NOTES

Prevalence of Bartonella clarridgeiae and Bartonella henselae in Domestic Cats from France and Detection of the Organisms in Erythrocytes by Immunofluorescence

Jean-Marc Rolain, Caroline Locatelli, Luc Chabanne, Bernard Davoust, and Didier Raoult

Unité des Rickettsies CNRS UMR-A 6020, IFR 48, Faculté de Médecine, Université de la Méditerranée, 13385 Marseille Cedex 05, France.

Received 3 September 2003/Returned for modification 11 November 2003/Accepted 19 November 2003

The prevalence of Bartonella infection in a pet cat population from France was found to be 8.1% (8 of 99 cats). The intraerythrocytic location of Bartonella clarridgeiae is shown for the first time, and we show that immunofluorescence detection of the organism in erythrocytes correlates with the number of bacteria in blood.

Bartonella species are facultative intracellular bacteria highly adapted to their reservoir hosts, in which the bacteria can cause a long-lasting intraerythrocytic bacteremia (6, 10). Four species of the genus Bartonella have been recovered from the blood of cats: Bartonella henselae, the agent of cat scratch disease (20); B. clarridgeiae (16); B. bovis (“B. weissii”) (R. Regnery, N. Marano, P. Jameson, E. Marston, D. Jones, S. Handley, C. Goldsmith, and C. Greene, 15th Meet. Am. Soc. Rickettsiol., p. 15, 2000); and B. koehlerae (7, 22). The vector of B. henselae is the cat flea (Ctenocephalides felis) (5). The cat flea may also be the vector for B. clarridgeiae and B. koehlerae (23). The prevalence of Bartonella bacteremia in apparently healthy cats varies from 4 to 70%, depending on the geographical location and the cat population studied (stray cats or pet cats) (3, 18). In specific hosts, the target cells for Bartonella are either endothelial cells or red blood cells (6). Both B. henselae and B. koehlerae have previously been shown to be located in the erythrocytes of cats (13, 19, 22, 24, 27), and B. quintana and B. bacilliformis have been shown to be located in the erythrocytes of humans (1, 21, 25). In this study we have evaluated the prevalence of Bartonella infection in a well-defined urban pet cat population by blood culture and detection of the organism in erythrocytes by immunofluorescence.

The study was performed between March and November 2002 at the National Veterinary School of Lyon, Lyon, France. All pet cats were anesthetized before 3 ml of fresh blood was collected aseptically from the external jugular vein and placed in vials containing EDTA. Upon receipt of the samples, five thin blood smears were made for each cat and were air dried and stored at room temperature until the direct immunofluorescence assays were carried out. The immunofluorescence assay (Axioskop 20; Carl Zeiss, Göttingen, Germany) and laser confocal microscopy were performed as described previously (24) with a mouse monoclonal antibody (monoclonal antibody B3D4) specific for the Bartonella genus (monoclonal antibody titer, 1/1,600 diluted 1/400 in phosphate-buffered saline). Isolation of the bacteria was carried out by using Columbia 5% sheep blood agar plates (Biomerieux, Marcy l’Étoile, France) (24). When Bartonella-like colonies were observed, the numbers of CFU were recorded. Isolates were characterized by sequencing of their 16S-23S intergenic spacer region genes by using the BLAST (version 2.0) program (National Center for Biotechnology Information) (26). Student’s t test was performed with EpilInfo software (version 6) for comparison of the laboratory data. A difference was considered significant when P was <0.05.

A total of 99 domestic indoor cats from the area of Lyon, France, were included in the study. Fleas were found on only two cats. Most cats were European breeds (94%) and were presented for sterilization (74%). The sex ratio of the cats was 0.87 (males to females). The ages of the cats ranged from 3 months to 17 years (mean age, 2.97 ± 3.65 years) with 48.8% of the cats being under 1 year of age (young cats). Eight cats (8.1%) yielded a positive blood culture result, with six being infected with B. henselae Houston-1 (GenBank accession number L35101) and two being infected with B. clarridgeiae (GenBank accession number AF312497) (Table 1). The geometric mean number of CFU for the six B. henselae (22 CFU) isolates was significantly lower than that for the two B. clarridgeiae isolates (3,162 CFU; 95% confidence interval, [CI], 20 to 192). The ages of the bacteremic cats ranged from 9 months to 10 years (average, 4.11 ± 3.74 years), with only one bacteremic cat (which was positive for B. clarridgeiae) being under 1 year of age. Of the eight cats found to be bacteremic by culture, four, including the two B. clarridgeiae-infected cats, were also positive by immunofluorescence with the Bartonella genus-specific monoclonal antibody (Fig. 1). The mean percentage of infected red blood cells for the four positive cats was 1.02% ± 1.55% (range, 0.03 to 3.7%). The intraerythrocytic location of B. clarridgeiae was confirmed by laser confocal microscopy (Fig. 1).
The geometric mean number of CFU for cats that were positive by immunofluorescence (532 CFU) was significantly higher than that for cats that were negative (11 CFU; 95% CI, 9 to 274 CFU).

We found that prevalence of Bartonella bacteremia among cats in France was lower (8.1%) than that reported previously (9, 15). Factors which appear to influence the prevalence of bacteremia include geographical location, cat population, cat age, and levels of flea infestation. For example, a low prevalence (7.2%) of bacteremia in pet cats in Japan has been reported (17), whereas prevalences higher than 60% have been reported in the United States, Europe, and Southeast Asia (3, 9, 15). It has been shown that seropositivity for Bartonella correlates with increasing climatic temperatures and annual levels of precipitation (11). Such variations in the prevalence of bacteremic cats in areas with different climates have also been demonstrated in European countries (4). Variations in cat populations may explain the differences in the prevalences of bacteremia, with pet cats less likely to be bacteremic than stray cats (3). Although kittens and young cats have been found to be bacteremic more frequently than older cats (9, 12, 28), such a correlation was not found in the present study. However, our preliminary results are consistent with those of previous reports (12, 14) that showed that older animals more often have chronic infections with lower concentrations of bacteria. This difference in the levels of bacteremia in cats with acute and chronic infections has also been described in humans with trench fever due to B. quintana (2, 8). The low prevalence of infections found in our study might have been due to the fact that the cats that we studied were mostly indoor cats that were not infested with fleas (only two cats had fleas). In our study we were also able to demonstrate for the first time the intraerythrocytic location of B. clarridgeiae (Fig. 1). The sensitivity of immunofluorescence detection compared to that of culture detection of Bartonella was 50%, similar to that reported for the detection of chronic bacteremia due to B. quintana in homeless people (21).

In conclusion, our study has shown a low prevalence of bacteremia due to Bartonella species in a well-defined pet cat population. Our study confirms that indoor pet cats are less frequently infected than stray cats. We found that B. clarridgeiae infections result in greater bacteremic loads than B. henselae infections. Immunofluorescence detection of the bacteria in erythrocytes was correlated with the bacteremic load.

We thank Patrick Kelly for reviewing the manuscript.

REFERENCES

1. Benson, L. A., S. Kar, G. McLaughlin, and G. M. Ihler. 1986. Entry of Bartonella bacilliformis into erythrocytes. Infect. Immun. 54:347–353.
2. Brouqui, P., B. La Scola, V. Roux, and D. Raoult. 1999. Chronic Bartonella quintana bacteremia in homeless patients. N. Engl. J. Med. 340:184–189.
3. Chomel, B. B., R. C. Abbott, R. W. Kasten, K. A. Fordhawkins, P. H. Kass, C. A. Glaser, N. C. Pedersen, and J. E. Koehler. 1995. Bartonella henselae bacteremia in cats in California: risk factors and associations between bacteremia and antibody tilters. J. Clin. Microbiol. 33:2445–2450.
4. Chomel, B. B., H. J. Boulouis, H. Petersen, R. W. Kasten, K. Yamamoto, C. C. Chang, C. Gandoin, C. Bouillín, and C. M. Hew. 2002. Prevalence of Bartonella infection in domestic cats in Denmark. Vet. Res. 33:205–213.
5. Chomel, B. B., R. W. Kasten, K. Floyd-Hawkins, B. Chi, K. Yamamoto, J. Roberts-Wilson, A. Nikos Gurfield, R. C. Abbott, N. C. Pedersen, and J. E. Koehler. 1996. Experimental transmission of Bartonella henselae by the cat flea. J. Clin. Microbiol. 34:1952–1956.
6. Debio, C. 2001. Bartonella interactions with endothelial cells and erythrocytes. Trends Microbiol. 9:279–285.
7. Drou, S., B. Chi, E. Horn, A. G. Steigerwalt, A. M. Whitney, and D. J. Brenner. 1999. Bartonella koehlerae sp. nov., isolated from cats. J. Clin. Microbiol. 37:1117–1122.
8. Foucault, C., K. Barrau, P. Brouqui, and D. Raoult. 2002. Bartonella quintana bacteremia among homeless people. Clin. Infect. Dis. 35:684–689.
9. Heller, R., M. Artois, V. Xemar, D. Bre Briel, H. Gelin, B. Jaulhac, H. Monteil, and Y. Piemont. 1997. Prevalence of Bartonella henselae and Bartonella clarridgeiae in stray cats. J. Clin. Microbiol. 35:1327–1331.
10. Jacomo, V., P. J. Kelly, and D. Raoult. 2002. Natural history of Bartonella infections (an exception to Koch’s postulate). Clin. Diagn. Lab. Immunol. 9:8–18.
11. Jameson, P., C. Greene, R. Regnery, M. Dryden, A. Marks, J. Brown, J. Cooper, B. Glaus, and R. Greene. 1995. Prevalence of Bartonella henselae antibodies in pet cats throughout regions of North America. J. Infect. 272:1145–1149.
12. Koehler, J. E., C. A. Glaser, and J. W. Tappero. 1994. Rochalimaea henselae infection: a new zoonosis with the domestic cat as a reservoir. JAMA 271:531–535.
13. Kordick, D. L., and E. B. Breitschwerdt. 1995. Intraerythrocytic presence of Bartonella henselae. J. Clin. Microbiol. 33:1655–1656.
14. Kordick, D. L., K. H. Wilson, D. J. Sexton, T. L. Hadfield, H. A. Berkhoff, and E. B. Breitschwerdt. 1995. Prolonged Bartonella bacteremia in cats associated with cat-scratch disease patients. J. Clin. Microbiol. 33:3245–3251.
15. La Scola, B., B. Davoust, M. Boni, and D. Raoult. 2002. Lack of correlation between serology, Bartonella DNA detection within fleas and results of blood culture in a Bartonella-infected stray cat population. Clin. Microbiol. Infect. 8:345–351.
16. Lawson, P. A., and M. D. Collins. 1996. Description of Bartonella clarridgeiae sp. nov., isolated from the cat of a patient with Bartonella henselae septiciaemia. Med. Microbiol. Lett. 5:64–73.
17. Maruyama, S., Y. Nakamura, H. Kabeya, S. Tanaka, T. Sakai, and Y. Katsube. 2000. Prevalence of Bartonella henselae, Bartonella clarridgeiae and the 16S rRNA gene types of Bartonella henselae among pet cats in Japan. J. Vet. Med. Sci. 62:273–279.
18. Maruyama, S., S. Nogami, I. Inoue, S. Namba, K. Asanome, and Y. Katsube. 1996. Isolation of Bartonella henselae from domestic cats in Japan. J. Vet. Med. Sci. 58:81–83.
19. Mehock, J. R., C. E. Greene, F. C. Gherardini, T. W. Hahn, and D. C. Krause. 1998. Bartonella henselae infection of feline erythrocytes in vitro. Infect. Immun. 66:3462–3466.
20. Regnery, R. L., M. Martin, and J. G. Olson. 1992. Naturally occurring Rochalimaea henselae infection in domestic cat. Lancet 340:557–558.
21. Rolain, J. M., C. Foucault, R. Guieu, B. La Scola, P. Brouqui, and D. Raoult. 2002. Bartonella quintana in human erythrocytes. Lancet 360:226–228.
22. Rolain, J. M., P. E. Fournier, D. Raoult, and J. Y. Boncancud. 2003. First isolation and detection by immunofluorescence assay of Bartonella koehlerae in erythrocytes from a French cat. J. Clin. Microbiol. 41:4001–4002.
23. Rolain, J. M., M. Franc, B. Davoust, and D. Raoult. 2003. Molecular detection of Bartonella quintana, B. koehlerae, B. henselae, B. clarridgeiae, Rickettsia felis and Wolbachia pipientis in cat fleas. France. Emerg. Infect. Dis. 9:338–342.
24. Rolain, J. M., B. La Scola, Z. Liang, B. Davoust, and D. Raoult. 2001. Immunofluorescent detection of intraerythrocytic Bartonella henselae in naturally infected cats. J. Clin. Microbiol. 39:2976–2980.
25. Rolain, J. M., S. Novelli, P. Ventosilla, C. Maguina, H. Guerra, and D. Raoult. 2003. Immunofluorescence detection of Bartonella bacilliformis flagella in vitro and in vivo in human red blood cells as viewed by laser confocal microscopy. Ann. N. Y. Acad. Sci. 990:581–584.
26. Roux, V., and D. Raoult. 1995. Inter-and intraspecies identification of Bartonella (Rochaimea) species. J. Clin. Microbiol. 33:1573–1579.
27. Schulein, R., A. Seubert, C. Gille, C. Lanz, Y. Hansmann, Y. Piemont, and C. Dehio. 2001. Invasion and persistent intracellular colonization of erythrocytes. A unique parasitic strategy of the emerging pathogen Bartonella. J. Exp. Med. 193:1077–1086.
28. Zangwill, K. M., D. H. Hamilton, B. A. Perkins, R. L. Regnery, B. D. Plikaytis, J. L. Hadler, M. L. Cartter, and J. D. Wenger. 1993. Cat scratch disease in Connecticut—epidemiology, risk factors, and evaluation of a new diagnostic test. N. Engl. J. Med. 329:6–13.