NOTE *Public Health*

Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* in Urban and Rural Dogs in Turkey

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ABSTRACT. The seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* was investigated in stray urban dogs and shepherd and farm guard dogs from rural areas sampled from 10 provinces of Turkey. Sera from 855 dogs were examined for the presence of anti-*B. vinsonii* subsp. *berkhoffii* antibodies by indirect fluorescent antibody test. Overall, 56 (6.6%) of the 855 dogs examined, including 16 (3%) of the 522 stray dogs and 40 (12%) of the 333 rural dogs, were seropositive. This is the first report on prevalence of antibodies to *B. vinsonii* subsp. *berkhoffii* in dogs in Turkey.

KEY WORDS: *Bartonella*, canine, Turkey.

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Members of genus *Bartonella* are fastidious hemotropic, gram-negative organisms that are arthropod-borne pathogens of growing medical importance. Genus *Bartonella* consists of 20 species and 3 subspecies, and several species have been identified in a wide range of domestic and wild mammals [3]. For the first time, *B. vinsonii* subsp. *berkhoffii* was isolated from a dog with endocarditis in 1993 and later identified using a 16S rRNA sequence [15]. In subsequent studies, it was also isolated from coyotes and foxes [10, 13]. Endocarditis is the most common manifestation of *B. vinsonii* subsp. *berkhoffii* in dogs [2, 3, 20]. It is also the causative agent of myocarditis, fever, granulomatous lymphadenitis, rhinitis, epistaxis, cutaneous vasculitis, anterior uveitis and immune mediated hemolytic anemia in dogs [3, 4, 11, 16, 19]. However, Kordick and Breitschwerdt described a persistent infection in a subclinically infected dog during a 14-month testing period [14]. *B. vinsonii* subsp. *berkhoffii* was isolated from an endocarditis lesion on the heart valve of a man [21] and in neurological and neurocognitive dysfunction and immunocompetent human patients [5, 6], raising new concerns about the zoonotic potential of this organism. The first isolation of *B. vinsonii* subsp. *berkhoffii* in dogs in Turkey was achieved by Celebi *et al.*, and a high prevalence of bacteremia was found in stray (5%) and sheltered dogs (12.4 %) in Ankara province [8]. In another study, *B. vinsonii* subsp. *berkhoffii* was not isolated in sheltered dogs in Istanbul province [9].

The aim of this study was to assess the seroprevalence of *B. vinsonii* subsp. *berkhoffii* in stray dogs from urban areas as well as in shepherd and farm guard dogs from rural areas of Turkey. The dog sera, used in this study were obtained from the sera bank of Refik Saydam National Public Health Agency and were collected from 855 dogs in ten different provinces of Turkey; The stray dogs (n=522) from urban areas were from Adana (n=84), Amasya (n=19), Aydın (n=60), Bursa (n=106), Hatay (n=84), İstanbul (n=100) and Urfa (n=69). The 333 Rural dogs were from Kars (n=179), Kırıkkale (n=59) and Sivas (n=95). These dogs were raised as shepherd and farm guard dogs but were roaming freely (Fig. 1).

The presence of IgG antibodies to *B. vinsonii* subsp. *berkhoffii* was evaluated by IFA, and the IFA antigen was prepared as described by Chang *et al.* with some modifications [10]. The *B. vinsonii* subsp. *berkhoffii* strain *(ATCC 51672)* 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* PBS. The suspension was frozen in –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 a 15 well, 4 mm Teflon printed slides (Immuno-Cell Int.), 2 μ*l* 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-dog 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 dilution of 1:64 was con-

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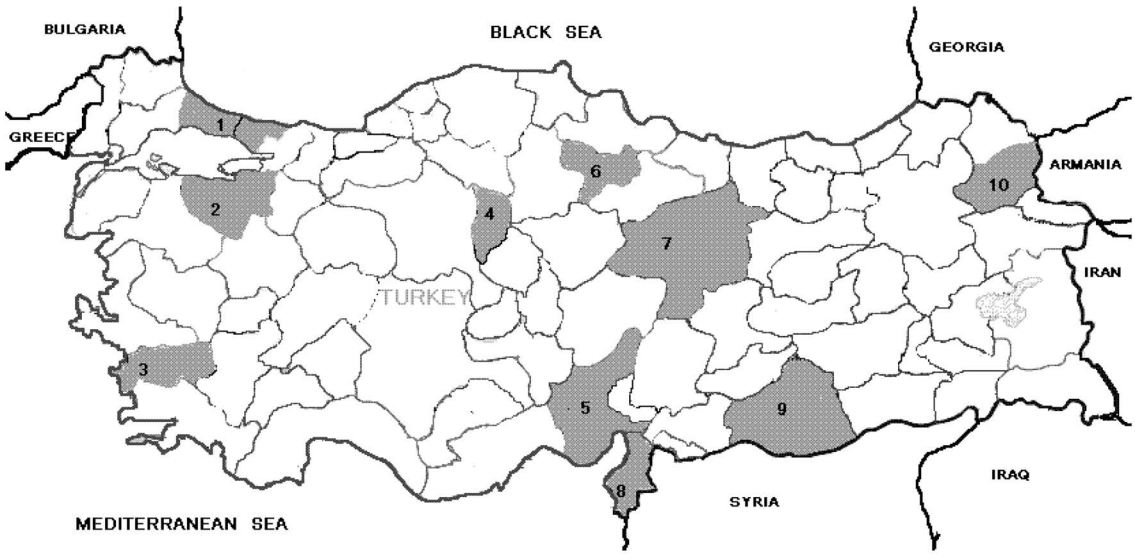

Fig. 1. Distribution of sera samples collected from different parts of Turkey. 1, Istanbul; 2, Bursa; 3, Aydın; 4, Kırıkkale; 5, Adana; 6, Amasya; 7, Sivas; 8, Hatay; 9, Urfa; 10, Kars.

Table 1. Seroprevalence of *B. vinsonii* subsp. *berkhoffii* in relation to geographical location

Dogs	Province	Seropositivity n (%)	IFA titers 1/64	1/128	1/256	1/512	1/1024
Stray Dogs	Adana	84 (2.4)	1	1			
	Amasya	19 (0)					
	Aydın	60 (5)	2	1			
	Bursa	106 (4.7)	2	2	1		
	Hatay	84 (2.4)	2				
	İstanbul	100 (3)	2	1			
	Urfa	69 (1.5)	1				
Rural Dogs	Kars	179 (10.1)	4	6	3	2	3
	Kırıkkale	59 (13.6)	3	2	2	1	
	Sivas	95 (14.7)	2	3	5	4	
	Total	855 (6.6)	19	16	11	7	3

sidered to be positive. 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 positive serum control samples were used in each slide. The positive control was serum sample obtained from a Turkish *B. vinsonii* subsp *berkhoffii* bacteremic dog [8].

Overall, 56 (6.6%) of the 855 dogs tested were seropositive for *B. vinsonii* subsp. *berkhoffii* (Table 1). The titers among the 56 seropositive dogs ranged from 1:64 to 1:1024. Seropositivity was 3% (16/522) in the stray dogs and 12% (40/333) in the rural dogs, respectively. There was a significant difference of seropositivity between the stray and rural dogs ($P<0.05$). In the stray dogs, seropositivity was as follows: 2.4% for Adana, 5% for Aydın, 4.7% for Bursa, 2.4% for Hatay, 3% for İstanbul and 1.5% for Urfa. In Amasya, none of the stray dogs were seropositive. Seropositivity in the rural dogs was observed in 10.1%, 13.6% and 14.7% of the dogs in Kars, Kırıkkale and Sivas, respectively (Table 1, Fig. 1).

In the present study, the overall seroprevalences of the dogs to *B. vinsonii* subsp. *berkhoffii* were 6.6% and 3% for the stray dogs in urban areas and 12% of the rural dogs were seropositive for *B. vinsonii* subsp. *berkhoffii*. In similar studies, higher seroprevalences were reported in Sudan (65%), Morocco and Thailand (38%), similar percentages were reported in Israel (10%) and lower seroprevalences were reported in the U.S.A. (2%) and Spain (1.1%) [1, 11, 12, 22, 23]. Low level bacteremia but high seroprevalence to *B. vinsonii* subsp. *berkhoffii* in dogs has been observed in tropical countries [7], while the highest positive rates for showing bacteremia and seroprevalence have been observed in coyotes in California [10]. Celebi *et al.* did not succeed in isolating *Bartonella* from the blood of shelter dogs from İstanbul [9]. This finding is in agreement with

our own data, showing a low seroprevalence (3%) in stray dogs from İstanbul.

A previous study has implicated ticks as potential vectors of these bacteria. Although *Rhipicephalus sanguineus* is believed to be a vector for transmission of *B. vinsonii* subsp. *berkhoffii*, fleas cannot be ruled out as potential vectors of the agent [18].

In the present study, a higher seroprevalence was observed in rural dogs than in urban dogs, as shown previously in a serosurvey in Morocco [12]. This result suggests that rural dogs may be more commonly exposed to vectors or a wildlife reservoir of *B. vinsonii* subsp. *berkhoffii* than urban dogs. While half of the seropositive rural dogs (20/40) had higher antibody titers (1:256 and higher) against *B. vinsonii* subsp. *berkhoffii*, only a stray dog among the urban dogs could achieve this titer (Table 1).

The present study shows that *B. vinsonii* subsp. *berkhoffii* is present in dogs throughout Turkey. Due to the zoonotic potency of the pathogen and the close relations between dogs and humans, further studies are needed to understand the mechanism of transmission and human exposure in Turkey.

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