Prevalence of *Bartonella henselae* and *Bartonella clarridgeiae* in an Urban Indonesian Cat Population

ERIC L. MARSTON, BARBARA FINKEL, RUSSELL L. REGNERY, IMELDA L. WINOTO, R. ROSS GRAHAM, STEVEN WIGNAL, GINDO SIMANJUNTAK, AND JAMES G. OLSON

Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia, and United States Naval Medical Research Unit-2 (US NAMRU-2), Jakarta Detachment, and Center for Infectious Diseases Research, National Institutes of Health Research and Development, Ministry of Health, Jalan Pencetakan Negara 29, Jakarta, Indonesia

Received 28 May 1998/Returned for modification 22 September 1998/Accepted 10 November 1998

We studied evidence of *Bartonella henselae* and *Bartonella clarridgeiae* infection in 54 cats living in Jakarta, Indonesia. By using an indirect immunofluorescence assay, we found immunoglobulin G antibody to *B. henselae* in 40 of 74 cats (54%). The blood of 14 feral cats was cultured on rabbit blood agar plates for 28 days. *Bartonella*-like colonies were identified as *B. henselae* or *B. clarridgeiae* by using restriction fragment length polymorphism analysis and direct sequencing of the PCR amplicons. Of the cats sampled in the study, 6 of 14 (43%; all feral) were culture positive for *B. henselae*; 3 of 14 (21%; 2 feral and 1 pet) culture positive for *B. clarridgeiae*. This is the first report that documents *B. henselae* and *B. clarridgeiae* infections in Indonesian cats.

The genus *Bartonella* consists of 11 validated species, of which 2 are associated with cats and 4 have been shown to cause human disease. *Bartonella bacilliformis*, the type strain, is the etiologic agent of Carrión’s disease and is thought to be transmitted by sand flies of the genus *Lutzomyia* (14). *Bartonella elizabethae* has been isolated only once (8), and its vector and reservoir are unknown. *Bartonella quintana*, the etiologic agent of trench fever (34), is thought to be transmitted by the human body louse (* Pediculus humanus corporis*) (18, 31), and any potential natural reservoirs, other than humans, have not been demonstrated. *Bartonella henselae*, the etiologic agent of cat scratch disease (CSD), has been identified as a cause of bacillary angiomatosis in immunocompromised persons (22, 27). The domestic cat is the reservoir and vector for *B. henselae*, the type strain, is thought to be transmitted by sand flies of the genus *Lutzomyia* (14). *Bartonella elizabethae* has been isolated only once (8), and its vector and reservoir are unknown. *Bartonella quintana*, the etiologic agent of trench fever (34), is thought to be transmitted by the human body louse (*Pediculus humanus corporis*) (18, 31), and any potential natural reservoirs, other than humans, have not been demonstrated. *Bartonella henselae*, the etiologic agent of cat scratch disease (CSD), has been identified as a cause of bacillary angiomatosis in immunocompromised persons (22, 27). The domestic cat is the reservoir and vector for human *B. henselae* disease, and cat fleas (*Ctenocephalides felis*) may serve as a putative arthropod vector (6, 17, 18, 32). *Bartonella clarridgeiae*, a recent addition to the genus (21), has been isolated several times from pet and feral cats (12, 13, 19) and is suspected of having the same feline host as *B. henselae*. Although an association of *B. clarridgeiae* with human cases has been reported twice (19, 23), the role of this organism in causing human disease is unclear.

The objectives of this study were to estimate both the prevalence of *B. henselae* IgG antibody and *Bartonella* species bacteremia in a sample of the cat population of Jakarta, Indonesia.

MATERIALS AND METHODS

Bacterial strains. The following *Bartonella* type strains used in this study were obtained from the American Type Culture Collection (ATCC; Rockville, Md.): *B. bacilliformis* KCS84 (ATCC 53586), *B. clarridgeiae* Houston-2 (ATCC 51734), *B. elizabethae* F9251 (ATCC 49927), *B. henselae* Houston-1 (ATCC 49882), *B. quintana* OK90-268 (Fuller strain), *Bartonella vinsonii* Baker (ATCC VR-152), and *Bartonella vinsonii* herkoffii 93-C01 (ATCC 51672). *B. grahamii* V2 and *B. doshiae* R18 were kindly provided by Richard Birtles.

Blood and serum collection. Between October 1995 and October 1996, EDTA-treated whole blood and serum samples were collected from 74 cats (both feral and pet) residing in areas proximal to the United States Navy Medical Research Unit Number 2 (NAMRU-2) and from Center for Infectious Diseases Research at the National Institutes of Health Research and Development (PIM) facilities in Jakarta (West Java), Indonesia (6°10' S/105° E). Samples were sent to the Centers for Disease Control and Prevention (Atlanta, Ga.) for culture and serological testing. Feral cats were trapped and their ages were determined, based upon the level of erosion of permanent teeth. Pet cats were enrolled through a local veterinary clinic.

Microbiology. Blood samples were directly plated on commercially available rabbit blood-heart infusion agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.), followed by incubation at 32°C in a humidified CO₂-enriched environment (27, 35), and kept for 28 days. Cultures identified by using colony morphology consistent with *Bartonella* species were harvested from the plates by using sterile Dacron-tipped swabs and 2 ml of brain heart infusion broth (Becton Dickinson Microbiology Systems) and stored at −70°C. Organisms were identified by using Gram stain, oxidase and catalase tests, and substrate utilization as measured by RapID ANAII diagnostic panels (Innovative Diagnostics Systems, Norcross, Ga.).

Controls. Experimental controls included the purified genomic DNA of the established *Bartonella* species. Controls also included blood from bacteremic cats naturally infected with *B. henselae* and blood from nonbacteremic cats studied in our lab. The specificities of the amplified PCR products were confirmed by restriction endonuclease fragment length polymorphism (RFPL) and direct dideoxy sequencing.

Isolate identification. DNA was extracted from the harvested bacterial isolate by using the QiAamp blood kit (Qiagen, Inc., Chatsworth, Calif.) in accordance with the manufacturer’s recommendations. Two oligonucleotides homologous to the citrate synthase (gltA) gene sequences of *B. henselae* Houston-1 (GenBank accession no. L38987) were used as...
antibody status and ownership status, with pet cats testing B. henselae antibody positive at a higher prevalence (82%) than feral cats (52%). However, when the data were split by age group, <1 year versus ≥1 year, the statistically significant association between B. henselae IgG antibody status and ownership status held true only for cats younger than 1 year of age, with 100% (9 of 9) of the pet cats testing antibody positive but only 45% (20 of 44) of the feral cats testing antibody positive (P = 0.003). Thus, generalizing that pet cats test positive more often than feral cats appears to hold true only when cats are less than 1 year of age. In fact, in the sample of cats greater than or equal to 1 year of age, the opposite appeared to be true, with a higher percentage of feral cats testing B. henselae positive (77%) than pet cats (63%). However, the difference was not statistically significant.

The B. henselae antibody titer values for all cats ranged from 31 to 2,048 with a GMT of 95.11. The B. henselae GMT for pet cats (45.33) was lower than the GMT for feral cats (119.25). The comparison of normalized mean GMT values for the pet and feral cats, after a log transformation, was statistically significant with t(31) = 3.55 at a P of 0.001. Although cats of <1 year of age had a higher B. henselae IgG GMT than cats of ≥1 year of age, this difference held true statistically for feral cats only [with t(38) = 3.00 at P of 0.005], not pet cats [t(9) = -1.397 at P of 0.197]..

Six of 14 cats (43%; all feral) were culture positive for B. henselae and 3 of 14 (21%) were culture positive for B. claridgeiae (2 feral and 1 pet) (Table 2). Of the cats that were culture positive for B. henselae, all had IgG antibodies to B. henselae. No cats were found to be doubly infected. Organisms resembling Bartonella species were isolated from 9 of 14 blood cultures (64%), in most cases after 7 to 14 days for B. henselae (range, 7 to 19 days) and 12 to 20 days for B. claridgeiae (range, 12 to 28 days). Organisms were identified as being similar in enzymatic profile to Bartonella species with a RapID ANA II panel score of 000671. Catalase and oxidase tests, Gram stain, growth requirements and characteristics, and colony morphology were also consistent with Bartonella species identification. The identities of the cultured organisms were confirmed to the species level with both RFLP analysis and dideoxynucleotide sequencing of the PCR amplicons. No differences were observed between the sequences obtained in this study and those found in GenBank (release 101.0) in which all B. henselae sequences and B. claridgeiae sequences were identical to previously released sequences (accession no. L38987 and U84386, respectively). The cats that were culture positive for B. claridgeiae were found to have negative titers (<64) to B. henselae antigen.

### RESULTS

Of the cats included in this study, 53 of 74 (72%) were under 1 year of age and 21 (28%) were judged to be older than 1 year of age; 57 of 74 (77%) were feral and 17 (23%) were pets; and 42 of 74 (57%) were female and 10 (14%) were male. For 22 (30%), gender was not ascertained.

Of the 74 cats tested for B. henselae IgG antibodies, 44 were positive by IFA (Table 1). Overall, there was a statistically significant association (P = 0.047) between B. henselae IgG antibody status and ownership status, with pet cats testing B. henselae antibody positive at a higher prevalence (82%) than feral cats (52%). However, when the data were split by age group, <1 year versus ≥1 year, the statistically significant association between B. henselae IgG antibody status and ownership status held true only for cats younger than 1 year of age, with 100% (9 of 9) of the pet cats testing antibody positive but only 45% (20 of 44) of the feral cats testing antibody positive (P = 0.003). Thus, generalizing that pet cats test positive more often than feral cats appears to hold true only when cats are less than 1 year of age. In fact, in the sample of cats greater than or equal to 1 year of age, the opposite appeared to be true, with a higher percentage of feral cats testing B. henselae positive (77%) than pet cats (63%). However, the difference was not statistically significant.

The B. henselae antibody titer values for all cats ranged from 31 to 2,048 with a GMT of 95.11. The B. henselae GMT for pet cats (45.33) was lower than the GMT for feral cats (119.25). The comparison of normalized mean GMT values for the pet and feral cats, after a log transformation, was statistically significant with t(31) = 3.55 at a P of 0.001. Although cats of <1 year of age had a higher B. henselae IgG GMT than cats of ≥1 year of age, this difference held true statistically for feral cats only [with t(38) = 3.00 at P of 0.005], not pet cats [t(9) = -1.397 at P of 0.197]..

Six of 14 cats (43%; all feral) were culture positive for B. henselae and 3 of 14 (21%) were culture positive for B. claridgeiae (2 feral and 1 pet) (Table 2). Of the cats that were culture positive for B. henselae, all had IgG antibodies to B. henselae. No cats were found to be doubly infected. Organisms resembling Bartonella species were isolated from 9 of 14 blood cultures (64%), in most cases after 7 to 14 days for B. henselae (range, 7 to 19 days) and 12 to 20 days for B. claridgeiae (range, 12 to 28 days). Organisms were identified as being similar in enzymatic profile to Bartonella species with a RapID ANA II panel score of 000671. Catalase and oxidase tests, Gram stain, growth requirements and characteristics, and colony morphology were also consistent with Bartonella species identification. The identities of the cultured organisms were confirmed to the species level with both RFLP analysis and dideoxynucleotide sequencing of the PCR amplicons. No differences were observed between the sequences obtained in this study and those found in GenBank (release 101.0) in which all B. henselae sequences and B. claridgeiae sequences were identical to previously released sequences (accession no. L38987 and U84386, respectively). The cats that were culture positive for B. claridgeiae were found to have negative titers (<64) to B. henselae antigen.

### Table 1. Prevalence of B. henselae IgG antibodies, as determined by IFA, in cats from Jakarta, Indonesia, by ownership status, age, and gender

<table>
<thead>
<tr>
<th>Age (yr) and gender</th>
<th>No. of cats positive/total no. tested (%)</th>
<th>Pet</th>
<th>Feral</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9/11 (82)</td>
<td>5/6 (83)</td>
<td>5/6 (83)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12/16 (75)</td>
<td>21/27 (78)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undetermined</td>
<td>3/22 (14)</td>
<td>3/22 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>9/11 (82)</td>
<td>20/44 (45)</td>
<td>29/55 (53)</td>
<td></td>
</tr>
<tr>
<td>≥1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1/2 (50)</td>
<td>2/4 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>4/6 (67)</td>
<td>9/12 (82)</td>
<td>13/17 (76)</td>
<td></td>
</tr>
<tr>
<td>Subtotal</td>
<td>5/8 (63)</td>
<td>10/13 (77)</td>
<td>15/21 (71)</td>
<td></td>
</tr>
<tr>
<td>Grand total</td>
<td>14/19 (74)</td>
<td>30/57 (53)</td>
<td>44/76 (58)</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2. Identification of bacteremia by Bartonella isolate and gender of cat from Jakarta, Indonesia

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of cats positive/total no. positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. henselae</td>
</tr>
<tr>
<td>Male</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>Female</td>
<td>2/6 (33)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>6/14 (43)</td>
</tr>
</tbody>
</table>
DISCUSSION

Cats are the zoonotic reservoir for B. henselae (17, 18, 26, 32). We demonstrated an overall B. henselae IgG antibody prevalence of 54% in our sample of cats living in Jakarta, Indonesia. The prevalence of B. henselae IgG antibody was highest among pet cats (74%), although this group had overall lower titers (GMT = 45). The prevalence observed among feral cats (53%) was lower than that among pet cats, although the titers were highest among this group (GMT = 119). The B. henselae IgG antibody prevalence data do not mirror previous observations that cats of >1 year of age have higher prevalences than cats of ≤1 year of age; however, the titers for these two groups do mirror these observations (GMTs, 117 versus 70). Feral cats of >1 year of age had much higher titers generally than pet cats of the same age group (GMTs, 154 versus 34), which is supported by previous prevalence findings. Other authors have indicated that feral cats tend to have higher prevalences of B. henselae IgG antibodies than pet cats do (4, 5, 17). The B. henselae IgG antibody prevalence in the United States has been observed to range from 41 to 85% (17, 36). The only IgG prevalence data presently available for cats other than those with CSD patients (81%) had antibodies to B. henselae. Those authors also found that people who owned at least one kitten of ≤1 year old were 15 times more likely to contract CSD than those who owned older cats. Individuals who had been scratched or bitten by a kitten were 27 times more likely to become infected, and people who had at least one kitten with fleas were 29 times more likely to become infected than people whose animals were free of fleas.

The majority of cats in our study were 1 year of age or younger. The high prevalence of B. henselae antibody is consistent with previous observations in young cats (5, 17). Despite the limitations resulting from the small sample size of our study, the data indicate that B. henselae infection in pet and feral cats is common in the urban center of Jakarta, Indonesia. This is the first report to document a distribution of B. clarridgeiae beyond North America and Europe, suggesting that, like B. henselae, B. clarridgeiae may also have a cosmopolitan distribution among Felis domestica.

ACKNOWLEDGMENTS

We thank Jane Rooney for her contributions to the serologic portion of this study and Barbara Ellis and Kent Wagoner for their critical review of the manuscript.

REFERENCES

27. Regnery, R. L., B. E. Anderson, J. E. D. Claridge, M. C. Rodriguez-Barra-


