

Seroprevalence of *Coxiella burnetii* Antibodies in High Risk Groups in Eastern Turkey

Türkiye'nin Doğusundaki Yüksek Riskli Gruplarda *Coxiella burnetii* Antikor Seroprevalansı

Mustafa BERKTAŞ, MD,^a
Ebubekir CEYLAN,^b
Görkem YAMAN, MD,^a
İ. Hakkı ÇİFTÇİ, MD^a

^aDepartment of Microbiology and Clinical Microbiology, Yüzüncü Yıl University Faculty of Medicine,

^bDepartment of Veterinary Internal Medicine, Yüzüncü Yıl University Faculty of Veterinary, Van

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Yazışma Adresi/Correspondence:
Mustafa BERKTAŞ, MD
Yüzüncü Yıl University
Faculty of Medicine,
Department of Microbiology and Clinical Microbiology, Van,
TÜRKİYE/TURKEY

ABSTRACT Objective: The objective of this study is to estimate and evaluate the prevalence of antibodies of *Coxiella burnetii* (*C. burnetii*) in people considered to be at risk; such as farmers, slaughterhouse-workers and butchers in Eastern region of Turkey. The number of relevant studies concerning the subject is inadequate especially in our region. **Material and Methods:** Five hundred fifty two serum samples were collected from the people considered to be at risk of contacting with *C. burnetii*, such as farmers, abattoir workers and butchers in four cities and their 14 districts in the Eastern Turkey. Serum samples were centrifuged and stored at -20°C. The serum samples were tested with an ELISA kit to detect IgG antibodies against *C. burnetii* phase II antigen in order to determine the prevalence of Q fever. **Results:** The mean seropositivity rate across the region was detected as 36.6%. Among different high risk professions, the highest prevalence rates were detected in abattoir workers (65.9%), followed by butchers (42.9%), and farmers (32.8%). The prevalence rates among butchers were detected as 63.2% in Bitlis, 39.1% in Van and 25% in Muş. Among farmers, the prevalence rates were 36.6% in Bitlis, 34% in Van, and 18.2% in Muş. **Conclusion:** Significantly high seropositivity rates among the people with high risk professions in the Eastern Anatolia is reported for the first time in this study. It is essential to identify the reservoirs in order to diagnose *C. burnetii* infection correctly. It is essential to take the necessary control and prevention precautions in high-risk occupations due to high seropositivity rates determined in this study.

Key Words: *Coxiella burnetii*; epidemiology; Q fever; zoonoses

ÖZET Amaç: Bu çalışmanın amacı Türkiye'nin doğu bölgesindeki çiftçiler, mezbaha-işçileri ve kasaplar gibi risk altında kabul edilen insanlarda *Coxiella burnetii* (*C. burnetii*) antikor prevalansını tahmin etmek ve değerlendirmektir. Özellikle bölgemizde konu ile ilgili çalışmaların sayısı yetersizdir. **Gereç ve Yöntemler:** Türkiye'nin doğu bölgesindeki dört il ve 14 ilçede, çiftçiler, mezbaha işçileri ve kasap gibi *C. burnetii* ile temas riski olduğu düşünülen insanlardan 552'sinin serum örneği toplandı. Serum örnekleri santrifüj edildi ve -20°C'de saklandı. Q ateşi yaygınlığını belirlemek için serum örnekleri *C. burnetii* faz II antijenine karşı IgG antikorları tespit eden bir ELISA kiti ile test edildi. **Bulgular:** Bölgede ortalama seropozitivite oranı %36.6 olarak saptandı. Farklı yüksek riskli meslekler arasında, en yüksek yaygınlık oranları mezbaha işçilerinde (%65.9), sonrasında kasaplarda (% 42.9) ve çiftçilerde (%32.8) tespit edildi. Kasaplar arasında yaygınlık oranları Bitlis'te %63.2, Van'da %39.1 ve Muş'ta % 25 olarak tespit edildi. Çiftçiler arasında yaygınlık oranları Bitlis'te %36.6, Van'da %34 ve Muş'ta %18.2 idi. **Sonuç:** Doğu Anadolu Bölgesi'nde yüksek riskli mesleklerdeki insanlar arasındaki anlamlı derecede yüksek seropozitiflik oranlarına ilk olarak bu çalışma ile işaret edilmektedir. *C. burnetii* enfeksiyonunu doğru teşhis etmek için rezervuarları belirlemek önemlidir. Riskli meslek gruplarındaki yüksek seropozitiflik nedeniyle gerekli kontrol ve koruma önlemlerinin alınması gerekmektedir.

Anahtar Kelimeler: *Coxiella burnetii*; epidemiyoloji; Q-ateşi; zoonozlar

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The genus *Coxiella* is composed of a single species, *Coxiella burnetii* (*C. burnetii*), and this obligate intracellular microorganism is the causative agent of the widespread zoonosis known as Q fever. *C. burnetii* is a highly infectious microorganism which resists elevated temperature, desiccation, osmotic shock, ultra-violet light and disinfectants, and consequently it is very stable in the environment. This bacterium can infect a broad spectrum of susceptible hosts including domestic animals (livestock and pets), wildlife and even non-mammalian species including reptiles, fish, birds and ticks. Human infection is usually via shedding of *C. burnetii* in milk, feces, urine and birthproducts from infected ruminants. Infection is mainly acquired through inhalation of infectious aerosols, at parturition (including normal births) or at slaughter.¹⁻³ Clinical signs of Q fever are often subclinical or extremely mild. *C. burnetii* infections may be acute or chronic. Acute Q fever is a systemic disease and its clinical signs vary greatly from patient to patient. The most important diagnostic clue is the epidemiological circumstances. Acute Q fever appears most commonly like a self limited flu-like syndrome with its most frequent symptoms usually following a sudden onset, as high grade fever, fatigue, headache, and myalgias. The other two major presentations of acute infection are atypical pneumonia and hepatitis. Atypical pneumonia is characterized by a nonproductive cough, fever, and minimal auscultatory abnormalities, however in some cases present with acute respiratory distress. Hepatitis may be encountered as an infectious hepatitis-like form, clinically asymptomatic hepatitis or prolonged fever of unknown origin with characteristic granulomas on liver biopsy. Chronic Q fever occurs in approximately 5% of patients infected with *C. burnetii*, and may develop insidiously months to years after the acute disease. Typically, the heart is the most commonly involved organ followed by arteries, bones, and liver. Endocarditis usually occurs in patients with previous valvular damage or those who are immunocompromised. Both acute and chronic Q fever has been described during pregnancy. Although most cases seem to be asymptomatic, complicati-

ons may complicate the course of the disease, such as in utero fetal death, placentitis, or thrombocytopenia.⁴⁻⁶ Q fever is one of the causes of systemic amyloidosis.⁷

Q fever is difficult to diagnose clinically, radiologically, or by traditional culture procedures. The diagnosis, however, can be made serologically through the demonstration and titration of the antibodies against the causative agent.⁸ The acute form of Q fever is often underdiagnosed because of the nonspecific clinical picture, and thus the serology is extremely important for diagnosis of the disease. Antibodies against phase II antigen are predominant in the acute phase of the diseases. Specific high levels of anti-phase I antibodies are normally found in chronic Q fever patients, however, both phase I and phase II antibodies can be detected in high levels for years.^{8,9}

The diagnostic tests usually employed are complement fixation (CF), indirect immunofluorescent assay (IFA) and ELISA. ELISA based tests, due to their high specificity and sensitivity, were proven to be greatly useful both for epidemiologic screening and as diagnostic tests for human Q fever.^{10,11}

Because of the polymorphism of the clinical outcome and the diagnosis being based exclusively on serology, the prevalence of *C. burnetii* infection among humans is largely unknown. The purpose of this seroepidemiological study is to estimate the prevalence of antibodies to *C. burnetii* in the high risk groups of Eastern region of Turkey.

MATERIAL AND METHODS

SAMPLE COLLECTION

A total of 552 blood samples were collected from farmers, butchers, and slaughterhouse workers in 18 different locations in Eastern Anatolia Region of Turkey, between 2005-2006 (Figure 1).

The numbers of the obtained blood samples were in proportion with the population of each location. Samples of the slaughterhouse workers were only collected from Van city, and samples of the butchers were collected from Van, Muş and Bitlis

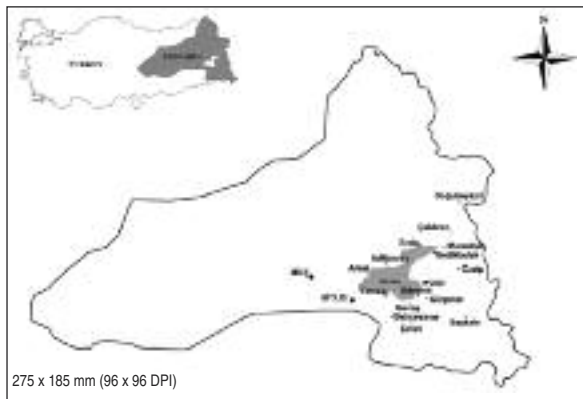


FIGURE 1: Locations of the collected blood samples in Eastern Turkey.

cities. The farmers' samples were collected from four cities and their 14 districts (Table 1).

The blood samples were collected aseptically by the qualified staff without using an anticoagulant, and transferred in accordance with the cold chain. The sera were obtained, destined for serological evaluation, and kept at -20°C until processing. A query was completed in order to record the sex, age and occupations of the study group.

ELISA TEST PROCEDURE

An ELISA kit (Vircell SL, Spain) was used to detect IgG antibodies against *C. burnetii* phase II antigen. The test was carried out according to the instructions of the manufacturer. The mean OD for the cutoff serum and antibody index was calculated.

Antibody index (AI) was calculated according to following formula: $(\text{AI}) = (\text{sample OD}/\text{cutoff serum mean OD}) \times 10$.

TABLE 1: Distribution of the blood samples according to occupation groups.

City	Occupations and number of the blood samples			Total
	Farmer	Butcher	Slaughterhouse worker	
Van	306	46	41	393
Bitlis	93	19	-	112
Muş	22	12	-	34
Ağrı	13	-	-	13
Total	434*	77	41	552

* Van 306 (Centrum 85, Bahcesaray 12, Baskale 10, Caldıran 7, Catak 20, Edremit 16, Ercis 24, Gedikbulak 52, Gevas 35, Gurpinar 22, Muradiye 12, Ozalp 11), Bitlis 93 (Centrum 39, Adilcevaz 12, Ahlat 22, Tatvan 20), Muş 22 and Ağrı Doğubeyazit 13.

Samples with indexes <9.0 were considered as negative for IgG antibodies, samples with equivocal results ($\text{AI} = 9.0-11.0$) were retested for confirmation, and samples with indexes >11 were considered as positive for IgG antibodies against *C. burnetii*.

STATISTICAL ANALYSES

The emphasized descriptive statistical values were stated as numericals and percentages (%). Fisher's absolute probability test, Z-Test and Chi-square tests were used in the comparisons made for these characteristics. Statistical significance level at the computations was taken as 5%.

The sample volume was determined according to the $n = N \times p \times (1-p) / (N-1) \times \sigma^2 p + p(1-p)$ equation used for estimating the ratios of finite populations.¹²

In this equation, the abbreviations used were follows; N: population volume (2.486.393), p: frequency ratio (33%) and σp : Standart deviation (2%).

RESULTS

The obtained blood samples were from 348 (63%) males, and 204 (37%) females. The high risk population included in this study were as follows; 434 farmers, 77 butchers, and 41 slaughterhouse workers. Their ages ranged from 17 to 63 years, and the mean age of the participants was 39.4 years. A total of 202 (36.6%) samples were detected as positive against *C. burnetii* phase II antigen. Seropositivity rates were 65.9% for slaughterhouse workers, 42.9% for butchers, and 32.8% for farmers (Table 2). The lowest prevalence rate of the disease in butchers was observed in Muş with 25%, this value was in Van 39.1% and highest in Bitlis with 63.2% (Table 2).

There were not any statistically significant differences among the butchers in different provinces concerning the seropositivity rates.

In farmers; the highest seroprevalence rate was detected in Bitlis with 36.6%, followed by Van with 34%, and Muş with 18.2%. There was not any seropositivity detected among 13 serum samples

TABLE 2: Results of the butchers' group according to cities.

City	n	Positive n	% Seroprevalence
Van	46	18	39.1
Muş	12	3	25
Bitlis	19	12	63.2
Total	77	33	42.9

collected from Dogubeyazit district of Ağrı city. Seropositivities in farmers' study group and their distribution among residential areas are shown in Table 3.

DISCUSSION

The interest for Q fever is increasing worldwide as indicated by the rising number of reviews published even in countries where its incidence is supposed to be very low. Indeed, the disease is considered as a re-emerging zoonosis in many countries. This could be due to the evolution of its epidemiology, or of the agent, which could become

more virulent, to modifications of its clinical signs, to an improvement of the sensitivity of diagnostic tests, or because practitioners are better informed and look for it more often.¹³

The present study was designed primarily to address the lack of data on the epidemiological features of Q fever in a high risk population in Eastern region of Turkey where livestock presence and farming activities are common. In the present study, the seropositivity against *C. burnetii* phase II antigen in the high risk population was 36.6%, indicating that antibody seropositivity was high in Eastern Turkey. There are several studies conducted in different regions of Turkey evaluating the seropositivity for *C. burnetii* in the high risk populations. In 1953, Payzın, reported that Q fever cases were distributed throughout Turkey and there were cases from Van but neither the numbers nor the seropositivity rates were mentioned.¹⁴ In another earlier study from Istanbul, a total of 79 sera samples were collected from the high risk population including veterinary surgeons, veterinary technicians, slaughterhouse workers, and farmers. The antibodies for *C. burnetii* were detected in 41 (51.8%) sera with ELISA method indicating a very high seroprevalence.¹⁵ In another study from the Aegean Region of Turkey, a total of 92 veterinarians and butchers were evaluated for *C. burnetii* IgG antibodies with ELISA method, and 34.8% were detected as seropositive.¹⁶ In a study from Erzurum, Eastern Turkey, 19.5% of healthy farmers were detected as *C. burnetii* IgG positive with ELISA method.¹⁷ In another study from Elazığ, Eastern Turkey, 12% of farmers and slaughterhouse workers were found to be seropositive, and all seropositive farmers were detected to have seropositive animals.¹⁸ According to the general seroprevalence studies in Turkey, the highest rates were found in Central Anatolia (Ankara, 28.0%), and in Eastern Turkey (Diyarbakir, 40.3%) where the livestock farming is an important occupation. When compared to the central and Eastern regions, Q fever is less prevalent in the Northern Turkey (Samsun, 1.8% and Trabzon, 11.2%) and in the Southern Turkey (Antalya, 13.2%).¹⁸ It has been considered that Q fever prevalence was higher in rural areas,

TABLE 3: Results of the farmers' group according to cities and their districts.

City / District	Samples	Positive n	Seroprevalence (%)
Van (total)	306	104	34
Centrum	85	44	51.8
Bahçesaray	12	5	41.7
Başkale	10	2	20.0
Çaldıran	7	-	0
Çatak	20	12	60.0
Edremit	16	6	37.5
Erciş	24	2	8.3
Gedikbulak	52	17	32.7
Gevaş	35	6	17.1
Gürpınar	22	6	27.3
Muradiye	12	4	33.3
Özalp	11	-	0
Bitlis (total)	93	34	36.6
Centrum	39	22	56.4
Adilcevaz	12	-	0
Ahlat	22	8	36.4
Tatvan	20	4	20.0
Muş Centrum	22	4	18.2
Ağrı Doğubeyazit	13	-	0
Total	434	142	32.8

because Q fever has been considered to be an occupational disease which affects people in contact with livestock and their products.¹⁹ In our study, with the participants in contact with livestock, we obtained seropositivity rates up to 65.9% indicating that occupations involved contacting with animals are at high risk of infection with *C. burnetii*, in Eastern region of Turkey. This finding is concordant with the studies from United Kingdom and Spain that found significantly higher seroprevalence among those working in a farm environment, working in agriculture or animal husbandry.²⁰

In our study the prevalence of *C. burnetii* infection was significantly higher in men (63%) than women (37%), as expected. This is because, in this area, mostly men are involved in occupations demanding close contact with livestock. Similar reports have been published regarding the prevalence of *C. burnetii* infection being more frequent in men in Spain, France, Australia and Tunisia.²¹⁻²³

The prevalence of *C. burnetii* infection seems to vary in different geographic areas and populations. The prevalence rates may also vary according to the techniques used for the detection of antibodies and interpretation of the positive results.¹⁹ In a study from Eastern Cantabria region of Spain, the prevalence of anti-phase II Coxiella IgG was 49% with higher rates in rural zones where more people were involved in farming activities.²² In a study from France, 71% of a population of 208 subjects comprising of goat farmers and veterinarians had antibodies against phase II epitopes.²⁴

A retrospective study carried out in Crete island of Greece examined 1298 patients over a period of five years and the seropositivity rate was

found to be 8% with the major risk factor reported as being in contact with farm animals.²⁵ Another study from North Dakota, USA, a total of 17 cases (3%) among 496 sheep producers, their family members, and hired helpers were identified, and lambing outdoors and frequent physical contact with sheep during lambing were associated with a higher risk.²⁶ In a seroepidemiological study comparing study and control cohorts from the UK, the prevalence of *C. burnetii* phase II antibodies were significantly higher in the study cohort (27%) than the control cohort (11%).²⁰ Generally, studies indicate that the Q fever prevalence is higher in rural zones compared to urban zones,^{14,20,22,27-29} however there are some studies reporting no significant correlation between seropositivity and the place of residence.^{19,23} In our study, the samples were obtained mostly from rural zones. However, our population only included the high-risk occupations; in this respect such a comparison would be unappropriate.

In conclusion, this is the first detailed study that evaluated a high risk cohort including slaughterhouse workers, butchers, and farmers. The prevalence rates of *C. burnetii* phase II antibodies were detected between 32.8% and 65.9%, confirming the common nature of this infection in the Eastern Anatolia Region of Turkey. These results indicate that it is essential to identify the reservoirs in order to diagnose *C. burnetii* infection correctly. It is necessary to take the regional epidemiologic studies seriously, make the routine controls on animals and animal products, educate the employers who are in the risk groups and cooperate with veterinarians in the region in order to take control precautions for this zoonotic infection.

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