

Seropositivity of *Bartonella henselae* in Risky Human Population, Cats and Dogs

Kedi, Köpek ve Risk Grubu İnsanlarda *Bartonella Henselae* Seropozitifliği

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Abstract

Objective: *Bartonella* species cause several diseases in humans such as cat scratch disease, bacillary angiomatosis, peliosis hepatis, endocarditis, Carrion disease and trench fever. There have been cat scratch disease and bacillary angiomatosis cases reports in Turkey. The aim of this study is to determine the seropositivity against *Bartonella henselae* in cat/dog owners who are in the risk group, cats and dogs in Western Aegean region, Turkey.

Materials and Methods: In this study, *B. henselae* immunoglobulin (Ig) G positivity was investigated in a total of 281 samples including a total of 131 people, 34 of whom are pet cat/dog owners and 97 of whom are stray cat/dog owners; as well as a total of 105 cats, of which 57 pet cats, 48 shelter cats, and 45 pet dogs. Sera tested for the presence of antibodies against *B. henselae* IgG using immunofluorescence assay with two commercial kits.

Results: *B. henselae* seropositivity of pet owners was significantly higher than the stray cat/dog owners (26.5% vs 6.8%). *B. henselae* IgG was found positive in 36.2% of total cats, 22.8% of pet cats, 52.1% of shelter cats. *B. henselae* seropositivity was found statistically higher in shelter cats than pet cats. No positivity was detected in the samples taken from the dogs.

Conclusion: It is concluded that being pet owner at home poses a risk for *B. henselae*. For the differential diagnosis, especially in patients in close contact with cats, *B. henselae* infection should be considered.

Keywords

Bartonella henselae, risky human, cat, dog, seropositivity

Anahtar Kelimeler

Bartonella henselae, risk grubu insan, kedi, köpek, seropozitiflik

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Öz

Amaç: *Bartonella* türleri insanlarda kedi tırmağı hastalığı, basiller anjiomatozis, basiller peliozis, Carrion hastalığı, enfektif endokardit ve siper ateşi gibi pek çok hastalığa yol açmaktadır. Türkiye'de *Bartonella*'lara bağlı kedi tırmağı hastalığı ve basiller anjiomatozis olgu sunuları bulunmaktadır. Bu çalışmada, Türkiye'nin batısında, kedi/köpek sahibi riskli insan, kedi ve köpeklerdeki *Bartonella henselae* seropozitivitesinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: Bu çalışmaya, 34'ü ev, 97'si sokak olmak üzere toplam 131 kedi/köpek sahibi ve 57'si ev kedisi, 48'i barınak kedisi olmak üzere toplam 105 kedi ile 45 ev köpeğinin serum örnekleri alınmıştır. Serum örneklerinde *B. henselae* IgG antikorları iki ticari kit kullanılarak immunfluoresan antikor yöntemi ile belirlenmiştir.

Bulgular: *B. henselae* seropozitifliği, ev kedi/köpek sahiplerinde sokak kedi/köpek sahiplerine göre (sırasıyla %26,5 ve %6,8). Seropozitiflik, istatistiksel olarak anlamlı

şekilde yüksek bulunmuştur. Kedilerde *B. henselae* seropozitifliği ortalama olarak %36,2 saptanmış olup, seropozitiflik barınak kedilerinde (%52,1) ev kedilerine (%22,8) göre istatistiksel olarak anlamlı oranda yüksek olarak belirlenmiştir. Köpeklerden alınan örneklerde herhangi bir seropozitiflik bulunmamıştır.

Sonuç: *B. henselae* için evde evcil hayvan sahibi olma risk oluşturmakta olup, özellikle kedilerle yakın temasta olan hastalarda, *B. henselae* enfeksiyonu ayırıcı tanıda düşünülmelidir.

Introduction

Bartonella genus received its name in 1909 from Alberto L. Barton who was working with and who also identified *Bartonella bacilliformis*, the cause of Carrion disease. *B. bacilliformis* has been accepted as the only member of this genus until a recent classification. *Bartonella* genus, which had previously been in Rickettsiales order, *Rickettsiaceae* family, was included in *Protobacteria* class, *Alphaproteobacteria* sub-group, *Bartonellaceae* family in 1993 (1). *Bartonella* species have adapted to many vertebrate hosts; humans, carnivores, rodents, ruminants, marine mammals, primates and bats. This group of bacteria has a high genetic diversity and adapt to changes in the ecological conditions. In recent years, new isolated species have been continually added to *Bartonella* genus (2). It is reported that seventeen of these species and 3 subspecies are associated with human disease; ([2] <http://www.bacterio.net/bartonella.htm>) (3-8).

All *Bartonella* species have specific mammalian hosts, in which they cause a long-lasting infection known as intraerythrocytic bacteraemia. Domestic cats are the principle reservoir for *B. henselae*, *B. clarridgeiae* and *B. koehlerae* (5,9,10). Among them, *B. henselae* is the most important zoonotic species to cause human disease.

B. henselae is continuously expanding and includes cat scratch disease (CSD), bacillary angiomatosis and peliosis hepatis, endocarditis, myocarditis, prolonged bacteremia and fever, ocular manifestations, encephalopathy, aseptic meningitis, acute hemiplegia, dementia, acute psychiatric symptoms, hepatosplenic abscesses, asymptomatic bacteremia, osteomyelitis, erythema nodosum, other skin lesions in human (11). Domestic cats represent the natural reservoir for the bacteria (12). Infected cats develop relapsing bacteremia, which may persist for up to two years (13,14). Cat flea (*Ctenocephalides felis*) transmits the bacteria from cats to new hosts. Although some of these animals may be bacteremic over a period of

more than one year, cats have relatively asymptomatic infection (13). Transmission from cats to human mainly occurs by cat scratch or bite or possibly by flea bite.

According to the studies in humans, *B. henselae* immunoglobulin (Ig) G positivity is 8.7-19.8% in healthy children and adolescents, 10.3-28.9% in risky group humans, 11-16% in human immunodeficiency virus infected patient and 3.3-6% in blood donors (15-24). In Turkey, related to *Bartonella*, there are case reports of CSD and bacillar angiomatosis, and seroprevalence studies conducted in blood donors, cats and dogs samples (23,25-30). The objective of this study is to determine the prevalence of serum antibodies against *B. henselae* which has gained importance in recent years, in risky human, cats and dogs in Western Aegean region, Turkey.

Materials and Methods

This research was conducted by a team of researchers of Aydın Adnan Menderes University Veterinary and Medicine Faculties, Aydın, Turkey in blood samples collected from adults who are cat/dog owners, pet cats/dogs and shelter cats in Aydın and İzmir province. The permissions of both human and animal ethics committees were taken.

In this study, *B. henselae* IgG positivity was investigated in a total of 281 samples including a total of 131 people, 34 of whom are pet cat/dog owners and 97 of whom are stray cat/dog owners; as well as a total of 105 cats, of which 57 pet cats, 48 shelter cats, and 45 pet dogs. *Bartonella henselae* IgG antibodies were investigated in the all sera with indirect immunofluorescence antibody (IFA) method by using two commercial kits which were double compartmented *B. henselae* & *quintana* IFA IgG (Vircell, Granada, Spain) kits containing *B. henselae* cepa Houston-1 (ATCC 49882) and *B. quintana* (CIP 107 027 N) grown on Vero cells; and compartmented *Bartonella* IFA IgG (Focus, California, U.S.A) kits containing *B. henselae* and *B. quintana* bacteria

produced in yolk sac cells. Antibodies were investigated in cat samples by using fluorescein labeled goat anti-feline IgG as conjugate, and in dog samples by using fluorescein labeled rabbit anti-canine IgG (Santa Cruz Biotechnology, Texas, USA).

All sera samples were tested with *B. henselae* & *quintana* IFA IgG (Vircell) kits in 1/32 and 1/64 dilutions. The samples which were positive in 1/64 titers were consecutively tested with both kits in 1:64, 1:128, 1:256, 1:512 dilutions, and positive titrations were determined. Slides were viewed at a final magnification of 400× on fluorescent microscope by two different assigned researchers. Fluorescent intensity was graded as +1-+4, and the samples with fluorescence \geq +2 were considered as positive. Positive and negative controls were used for each study.

Results

B. henselae IgG positivity was on average 11.5% for risk group humans (cat/dog owners) (Table 1). *B. henselae* seroprevalence of pet owners was significantly higher than the stray cat/dog owners (26.5% vs 6.8%). *B. henselae* seropositivity was found in 36.2% of total cats, 22.8% of pet cats and 52.1% of shelter cats (Table 2). *B. henselae* seropositivity was found statistically higher in shelter cats than pet cats. The positivity in cats in 1/64 dilution was 20.0%, in

Group	Number	<i>Bartonella henselae</i> immunoglobulin G positivity	
		Number	%
Pet cat/dog owners	34	9	26.5
Stray cat/dog owners	97	6	6.2
Total	131	15	11.5

Group	Number	<i>Bartonella henselae</i> immunoglobulin G positivity	
		Number	%
Pet cats	57	13	22.8
Shelter cats	48	25	52.1
Total	105	38	36.2

Table 3. *B. henselae* immunoglobulin G positivity in cats according to the dilutions

Titer	<i>Bartonella henselae</i> immunoglobulin G positivity	
	Number	%
1/64	21	20.0
1/128	14	13.3
1/256	3	2.9
Total	38	36.2

1/128 dilution was 13.3%, and in 1/256 dilution was 2.8% (Table 3). All dog samples were found negative for *B. henselae* IgG.

The seropositivity of *B. quintana* IgG in some samples were found lower than *B. henselae* IgG titers and this was considered as cross reactivity (31).

Discussion

In this study, *B. henselae* seropositivity was determined in risk group human and both pet and stray cats in Western Aegean region of Turkey. *B. henselae* IgG positivity was 11.5% for risk group human (cat/dog owners), 26.5% for pet cat/dog owners and 6.2% for stray cat owners (Table 1). It was found that owning pet cats increases seropositivity significantly. Having a pet cat poses a higher risk to *B. henselae* infections than having a stray cat. In Turkey, related to *Bartonella*, there are case reports of CSD and bacillary angiomatosis (25-28). In a study conducted in Aydin province, Turkey, *Bartonella* seroprevalence in blood donors was found 3,3% (24), and another study was conducted in Denizli, a neighbour city of Aydin, with blood donors *B. henselae* seroprevalence was found as 6.0% (23). The presence of similar seroprevalence rates on the two adjacent provinces suggests that *B. henselae* infections are undergoing in this area. *Bartonella* spp seropositivity has been reported to be between 1.2% and 19.6% in healthy individuals and between 2.6% and 65% at different risk groups in various countries (32-35).

B. henselae seropositivity was found positive in 36.2% of total cats, 22.8% of pet cats and 52.1% of shelter cats. The seropositivity was lower in stray cat owners compared to pet cat owners. This result suggests that the seropositivity in humans may be associated with close contact to cats. Domesticated

cats are main reservoirs for *B. henselae* (9,36,37). Seroprevalence in cats varies according to geographical regions and climatic characteristics (18,38). *B. henselae* seroprevalence of domestic cats has been reported between 5 and 81% (36,38,39). In a similar study conducted in Ankara-Turkey, seroprevalence in cats was found as 18.8% (29). Seropositivity of *B. henselae* in cats is observed to be high in temperate and humid regions and low in cold and arid regions. This is due to the increased proliferation of cat fleas in temperate climates, so that in humid, temperate climates, cat fleas produce intensive infestation in the cats, resulting in the rise of *B. henselae* prevalence (36,38-40). Seropositivity against *Bartonella* species in humans is an expected outcome due to warm and mild climate of Aydın and İzmir provinces, where this study is conducted.

In dogs, *Bartonella* species may lead to clinical disorders such as endocarditis, bacillary angiomatosis, granulomatous hepatitis, lymphadenitis and granulomatous rhinitis (41-43). *B. vinsonii* subsp. *berkhoffii* is the first *Bartonella* type isolated from dogs, the isolation of *B. henselae*, *B. clarridgeiae* and other species has showed a significant increase (42). Although the role of dogs for transmission of *Bartonella* species is unclear, they are important due to the possibility that they may become reservoirs. In two studies conducted in the US, *B. henselae* seropositivity have been reported in 10.1% of healthy dogs and 27.2-32% of the ill dogs (44,45). In another study conducted in Spain, *B. henselae* seropositivity was found as 16.8%, whereas *B. vinsonii* subsp. *berkhoffii* was found as 1.1% (46). In our study, all pet dogs samples were negative in *B. henselae* IgG. This result may be related to the fact that the study was conducted in pet dogs, and in order to suggest that *B. henselae* is not present in dogs in our region, it is necessary to conduct a research on both stray and shelter dogs. There has been no study on *B. henselae* isolation in neither pet nor stray/shelter dogs in Turkey. However, in a study all species which has been isolated was *B. vinsonii* subsp. *berkhoffii* and *B. vinsonii* subsp. *berkhoffii* IgG seroprevalence has been reported as 3% in stray dogs and 12% in rural dogs (30).

Study Limitation

The limitations of this study include several topics since it was not possible to distinguish

individuals as stray cat/dog owners. Moreover, since the maintenance of contact, sampling, disease anamnesis and screening of stray cats and dogs were difficult, the use of these animal samples were limited in this research. The recent *Bartonella* infection could not be diagnosed because *Bartonella* IgM did not be investigated in the sera samples.

Conclusion

In humans, CSD is usually clinically suspected and diagnosed by the determination of antibodies against *B. henselae* or the bacterial DNA from the tissue kept. Since it is difficult to culture *Bartonella* species from human samples, serological diagnosis is the first step to confirm the preliminary diagnosis. Many tests have been developed for the serological diagnosis with varying in sensitivity and specificity (IFA, enzyme immunoassay, immunoblot). Indirect IFA is one of the most commonly used methods of serological tests. IgG titer is 1/512 and above in CSD. However, low antibody titers, such as 1/64 and 1/128 titers, can be found in both patients and healthy controls. As low antibody titers can be found at the beginning or the end of disease, they also can only be related to the exposure to the causative agent (18,47). Two different commercial kits were used in this study. First, all sera were run in 1/64 dilution with *B. henselae* & *quintana* IFA IgG (Vircell) kit. The sera which were subsequently positive were also studied with the *Bartonella* IFA IgG (Focus) kit. The difference between these two kits is that the wells on the slides of the screening kits contain *B. henselae*, cepa Houston-1 (ATCC49882) and *B. quintana* (N CIP 1070271) bacteria produced in Vero cells, while the other kit contains bacteria-infected Vero cells. As the production methods of the kits differ, there are also differences in terms of cost. Direct and dilution results were found to be compatible between the kits used in this study. Regnery and colleagues used Vero cells infected with *B. henselae* as antigens in CSD serology (48). Many researchers have used *B. henselae* and infected Vero cells as IFA antigens based on the work of Regnery and colleagues (20,21,49,50)

Bartonella species cause many different types of clinical findings in human and animals. Especially the role of cats spreading CSD to people by being reservoirs is well known. Isolation of *Bartonella* species, which are increasingly important zoonotics, in cats and dogs in a geographical region may be a sign

of humans in that having the disease, and therefore epidemiological studies in human and animals are required. This study is the first report of the *B. henselae* positivity seropositivity in cats, dogs and pet owners in Western Aegean region of Turkey. We found out that being pet owner at home poses a risk for *B. henselae*. Especially in patients in close contact with cats, *B. henselae* infection should be considered for the differential diagnosis.

Ethics

Ethics Committee Approval: HEK/2008/006, EK/2008/00224.

Informed Consent: Written informed consent was obtained from all participants.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Not made Concept: N.A., B.K., S.K., M.T., M.E., Design: N.A., M.E., S. K., B.K., M.T., Data Collection or Processing: S.K., U.P., S.T., B.K., M.E., A.M.A., Analysis or Interpretation: N.A., B.K., S.K., M.E., A.M.A., M.T., Literature Search: N.A., B.K., M.E., S.K., A.M.A., Writing: N.A., B.K., M.E., S.K.

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