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Original Investigation / Özgün Araştırma

Investigation of *Brucella* and *Coxiella burnetii* antibodies among humans at risk and control groups living in southeastern Turkey

Türkiye'nin güneydoğusunda yaşayan risk ve kontrol grubundaki kişilerde *Brucella* ve *Coxiella burnetii* antikorlarının araştırılması

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ABSTRACT

Objective: *Brucella* and *Coxiella* are zoonotic pathogens with a broad geographic distribution. In this study, we investigated the prevalence of *Brucella* and *Coxiella burnetii (C. burnetii)* antibodies among at-risk and control groups living in southeastern Turkey.

Methods: Cross-sectional study. Age, gender, symptoms, and risk factors of subjects were obtained by questionnaire. The presence of *Brucella* antibodies was determined by *Brucellacapt* tests. *C. burnetii* IgM and IgG antibodies were detected by enzyme-linked immunosorbent assay. Positive and equivocal samples were confirmed with an indirect fluorescent-antibody test.

Results: The risk group was composed of farmers (27.7%), butchers (27.3%), laboratory workers (22%), slaughterhouse workers (20%), and veterinarians (3%). The control group was comprised of housewives (36.7%), tradesmen (35%), and office workers (28.3%). For *Brucella*, 22% of the risk group and 14.7% of the control group had a titer≥1:40 (p=0.020). Of the risk and control groups, 6.7% and 2.7%, respectively, had a titer ≥1:160 (p=0.020). *C. burnetii* IgM and IgG antibodies were detected in 2% and 40% of the risk group subjects and in 0.7% and 37.3% of the control group subjects, respectively (p=0.285 and p=0.502).

Conclusion: The high prevalence of brucellosis in risk groups compared to the control group and the probability of exposure to *C. burnetii,* in several sections of the community, especially the risk groups, show the importance of the control of zoonotic diseases.

Keywords: *Brucella* spp., *Coxiella burnetii*, *Brucellacapt* test, Enzyme-linked immunosorbent assay (ELİSA), Immunofluorescent assay (IFA)

ÖZ

Amaç: *Brucella* ve *Coxiella* geniş bir coğrafik dağılım gösteren zoonotik patojenlerdendir. Bu çalışmada, Türkiye'nin güneydoğusunda yaşayan risk ve kontrol grubunda bulunan kişilerde *Brucella* ve *Coxiella burnetii* antikorlarının prevalansının araştırılması amaçlanmıştır.

Yöntemler: Kesitsel bir çalışmadır. Çalışma kapsamına alınan kişilere yaş, cinsiyet, semptom ve risk faktörlerine ilişkin anket formları uygulanmıştır. *Brucella* antikorunun varlığı *Brucellacapt* testi ile araştırılmıştır. *Coxiella burnetii (C. burnetii)*, immunoglobulin M (IgM) ve immunoglobulin G (IgG) antikorları Enzyme-linked immunosorbent assay (ELİSA) ile araştırılmıştır. Pozitif ve kuşkulu saptanan örnekler Immunofluorescent assay (IFA) testi ile doğrulanmıştır.

Bulgular: Risk grubunda bulunanların %27,7'si çiftçi, %27,3'ü kasap, %22'si laboratuvar personeli, %20'si mezbaha işçisi, %3'ü veteriner, kontrol grubunda bulunanların %36,7'si ev hanımı, %35'i esnaf, %28,3'ü memurlardan oluşmuştur. *Brucella* antikor pozitifliği ≥ 1:40 titrede, risk grubunda %22, kontrol grubunda %14,7 (p=0,020), ≥1:160 titrede, risk grubunda %6,7, kontrol grubunda %2,7 oranında saptanmıştır (p=0,020). *C. burnetii* IgM pozitifliği risk grubunda %2, kontrol grubunda %0,7, IgG pozitifliği risk grubunda %40, kontrol grubunda %37,3 oranında saptanmıştır. Risk ve kontrol grubu arasında *C. burnetii* IgM ve IgG pozitifliği açısından anlamlı bulunmamıştır (p=0,285, p=0,502).

Sonuç: Risk grubunda kontrol grubuna göre yüksek oranda brucellozis prevalansı saptanması ve özellikle risk grubundakiler olmak üzere ciddi bir toplum kesiminin *C. burnetti* ile karşılaşma ihtimalinin bulunması, bize zoonotik hastalıkların kontrolünün önemini göstermektedir.

Anahtar kelimeler: *Brucella* spp., *Coxiella burnetii*, *Brucellacapt*, Enzyme-linked immunosorbent assay (ELİSA), Immunofluorescent assay (IFA)

INTRODUCTION

Brucellosis is an endemic zoonosis in some developing countries (1). *Brucella* spp. may be transmitted to humans through consumption of the meat, body fluids such as milk and urine, dairy products prepared with infected milk, or the placenta of infected animals (2). Transmission may also occur through sexual contact, through transfusion of infected blood, or in the laboratory through accidental transmission by inhalation (2). In humans, it

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can cause chills, undulant fever, perspiration, stomachache, arthralgia miscarriage, and orchitis and sterility in men (3). Conventional microbiological methods (culture and identification), serological tests, and enzyme-linked immunosorbent assay (ELISA) tests are mainly used to diagnose brucellosis (2). It is common as an occupational disease among veterinarians, farmers, animal breeders, herdsmen, butchers, and slaughterhouse workers, who may become infected through direct contact with animals (2).

Q fever is an infection caused by *Coxiella burnetii (C. burnetii)*. The most common sources of transmission to humans are farm animals such as sheep, goats, and cattle (4). Infected animals pass the microorganisms to the environment through their urine, feces, milk, and birthing products (5). The organism is transmitted to humans through the digestive system upon consumption of raw or unpasteurized milk and dairy products, through the skin and mucosa, or through inhalation of contaminated dust. The most common mode of transmission of *C. burnetii* to humans is inhalation (4, 6). Q fever may cause asymptomatic acute disease or a chronic infection (7). The diagnosis of Q fever is made by detecting antibodies against *C. burnetii* using complement fixation, indirect fluorescent antibody (IFA), micro-immunofluorescence, ELISA, or micro-agglutination tests. The IFA technique has been suggested as the reference method (gold standard) (8). Q fever is generally considered an occupational disease among people working with farm animals, among laboratory staff working with infected animals, and among veterinarians (9).

In this study, we aimed to investigate the seroprevalence of *Brucella* and *C. burnetii* among various occupational groups in Gaziantep and the possible risk factors.

METHODS

Ethical Approval

This study was approved (Resolution No. 05-2010/2) by the Ethics Committee of Gaziantep University School of Medicine. Informed consent was obtained from the persons involved in the study.

Risk and Control Groups

The study was carried out in Gaziantep, between October 2010 and July 2011. In this cross-sectional study, blood samples were simultaneously collected from the risk and control groups. Information about age, gender, clinical diagnosis, risk factors, and symptoms of participants were recorded. The study included 300 at-risk subjects and 300 controls.

Sample Collection

Our laboratory tests were carried out at the laboratory of the Division of Clinical Microbiology. After collection, the samples were centrifuged at 1.500 rpm for 10 minutes and the serum was stored at - 20°C until use. Hemolyzed and lipemic samples were rejected.

Laboratory Tests

Brucella antibodies were detected using the Brucellacapt test (Vircell, Spain). The results were categorized as negative, having an antibody titer ≥1:40, or having an antibody titer ≥1:160. Because blocking antibodies were detectable with the *Brucellacapt* test, all the antibody titers were determined using the *Brucellacapt* test (10, 11).

Detection of IgM and IgG antibodies against *C. burnetii* Phase II antigens was performed using an ELISA kit (Vircell, Spain). All equivocal and positive ELISA tests were evaluated with an IFA test, (Vircell, Spain) following the manufacturer's instructions. Titers of ≥1:24 and ≥1:64 with Phase I and II IgM antibodies and IgG antibodies, respectively, were considered positive. *C. burnetii* IgM and IgG antibody results given in the study showed Phase II antibody results.

Statistical Analysis

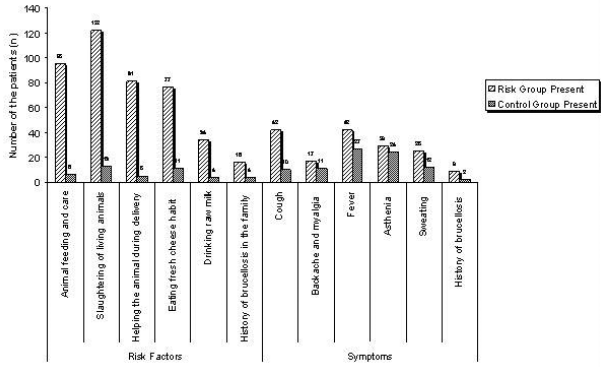
The data were analyzed using the chi-square test with Statistical Package for Social Sciences (SPSS), version 11.5 (SPSS Inc., Chicago, IL, USA).

RESULTS

Out of the 600 participants, 428 (71.3%) were male. The distribution according to age was as follows: 12.3% were 15-24 years, 38% were 25-34 years, 35.9% were 35-44 years, 11% were 45-54 years, and 2.8% were ≥55 years. In the risk group, 27.7% were farmers, 27.3% were butchers, 22% were laboratory workers, 20% were slaughterhouse workers, and 3% were veterinarians. In the control group, 36.7% were housewives, 35% were tradesmen, and 28.3% were office workers. In the risk group, 44.3% had worked more than 10 years, 28.3% 6 to 10 years, 22.7% had worked 2 to 5 years, and 4.7% had worked less than 2 years at their current occupation.

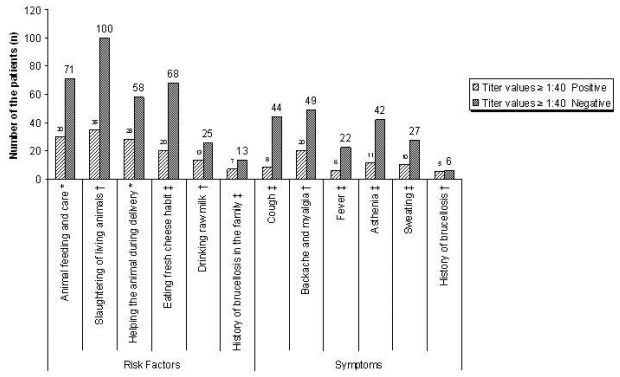
The risk factors and symptoms in the risk and control groups are shown in Figure 1. *Brucellacapt* titer results for the risk and control groups are shown in Table 1. Twenty-two percent of the risk group and 14.7% of the control group showed antibody titers (≥1:40) indicating previous exposure to *Brucella*, and the difference between the two groups was significant (p=0.02). Previous exposure was found among veterinarians (55.5%), farmers (31.3%), slaughterhouse workers (25.0%), tradesmen (17.2%), butchers (17.1%), housewives (15.4%), office workers (10.6%), and laboratory workers (9.1%). Because the number of people

Figure 1. The distribution of the risk factors and symptoms in the risk and control groups



in the various occupational groups differed, statistical comparison was not done. Previous exposure to *Brucella* determined by age groups was as follows: 13.6% among those aged 15-24 years, 20.2% among those aged 25-34 years, 18.6% among those aged 35-44 years, 19.7% among those aged 45-54 years, and 5.8% among those aged ≥55 years, and the differences were not significant (p=0.475). Previous exposure of males was 18.9% and of females was 16.8%, and the difference was not significant (p=0.554). Previous history of exposure was significantly (p=0.04) associated with the number of years worked in current occupation, and 27.1% of people who worked more than 10 years, 24.8% who worked 6 to 10 years, 11.8% who worked 2 to 5 years, and 7.1% who worked less than 2 years had elevated antibody titers. The distribution of *Brucella* antibody positivity at titers ≥1:40 among individuals according to having risk factors and symptoms (positive or negative) are shown in Figure 2.

Figure 2. The distribution of Brucella antibody positivity at titers ≥1:40 among individuals having risk factors and symptoms



Among the risk group, 6.7% had *Brucella* antibody titers ≥1:160, and among the control group 2.7% had *Brucella* antibody titers ≥1:160 (p=0.02). The distribution of participants with antibody titers ≥1:160 according to their occupation was as follows: 22.2% of veterinarians, 10.8% of farmers, 6.7% of slaughterhouse workers, 6.1% of butchers, 4.7% of tradesmen, 1.8% of housewives, and 1.2% of office workers. None of the laboratory workers had antibody titers ≥1:160. The distribution of participants with antibody titers ≥1:160 according to their age groups was as follows: 6.8% of people aged 15-24 years, 4.4% of people aged 25-34 years, 3.7% of people aged 35-44 years, and 7.5% of people aged 45-54 years. None of the subjects aged ≥55 years had titers ≥1:160 (p=0.511). Of all the participants with antibody titers ≥1:160, 5.8% were males and 1.7% were females (p=0.03). When *Brucella* antibody positivity (≥1:160) was investigated by gender, positive results were obtained in 25 (5.8%) of 428 males but in

Table 1. The results of *Brucellacapt* tests in the risk and control groups

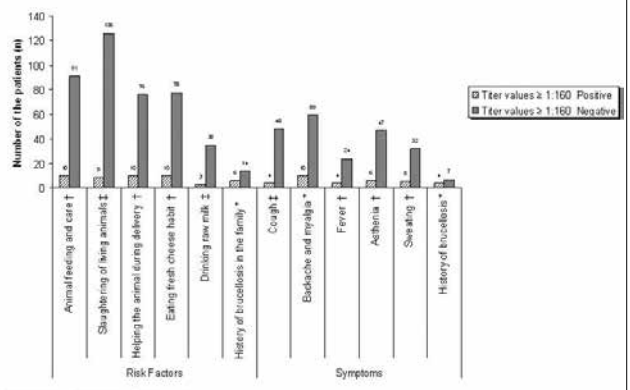
		***Brucellacapt* titer**								
		1:40	**1:80**	**1:160**	**1:320**	**1:640**	**1:1280**	**1:2560**	**1:5120**	**Total**
Risk Group n (%)	Butcher	4 (17.4)	5 (21.8)	4 (36.3)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	14 (21.2)
	Slaughterhouse worker	6 (26.1)	5 (21.8)	3 (27.3)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (22.7)
	Farmer	8 (34.8)	9 (39.1)	3 (27.3)	3 (75.0)	0 (0.0)	1 (50.0)	1 (100.0)	1 (100.0)	26 (39.4)
	Laboratory staff member	5 (21.7)	1 (4.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (9.1)
	Veterinarian	0 (0.0)	3 (13.0)	1 (9.1)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	5 (7.6)
	Total	23 (100.0)	23 (100.0)	11 (100.0)	4 (100.0)	1 (100.0)	2 (100.0)	1 (100.0)	1 (100.0)	66 (100.0)
Control Group n (%)	Office workers	6 (28.6)	2 (13.3)	0 (0.0)	1 (33.3)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	9 (20.5)
	Housewife	9 (42.8)	6 (40.0)	1 (33.3)	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.0)	0 (0.00)	17 (38.6)
	Tradesman	6 (28.6)	7 (46.7)	2 (66.7)	2 (66.7)	0 (0.00)	1 (100.0)	0 (0.00)	0 (0.00)	18 (40.9)
	Total	21 (100.0)	15 (100.0)	3 (100.0)	3 (100.0)	0 (0.00)	1 (100.0)	1 (100.0)	0 (0.00)	44 (100.0)

Table 2. The results of *Coxiella burnetii* ELISA in the risk and control groups

	ELISA IgM				ELISA IgG			
Study group	Positive n (%)	Equivocal n (%)	Negative n (%)	Total n(%)	Positive n (%)	Equivocal n (%)	Negative n (%)	Total n (%)
Risk group	1 (0.3)	6 (2)	293 (97.7)	300 (100)	74 (24.7)	47 (15.7)	179 (59.7)	300 (100)
Control Group	1 (0.3)	1 (0.3)	298 (99.3)	300 (100)	87 (29)	31 (10.3)	182 (60.7)	300 (100)
Total	2 (0.3)	7 (1.2)	591 (98.5)	600 (100)	161 (26.8)	78 (13)	361 (60.2)	600 (100)

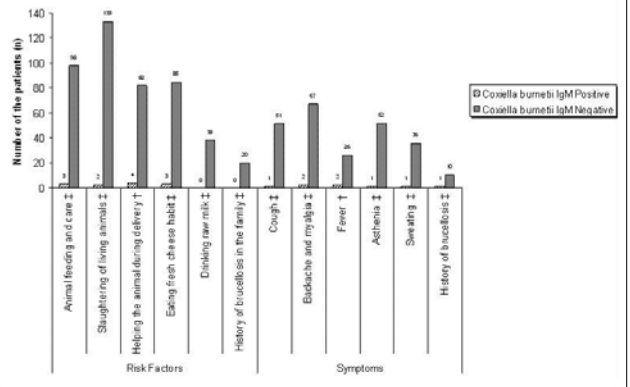
ELİSA: enzyme–linked immunosorbent assay; IgM: immunoglobulin M; IgG: immunoglobulin G

Figure 3. The distribution of *Brucella* antibody positivity at titers ≥1:160 among individuals having risk factors and symptoms



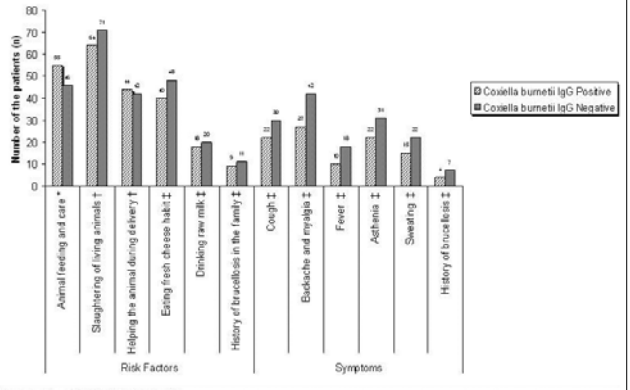
* p≤0.001, † p≤ 0.05, ‡ p>0.05

Figure 4. The distribution of *C. burnetii* IgM antibodies among individuals having risk factors and symptoms



† p≤ 0.05, ‡ p>0.05

Figure 5. The distribution of *C. burnetii* IgG antibodies among individuals having risk factors and symptoms



* p≤0.001, † p≤ 0.05, ‡ p>0.05

3 (1.7%) of 172 females. It was found statistically significantly more frequent in males (p=0.03). Brucellosis was not significantly associated with the duration of work in the current occupation (p=0.10). The distribution of *Brucella* antibody positivity at titers ≥1:160 among individuals according to having risk factors and symptoms (positive or negative) is shown in Figure 3.

Regarding *C. burnetii*, 0.3% of participants had a positive ELISA test for IgM antibodies, 1.2% had an equivocal test, and 98.5% had a negative test, whereas 26.8% had a positive ELISA test for IgG antibodies, 13% had an equivocal test, and 60.2% had a negative test (Table 2).

When ELISA and IFA antibody results for *C. burnetii* positivity were evaluated, IgM was found positive in 2% and 0.7% of the risk and control groups, respectively, whereas IgG was found positive in 40% and 37.3% of the risk and control groups, respectively. No significance was found between the risk and control groups in terms of IgM and IgG positivity (Fisher p=0.285 and p=0.502, respectively). *C. burnetii* IgM positivity was detected in 3.3% of slaughterhouse workers, 22.2% of veterinarians, 1.8% of housewives, 1.2% of farmers, and 1.5% of laboratory staff members, whereas no positivity was recorded among butchers, office workers, or tradesmen. *C. burnetii* IgG positivity was determined in 37.8% of butchers, 51.7% of slaughterhouse workers, 59% of farmers, 40% of tradesmen, 34.5% of housewives, 37.6% of office workers, 10.6% of laboratory staff members, and 22.2% of veterinarians. *C. burnetii* IgM and IgG antibodies were positive in 2.7% and 32.4% of participants aged 15-24 years, in 1.3% and 36.8% of those aged 25-34 years, and in 1.4% and 41.4% of those aged 35-44 years, respectively. While IgG antibodies were positive in 39.4% of those aged 45-54 years and in 52.9% of those aged 55 years and over, no IgM positivity was detected in those aged 45 years and over. No significant relationship was found between the age groups regarding IgM and IgG positivity (p=0.702 and p=0.450, respectively). When *C. burnetii* IgM and IgG antibodies were evaluated by gender, 2.3% and 36% of females and 0.9% and 39.7% of males were found to be positive, respectively. No significant relationship was detected between the two genders (Fisher p=0.234 and p=0.403, respectively). According to the duration of work in the risk group, no IgM positivity was determined for less than 2 years of work, whereas positivity was detected in 1.4% for 2 to 5 years, 4.7% for 6 to 10 years, and 0.8% for more than 10 years. IgG positivity was detected in 35.8% working for less than 2 years, in 30.9% working for 2 to 5 years, in 31.8% working for 6 to 10 years, and in 50.3% for working more than 10 years. Working time was not found to be significant in terms of IgM positivity (p=0.202), whereas it was significant for IgG (p=0.013). The distribution of *C. burnetii* IgM and IgG antibodies among individuals having risk factors and symptoms are shown in Figures 4 and 5.

DISCUSSION

Although brucellosis is seen in every region of the world, it is hyperendemic in the Mediterranean countries, the Arabian Peninsula, India, Mexico, and Central and South America (12). In our study, *Brucella* antibody positivity (titer≥1:40) was detected in 18.3% of 600 participants, and the titers of *Brucella* antibodies were ≥1:160 in 4.7% of the 600 participants. In this study, *Brucella* antibody positivity (titer≥1:40) in persons in the at-risk group (22%) was significantly higher than the control group (14.7%). Kılıç et al. (13)

detected 19% positivity among veterinarians and 4.7% among veterinary students and slaughterhouse workers in the province of Hatay using a micro-agglutination test. In a study carried out in the south of Iran, there was 7.8% antibody positivity (titer≥1:40) among people in the at-risk group for brucellosis with standard tube agglutination test (14). Similar to our findings, they reported that profession was the main factor for seropositivity.

Altındiş (15) reported 13.3% positivity (titer≥1:160) among fatteners, 8.6% positivity among butchers and sausage manufacturers, and 15.7% positivity among milk collectors and workers in the dairy product workshops in Afyon. In our study, the rate of *Brucella* antibodies was also high among farmers depending on these risk factors. The high prevalence among butchers and slaughterhouse workers might be accounted for by their dealing with animals with bare hands, slaughtering of animals, cuts on the skin, and inhalation of the agent.

In our study, a significant difference was found between the behaviors constituting the risk factors for the disease-such as animal feeding and care, slaughtering of living animals, helping the animal during delivery, and drinking raw milk and *Brucella* antibody positivity in terms of the indication of contact in a person (Figure 2). In their study in the province of Sivas in Central Anatolia, Alim *et al*. (16) regarded such features as direct contact with animals, the use of unhygienic meat, unpasteurized milk and their products, and occupation as the risk factors and reported 21.5% positivity among those with risk factors but 4.9% positivity among those with no risk factors using the Brucella agglutination test (Rose Bengal and Wright). The main source of transmission for brucellosis in Turkey is the consumption of unpasteurized milk and dairy products (17). In an epidemiological study carried out in Bosnia and Herzegovina, it was stated that in villages human brucellosis was transmitted mostly by contact with infected animals and their products, and in cities by consumption of dairy products made from contaminated, unpasteurized milk (18).

Q fever is an essential zoonotic infection that affects both animals and human beings. In our study, *C. burnetii* IgM positivity was found to be 2% and 0.7% in risk and control groups, while IgG positivity was found to be 40% and 37.3% in risk and control groups (Fisher p=0.285 and p=0.502, respectively). Aydın, Eyigör et al. (19) observed *C. burnetii* IgM positivity of 7.6% and IgG positivity of 42.3% in all study groups in their study with IFA and ELISA tests. Three occupational groups were included in the study (veterinarians, cattle-dealers, and butchers). In their study, they had collected serum samples from healthy people randomly in the city centers of Antalya, Diyarbakır, and Samsun, Berberoğlu *et al.* (20) reported 13.2%, 6%, and 1.8% IgG positivity, respectively, with IFA tests. Kılıç et al. (13) detected 20.6% IgG positivity against *C. burnetii* with IFA tests in their study on the at-risk groups in the province of Hatay. In the high-risk groups in eastern Turkey, Berktaş et al. (21) reported the rate of *C. burnetii* IgG seropositivity as 36.6% with ELISA tests. Sertpolat et al. (22) reported 39.3% IgG positivity using IFA tests in their study with healthy donors living in and around İzmir located in the western part of Turkey. In a study carried out in the province of Samsun in northern Turkey (23), the authors worked with 407 subjects in total, and 8.1% of them were identified as having past evidence of infection and 5.4% of them were considered to have the evoluative form of Q fever (17 acute and 5 chronic forms) by the microimmunofluorescent antibody test. They found 13.5% total seropositivity among healthy people, confirming that Q fever is prevalent in their region and is often asymptomatic. We think that the rates of *C. burnetii* seropositivity in the risk and control groups are close to each other due to its resistance to environmental conditions and due to its ability to be easily carried by air.

In our study, the highest *C. burnetii* IgG positivity (59%) was found among farmers. In Turkey, Kılıç et al. (13) reported 23.3% positivity among slaughterhouse workers, 28.6% positivity among veterinarians, and 14% positivity among veterinary students in Hatay. Berktaş et al. (21) detected the highest prevalence in the eastern region of Turkey being 65.9% among slaughterhouse workers, followed by 42.9% among butchers and 32.8% among farmers. In their study in and around İzmir, Sertpolat et al. (22) reported that the highest positivity among the occupational groups was 53.3% among farmers and butchers. In a study carried out in Southern Italy, serological testing revealed that 73.4% of subjects exposed to farm animals (cattle and sheep) were positive for anti-*C. burnetii* IgG (titer≥20) compared to 13.6% of control subjects (p<0.0001). In particular, the IgG seroprevalence for *C. burnetii* was 84% in the group of animal breeding workers, 60.6% in the group of agriculture/animal breeding, and 100% in the group of veterinarians (24).

Sertpolat et al. (22) reported that anti-*C. burnetii* IgG positivity was the highest (47.3%) in the age group of 40 years and over. They thought that this resulted from reinfection as those who were older than 40 years had been exposed to the infection for a longer period of time. *Coxiella burnetii* IgG positivity was statistically correlated to the number of years of working in the occupation (p=0.013). Karabay et al. (25) reported that the seroprevalence of *C. burnetii* was 23.8% among the participants above 18 years of age and 4.4% among those younger than 18 years of age by IFA tests (p<0.01). There was a significant relationship between *C. burnetii* seropositivity and direct contact with the birth products of farm animals (p<0.001); however, there was no significant difference between genders (25). These data show that long-term contact with animals is a real risk factor for *C. burnetii*. Human beings are often infected by the feces, milk, placenta, and body fluids of infected animals and by the inhalation of contaminated aerosols (8).

CONCLUSION

In order to prevent brucellosis in human beings in the province of Gaziantep, measures must be taken for the control and eradication of the disease in animals; unpasteurized milk and dairy products must not be consumed; and the people in the at-risk group must be informed about the need for taking protective measures while contacting animals or their waste materials. Because *C. burnetii* antibody positivity was detected at a high rate in our society in general, it was concluded that our people should be made conscious of zoonotic infections and that the epidemiological properties of the zoonotic infections should be clarified in the region.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gaziantep University School Medicine (05.2010/02).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

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