cats showed that seroprevalence varies widely between different countries or between geographic regions within countries. Seroprevalences of 4 to 81% in the United States, 6 to 22% in Japan, 33% in Austria, 12% in Egypt, 7% in Portugal, 5% in Alaska, and 0% in western Canada have been found (7, 9, 10, 17, 31, 32). Ueno et al. (31) found no significant differences in seroprevalence between old and young cats or between male and female cats. Determination of antibody titers in 628 feline serum samples from 33 geographic locations throughout the United States and western Canada revealed that seropositivity correlates with increasing climatic warmth and annual precipitation (17). These warm, humid areas would also have the highest number of potential arthropod vectors. Risk factors associated with B. henselae bacteremia and seropositivity in cats were determined by Chomel et al. (10). They found that impounded and former stray cats were 2.86 times more likely to be bacteremic than pet cats. Furthermore, young cats (<1 year old) were 1.64 times more likely than adult cats to be bacteremic, and bacteremic cats were 1.64 times more likely than nonbacteremic cats to be infested with fleas (10).

In this study we determined the prevalence of Bartonella bacteremia and of B. henselae seroprevalence in 113 Dutch cats from shelters (sheltered cats), 25 specific-pathogen-free (SPF) cats, and 50 Dutch pet cats. We analyzed the Bartonella strains isolated from the cats by a Bartonella-specific PCR and hybridization assay and by 16S-23S spacer PCR-restriction fragment length polymorphism (RFLP) analysis and correlated B. henselae seropositivity and bacteremia with factors such as flea infestation, age, sex, sex.

MATERIALS AND METHODS

Animals. In October 1995, 113 cats residing in four shelters in different regions of The Netherlands were enrolled in the study. The shelters were in Zaandam (northwest), Amerongen (central), Eindhoven (southeast), and Zoetermeer (southwest) and are referred to as shelters I to IV, respectively. Thirty-four cats were from shelter I, 33 cats were from shelter II, 11 cats were from shelter III, and 35 cats were from shelter IV. All cats were examined physically and received antilice treatment upon arrival in the shelter. Subsequently, they were kept in quarantine for 2 to 3 weeks before entrance into a group of cats. Of the 113 sheltered cats, 53% were females. The ages of the cats ranged from 8 weeks to 14 years, with a mean age of 3.6 years and a median age of 2.8 years. Eighty-three cats (74%) were more than 1 year old. Sixty-five percent were stray cats, but most Dutch “stray” cats are formerly owned pet cats. Eighteen of the 113 sheltered cats (16%) belonged to a household before arrival in the shelter, and the other cats (88%) were transferred from another shelter. Seventy-five of the cats lived completely indoors at the time of the survey, while three of the 11 cats in shelter III and all 35 cats in shelter IV daily spent a few hours in a fenced-off, outdoor place. Twenty-seven cats (24%) were infested with fleas at the time of the blood sampling (see Table 2). When possible, a total volume of 3 ml of blood was taken from the jugular vein of each cat. One milliliter of blood was collected in 70% ethanol. From each sheltered cat, data concerning estimated age, sex, neutering history, life history, date of arrival in the shelter, presence of flea infestation, and breed were recorded.

Additionally, in May 1995, blood samples were collected from 25 SPF cats.
RESULTS

Bacteriological data. B. henselae was cultured from the blood of 25 (22%) of the 113 sheltered cats. The degree of bacteremia varied from 1 to more than 2,000 CFU per ml of blood (Table 1). Prevalences of bacteremia in the different shelters are listed in Table 2. Of the 73 stray cats, 19 (26%) were bacteremic, and the 20 former household cats showed a prevalence of 25%. Of the 20 cats that were moved in from another shelter, only 1 (5%) was culture positive.

In shelters I, II, and IV, the prevalence of flea infestation was higher in cats that had just arrived in the shelter (less than 3 weeks earlier) than in cats that had arrived more than 3 weeks earlier. Shelter III was shown to have ineffective antiflea treatment, because three cats that had resided in the shelter for 2 to 4 months before sampling carried fleas. Eight of the 25 bacteremic cats (32%) and 19 of the 88 nonbacteremic cats (22%) had fleas. The prevalence of bacteremia was highest among cats less than 1 year old (9 of 30; 30%). Ten of the 45 cats between 1 and 4 years of age (22%) and 6 of the 38 cats more than 4 years old (16%) were bacteremic. The prevalence of bacteremia was higher in male cats (28%) than in female cats (17%).

All cultures of blood from the 25 SPF cats remained negative until the end of the 4-week incubation period.

Molecular detection and subtyping of Bartonella. Cultured isolates from all cats were confirmed to be Bartonella species by Alu I-PCR-RFLP analysis of the 16S-23S rRNA intergenic spacer region as described previously (6, 22).

Detection and analysis of Bartonella DNA in cat blood and in fleas. One milliliter of citrate-treated cat blood was centrifuged at 12,000 × g for 1 min in a microcentrifuge to separate the plasma from the cells. Total DNA was extracted from the blood of 27 cats described earlier for the extraction of DNA from tissue (5, 27). The DNA was suspended in 25 μl of water, and 1 μl of this DNA extract was used as a template in a 25-μl PCR mixture. A fragment of the Bartonella 16S rRNA gene was amplified with primers p24E and p12B and was subsequently hybridized with a probe specific for B. henselae (both RFLP patterns A and B) or with a newly designed probe (5’-GATTAGCTCGACCTTGCGA-3’) specific for Bartonella species with RFLP pattern G (5, 27). Only the PCR products obtained from the flea extracts were subsequently hybridized with a newly designed probe specific for Bartonella quintana (5’-ATTAAGTTGGGACCTATTGAGGG-3’). The detection of B. henselae in the fleas described earlier for the extraction of DNA from tissue (5, 27). The DNA was suspended in 25 μl of water, and 1 μl of a 1/15 or 1/30 dilution of this DNA suspension was used as a template in the Bartonella PCR-hybridization assay as described previously and in the Bartonella type-specific PCR described above (5, 27).

DNA sequence analysis of the 16S rRNA gene of Bartonella RFLP type G. The virtually complete 16S RNA genes (1,400 bp) of two type G Bartonella strains isolated from two different cats were amplified by PCR. The DNA sequences of the ends of these products were determined with the use of the fluorescent dye terminator sequencing kit of the cycling sequencing system (Applied Biosystems Division, Perkin-Elmer, Nieuwerkerk aan de IJssel, The Netherlands). The sequence was compared with the DNA sequences present in the nucleotide sequence databases with the FastA DNA comparison program (23).

B. henselae EIA. Antibodies to B. henselae in plasma or serum samples from all 188 cats were determined by an enzyme-linked immunoassay (EIA) as described by Barka et al. (5), with minor modifications. Briefly, B. henselae ATCC 49882 was grown on Columbia agar plates (Oxoid) containing 5% sheep blood for 7 days at 35°C with 5% CO2. The colonies were scraped from the plates, suspended in 0.04 M Na2CO3 and diluted to an absorbance of 1.0 to 1.4 at 620 nm, and incubating the suspension for 30 min at 60°C. The bacterial lysates were stored at −20°C. One microliter of these lysates was used in a 25-μl PCR mixture. Bacterial isolates and subcultures were determined as Bartonella species by Alu I-PCR-RFLP analysis of the 16S-23S rRNA intergenic spacer region as described previously (6, 22).
seropositivity rate of 35%, and of the 20 cats that came from another shelter, 60% were seropositive.

Forty-six of the 86 cats without fleas (53%) and 11 of the 27 cats with fleas (41%) were seropositive. The seroprevalence was 40% among cats less than 1 year of age, 58% among cats between 1 and 4 years of age, and 50% among cats older than 4 years. Seroprevalence was a little higher among male cats (55%) than among female cats (47%). Twenty-eight of the 50 serum samples from pet cats (56%) were positive. The OD_{450} among sera from negative pet cats varied from 0.059 to 0.284 (mean, 0.180; median, 0.184), and the OD_{450} among sera from positive pet cats varied from 0.303 to 1.108 (mean, 0.504; median, 0.438).

Comparison of bacteriological and serological data. Twenty-three of the 113 cats (20%) were bacteremic and had anti-*B. henselae* antibodies. Thirty-four of the 113 cats (30%) had antibodies to *B. henselae* but were not bacteremic, whereas only 2 of the 113 cats (2%) were bacteremic but seronegative. The remaining 54 cats (48%) were both bacteriologically and serologically negative. Antibody titers were higher among bacteremic cats (mean OD_{450}, 0.649; median OD_{450}, 0.638) than among nonbacteremic cats (mean OD_{450}, 0.330; median OD_{450}, 0.253).

The positive predictive value of the serologic assay for bacteremia, i.e., the percentage of seropositive cats that are bacteremic, is 96% (54 of 56). The 16S rRNA gene sequences of two *Bartonella* strains with *AluI* RFLP pattern G (Table 1, cats 22 and 24) were amplified with universal primers. DNA sequence analysis showed that the 1,399-bp 16S rRNA gene sequences of the two isolates were identical. Comparison of the sequence with those present in the nucleotide sequence databases revealed a 99.3% identity with the 16S rRNA gene sequence of *Bartonella claridgeiae*, a

### TABLE 1. Bacteriologic, serologic, and molecular data for 25 Dutch sheltered cats infected with *Bartonella* species

<table>
<thead>
<tr>
<th>Cat no.</th>
<th>Blood culture</th>
<th>EIA IgG serology</th>
<th>Result of PCR of blood</th>
<th><em>Bartonella</em> type in fleas</th>
<th>No. of fleas</th>
<th>Result of PCR of fleas</th>
<th><em>Bartonella</em> type in fleas</th>
<th>Age (yr)</th>
<th>Shelter</th>
<th>Life history</th>
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<td>A</td>
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<td>–</td>
<td>B</td>
<td>9</td>
<td>I</td>
<td>Stray</td>
</tr>
<tr>
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<td>++</td>
<td>+</td>
<td>–</td>
<td>A</td>
<td>0</td>
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<td>2</td>
<td>II</td>
<td>Household</td>
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<td>+</td>
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<td>+</td>
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<td>I</td>
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<td>Stray</td>
<td>IV</td>
<td>Other shelter</td>
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<td>+</td>
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<td>Stray</td>
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</table>

* a, 1 to 50 CFU/ml; +, 50 to 250 CFU/ml; ++, 250 to 2,000 CFU/ml; ++++, >2,000 CFU/ml.

* b, OD_{450} in *B. henselae* EIA of $\geq 0.3$; –, OD_{450} of <0.3.

* c, Combination of results obtained by 16S-23S rRNA spacer RFLP analysis of isolates and by 16S rRNA type-specific PCRs and hybridizations of blood cells.

* d, Results of 16S rRNA type-specific PCRs and hybridizations of DNA extracts.

### TABLE 2. *B. henselae* bacteremia, antibody prevalences, and other data by shelter among 113 Dutch sheltered cats

<table>
<thead>
<tr>
<th>Shelter</th>
<th>Total Sero-positive</th>
<th>Bacteria</th>
<th><em>Bartonella</em> type</th>
<th>Flea infected</th>
<th>PCR positive</th>
<th>Life history</th>
<th>Age</th>
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<tbody>
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<td>A</td>
<td>B</td>
<td>A+G</td>
<td>B+G</td>
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<tr>
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<td>34</td>
<td>22</td>
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<td>6</td>
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<td>5</td>
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<td>2</td>
<td>1</td>
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<td>2</td>
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</tr>
<tr>
<td>Total</td>
<td>113</td>
<td>57</td>
<td>25</td>
<td>6</td>
<td>10</td>
<td>4</td>
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</table>
Bartonella species recently isolated from the blood of a cat in North America (GenBank accession no. X89208).

**DISCUSSION**

*B. henselae* has recently been implicated as the major cause of CSD and BA, and its clinical spectrum is still expanding. Cats are the only known reservoir of *B. henselae* (19). Several investigators have reported a high seroprevalence and asymptomatic *B. henselae* bacteremia among naturally infected cat populations (7, 9, 10, 17, 19, 20, 31–33).

In the study presented here the prevalence of Bartonella species in Dutch domestic cats was determined. Our results indicate a prevalence of Bartonella bacteremia of 22% in Dutch sheltered cats. This percentage is lower than those found by Chomel et al. (10) in California (40%) and Kordick et al. (20) in North Carolina (28%). This difference may be explained by the fact that in The Netherlands the average daily temperature is lower than the average daily temperatures in California and North Carolina. Jameson et al. (17) reported that *B. henselae* seroprevalences in cats paralleled increasing climatic warmth, annual precipitation, and estimated cat flea prevalences in different regions of North America.

We found a *B. henselae* seropositivity rate of 50% in the cat population in Dutch shelters, compared to a seroprevalence of 56% in Dutch pet cats, which indicates that stray cats in The Netherlands are at the same risk of becoming infected with Bartonella as pet cats. The seropositivity rate among Dutch cats is high in comparison to the seropositivity rate among cats in other countries, but the ratio between the incidence of bacteremia and seropositivity (22 versus 50%) is in the same range as the relation found by Chomel et al. (10) (40 versus 81%) and Kordick et al. (20) (28 versus 48%). The high prevalences of bacteremia and seropositivity in the cats tested by Chomel et al. (9) may be explained by the fact that of the 205 cats that they tested, 44 cats that were heavily infested with fleas belonged to the same owner. All of these cats had antibodies, and 70% of them were bacteremic. If data for those 44 cats are omitted, both the incidence of bacteremia and seroprevalence are lower (31 and 76%, respectively). Two bacteremic cats in our study did not have *B. henselae* antibodies. Possibly, these cats were in an early stage of infection, most probably acquired during their stay in the shelter. Another explanation may be that these cats were immunosuppressed due to infection with a feline immunodeficiency virus. Alternatively, some cats may be poor responders to *B. henselae* antigens.

Three different Bartonella species or subspecies were detected in Dutch cats. *Atu* RFLP type B was predominant over *Atu* RFLP types A and G. In Dutch CSD patients, only types A and B have been detected so far. About 75% of Dutch *I RFLP* types A and G. In Dutch CSD patients, only types tested in Dutch cats.

In the study presented here the prevalence of Bartonella species in Dutch domestic cats was determined. Our results indicate a prevalence of Bartonella bacteremia of 22% in Dutch sheltered cats. This percentage is lower than those found by Chomel et al. (10) in California (40%) and Kordick et al. (20) in North Carolina (28%). This difference may be explained by the fact that in The Netherlands the average daily temperature is lower than the average daily temperatures in California and North Carolina. Jameson et al. (17) reported that *B. henselae* seroprevalences in cats paralleled increasing climatic warmth, annual precipitation, and estimated cat flea prevalences in different regions of North America.

We found a *B. henselae* seropositivity rate of 50% in the cat population in Dutch shelters, compared to a seroprevalence of 56% in Dutch pet cats, which indicates that stray cats in The Netherlands are at the same risk of becoming infected with Bartonella as pet cats. The seropositivity rate among Dutch cats is high in comparison to the seropositivity rate among cats in other countries, but the ratio between the incidence of bacteremia and seropositivity (22 versus 50%) is in the same range as the relation found by Chomel et al. (10) (40 versus 81%) and Kordick et al. (20) (28 versus 48%). The high prevalences of bacteremia and seropositivity in the cats tested by Chomel et al. (9) may be explained by the fact that of the 205 cats that they tested, 44 cats that were heavily infested with fleas belonged to the same owner. All of these cats had antibodies, and 70% of them were bacteremic. If data for those 44 cats are omitted, both the incidence of bacteremia and seroprevalence are lower (31 and 76%, respectively). Two bacteremic cats in our study did not have *B. henselae* antibodies. Possibly, these cats were in an early stage of infection, most probably acquired during their stay in the shelter. Another explanation may be that these cats were immunosuppressed due to infection with a feline immunodeficiency virus. Alternatively, some cats may be poor responders to *B. henselae* antigens.

Three different Bartonella species or subspecies were detected in Dutch cats. *Atu* RFLP type B was predominant over *Atu* RFLP types A and G. In Dutch CSD patients, only types A and B have been detected so far. About 75% of Dutch patients with CSD were infected with *B. henselae* of type A and 25% were infected with *B. henselae* of type B (6). However, the minority of the Dutch cats that we tested (28%) were infected with type A, and the majority were infected with type B (56%). It is possible that *B. henselae* of type A is more infectious for humans than type B, which may result in a higher prevalence of type A in CSD patients.

Bartonella RFLP type G was mainly found in former stray cats. It is possible that Bartonella type G has another reservoir in animals living in the wild from which stray cats acquire the bacterium, with fleas or other arthropods as a vector. Bartonella type G was found earlier in the blood of a Dutch cat whose owner had CSD, but in a lymph node specimen from that patient, *B. henselae* of RFLP type B was found (6). The recently described *B. claridgeiae* was also isolated from a cat whose owner was infected with another Bartonella species, i.e., *B. henselae* (12, 21). The 16S rRNA gene sequences of two strains of the Bartonella type G were identical and had 99.3% identity with the 16S rRNA gene sequence of *B. claridgeiae*. Almost all differences were located in regions of the 16S rRNA gene that are variable within the Bartonella genus. Therefore, Bartonella type G may represent another *B. claridgeiae* subspecies or another Bartonella species closely related to *B. claridgeiae*.

All cats that were infected with Bartonella type G only (16% of all bacteremic cats) had high antibody titers to *B. henselae*. This type may be nonpathogenic for humans because it has not yet been found in patients. Thus, some seropositive cats may not provide any risk of infections for humans. The antibodies might also be the remains of an immune response to a previous, yet cleared infection with *B. henselae* of type A or B, or the load of *B. henselae* of type A or B might have been below the detection limit of culture at the time of sampling. Therefore, the IgM serology of cats may be necessary to obtain evidence of an active infection with *B. henselae*. In addition, there is a need for serologic assays that can discriminate between *B. henselae* type A or B and other Bartonella species, especially type G, in cats.

The occurrence of double infections with different Bartonella types in cats, as determined by *Atu* RFLP analysis and by Bartonella type-specific PCRs and probe analysis, was striking. Four cats were found to be infected with both *B. henselae* RFLP type B and Bartonella RFLP type G, and one cat was infected with both *B. henselae* type A and Bartonella type G. *B. henselae* type A together with *B. henselae* type B was not found in any of the tested cats. It is possible that infection with type G does not raise a protective immune response against infection with type A or B, or vice versa. Another explanation for the occurrence of double infections may be that flea carry two Bartonella species which are transmitted simultaneously when cats sustain a flea bite. Double infection with two very closely related bartonellas such as *B. henselae* types A and B apparently does not occur in humans or in cats. The immune response elicited by infection with one of the latter *B. henselae* types may provide protection against infection with the other *B. henselae* type.

Culturing of Bartonella species from cat blood was more successful than detection of Bartonella DNA in cat blood by PCR. This can be explained by the fact that for culturing, 1 ml of blood was used, while in the PCR assay DNA from 50 μl of blood was used. Bartonella type G grows more slowly and yields smaller colonies than *B. henselae* types A and B. These findings are in contrast to those described by Clarridge et al. (12) and Lawson and Collins (21) regarding the cultivation of *B. claridgeiae*, indicating that Bartonella type G differs from *B. claridgeiae*. The slower in vitro growth of Bartonella type G may explain the fact that type G was detected in two cats by PCR, whereas only *B. henselae* of type A had been cultured from one cat and *B. henselae* of type B had been cultured from the other cat.

The percentage of cats with flea infestation was higher among bacteremic cats than nonbacteremic cats, but it was lower among *B. henselae*-seropositive cats than seronegative cats. Cats from shelter III were 8 times more likely to be bacteremic than cats from shelter IV and 2.5 times more likely to be bacteremic than cats from shelter II. The four shelters had different regimens for antiflea treatment, with different degrees of effectiveness. We do not know how many cats were infested with fleas at the time of arrival in the shelter, and therefore, it is difficult to draw conclusions about the correlation between flea infestation and bacteremia. Strikingly, only 5% of the cats that were moved from another shelter were...
bacteremic, while the seropositivity rate among these cats was rather high (60%). This indicates that many of those cats that were moved had been bacteremic before sampling. Shelter IV, where antiflea treatment was very effective, harbored the largest number of cats that were moved from one shelter to the other. Thus, it is plausible that reinfections of cats with *Bartonella* species occurs, but only in the presence of fleas. This would also explain the high incidence of bacteremia in shelter III (45%), where 82% of the cats were infested with fleas.

The presence of *Bartonella* DNA in 26% of the fleas that we analyzed and the results of other investigators (19) also suggest that fleas play a role in the transmission of *B. henselae* among cats. Chomel et al. (11) recently obtained strong evidence that the cat flea is a vector for *B. henselae* by establishing experimental transmission of the organism between cats in the presence of fleas, but not in the absence of fleas. Our finding that one flea extract contained *Bartonella* DNA that strongly reacted with a *B. quintana*-specific probe indicates that fleas also carry bartonellas that are at least closely related to *B. quintana*. Sequence analysis of the PCR product is necessary to determine the nature of the *Bartonella* species present in the flea extract.

The higher prevalence of bacteremia in young cats than in adult cats (>1 year old) is consistent with the findings of Chomel et al. (10) and supports the epidemiologic link between human exposure to young cats and the development of CSD or BA (2, 8, 19, 30, 33). Among the bacteremic cats, young cats were two times more likely to have a high degree of bacteremia (≥250 CFU/ml) than adult cats. Moreover, 13 of the 19 bacteremic cats ≤4 years old (68%) had high levels of bacteria, while none of the 6 bacteremic cats >4 years old did. The higher seropositivity in Dutch adult cats than in cats younger than 1 year is in contrast to the findings of Chomel et al. (10), but the relatively high seroprevalence in cats of 1 to 2 years of age (64%) is similar to that found by Chomel et al. (10).

The *B. henselae* EIA was shown to have a positive predictive value for *Bartonella* bacteremia in cats of 39% and therefore is not useful. In contrast, *B. henselae* seronegativity was highly predictive of the absence of *Bartonella* bacteremia in cats (negative predictive value, 96%). Thus, seronegativity is a reliable indicator of the absence of *Bartonella* bacteremia in cats. Similar positive and negative predictive values were found for an immunofluorescence test described by Chomel et al. (10), who reported values of 46 and 90%, respectively. Thus, prospective cat owners who are immunocompromised should acquire *B. henselae*-seronegative cats. If the individual already owns a seropositive cat, measures such as effective antifea or antibiotic treatment should be taken to reduce the risk of getting infected by the cat.

The cat population in The Netherlands is approximately 2 million. An incidence of *Bartonella* bacteremia of approximately 22% would indicate that more than 400,000 cats are infected. Nevertheless, the estimated incidence of CSD in The Netherlands is 2,000 cases per year. This means that the chance of developing CSD after a cat scratch or bite is relatively low. A number of factors may play a role in transmission. The first is the bacterial load in cat blood. Strong fluctuations in the levels of bacteremia have been shown (4, 14, 19). It is possible that only cats with high levels of bacteremia are a risk factor for acquiring CSD. The second factor that may play a role is the mode of transmission. The bacteria may be excreted via saliva or feces or by blood originating from scratching wounds and may contaminate the fur and claws of the bacteremic cat. Another possibility is that transmission occurs only via the fleas of infected fleas, as suggested by Chomel et al. (11). The latter alternative is supported by the previous findings with *Rickettsia typhi*, which is transmitted via flea feces when the host subsequently scratches the flea bite (16). Another *Bartonella* species, *B. quintana*, is transmitted via louse feces and can cause trench fever, BA, and endocarditis (16, 18, 29). No data on the relative importance of the possible factors that may play a role in the transmission of *B. henselae* are available.

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**REFERENCES**


