NOTE *Public Health*

A Serological Investigation of *Bartonella henselae* Infection in Cats in Turkey

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(Received 6 May 2011/Accepted 23 June 2011/Published online in J-STAGE 7 July 2011)

ABSTRACT. *Bartonella henselae* is the causative agent of cat scratch disease (CSD) in humans. Cats are the main reservoir of this bacterium and may infect humans through scratches and bites. The purpose of this study was to determine the *B. henselae* seroprevalence in cats in Turkey. A total of 298 cats blood samples were collected from six different provinces of Turkey. Sera were tested for the presence of anti-*B. henselae* IgG antibodies by indirect fluorescent antibody test (IFA). The seroprevalence of *B. henselae* was 27.9% (83/298) for the cats examined in this study. The seroprevalence of cats by province was significantly higher in Bursa (41.3%), Adana (33.9%), Aydin (27.5%) and Burdur (32.3%) than in Kayseri (17.9%) and Istanbul (12.5%). Statistically significant differences were not observed between cat sexes and living conditions of cats. The results revealed that *B. henselae* is an important zoonotic pathogen in Turkey.

KEY WORDS: *Bartonella henselae*, feline, IFA, Turkey.

J. Vet. Med. Sci. 73(11): 1513–1516, 2011

The genus *Bartonella* is comprised of fastidious hemotropic Gram-negative bacteria that have been increasingly identified in a wide range of domestic and wild animals. *Bartonella* species have been recognized as zoonotic agents, including *B. henselae*, *B. elizabethae*, *B. grahamii*, *B. vinsonii* subsp. *arupensis*, *B. vinsonii subsp. berkhoffii*, *B. washoensis, B. koehlerae*, *B. clarridgeiae* and *B. rochalimae* [4, 13, 14]. Domestic cats are the primary reservoir of *B. henselae*, *B. clarridgeiae* and *B. koehlerae* [19, 26]. *B. henselae* is the causative agent of cat scratch disease (CSD) and causes bacillary angiomatosis, peliosis hepatis, splenic peliosis and chronic bacteriemia, endocarditis or neurological disease in immunocompromised patients [3]. Most naturally infected cats appear to be asymptomatic, although bacteremia over a period of more than one year has been reported in asymptomatic infected cats [7]. Since cats are the major source of the infection, it is of interest to determine the seroprevalence to *B. henselae* infection among cats. Previous epidemiological studies of *Bartonella* infection in cats have shown a 4 to 81% range worldwide [9]. The purpose of this study was to investigate the seroprevalence of *B. henselae* infection in domestic cats in Turkey.

This study was carried out between March 2007 and August 2008 in 4 geographical regions (The Marmara, Aegean, Mediterranean and Central Anatolia Regions) of Turkey (Fig. 1). A total of 298 cat blood samples were collected from the following six different province: Aydin (n=58) in the Aegean Region, Bursa (n=58) and Istanbul (n=56) in the Marmara Region, Adana (n=53) and Burdur (n=34) in the Mediterranean Region and Kayseri (n=39) in the Central Anatolia Region (Fig. 1). According to the living condition of the cats, 131 (44.0%) were pet cats and the remaining 167 (56.0%) were stray/shelter cats. According to sex**,** 153 (51.3%) were female and 145 (48.7%) were male. The ages ranged from 4 months to 14 years.

The presence of IgG antibodies to *B. henselae* was evaluated using the IFA described by Regnery *et al.* [24]. Antigen was prepared at National Public Health Agency laboratories with some modifications. *B. henselae* (ATCC 49882) was cocultivated for 4–5 days with Vero cells to prevent autoagglutination of organisms. Infected cells were harvested and centrifuged at 200 × g for 10 min. The supernatant was discarded, and the infected cells were resuspended in 2 m*l* phosphate buffered saline (PBS). The suspension was frozen at –80°C for 10 min and thawed in an ultrasonic bath for 2 min to destroy the infected Vero cells. The suspension was centrifuged at 500 × g for 10 min in order to separate it from artifacts. To each well of 15-well 4 mm Teflon printed slides (Immuno-Cell Int.), 2 μl of supernatant was added, and the slides were then air-dried, fixed in acetone and stored at –20°C until they were used. Serum samples were initially screened at dilutions of 1:32 and 1:64. The secondary antibodies used were fluorescent-labeled goat anti-cat immunoglobulin G (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD, U.S.A.). The intensity of the bacillus-specific fluorescence was scored subjectively from 1 to 4, and a fluorescence score of 2 at a dilution of 1:64 was considered to be positive [24]. Samples shown to be positive by testing at a dilution of 1:64 were titrated in serial two-fold dilutions to the end point. Negative and pos-

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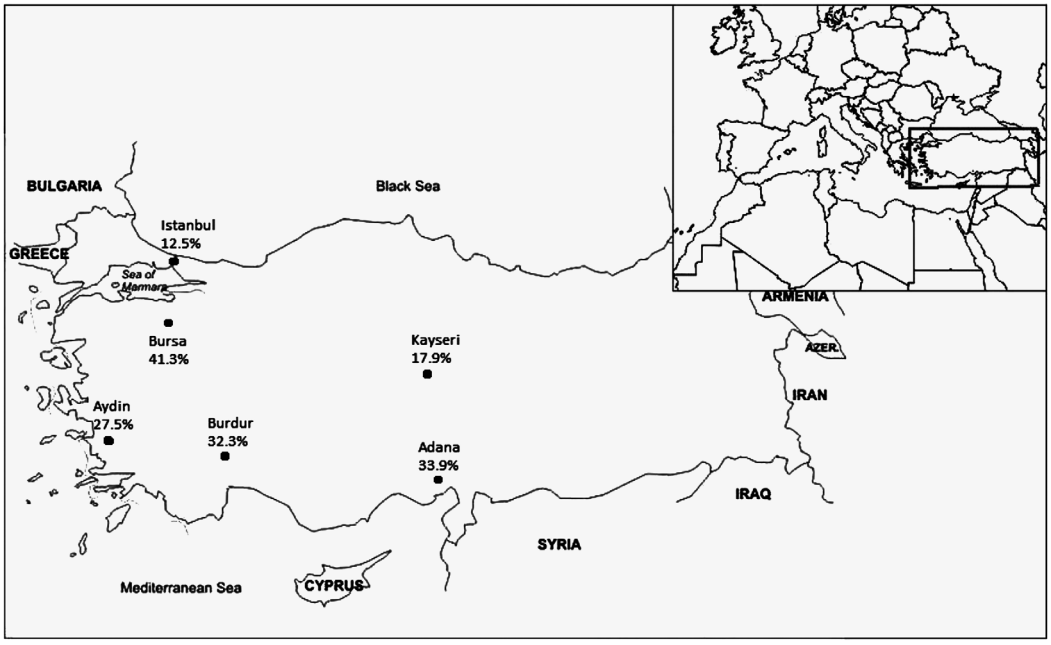

Fig. 1. Distribution and seropositivity of serum samples collected from different parts of Turkey.

Table 1. *B. henselae* seropositivity in cats according to living conditions and provinces in Turkey

Living conditions	Marmara		Mediterranean		Aegean	Central Anatolia	Total
	Bursa	Istanbul	Adana	Burdur	Aydin	Kayseri	
Pet cats	35.0% (7/20)	12.5% (7/56)	31.0% (9/29)	–	26.9% (7/26)	–	22.9% (30/131)
Stray / Shelter cats	44.7% (17/38)	–	37.5% (9/24)	32.3% (11/34)	28.1% (9/32)	17.9% (7/39)	31.7% (53/167)
Total	41.3% (24/58)[a)]	12.5% (7/56)[b)]	33.9% (18/53)[a)]	32.3% (11/34)[a)]	27.5% (16/58)[a)]	17.9% (7/39)[b)]	27.9% (83/298)

a, b) Different letters indicate a significant difference among groups by chi-square test ($P<0.05$).

itive serum control samples were used in each slide.

The chi-square test was performed to compare the seroprevalence of *B. henselae* according to sex, living conditions and province. The significance level was set as $P<0.05$.

In this study, the average seroprevalence of *B. henselae* was detected 27.9% (varied in the different provinces from 12.5 to 41.3%) in Turkey (Table 1). The prevalence rate was similar to those found in other countries, such as Austria 33.3% [1] and France 36.0% [11]. However, the prevalence rate was higher than those observed in Switzerland (8.3%) [16], Czech Republic (8.0%) [22], Japan (9.1%) [20], United Kingdom (9.4%) [8] and Germany (13.0%) [25]. In Europe, the highest reported *Bartonella* seroprevalences were found in The Netherlands, with 56.0% of 50 pet cats and 50.0% of 113 sheltered cats [6], and Poland (86.0%) [23].

When evaluating *B. henselae* infection in different regions of Turkey, higher seroprevalences of infection were detected in Bursa (41.3%), Adana (33.9%), Aydin (27.5%) and Burdur (32.3%) than in Kayseri (17.9%) and Istanbul (12.5%). Celebi *et al.* had previously reported that the seroprevalence of *B. henselae* in cats Ankara in the Central Anatolia Region of Turkey was 18.6% [10]. The data is similar to that in shelter cats in Kayseri in the Central Anatolia Region. Turkey is a Mediterranean country; however, there are differences in climate and humidity according to region. In the study areas, the Mediterranean, Aegean, and Marmara Regions which are coastal areas, have high temperatures and humidity; however, Kayseri (Central Anatolia Region), which is an inland area, has a cold and dry climate. Jameson *et al.* suggested that cats in a warm and humid environment were associated with higher seroprevalence of *B. henselae* than those in a cold and dry environment [18]. Furthermore, it was reported that the cat flea is an important vector for the transmission of *B. henselae* between cats [12]. In the present study, though the authors did not examine the flea infestation in cats, the infestation status of fleas in cats may influence the difference in seroprevalence by according to region. In this study, higher seroprevalences of *B. henselae* were detected in Adana, Burdur (Mediterranean Region), Aydin (Aegean Region), and Bursa (Marmara Region) than

in Kayseri (Central Anatolia Region). However, the lowest seroprevalence rate was detected in Istanbul (Marmara region). It is commonplace knowledge that the result of epidemiological studies are influenced by many factors such as the number of sampled animals, the age of the animals, the time of sampling, individual differences and so on.

In the present study, there was no significant difference in *B. henselae* seroprevalence between pet cats (22.9%) and stray or shelter cats (31.7%). Previously, Celebi *et al.* determined the seroprevalence of Bartonella infection to be 27.5% in stray cats and 13.0% in pet cats in the Ankara Province of Turkey [10]. Fabbi *et al.* determined the seroprevalence of stray cats in Italy to be 39.0% [15]. In France, the seroprevalence of indoor cats was found to be 41.0% in Paris by Gurfield *et al.* [17]. Baneth *et al.* determined the seroprevalence of indoor cats in Israel to be 39.5% [5]. Maruyama *et al.* investigated the relation between *B. henselae* positivity and living conditions in cats. Seroprevalence was determined to be 14.5% in outdoor domestic cats and 7.0% in indoor domestic cats in Japan [21].

In regard to the seroprevalence of *Bartonella* infection in cats in terms of sex, there are contradictory results. According to some authors, no significant differences have been detected between the sexes [2, 16, 21]. However, some researchers [6, 20] have reported that male cats had higher infection rates, whereas Sander *et al*. found that female cats had a higher prevalence [25]. In our study, there was no statistically significant difference in seroprevalence between male (28.1%) and female cats (26.1%).

In conclusion, our results showed that *B. henselae* infection is common in domestic cats (27.9%) in Turkey, that it represents an important reservoir of infection for human CSD and that it therefore might pose some risk to public health.

REFERENCES

1. Allerberger, F., Schonbauer, M., Zangerle, R. and Dierich, M. 1995. Prevalence of antibody to Rochalimaea henselae among Austrian cats. *Eur. J. Ped.* **154**: 165.
2. Al-Majali, A. M. 2004. Seroprevalence of and risk factors for *Bartonella henselae* and *Bartonella quintana* infections among pet cats in Jordan. *Prev. Vet. Med*. **64**: 63–71.
3. Anderson, B. E. and Neuman, M. A. 1997. *Bartonella* spp. as emerging human pathogens. *Clin. Microbiol. Rev.* **10**: 203–219.
4. Avidor, B., Graidy, M., Efrat, G., Leibowitz, C., Shapira, G., Schattner, A., Zimhony, O. and Giladi, M. 2004. *Bartonella koehlerae*, a new cat-associated agent of culture-negative human endocarditis. *J. Clin. Microbiol.* **42**: 3462–3468.
5. Baneth, G., Kordick, D. L., Hegarty, B. C. and Breitschwerdt, E. B. 1996. Comparative seroreactivity to *Bartonella henselae* and *Bartonella quintana* among cats from Israel and North Carolina. *Vet. Microbiol*. **50**: 95–103.
6. Bergmans, A. M., De Jong, C. M., Van Amerongen, G., Schot, C. S. and Schouls, L. M. 1997. Prevalence of *Bartonella* species in domestic cats in The Netherlands. *J. Clin. Microbiol.* **35**: 2256–2261.
7. Breitschwerdt, E. B. and Kordick, D. L. 2000. *Bartonella* infection in animals: Carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clin. Microbiol. Rev.* **13:** 428–438.
8. Birtles, R .J., Laycock G., Kenny, M. J., Shaw, S. E. and Day, M. J. 2002. Prevalence of Bartonella species causing bacteremia in domesticated and companion animals in the United Kingdom. *Vet. Rec.* **151:** 225–229.
9. Boulouis, H. J., Chang, C. C., Henn, J. B., Kasten, R. W. and Chomel, B. B. 2005. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. *Vet. Res*. **36:** 383–410.
10. Celebi, B., Kilic, S., Aydin, N., Tarhan, G., Carhan, A. and Babur, C. 2009. Investigation of *Bartonella henselae* in cats in Ankara, Turkey. *Zoon. Public Health* **56:** 169–175.
11. Chomel, B. B., Gurfield, A. N., Boulouis, H. J., Kasten, R. W. and Piemont, Y. 1995. Reservoir felin de l'agent de la maladie des griffes du chat, Bartonella henselae, en region parisienne: resultats preliminaires. *Rec. Med. Vet.* **171**: 841–845.
12. Chomel, B. B., Kasten, R. W., Floyd-Hawkins, K., Chi, B., Yamamoto, K., Roberts-Wilson, J., Gurfield, A. N., Abbott, R. C., Pedersen, N. C. and Koehler, J. E. 1996. Experimental transmission of *Bartonella henselae* by the cat flea. *J. Clin. Microbiol.* **34**: 1952–1956.
13. Chomel, B. B., Boulouis, H. J. and Breitschwerdt, E. B. 2004. Cat scratch disease and other zoonotic *Bartonella* infections. *J. Am. Vet. Med. Assoc*. **224**: 1270–1279.
14. Eremeeva, M. E., Gerns, H. L., Lydy, S. L., Goo, J. S., Ryan, E. T., Mathew, S. S, Ferraro, M. J., Holden, J. M., Nicholson, W. L., Dasch, G. A. and Koehler, J. E. 2007. Bacteremia, fever, and splenomegaly caused by a newly recognized *bartonella* species. *New Engl. J. Med.* **356**: 2381–2387.
15. Fabbi, M, Vicari, N., Tranquillo, M., Pozzi, C., Prati, P., De Meneghi, D., Bertoletti, I., Lauzi, S., Guiso, P. and Genchi, C. 2004. Prevalence of *Bartonella henselae* in stray and domestic cats in different Italian areas: evaluation of the potential risk of transmission of *Bartonella* to humans. *Parassitologia* **46**: 127–129.
16. Glaus, T., Hofmann-Lehmann, R., Greene, C., Glaus, B., Wolfensberger, C. and Lutz, H. 1997. Seroprevalence of *Bartonella henselae* infection and correlation with disease status in cats in Switzerland. *J. Clin. Microbiol*. **35**: 2883–2885.
17. Gurfield, A. N., Boulouis, H. J., Chomel, B. B., Kasten, R. W., Heller, R., Bouillin, C., Gandoin, C., Thibault, D., Chang, C. C., Barrat, F. and Piemont,Y. 2001. Epidemiology of *Bartonella* infection in domestic cats in France. *Vet. Microbiol*. **80**: 185–189.
18. Jameson, P., Greene, C., Regnery, R., Dryden, M., Marks, A., Brown, J., Cooper, J., Glaus, B. and Greene, R. 1995. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. *J. Infect. Dis.* **172**: 1145–1149.
19. Koehler, J. E., Glaser, C. A. and Tappero, J. T. 1994. *Rochalimaea henselae* infection: A new zoonosis with the domestic cat as reservoir. *J. Am. Med. Assoc.* **271**: 531–535.
20. Maruyama, S., Hiraga, S., Yokoyama, E., Naoi, M., Tsuruoka, Y., Ogura, Y., Tamura, K., Namba, S., Kameyama, Y., Nakamura, S. and Katsube, Y. 1998. Seroprevalence of *Bartonella henselae* and *Toxoplasma gondii* infections among pet cats in Kanagawa and Saitama Prefectures. *J. Vet. Med. Sci.* **60**: 997–1000.
21. Maruyama, S., Kabeya, H., Nakao, R., Tanaka, S., Sakai, T., Xuan, X., Katsube, Y. and Mikami, T. 2003. Seroprevalence of *Bartonella henselae, Toxoplasma gondii, FIV* and *FeLV* infections in domestic cats in Japan. *Microbiol. Immunol.* **47**: 147–153.

22. Melter, O., Hercik, K., Weyant, R.S., Janecek, J., Nemec, A., Mecera, J., Gonzorova, L. and Branny, P. 2003. Detection and characterization of feline Bartonella henselae in the Czech Republic. *Vet. Microbiol*. **93**: 261–273.
23. Podsiadly, E., Sokolowska, E. and Tylewska-Wierzbanowska, S. 2003. Seroprevalence of *Bartonella henselae* and *Bartonella quintana* infections in Poland in 1998–2001. *Ann. New York Acad. Sci.* **990**: 407–408.
24. Regnery, R. L., Olson, J. G., Perkins, B. A. and Bibb, W. 1992. Serological response to "*Rochalimaea henselae*" antigen in suspected cat scratch disease. *Lancet* **339**: 1443–1445.
25. Sander, A., Buhler, C., Pelz, K., Von Cramm, E. and Bredt, W. 1997. Detection and identification of two *Bartonella henselae* variants in domestic cats in Germany. *J. Clin. Microbiol.* **35**: 584–587.
26. Zangwill, K. M., Hamilton, D. H., Perkins, B. A., Regnery, R. L., Plikaytis, B. D., Hadler, J. L., Carter, M. L. and Wenger, J. D. 1993. Cat scratch disease in connecticut: epidemiology, risk factors, and evaluation of a new diagnostic test. *New Eng. J. Med*. **329**: 8–13.