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

ERIC L. MARSTON,1 BARBARA FINKEL,1 RUSSELL L. REGNUERY,1 IMELDA L. WINOTO,2 R. ROSS GRAHAM,2 STEVEN WIGNAL,2 GINDO SIMANJUNTAK,3 AND JAMES G. OLSON3*

Centers for Disease Control and Prevention, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia,1 and United States Naval Medical Research Unit-2 (US NAMRU-2), Jakarta Detachment,2 and Center for Infectious Diseases Research, National Institutes of Health Research and Development, Ministry of Health, Jalan Percetakan Negara 29,3 Jakarta, Indonesia

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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. *B. clarridgeiae* and is suspected of having the same feline host as *B. henselae*. This organism has been identified as a recent addition to the genus (21), has been reported twice (19, 23), the role of this organism in causing human disease is unclear.

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 55886), *B. clarridgeiae* Houston-2 (ATCC 51734), *B. elizabethae* F3251 (ATCC 49927), *B. henselae* Houston-1 (ATCC 49882), *B. quintana* OK00-268 (Fuller strain), *Bartonella vinsonii* Baker (ATCC VR-152), and *Bartonella vinsonii* herkoffi 93-C01 (ATCC 51672). *Bartonella heniama* V2 and *Bartonella doshiae* K18 were kindly provided by Richard Britles. 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 (P3M) facilities in Jakarta (West Java, Indonesia (6°10′ S/106°50′ 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-agar 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 as having 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 Dickson 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 (RFLP) and direct dideoxy sequencing.

Isolate identification. DNA was extracted from the harvested bacterial isolate using the QIAamp blood kit (Qiagen, Inc., Chatsworth, Calif.) in accordance with the manufacturer’s instructions. Of the 11 validated species, two oligonucleotides homologous to the citrate synthase (gltA) gene sequences of *B. henselae* were used as primers.
TABLE 1. Prevalence of B. henselae IgG antibodies, as determined by IFA, in cats from Jakarta, Indonesia, by age, ownership status, and gender

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

The comparison of normalized mean GMT values for the pet and feral cats, after a log10 transformation, was statistically significant with r(51) = 3.55 at P = 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 r(38) = 3.00 at P(0.005), not pet cats [(r(9) = −1.397 at P(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 28 days for B. claridgeiae (range, 12 to 28 days). Organisms were identified as being similar in enzymatic profile to Bartonella species with a RapID ANAII 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.

| Gender         | No. positive for isolate/total no. positive (%) |
|----------------|-----------------------------------------------|
|                | B. henselae                        | B. claridgeiae                  |
| Male           | 2/6 (33)                           | 2/6 (33)                          |
| Female         | 2/6 (33)                           | 1/6 (17)                          |
| Undetermined   | 2/2 (100)                          | 0/2 (0)                           |
| Total          | 6/14 (43)                          | 3/14 (21)                         |

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 log10 transformation, was statistically significant with t(51) = 3.55 at P = 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(0.005), not pet cats [(t(9) = −1.397 at P(0.197)].

Twelve microliters of each PCR-amplified gltA product was used for gltA RFLP analysis. A panel of three restriction endonucleases was used as described in the manufacturer’s specifications in a 20-μl final volume: HindIII, Msel, and TaqI (Promega, Madison, Wis.). (24). The digest was analyzed by electrophoresis on a 2% agarose gel in 1× Tris-borate-EDTA buffer. Gels were stained with ethidium bromide and visualized by UV fluorescence (29). RFLP patterns were compared to the RFLP patterns from all recognized Bartonella species.

The specificities of the amplified products were confirmed by direct sequencing. The primers BCS781.p and BCS1137.n were used to sequence the PCR products. All PCR products were sequenced in both directions with the Prism dye terminator kit (Applied Biosystems Incorporated, Foster City, Calif.) by using an ABI-Prism model 377 autosequencer (Applied Biosystems Incorporates, Foster City, Mass.). The PCR amplification was performed with 10 μl of sample in a 100-μl reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 0.001% gelatin, 0.1% Brij 35, 200 μM (each) deoxy-nucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 0.5 μM (each) primer BCS781.p and BCS1137.n, and 0.2 U of thermostable Ampli-Taq DNA polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). Reaction conditions have been described previously in detail (24).
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 role of mild CSD-like symptoms (19), while a more seriously infected person is 29 times more likely to become infected than those who owned older cats. Individuals who had at least one kitten or puppy were 27 times more likely to become infected than people whose animals were free of fleas. People whose animals were free of fleas.

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