Journal of Scientific Research & Reports
3(6): 764-771, 2014; Article no. JSRR.2014.001

SCIENCEDOMAIN *international*
www.sciencedomain.org

Seroprevalence of *Bartonella vinsonii* subsp. *berkhoffii* and *Bartonella henselae* in Urban Stray Dogs in Eskisehir, Turkey

Nihal Dogan[1*], Bekir Celebi[2], Mesut Mungan[2], Cem Kose[3] and Aysegül Taylan Ozkan[2,4]

[1]Eskisehir Osmangazi Üniversty, Medical Faculty, Microbiology Department, Eskisehir, Turkey.
[2]National Reference Laboratory for Parasitic Diseases, Department of Public Health Agency, Turkey.
[3]The Tepebasi Municipiality and Animal Shelter, Eskisehir, Turkey.
[4]Hitit Üniversty, Medical Faculty, Microbiology Department, Corum, Turkey.

Authors' contributions

This work was carried out in collaboration between all authors. Author ND designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BC and MM managed the analyses of the study. Author CK collected samples. Author ATO managed the literature searches and analyses. All authors read and approved the final manuscript.

Original Research Article

Received 6th November 2014
Accepted 18th January 2014
Published 29th January 2014

ABSTRACT

Aims: *Bartonella* species are significant emerging infectious bacterial pathogens in dogs and humans. They are vector-transmitted, blood-borne, intracellular, gram-negative bacteria that can induce prolonged infection in the host. In this study, the seroprevalence of *Bartonella vinsonii* subsp. *berkhoffi (Bvb)* and *Bartonella henselae (Bh)* infections in healthy looking urban stray dogs in Eskisehir, Turkey was determined.
Methodology: One hundred eighty six sera were collected randomly from clinically healthy looking shelter dogs during July 2011 - November 2012. Serological tests were performed using (*Bh*) and (*Bvb*) antigens using the Indirect Fluorescein Antibody test (IFAT).
Results: Twenty (10.8%) samples were positive for *Bvb* IgG and 39 (21.1%) for *Bh* IgG, showing that *Bvb* and *Bh* are significant zoonotic pathogens in dogs in this region. Due to

**Corresponding author: E-mail: nihaldogan42@gmail.com;*

the se zoonotic potency of these pathogens and the close relationship between dogs and humans, further studies are required to understand the mechanism of transmission.
Conclusion: *Bh* and *Bvb* infections are common in stray dogs (21.1%-10.8%) in Eskişehir/Turkey. As a result, veterinarians play an important role in advising for the public health of epidemiologic and zoonotic pathogens that indicate a significant risk of artropod born diseases. Eliminating or minimizing of flea and tick exposures is very important for public health.

Keywords: Bartonella vinsonii subsp. Berkhoffii; Bartonella henselae; Stray dogs; IFA; Zoonoses; Turkey.

1. INTRODUCTION

Bartonella spp. is gram-negative bacteria that are highly adapted to a mammalian reservoir host and within which the bacteria usually cause a long-lasting intra erythrocytic infection. Throughout the world *Bartonella* species are considered emerging pathogens in veterinary and human medicine [1-4]. These facts are of particular importance to veterinarians and physicians as an increasing number of animal reservoir hosts have been identified for various *Bartonella* species, e.g *Bartonella henselae* (*Bh)* has co-evolved with cats and *Bartonella vinsonii* subsp. *berkhoffii* (*Bvb*) has co-evolved with canines, such as dogs. Bvb has been characterized as an important pathogen in dogs and is an emerging pathogen in people with zoonotic potential. Since 1993, many *Bartonella* species have been associated with endocarditis in humans and dogs [1,4-8].

However these organisms were also be found in healthy dogs. It has been isolated from a human endocarditis patient and from immune competent veterinary professionals [5,7,9]. The extent to which dogs can serve as a reservoir host for *Bvb* or other *Bartonella* spp., such as *B. henselae, B. clarridgeae* or *B. elizabethae* is poorly characterized. Although dogs have been implicated by the direct transmission of *B.* henselae, humans can only be infected by a scratch or bite of cats [3]. Cat scratch disease caused by *Bh*, has a worldwide distribution and are mainly from reservoirs domestic cats, which also act as reservoirs. As for many vector-borne disease agents, *Bartonella* species are transmitted by a vector to new susceptible hosts. Transmission of the diseases are usually by ticks, flies, lice and also by bites and scratches of host reservoirs and perhaps even by infected needles [1,4]. However, it is not a vailable disease in humans in most countries. Accordingly, sufficient data to determine the exact incidence or prevalence of *Bartonella* infection are not obtainable. Confirmation of cat scratch disease is mainly established on the basis of results of serologic testing. High IFA antibody titers of *Bh* are associated with endocarditis in humans. The gold standard for diagnosis is isolation of the bacterium, but the prevalence of infections in healthy animals in endemic areas is high, therefore a positive culture is confirmatory for the diagnosis IFA and ELISA are also useful serological methods for confirmation. According to transmission studies, vector control is the best method for the prevention of disease [6,7,9].

Canine vector-borne diseases are worldwide emerging problems due to their frequency morbidity and zoonotic relevance with dogs potentially serving as sentinels for human infections [1]. Environmental changes, especially global warming have an impact on the geographical distribution, abundance and vectorial capacity of arthropods. In addition to human and animal population dynamics, including the increased mobility of dogs, climatic changes may affect the occurrence and spread of zoonotic diseases. For this reason, updated information on the epidemiological zoonotic and vector-born diseases in all

countries is required to determine the regional risk map and identify new areas of endemicity and forecast [3,5,6,10,11].

The aim of this study was to assess the seroprevalence of *B. vinsonii* subsp*. berkhoffii* and *B. henselae*, which are zoonotic agents, in stray dogs in Eskisehir, Turkey.

2. MATERIALS AND METHODS

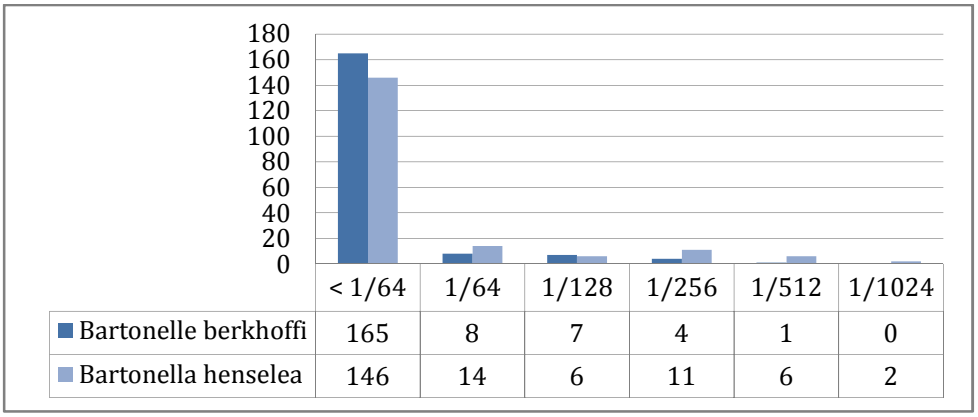
Blood samples were collected from 185 clinically healthy looking stray dogs in the province of Eskisehir between July 2011and November 2012. They were captured by municipality workers for sterilization and rabies vaccination purposes from residential areas througt the city. These sheltered dogs were kept under the control of the local municipality and controlled for tick infestation (usually infested with *Rhipicephalus* spp.) during the summer. Two ml of blood was collected from each dog. Sera were separated and stored at – 20ºC until use. The presence of IgG antibodies to *Bvb* and *Bh* were evaluated by IFA, while the antigen was prepared as described by Chang et al. with some modifications [12,13]. *Bvb* strain (ATCC 51672) was co cultivated for 4–5 days with Vero-cells to prevent auto agglutination 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 ml PBS. The suspension was frozen at –80ºC for 10 min and thawed in an ultrasonic bath for 1 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 every 15 well, 4 mm teflon coated slides (Immuno-Cell Int.City, Country), 2 µl of supernatant was added and the slides were then air-dried, fixed in acetone and stored at –20ºC until they were used*. Bh* (ATCC 49882) IFA antigen was prepared by the same method. 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 (Sigma – Aldrich, Steinheim, Germany). 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 considered 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 statistical analyses of associations between the biometric parameters and the disease were performed by Chi-squared analysis using the SPSS 17.0, while we values of $p<0.05$ was considered as statistically significant.

This research was carried out with Osmangazi University, Medical Faculty, Parasitology Department in Eskisehir, Refik Saydam National Public Health Agency, Communicable Diseases Research Department and Municipiality Animal shelter of Eskişehir.

3. RESULTS

The seroprevalence of *Bvb* and *Bh* IgG antibodies was 10.8% (20/185) and 21.1% (39/185), respectively, with IFA test. The titers among the seropositive dogs ranged from 1:64 to 1:1024 (Fig. 1). The age distribution of the dogs was in the range of 6 and 180 months (mean 30 months), while 51.4% of the dogs were females and 48.6% were males. The seroprevalence increased at 2 years and older dogs, but there was no significant difference (Table 1). The seroprevalence of *Bvb* and *Bh* in female dogs were 12.6% respectively and 22.1%, in male dogs as 8.9% and 20.0%, in male dogs, however the difference were not statistically significant $P<0.05$ (Table 2).



**DOGS WİTH RECİPROCAL TİTERS >64 ARE CONSİDERED IFA*

Fig. 1. Distribution of IFAT IgG titers against *Bvb* and *Bh* in stray dogs in Eskisehir (n=185)

Table 1. The distribution of age groups for the seroprevalance of *Bvb* and *Bh* in stray dogs

Features		*B. vinsonii subsp. berkhoffii*		*B. henselae*	
		Positive		Positive	
Age	**n**	**n**	**%**	**n**	**%**
<1	53	4	7.5	9	17
1-2	50	3	6	10	20
2-4	44	7	15.9	12	27.3
4>	38	6	15.8	8	21.1
TOTAL	185	20	10.8	39	21.1

** p<0.05; **Dogs with reciprocal titers >64 by IFAT are considered as positive*

Table 2. The distribution of gender for the seroprevalance of *Bvb* and *Bh* in stray dogs

Features		*B. vinsonii subsp. berkhoffii*		*B. henselae*	
		Positive		Positive	
Gender	**n**	**n**	**%**	**n**	**%**
Female	95	12	12.6	21	22.1
Male	90	8	8.9	18	20
TOTAL	185	20	10.8	39	21.1

** p<0.05; **Dogs with reciprocal titers >64 by IFAT are considered as positive*

In addition 14, out of 39 seropositive dogs were positive for both *Bvb* and *Bh* when 70% (14/20) of *Bvb* seropositive dogs were also *Bh* positive and 36% (14/39) of *Bh* seropositive dogs were *Bvb* positive. In 11 of 14 seropositive dogs, *Bh* IgG antibody titers were higher than *Bvb* IgG titers; and in 3 of 14 seropositive dogs, *Bvb* IgG antibody titers were higher than *Bh* IgG titers.

4. DISCUSSION

Domestic pets are susceptible to infection with various species of *Bartonella* and can play a role in human *Bartonella* infections. According to previous studies, *Bh* bacteremia has been determined 41% of healthy cats [9]. Dogs may carry *Bvb, Bh,* and other *Bartonella* species and accordingly *Bartonella* infections are more likely to cause clinical symptoms in dogs than in cats. Clinical diagnosis of *Bartonella* infection in dogs is a considerable problem, because the clinical spectrum has not been understood. The low level of seroprevalence in dog populations worldwide suggests that, dogs are probably not a natural reservoir for *Bvb*. It is unknown so far, whether dogs can transmit infection to humans or not. In addition to dogs and cats, numerous domestic and wild animals, including bovine, canine, human and rodent species can serve as reservoir hosts for various *Bartonella* species [3,6,11,14-17].

The important role of dogs as a reservoir for *Bartonella* spp. is less clear than of cats because domestic dogs are more likely to be accidental hosts, at least in nontropical regions. Nevertheless, dogs are excellent sentinels for human infections because similar disease symptom also develops in dogs [6,10,11].

Bvb is considered the most frequent *Bartonella* species that causes disease in dogs. However, this conclusion may not be accurate, as sera from dogs has not been screened systematically against a large panel of other *Bartonella* species and few PCR examinations have been performed [15,16].

The first isolation of *Bvb* in dogs in Turkey was achieved in the province of Ankara by Celebi et al. [12] and a high prevalence of bacteremia in stray dogs (5%) and sheltered dogs (12.4%) was found. The authors investigated the seroprevalence of Bvb, in 855 urban stray dogs as well as in shepherd and farm guard dogs from rural areas of 10 different cities of Turkey. A seropositivity of 6.5% in dogs in general total and 3% of stray dogs and 12% of rural dogs, was found [12].

In the present study, using the IFAT test a Bvb IgG seroprevalence of 10.8% in healthy looking stray dogs in Eskisehir was observed. Similar studies were conducted in other countries. In Sudan the seroprevalence in dogs was 65%, in Morocco and Thailand 38%, in Israel 10% in the U.S.A. 2% and in Spain 1.1%, the highest positive rates of bacteremia and seroprevalence have been observed in coyotes in California. In tropical countries, low level of bacteremia but high seroprevalence to *Bvb* in dogs has been also observed [17-22].

The rate of antibodies to *Bvb* antigens is usually <4% in sick or healthy dog populations in endemic areas. Therefore, detection of Bvb antibodies in a sick dog provides a strong clinical evidence for prior exposure and potentially active infection. A reciprocal titer of 1:64 or greater is considered indicative of prior exposure to or active infection with *Bvb* or *Bh* in dogs. Due to the lack of sensitive conventional microbiological tests, nowadays bartonellosis is being diagnosed by PCR tests or specific DNA sequences [6,8,13,16,20,21].

There are limited data available on the prevalence of *Bh* in dogs. Demers et al reported that 2 out of 31 (6.5%) dogs in Hawaii were seropositive for *Bh* (titers of 1:64 and 1:128, respectively); however, bacteria were not isolated from blood samples taken, from these dogs, while a PCR assay was not performed [18]. In the UK, it was reported that 3 of 100 (3%) dogs were seropositive for *Bh* [22]. Tsukahara et al. performed IFA tests in 52 dogs and reported that 4 (7.7%) were seropositive against *Bh*. in Japan [23]. In the present study 21.1% of examined healthy looking dogs were positive for IgG antibodies against *Bh*

showing that *Bh* is an important pathogen with zoonic potential in Turkey. In the study of Guzel et al*,* blood samples were collected in an amount of 298 cats from six different provinces of Turkey and a *Bh* seroprevalence of 27.9% (83/298) was found [24].

In contrast to the large body of literature regarding *Bh* in humans and cats, there is little information about *Bh* as an infectious agent in dogs. Gallego et al. performed a cross-sectional serosurvey using *Bh* antigen in order to compare the seroprevalence between sick and healthy dogs from the south-eastern USA and found that. 10.1% of healthy dogs and in 27.2% of sick dogs were positive, while Bvb antibodies were detected in 1% and 4.7% of dogs, respectively [16]. In our study, we detected a higher Bh seroprevalence (21.1%) than the Bvb seroprevalence (10.8%) in healthy looking dogs. Furthermore, it was detected that 70% (14/20) of *Bvb* seropositive dogs were *Bh* seropositive and 36% (14/39) of *Bh* seropositive dogs were *Bvb* seropositive. It was suggested that these two agents can show cross-reactivity in serological tests.

Recent studies have shown that many dogs infected with vector-borne agents remain asymptomatic for months or even years, but diagnosis of subclinical infection is important, as those animals might still serve as reservoirs of pathogens to other hosts including humans. Infected healthy cats play important role for *Bh* in endemic areas. Some investigators recommended that an annual serological screening should be undertaken to promote early detection and treatment, in the areas of endemicity [10,12,25].

Spread of these diseases was associated with the spread of fleas and it was reported that climate changes affects the prevalence of these vectors as the fleas proliferations are affected by climate changes [11]. Moderate and humid areas are show especially dense flea infestations of cats. Therefore, even in the different climatic areas of the same country differences in vector prevalence can be found [7,11,19,21]. Jameson *et al*. suggested that cats in a warm and humid environment were associated with higher seroprevalence of *Bh* than those in a cold and dry environment [26]. Furthermore, it was reported that the cat flea is an important vector for the transmission of *Bh* between cats [5,26]. In our country, seropositivity of *Bartonella sp* in dogs and cats was higher in the Mediterranean climate regions than central Anatolia [12,24]. Eskisehir is located in central Anatolia region, which is an inland area has a cold and dry climate, however still a - but high *Bartonella* seropositivity was found in this area.

5. CONCLUSIONS

Our results showed that, *Bh* and *Bvb* infections are common in stray dogs (21.1%-10.8%) in Eskisehir, Turkey. Due to the zoonotic potency of the pathogen and the close relationship between dogs and humans, further studies are necessary to understand the mechanism of transmission and the relevance to humans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Breitschwerdt EB, Kordick DL. Bartonellosis. J Am Vet Med Assoc. 1995;206:1928-31.

2. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic Bartonella infections. Vet Res. 2005;36:383-410.
3. Breitschwerdt EB, Kordick DL. Bartonella infection in animals: carriership, reservoir potential, pathogenicity and zoonotic potential for human infection. Clin Microbiol Rev. 2000;13:428-38.
4. Breitschwerdt EB, Maggi RG, Lantos PM, Woods CW, Hegarty BC, Bradley JM. *Bartonella vinsonii* subsp*. berkhoffii* and *Bartonella henselae* in a father and daughter with neurological diseases. Parasites and Vectors. 2000;3:29.
5. Chomel BB, Boulouis HJ, Breitschwerdt EB. Cat scratch disease and other zoonotic *Bartonella* infections**,** J Am Vet Med Today. 2004;224:1270-79.
6. Chomel BB, Boulouis HJ, Maruyama S, Breitschwerdt EB. *Bartonella* spp. in pets and effect on human health. Emerg Infect Dis. 2006;12:389-94.
7. Guptill L. Bartonellosis. Vet. Microbiol. 2010; 140: 347–359.
8. La Scola B, Raoult D. Culture of Bartonella quintana and *Bartonella henselae* from human samples: a 5-year experience (1993 to 1998). J Clin Microbiol. 1999;37:1899-1905.
9. Gurfield AN, Boulouis HJ, Chomel BB, Kasten RW, Heller R, Bouillin C, *et al.* Gandoin C, Thibault D, Chang CC, Barrat F, Piemont Y. Epidemiology of *Bartonella* infection in domestic cats in France. Vet Microbiol. 2001;80:85-98.
10. Day MJ. One health. The importance of companion animal vector-borne diseases. Parasit Vectors. 2011;4:49.
11. Gray JS, Dautel H, Estrada-Peña A, Kahl O, Lindgren E. Effects of climate change on ticks and tick-borne diseases in Europe. Interdiscip Perspect Infect Dis. 2009;593232.
12. Celebi B, Carhan A, Kilic S, and Babur C. Detectiontion and genetic diversity of *Bartonella vinsonii* subsp. berkhoffii strains isolated from dogs in Ankara, Turkey. J Vet Med Sci. 2010;72:969–973.
13. Chang CC, Kasten RW, Chomel BB, Simpson DC, Hew CM, Kordick DL, *et al.,* Piemont Y, Breitschwerdt EB. Coyotes (*Canis latrans*) as the reservoir for a human pathogenic *Bartonella* sp.: Molecular epidemiology of *Bartonella vinsonii subsp. berkhoffii* infection in coyotes from central coastal California. J Clin Microbiol.2001;38:4193-4200.
14. Available: www.cdc.gov/bartonella/.../index.html In our study, there was no evidence of a clinically finding positive dogs with *Bartonella* spp.; 2012.
15. Smith BE, Tompkins MB, Breitschwerdt EB: Antinuclear antibodies can be detected in dog sera reactive to *Bartonella vinsonii subsp. berkhoffii, Ehrlichia canis, or Leishmania infantum* antigens. J Vet Intern Med. 2004;18:47-51.
16. Solano-Gallego L, Bradley J, Hegarty B, Sigmon B, Breitschwerdt E. *Bartonella henselae* IgG antibodies are prevalent in dogs from southeastern USA. 2004;35:585-95.
17. Baneth G, Breitschwerdt EB, Hegarty BC, Pappalardo B, Ryan JA. Survey of tick-borne bacteria and protozoa in naturally exposed dogs from Israel. Vet Parasitol. 1998;31:133-142.
18. Demers DM, Bass JW, Vincent JM, Person DA, Noyes DK, Stage CM, *et al.,* Samlaska CP, Lockwood NH, Regnery RL, Anderson BE. Cat-scratch disease in Hawaii: etiology and seroepidemiology. J Pediatr. 1995;127:23-26.
19. Diniz PP, Maggi RG, Schwartz DS, Cadenas MB, Bradley JM, Hegarty BC, *et al.,* Breitschwerdt EB. Canine bartonellosis: Serological and molecular prevalence in Brazil and evidence of co-infection with *Bartonella henselae* and *Bartonella vinsonii subsp. berkhoffii*. Vet Res. 2007;38:697-710.
20. Henn JB, Vanhorn B A, Kasten RW, Kachani M, Chomel BB. Antibodies to *Bartonella vinsonii subsp. berkhoffii* in Moroccan dogs. Am J Trop Med Hyg. 2006;74:222-223.

21. Suksawat J, Xuejie Y, Hancock SI, Hegarty BC, Nilkumhang P, Breitschwerdt EB. Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand. J Vet Intern Med. 2001;15:453-462.
22. Barnes A, Bell SC, Isherwood DR, BennettM, Carter SD. Evidence of *Bartonella henselae* infection in cats and dogs in the United Kingdom. Vet Rec. 2000;147:673-677.
23. Tsukahara M. Cat Strach disease in Japan. J Infect Chemother. 2002;8:321-5.
24. Guzel M, Celebi B, Yalcin E, Koenhemsi L, Mamak N, Pasa S, Aslan O. A serological investigation of *Bartonella henselae* infection in cats in Turkey. J Vet Med Sci. 2011;73:1513-6.
25. Boulouis HJ, Chang CC, Henn JB, Kasten RW, Chomel BB. Factors associated with the rapid emergence of zoonotic *Bartonella* infections. Vet Res. 2005;36:383–410.
26. Jameson P, Greene C, Regnery R, Dryden M, Marks A, Brown J, Cooper J, Glaus B. Greene R. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. J Infect Dis, 1995;172:1145–1149.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=417&id=22&aid=3470