

Seroprevalence of *Coxiella burnetii* in Stray Cats in Central Anatolia*

Selçuk KILIÇ^{1,**}, Tomoyoshi KOMIYA², Bekir ÇELEBİ¹, Nejat AYDIN³, Junko SAITO², Hiroko TORINIWA²,
Bilge KARATEPE⁴, Cahit BABÜR¹

¹Refik Saydam National Hygiene Center, Department of Communicable Diseases Research, Ankara - TURKEY

²Research Center for Biologicals, The Kitasato Institute, JAPAN

³Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Ankara - TURKEY

⁴Institute of Sciences, Niğde University, Niğde - TURKEY

Received: 06.07.2008

Abstract: *Coxiella burnetii* causes Q fever (Coxiellosis) in humans and animals worldwide. The present study was carried out to determine the seroprevalence of Q fever among stray cats in 3 providences (Ankara, Niğde, and Kayseri) in Central Anatolia, Turkey. A total of 143 sera from stray cats were examined for the presence of IgG against *C. burnetii* phase II antigen by indirect fluorescent antibody test (IFAT). Seven out of the 143 (4.9%) stray cats were seropositive for Q fever, with titers of 1:64 to 1:256. Seroprevalences in Ankara, Niğde, and Kayseri provinces were 1.6%, 7.4%, and 8.3%, respectively. This is the first report of the presence of *C. burnetii* in cats in Turkey.

Key Words: *Coxiella burnetii*, cat, seroprevalence, IFAT

İç Anadolu Bölgesi Sokak Kedilerinde *Coxiella burnetii* Seroprevalansı

Özet: *Coxiella burnetii*, tüm dünyada insan ve hayvanlarda Q hummasının (Coxiellosis) etkenidir. Bu çalışma, İç Anadolu bölgesinde Ankara, Niğde ve Kayseri illerindeki sokak kedilerinde Coxiellosis seroprevalansını saptamak amacıyla yapılmıştır. 143 sokak kedisinin serumunda *C. burnetii* faz II antijenine karşı şekillenen IgG antikorları İndirekt Floresan Antikor Testi (IFAT) ile araştırılmıştır. 143 kedinin yedisi (% 4,9) 1:64 ile 1:256 titrelerde pozitif olarak bulunmuştur. Ankara, Niğde ve Kayseri illerindeki seropozitiflik oranları sırasıyla % 1,6, % 7,4 ve % 8,3 olarak saptanmıştır. Bu araştırma ülkemizde kedilerde *C. burnetii*'nin varlığına ait ilk bildirimdir.

Anahtar Sözcükler: *Coxiella burnetii*, kedi, seroprevalans, IFAT

Q fever, caused by the rickettsial organism *Coxiella burnetii*, is a zoonosis with a worldwide distribution in a great variety of vertebrates and invertebrates (1). Animals typically acquire the bacterium through exposure to other infected animals, or by direct contact with contaminated body fluids or aerosol. *C. burnetii* localizes in the mammary glands, supramammary lymph nodes, uterus, and placenta in domestic ruminants and other susceptible species. Although *C. burnetii* can induce abortion and still birth in domestic mammals and

ruminants, most animals infected with *C. burnetii* are rarely symptomatic. However, infected animals can shed massive numbers of bacteria at parturition, and intermittently in various secretions and excreta (1,2). Infected placental tissue, postpartum discharges, and feces are presumed to be the principal sources of transmission to other animals and to humans via inhalation of infectious aerosols or air-borne contaminated dust (1,3).

* This study was presented as a poster presentation at the VIIth Veterinary Microbiology Congress 26-28 September, 2006; Side, Antalya, Turkey.

** E-mail: selcuk.kilic@rshm.gov.tr

The most common reservoirs for infection in humans are domesticated ruminants, primarily cattle and sheep, and to a lesser extent goats (3,4). Companion animals such as cats and dogs as well as rabbits are known to be potential sources of urban outbreaks (5-9). Since the domestic cat has recently been implicated in several outbreaks of human Q fever in urban areas, it has been suggested that in some regions of the world cats may be more commonly implicated than domestic ruminants in the spread of *C. burnetii* to humans (10).

In Turkey, Q fever was first recognized in 1947 when a small outbreak occurred in a rural community (11). Since the importance of Q fever became apparent, serological surveys have been performed to trace the prevalence of the disease in humans and domestic animals. Serological evidence of *C. burnetii* infection in ungulate mammals and humans has been reported (12,13). However, there is no record regarding Q fever in cats in Turkey. In this study, therefore, we aimed to investigate the prevalence of *C. burnetii* infection in stray cats in Central Anatolia, Turkey.

Peripheral blood samples were collected from 143 stray cats in 3 provinces, Ankara (n = 63), Niğde (n = 68), and Kayseri (n = 12), in Central Anatolia in March and April 2005. The serum was immediately separated by centrifugation and stored at -20 °C until serological analyses were performed.

The age and gender were recorded for each animal. The age of cats varied from 4 months to 14 years old. Most of the cats (71.3%) were < 3 years old and females accounted for 57.6% of the 143 cats.

The presence of IgG against *C. burnetii* was measured by the indirect fluorescent antibody test (IFAT) with the antigen of *C. burnetii* phase II, Nine Mile strain-ATCC 616 VR using the method described by Komiya et al. (14). In brief, the sera were treated at 56 °C for 30 min in order to inactivate them. Two-fold dilutions in phosphate-buffered saline (pH 7.2) from 1:16 to 1:1024 were tested against fixed and purified antigens. Fluorescein-conjugated goat anti-cat IgG (Cappel Products, Organon Teknika Corp, USA) was used as a second antibody. Sera with *C. burnetii* phase II antibody titers 1/64 and 1/512 were used as positive controls and those with negative serology were used as negative controls. Positive and negative controls were run for each test.

Considering the relatively limited information about interpretation of the serology of *C. burnetii* infection in cats, particularly diagnostic titers as in humans, we considered cats with a result of ≥1:64 for phase II antibodies as positive.

Out of 143 sera examined, 7 cats (4.9%) were seropositive at the titers ≥1:64. Among the 7 seropositive cats, *C. burnetii* phase II IgG antibodies were detected at dilutions of 1:64 and 1:256 in 6 (85.7%) cats and 1 (14.3%) cat, respectively.

Seroprevalences in Ankara, Niğde, and Kayseri provinces were 1.6%, 7.4%, and 8.3%, respectively (Table 1).

Table 2 demonstrates gender and age related prevalence obtained using samples from 81 (56.6%) females and 62 (43.4%) males. The positive rate was 4.8% (3/62) in males and 4.9% (4/81) in females. Regarding the age of cats, seropositivity was 2.2% (1/46) in cats under 1 year old, 5.6% (2/36) in those 1 to 2

Table 1. Seroprevalence of *C. burnetii* in relation to geographical location.

Province	samples (n)	Reciprocal IFAT titer							
		16		32		64		256	
		n	(%)	n	(%)	n	(%)	n	(%)
Ankara	63	4	6.3	7	11.1	1	1.6	0	(0)
Niğde	68	12	17.6	5	7.4	4	5.9	1	1.5
Kayseri	12	1	8.3	1	8.3	1	16.7	0	(0)
Total	143	17	11.9	12	8.4	6	4.2	1	0.7

Table 2. Seroprevalence of Q fever according to gender and age.

Data	Samples	Reciprocal IFAT titer/n (%)				
		<16	16	32	64	256
Sex						
Female	81	57	12	8	4	-
Male	62	50	5	4	2	1
Age						
<1 years	46	39 (84.8)	3 (6.5)	3 (6.5)	1 (2.2)	0
1-2	36	24 (66.7)	7 (19.4)	3 (8.3)	2 (5.6)	0
2-3	20	14 (70)	2 (10)	3 (15)	0	1 (5)
3>	41	30 (73.2)	5 (12.2)	3 (7.3)	3 (7.3)	0
Total	143	107 (74.8)	17 (11.9)	12 (8.4)	6 (4.2)	1 (0.7)

years old, 5% (1/20) in those 2 to 3 years old, and 7.3% (3/41) in those over 3 years old, respectively. The positive rate in cats over 3 years old was slightly higher than that in younger cats.

Although serologic studies suggest that *C. burnetii* infection may be widespread in the animal population, lack of clinical data and diagnosis renders it impossible to estimate the true incidence of the infection in animals worldwide.

The prevalence of *C. burnetii* infection varies considerably among different geographic areas, seasons, and study populations, and depends on the techniques used for antibody detection and the criteria used to define positive results (1,4,15).

The seroprevalence of the disease in domestic and pet cats has been reported to be 0%-16.5% in Japan (16-19), 10.6% in Korea (14), 6.2%-19.2% in Canada (20), 2% in South Africa, and 13.5% Zimbabwe (21). A higher prevalence rate in stray cats (41.7%) was reported in Japan (14). However, Houwers and Richardus (22) failed to show even a single antibody positive among 26 cats in the Netherlands.

This is the first report of the presence of *C. burnetii* infection in cats in Turkey. As reported for cats in other countries, *C. burnetii* antibodies were detected in 4.9% of the cats, suggesting that this infection is not widespread in this sample of Turkish cats. However, most of these studies, like ours, were performed on convenient samples that may not be fully representative of the cat population of these various countries.

The seroprevalence (4.9%) in these cats was slightly lower than the values (6.2%-8.6%) previously reported in similar studies for pet cats in other countries (14,17,20). The lower seroprevalence rate found in this study may be related to the small number of samples, geographical variation, and criteria used to define positive results. It should be noted that there is not currently a universal consensus about an appropriate antibody cut-off level to use for seroepidemiologic surveys since the cut-off level is dependent on the antigen preparation being used, the population studied, and estimated prevalence rate (12,14,16,17,20). When compared to most of the previous studies, which used lower cut-off level by IFAT, we chose a higher cut-off antibody titer of 1:64 since the prevalence rate in cats was unknown. This may have led to the lower seroprevalence obtained in the present study.

In conclusion, the results of this study demonstrated the presence of anti *C. burnetii* antibodies in cats in Turkey. While the role of cats in transmitting *C. burnetii* would appear to be less important than that of ungulate mammals, it must not be disregarded because parturition products of cats coming into contact with other animals including outdoor cats and dogs could be considered possible sources of infection for humans. The data obtained from this study may be useful for reference in further studies. The need for further studies in collaboration between veterinary and medical services to elucidate the epidemiology of Q fever in order to shed more light on of *C. burnetii* infections in Turkey is obvious.

References

1. Maurin, M., Raoult, D.: Q fever. *Clin. Microbiol. Rev.*, 1999; 12: 518-553.
2. Stoenner, H.G.: Q fever. In: Steele, J.H., Stoenner, H., Kaplan, W., Torten, M., eds., *Handbook Series in Zoonoses. Sect. A: Bacterial, Rickettsial and Mycotic Diseases-v.1*. CRC Press Inc., Boca Raton, Florida. 1979; 337-340.
3. Aitken, I.D.: Clinical aspects and prevention of Q fever in animals. *Eur. J. Epidemiol.*, 1989; 5: 420-424.
4. Lang, G.H.: Coxiellosis (Q fever) in animals. In: Marrie, T.J. Ed., *Q Fever: The Disease*, Vol. 1. CRC Press, Boca Raton. 1990: 23-48.
5. Marrie, T.J., MacDonald, A., Durant, H., Yates, L., McCormick, L.: An outbreak of Q fever probably due to contact with a parturient cat. *Chest*, 1988; 93: 98-103.
6. Langley, J.M., Marrie, T.J., Covert, A., Waag, D.M., Williams, J.C.: Poker players' pneumonia. An urban outbreak of Q fever following exposure to a parturient cat. *N. Engl. J. Med.*, 1988; 319: 354-356.
7. Kosatsky, T.: Household outbreak of Q-fever pneumonia related to a parturient cat. *Lancet*, 1984; 22: 1447-1449.
8. Pinsky, R.L., Fishbein, D.B., Greene, C.R., Gensheimer, K.F.: An outbreak of cat-associated Q fever in the United States. *J. Infect. Dis.*, 1991; 164: 202-204.
9. Buhariwalla, F., Cann, B., Marrie, T.J.: A dog-related outbreak of Q fever. *Clin. Infect. Dis.*, 1996; 23: 753-755.
10. Marrie, T.J., Durant, H., Williams, J.C., Mintz, E., Waag, D.M.: Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada. *J. Infect. Dis.*, 1988; 158: 101-108.
11. Payzin, S., Golem, S.B.: Q fever in Turkey. *Turk. Bull. Hyg. Exp. Biol.* 1948; 3: 95-113. (article in Turkish with an abstract in English)
12. Cetinkaya, B., Kalender, H., Ertas, H.B., Muz, A., Arslan, N., Ongor, H., Gurcay, M.: Seroprevalence of coxiellosis in cattle, sheep and people in the East of Turkey. *Vet. Rec.*, 2000; 146: 131-136.
13. Berberoğlu, U., Gözalan, A., Kılıç, S., Kurtoğlu, D., Esen, B.: A seroprevalence study of *Coxiella burnetii* in Antalya, Diyarbakır and Samsun provinces. *Mikrobiyol. Bült.*, 2004; 38: 385-391. (article in Turkish with an abstract in English)
14. Komiya, T., Sadamasu, K., Kang, M.I., Tsuboshima, S., Fukushi, H., Hirai, K.: Seroprevalence of *Coxiella burnetii* infections among cats in different living environments. *J. Vet. Med. Sci.*, 2003; 65: 1047-1048.
15. Fournier, P.E., Marrie, T.J., Raoult, D.: Diagnosis of Q fever. *J. Clin. Microbiol.*, 1998; 36: 1823-1834.
16. Htwe, K.K., Amano, K., Sugiyama, Y., Yagami, K., Minamoto, N., Hashimoto, A., Yamaguchi, T., Fukushi, H., Hirai, K.: Seroepidemiology of *Coxiella burnetii* in domestic and companion animals in Japan. *Vet. Rec.*, 1992; 131: 490.
17. Hirai, K., To, H.: Advances in the understanding of *Coxiella burnetii* infection in Japan. *J. Vet. Med. Sci.*, 1998; 60: 781-790.
18. Morita, C., Katsuyama, J., Yanase, T., Ueno, H., Muramatsu, Y., Hohdatsu, T., Koyama, H.: Seroepidemiological survey of *Coxiella burnetii* in domestic cats in Japan. *Microbiol. Immunol.*, 1994; 38: 1001-1003.
19. Nguyen, S.A.V., To, H., Minamoto, N., Ogawa, M., Yamaguchi, T., Fukushi, H., Hirai, K.: Evaluation of the high-density agglutination test for *Coxiella burnetii* antibodies in animals. *Clin. Diagn. Lab. Immunol.*, 1997; 4: 676-680.
20. Higgins, D., Marrie, T.J.: Seroepidemiology of Q fever among cats in New Brunswick and Prince Edward Island. *Ann. N. Y. Acad. Sci.*, 1990; 590: 271-274.
21. Matthewman, L., Kelly, P., Hayter, D., Downie, S., Wray, K., Bryson, N., Rycroft, A., Raoult, D.: Exposure of cats in southern Africa to *Coxiella burnetii*, the agent of Q fever. *Eur. J. Epidemiol.*, 1997; 13: 477-479.
22. Houwers, D.J., Richardus, J.H.: Infections with *Coxiella burnetii* in man and animals in the Netherlands. *Zentralbl. Bakteriol. Mikrobiol. Hyg. (A)*, 1987; 267: 30-36.