**ARAŞTIRMA MAKALESİ /** *RESEARCH ARTICLE*

# Investigation of *Bartonella hanselae* Seroprevalence in the Northern Countryside of Denizli Province

*Denizli Kuzey Kırsalında Bartonella hanselae Seroprevalansının Araştırılması*

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## ABSTRACT

***Aim:*** *Bartonella species are zoonotic bacteria that are transmitted through gram negative coccobacillus/bacillus. The importance of the Bartonella species has increased with there cent epidemiological studies on human sand animals. The proportions differ according to the epidemiological studies conducted in different risk groups. The B.henselae ranges between 5.5% and 57.3% in seroprevalence studies conducted in differen tparts of the world. It is important to expand the seroprevalence studies of bartonellosis at Türkiye. The aim of this study was to determine the seroprevalence of in working adults at risk by occupational groups in northern rural area of Denizli.*

***Material and Method:*** *Antibodies against B.henselae ATCC 49882 (Houston-1) strain were detected using immunofluorescent antibody technique in serum samples collected from 477 healthy adult volunteers working in risky occupational groups in then norther countryside of Denizli. Serum samples were studied in laboratory of Medical Microbiology Department, Faculty of Medicine, Pamukkale University.*

***Results:*** *The prevalence of B.henselae seropositive was found to be 44.0%. Antibodiesof B.henselae were found in 26.8% volunteers at 1/64, in 13.8% at 1/128, in 2.7% at 1/256, in 0.6% at 1/512 dilutions. The analysis of the data revealed that statistical difference sexist for B.henselae according to tick, exposure to sandfly, live in wetlands, agriculture, and age groups (p<0.005).*

***Conclusion:*** *This study makes an important contribution in determining the seroprevalence of bartonellosis relevant to Türkiye. According to the screening of seroprevalence by risk occupational groups in this study, it is concluded that risk groups with different regional and geographical features should be preferred for bartonellosis seroprevalence examinations.*

**Keyword:** *Bartonella henselae; seroprevalence; zoonosis; risk occupational groups*

## ÖZET

***Amaç:*** *Bartonella türleri Gram negatif kokobasil/basil şeklinde, vektörlerle geçiş yapan, zoonotik bakterilerdir. Son yıllarda insanlarda ve hayvanlarda yapılan epidemiyolojik çalışmalar ile Bartonella türleri önem kazanmıştır. Risk gruplarında yapılan epidemiyolojik çalışmalarda oranlar değişkenlik göstermektedir. Dünyanın farklı bölgelerinde yapılan seroprevalans çalışmalarında B.henselae %5,5 ile %57,3, oranında saptanmıştır. B.henselae ülkemizde de görülmekte olan vektörel yayılımlı bir enfeksiyon etkenidir. Ülkemizin coğrafik ve iklimsel özellikleri gözönüne alındığında bartonellozun seroprevalans çalışmalarının yaygınlaştırılması önem arzetmektedir. Sunulan çalışmada Denizli'nin kuzey kırsalında risk oluşturan meslek gruplarında çalışan yetişkinlerde B.henselae seroprevalansının saptanması amaçlandı.*

***Materyal ve Metot:*** *Denizli'nin kuzey kırsalında risk oluşturan meslek gruplarında çalışan Sağlıklı 477 yetişkin gönüllüden toplanan serum örneklerinde immünfloresan antikor tekniği kullanılarak B.henselae ATCC 49882 (Houston-1) kökenine karşı oluşan antikorlar saptandı. Toplanan serum örnekleri Pamukkale Üniversitesi Tıp Fakültesi Mikrobiyoloji Anabilim Dalı laboratuvarında çalışıldı.*

***Sonuçlar:*** *B.henselae seropozitiflik oranı %44,0 olarak bulundu. Gönüllülerin %26,8'inde 1/64, %13,8'inde 1/128, %2,7'sinde 1/256, %0,6'sında 1/512 dilüsyonda B.henselae antikorları pozitif saptandı. B.henselae için kene teması, tatarcık maruziyeti, sulak alanda yaşama, tarım yapma ve yaş gruplarında istatistiksel farklılık saptandı (p<0,005).*

***Tartışma:*** *Türkiye'de seroprevalans çalışmaları sınırlı sayıdadır. Sunulan çalışma Türkiye'de bartonellozseroprevalansının saptanması açısından önemlidir. Risk oluşturan meslek gruplarında yaptığımız seroprevalans taraması ile bölgesel ve farklı coğrafik özellik gösteren risk gruplarında bartonellozseroprevalans çalışmalarının yapılması gerektiği sonucuna varıldı.*

**Anahtar Kelimeler:** *Bartonella henselae; seroprevalans; zoonoz; risk oluşturan meslek grupları*

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## Introduction

*Bartonella* are important vector transmitted inflammatory conditions in both animals and humans[1]. *Bartonella* are members of a small genus, pleomorphic, Gram-negative, weakly staining bacilli and coccobacilli, oxidase, and catalase negative microorganisms. There are more than 30 known types of these bacteria, which are members of the phylum *α-Protobacteria*[2,3]. Bartonellosis could often be overlooked while it progresses as a silent infection. Hence, seroprevalence methods have been favored when it comes to investigating the existence of regional illness. Seroprevalence values ranging from 5.5% to 60.5% have been detected in different risk groups in studies carried out in rural areas and different regions of the world[4–8]. In Türkiye, although there are case reports, a limited number of studies have reported seroprevalence in different risk groups[1,9,10].

## Material and Methods

This study presents the risk factors and seroprevalence of *Bartonella henselae* in a well-defined and confined geographical area.

### *Study Design and Area*

The study was carried out in Denizli province, Menderes Valley, North region. The region's main source of livelihood is agriculture (crops production and raising livestock). The area of study was divided into four main regions corresponding to the differences of altitude and settlement characteristics of the regions (Fig. 1). Population density is 25 person/km$^2$ in mountainous areas and valleys (I, II, III; 600–2000 m) and >200 person/km$^2$ in the central rural area (IV; <600 m). According to Köpplen-Geiger climate classification, the region is "CSA" (mild winter, very hot summers, and arid climate (Mediterranean climate) with temperatures ≥22°C)[11].

### *Study Groups*

A total of 477 adult volunteers from the northern rural area of Denizli province were included in the study. Detailed questionnaires were filled during the meetings with the adult volunteers. Demographic data of the adults included in the study were recorded. Collected serum samples were centrifuged the same day and were preserved in sterile eppendorf tubes at -20$^0$C until the experimental study.

### *Laboratory testing*

The lyophilized *B.henselae* ATCC 49882 (Houston-1) was plated in 5% defibrinated brain heart infusion agar media with horse blood by suspending it with sterile saline and was bred in a humid incubator at 37°C which has 10% $CO_2$. Control breeding was carried out using auramine–rhodamine fluorescence and Gram staining. Reproducing bacteria were co-cultured with Vero cell lines growing in 25 cm$^2$ flasks. Briefly, 2 ml trypsin was added to Vero cell lines which were attached to the flask surface through reproducing. After 3 minutes, 7 ml cell growth solution (100 ml Eagle's Medium, 10 ml Fetal Calf Serum, 2 ml L-glutamine, 1 ml HEPES solution and 0.4 ml amphotericin-B) was added to the medium. The suspension liquid with the cells were centrifuged for 5 minutes at 25°C 1000 g. The supernatant fluid floating after the centrifugation was added to the 2 ml growth solution pre-prepared on outer cell pellet. The cells added to flasks continued to incubate in incubators providing a humid atmosphere at 37°C which has 5–10% $CO_2$ until a monolayer cell line was formed with an inverted microscope. 100 µl of the *B.henselae* ATCC 49882 (Houston-1) cell species which has formed monolayer was added and taken to co-cultivation. Those cells were incubated in an incubator at 37°C, which was providing humidity and had 5–10% $CO_2$. After the incubation, co-culture cells were deactivated by making them sit in a water bath at 56°C for 30 minutes. After transferring 10 µl to commercially bought teflon coated microscope slides, they were dried using room temperature laminar air flow. Dried slides were immobilized in acetone at -20°C for 15 minutes. After the immobilization of slides using acetone, the slides were preserved in a container at -70°C until the time of study. The test for antibodies in the collected serum samples was carried out using the immunofluorescence antibody detection method described by Regnery et al.[12] During the study, the serum of a patient who was diagnosed with bacillary angiomatosis both pathologically and clinically was used as positive control. Specific immunofluorescence scoring of the serum sample examinations from 0 to +3 was done considering the pre-defined fluorescence reflection intensity subjectively[13]. For *B.henselae*, a positivity of +2 in ≥1/64 dilution was accepted as seropositive[3,13,14].

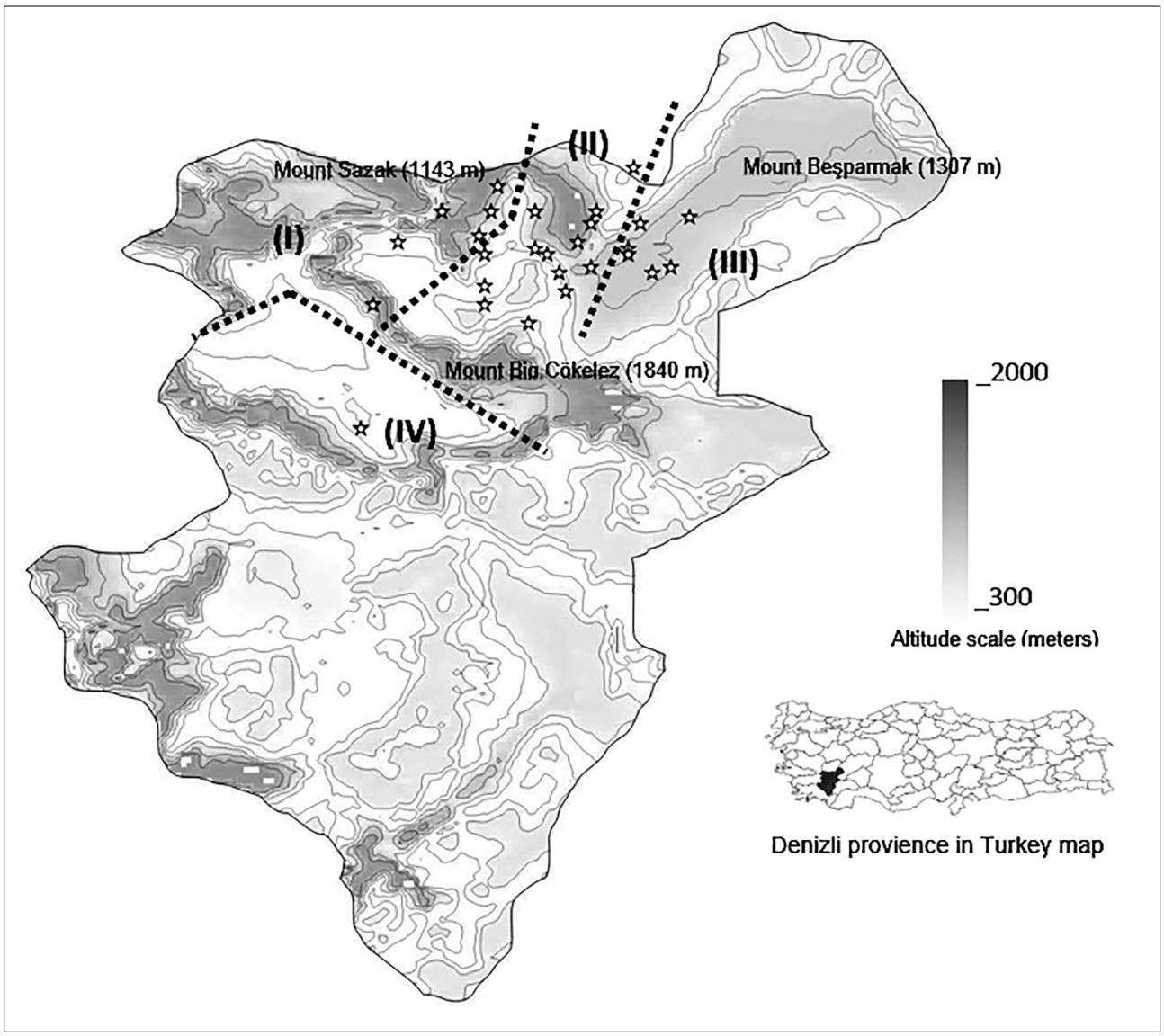


***Figure 1.*** *Geographical study parts of Denizli province, sampling points as (I) Bekilli villages area; (II) Çal villages area; (III) Baklan villages area; (IV) Denizli city center.*

### *Data Analysis*

IBM Statistical Package for Social Sciences (SPSS) program version 18.0 (IBM Corp, Armonk, NY, USA) was used for the statistical analyses with 95% confidence.

### *Ethical Consideration*

This research has been approved by Pamukkale University Medical Ethics Council with the number 2008/7-1.

## Results

The screening of 477 people included in the study demonstrated the existence of antibodies in ≥1/64 dilutions of 210 volunteers (44.0%) (Table 1).

Seropositivity-wise, there were no differences between male volunteers (111/242; 23.3%) and female volunteers (99/235; 20.7%) participating in the study. Among the regions included in the study, the highest amount of seropositivity was found in (Fig. 1-II) Çal region (Table 2; $p<0.05$).

The differences of antibody seroprevalence among people living in different altitudes (Fig. 1) are given in the Table 3. Antibody prevalence is higher in people living at altitudes ≥600 m but the estimated relative risk ratio drops as the altitude rises ($p<0.001$; Table 3). Further, the statistical analysis demonstrated the following:

***Table 1.*** *The distribution of Bartonella henselae antibodies in the study groups (N=477)*

| Percentage | Number of samples | Positive sample (%) | Cumulative positivity (%) |
|---|---|---|---|
| <1:64 | 267 | 56.0 | 0.0 |
| 1:64 | 128 | 26.9 | 26.9 |
| 1:128 | 66 | 13.8 | 40.7 |
| 1:256 | 13 | 2.7 | 43.4 |
| 1:512 | 3 | 0.6 | 44.0 |

***Table 2.*** *The distribution of Bartonella henselae antibodies in the study group in accordance with the sample collection region*

| Region | Sample number (N) | Seropositive sample n (%) |
|---|---|---|
| (I) Bekilli | 31 | 9 (1.8) |
| (II) Çal | 313 | 141 (29.5) |
| (III) Baklan | 78 | 43 (9.0) |
| (IV) Central rural area | 55 | 18 (3.8) |

***Table 3.*** *The distribution of Bartonella henselae antibodies in the study group according to the residency in altitudes*

| Altitude (m) | Sample number (N) | Seropositive sample n (%) | Odds percentage | p value |
|---|---|---|---|---|
| 300–600 | 22 | 9 (40.9) | 0.90 | 0.82 |
| 600–1600 | 214 | 113 (54.6) | 2.04 | <0.001 |
| 1600+ | 241 | 84 (34.9) | 0.49 | <0.001 |

***Table 4.*** *The distribution of Bartonella henselae antibodies in the study group in accordance with the age groups*

| Age group | Sample Number (N) | Seropositive sample n (%) |
|---|---|---|
| 10–19 | 16 | 7 (1.5) |
| 20–29 | 56 | 25 (5.2) |
| 30–39 | 116 | 45 (9.4) |
| 40–49 | 56 | 56 (11.7) |
| 50–59 | 83 | 44 (9.2) |
| 60–69 | 40 | 25 (5.2) |
| 70–79 | 15 | 7 (1.4) |
| 80+ | 5 | 1 (0.2) |

- Difference in *B.henselae* seropositivity based on age groups of the volunteers participating in the study was not significant (p>0.05; Table 4).
- Difference in *B.henselae* seropositivity and the job groups of the volunteers participating in the study was not found (p>0.05; Table 5).
- People with history of working or doing agriculture in wetlands and contact with ticks had higher *B.henselae* seroprevalence.

## Discussion

The aim of this study was to detect and examine the seroprevalence of *B.henselae* in occupational groups susceptible to bartonellosis among the locals living in the northern rural area of Denizli. Risk groups were formed using factors such as occupation, area of residence, characteristics of life, exposure to vector. In variations of the study, the *B.henselae* seropositivity was found as 44.0% in northern rural area of Denizli.

The studies carried out to determine *B.henselae* antibodies in healthy humans vary greatly in different regions of the world. These differences are attributed to the characteristics of life of societies and the ecological variabilities of the regions they live in. During the scanning of people living in rural areas, *B.henselae* seroprevalence has been detected as 5.5% in Thailand[6], 9.6% in China[15] in Tianjin region, 13.3% in Brazil[16], 15% in Korea[17], 15.9% in Crete[18], 19.6% in East China[19], 19.8% in Greece[20], 36.8% in Canada British Columbia[21], 0.8–9.3% in the United States of America[13], 57.3% in Croatia[6], 65% in Austria[22], 11.9–60.5% in Spain[23] and 30.4–48. % in Poland[2,24]. The amount of *B.henselae* seroprevalence of the healthy blood donors consulting in hospitals within the region of this study has been found to be 6.0% [14]. This percentage is relatively 96/9 low considering the result of the study (44%.0) conducted in rural areas.

In the study conducted in four regions according to their characteristics of life and geography, high seropositivity was detected in (II) Çal region (Fig. 1; Table 2; p<0.05). Similar results have also been achieved by the epidemiologic studies conducted in the world. Sun et al.[19] reported different percentages of seropositivity in the eastern regions of China. Studies conducted in the rural areas of Brodsko and Posavka of Crotia on *B.henselae* seroprevalence among the healthy population detected seropositivity to be 42.9% and 62.2% respectively[6].

The rural as well as the mild climatic nature of the region located in Northern part of Croatia serves as the primary reasons for the high *B.henselae* seropositivity

***Table 5.*** *The distribution of anti-Bartonella henselae antibodies in the study group in accordance with the occupational groups*

| Occupational group | Sample number (N) | Seropositive sample n (%) |
|---|---|---|
| Farmer | 401 | 174 (36.5) |
| Veterinary | 22 | 12 (2.5) |
| Shepherd | 18 | 6 (1.3) |
| Livestock breeder | 9 | 3 (1.3) |
| Retiree | 8 | 6 (1.3) |
| Veterinary technician | 6 | 2 (0.4) |
| Butcher | 4 | 3 (0.6) |
| Forester | 3 | 3 (0.6) |
| Other | 6* | 1 (0.2) |

* Student, janitor, worker.

***Table 6.*** *The distribution of Bartonella henselae seropositivity in accordance with the risk factors*

| Risk factor | Odds percentage | 95% Confidence interval | p value |
|---|---|---|---|
| Barn livestock raising | 1.277 | 0.67–2.41 | 0.450 |
| Domestic animal bite/scratch | 0.705 | 0.46–1.06 | 0.095 |
| Wild animal bite/scratch | 1.449 | 0.54–3.82 | 0.453 |
| Working at wetlands | 1.857 | 1.19–2.88 | 0.005* |
| Working at a farm | 0.633 | 0.41–0.96 | 0.032* |
| Hunting | 0.809 | 0.49–1.32 | 0.389 |
| Exposure to gnats | 0.635 | 0.34–1.15 | 0.138 |
| Exposure to fleas | 0.836 | 0.56–1.24 | 0.374 |
| Exposure to ticks | 0.670 | 0.45–0.99 | 0.046* |
| Exposure to (body/hair) lice | 1.324 | 0.90–1.93 | 0.143 |
| Exposure to mang | 0.962 | 0.32–2.81 | 0.944 |
| Travelling abroad (>1 month) | 1.078 | 0.62–1.86 | 0.785 |

* $p<0.05$

percentages. This data is in compliance with our findings. (II) Çal region, where high seropositivity was detected, is a low-altitude land compared to (I) Bekilli and (III) Baklan regions and it is located at a higher altitude than (IV) rural area of Denizli (Table 3). This finding shows that the role of fauna and vector transmissions are affected by the altitude.

Studies conducted in People's Republic of China, Thailand[5], Croatia [6], Greece [20] and Korea [17] report no difference of *B.henselae* seroprevalence between genders. This study confirms that finding by determining no difference in percentages of seropositive cases between genders.

When the occupational groups were evaluated, no statistical difference of *B.henselae* seropositivity was found ($p>0.05$). The highest percentage of seropositivity was found among farmers (36.5%; Table 5). *B.henselae* positivity was found in 65% of the healthy workers working in a zoo in Austria in Tianjin China among 365 agriculture workers, *B.henselae* was found in 79.8% of those who herd cattles, 14.5% of milkers, 14.5% of those who work at packaging and 1.7% of the vets [15,22]. Long durations of animal contact have been put forward as the reason for these increasing percentages. In Poland, it was found in 27.7% and 31.5% of farmers and foresters respectively, in 2015[24]. In a screening made using San Antonio 2 isolate in Spain's La Rioja region, it was found in 53.6% of health workers who frequently encountered cat scratch disease as well as in other bartonellosis patients[3].

While 2.5% seroprevalence was detected in vets, this percentage was reported as 12.5% among the vets who live in Denizli province center but work at a neighboring rural area[10]. The percentage found as 30% in Aydin, a neighboring province, is higher[10]. In different parts of the world, vets have been studied as risk group and seroprevalence was reported to range between 2.3 and 51.1%[10]. Chmielewski et al.[2]have researched the existence of *Bartonella spp.* in different occupational groups and while seropositivity was detected in 48.3% of homeless alcoholics, 45% of vets and 53.3% of cat breeders, no antibodies were found in people who take intravenous medicines. In this study, participants are farmers engaged in both crop and animal husbandry, therefore, similar to studies done in Austria[22] and China[15], this study considered long durations of contact with animals, as the primary reason for the high prevalence of seroprevalence among the farmers.

Among the people living in rural areas, no statistical difference of *B.henselae* antibodies between age groups were detected. Similar data were acquired in studies conducted in different parts of the world. Despite high percentages in this metric in various parts of the world such as 26.9% in the age group of 45–59 in China[19], 57.8% in the age group of 19–65 in Croatia[6] and 21.6% in the age group of 60 and over in Korea[17]. The analysis in this study did not find a statistical difference between age groups. On the other hand, Pons et al.[23] found a statistical difference in the age group of 30–64 compared to other groups in Northern Spain using IFA. These differenced could be explained by regional differences.

According to this study, *B.henselae* antibodies are higher in people who work on farms and wetlands than those who do not work in wetlands (Table 6; $p<0.05$). One of the big branches of Büyük Menderes lies in the (IV) north rural area of Denizli and this is where the sample is collected. This region also has large irrigation areas, which have branches of Büyük Menderes in them. The fact that there is a dam on this river and canals to use the river water for irrigation throughout the region is important because conducive environment for thriving is provided for vectors such as gnats. However, the *B.henselae* seroprevalence is not high among those who reported exposure to gnats ($p>0.05$; Table 6). In higher altitudes, the risk factor dropping and making an ecological restriction supports the vectoral transmission (Table 3). One finding of the current study is that people with exposure to ticks have high antibody levels ($p<0.05$; Table 6) and this is in line with the ecological restriction explanation.

Arthropod contact is reported to play a big role in *B.henselae* infections[24–28]. Different studies proposed that ticks are important vectors when it comes to *Bartonella* species' infecting humans and animals[24,25,27,28]. In this study, the amount of *B.henselae* antibodies was found to be statistically higher in people in contact with ticks (Estimated relative risk: 0.67; $p<0.02$). Studies conducted in Poland[24], Austria[26] and the US[28] proposed the infection via ticks as only one of the factors. In this study, statistical difference of contact with ticks was significant and this leads one to consider that it is necessary to assess the tick population in the rural areas of Denizli for *Bartonella spp*. isolation. Contact with ticks should be evaluated during bartonellosisprediagnosis.

There were no statistical differences in seropositivities of *B.henselae* between contact and bites of domesticated and wild animals ($p>0.05$; Table 6). Sun et al.[19] and Breitscwerdit et al.[26] have proposed that regular contact with domesticated and wild animals increase the *B.henselae* positivity percentages. In this study, there were no long periods of cat and dog contacts. Only short periods of contact outside of house have been indicated. In the rural areas of Denizli, dogs are raised as shepherd dogs and their contact with humans is limited to when their owners feed them. Cats are mostly raised outside in farms to catch mice in barns. Therefore, it was concluded that there were no long periods of contact in this study. Regional customs can be thought to have played a role in these contacts and affected the seropositivity.

As a result, the rural nature of the regions included in the study, the characteristics of common occupations, vector contacts, and region's geographical features indicate that encountering *B.henselae* is related to regional distribution. Acquiring epidemiological data from similar geographical areas of Türkiye will provide information about the up-to-date status of bartonellosis.

### *Conflict of Interest Statement*

We declare that we have no conflict of interest.


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