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Coxiella burnetii seroprevalence in the rural part of Bolu, Turkey*

Aim: This study was performed to determine the seroprevalence of *Coxiella burnetii* in the rural part of Bolu, a city located in the western part of the Black Sea region of Turkey.

Materials and Methods: The study population was chosen by cluster sampling to represent the rural population of the city. Serum samples were collected from the 9 rural districts of Bolu. Blood samples were taken to the laboratory while stored at 4 °C in ice-cooled boxes. The sera were then stored at −20 °C until testing. The presence of IgG antibodies against *C. burnetii* phase-II antigens in the sera was determined using IFA.

Results: The study included 293 cases and *C. burnetii* phase-II IgG was positive in 61 of the subjects (20.8%). The seroprevalence of *C. burnetii* was 23.8% (59/248) among the participants above 18 years of age and 4.4% (2/45) among those younger than 18 years of age ($P < 0.01$). 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 not a significant difference between genders.

Conclusions: Although the seroprevalence rate was high, Q fever was underreported and unrecognized in rural Bolu. We conclude that Q fever must be considered in patients with a suitable clinical course in this region.

Key words: *Coxiella burnetii*, seroprevalence, Bolu, Turkey

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Türkiye'nin Bolu ili kırsalında *Coxiella burnetii* seroprevalansının araştırılması

Amaç: Bu çalışmada Türkiye'nin Batı Karadeniz bölgesinde yer alan Bolu'nun kırsal bölgesinde *Coxiella burnetii* seroprevalansının belirlenmesi amaçlandı.

Yöntem ve Gereç: Çalışma popülasyonu Bolu'nun kırsal ilçelerini de temsil edebilmek amacıyla tabakalı örnekleme yöntemine göre belirlendi. Bolu'nun 9 ilçesinden serum örnekleri toplandı. Kan örnekleri +4 °C lik buzlu kutularda laboratuvara getirildi. Serum örnekleri ayrılarak çalışma zamanına kadar −20 °C 'de saklandı. Hastalardan alınan serum örneklerinden IFA yöntemiyle *C. burnetii* faz II antijenlerine karşı oluşan IgG antikorlarının varlığı araştırıldı.

Bulgular: Çalışmaya 293 olgu dahil edildi. Olguların 61'inde (% 20,8) *C. burnetii* faz II IgG pozitifliği saptandı. *Coxiella burnetii* seroprevalansı 18 yaş üzerindeki olgularda % 23,8 (59/248) ve 18 yaş altındakilerde % 4,4 (2/45) idi ($P < 0,01$). Coxiella seropozitifliği ve hayvanların gebelik ürünleriyle temas etme arasında istatistiksel anlamlı ilişki saptanmışken ($P < 0,001$), cinsiyetle *Coxiella burnetii* seropozitifliği arasında anlamlı bir ilişki saptanmadı.

Sonuç: Yüksek Coxiella seroprevalansına rağmen bölgemizdeki olgulara Q ateşi tanısı konulamamakta ve bildirim yapılmamaktadır. Bölgemizde yaşayan hastalardan, Coxiella enfeksiyonuyla uyumlu klinik seyir gösteren hastalarda, Q ateşinin ayırıcı tanıda düşünülmesi gerektiği kanısındayız.

Anahtar sözcükler: *Coxiella burnetii*, seroprevalans, Bolu, Türkiye

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Introduction

Coxiella burnetii is a small (0.3-0.7 μm) gram-negative coccobacillus. It is the etiological agent of Q fever. Q fever is a zoonosis that can be transmitted to humans by infected sheep, goats, and cattle (1). It is resistant to environmental conditions, and both chemical and physical inactivation due to the formation of metabolically inactive small cell variants. It can survive at 60 °C for 60 min and in a 5% formalin solution for 4 h. Infection in humans occurs primarily after inhalation of aerosolized organisms. Infection can be transmitted via the urine, feces, milk, and delivery products of infected animals. For this reason Q fever is most commonly seen in rural parts of Turkey and primarily in breeders of farm animals (2). Q fever is an acute infection that rarely becomes chronic. Symptoms of Q fever include fever with chills, headache, malaise, myalgia, and rarely atypical pneumonia, hepatitis or meningoencephalitis. Nonetheless, 60% of those infected with this agent are asymptomatic (3).

Only a few studies have investigated the prevalence of Q fever in Turkey and the seropositivity rate of *C. burnetii* was reported to be between 7.1% and 39.3% (4,5). Due to the lack of specific clinical signs and symptoms of disease, it is impossible to estimate the true incidence in Turkey, including the Bolu region. With the present study we aimed to determine the seroprevalence of *C. burnetii* in children and adults in the rural part of Bolu, which is located in the Black Sea region of Turkey, using the indirect immunofluorescence antibody method.

Materials and methods

Ethical approval

This study was approved by the Abant İzzet Baysal University Ethics Committee. Informed consent was obtained from each subject or their parents, in the case of children.

The city

Bolu is a city located in the western part of the Black Sea region of Turkey, with a population of 270,654 according to the general population census of 2000. It has 9 districts in which cattle and sheep breeding is a common activity.

Sample size

The study population was chosen by cluster sampling to represent the rural population of the city. Samples were chosen according to the population ratio of each district based on the general population census of 2000. The following formula was used to determine the sample size (6)

$$n = \frac{N.t^2.p.q}{d^2.(N-1)+t^2.p.q}$$

In this formula *N* represents the population size, *t* is the constant (1.96), and *p* is the a priori assumption of the population parameter (0.28 according to the results of recent studies in Turkey), *q* is equal to 1 - *p*, and *d* is the margin of error (2%).

According to the formula, a minimum of 220 subjects were necessary. The sample size from each district was determined by the relative proportion of the population density of the district.

Questionnaire and laboratory tests

Each subject completed a questionnaire that collected data on demographic parameters, and involvement with animal birth products, milking, inhalation of stable dust, assisting in the birth of animals, and tick bites. Afterwards, 10 mL of venous blood was obtained from each participant. These blood samples were immediately transferred to the microbiology laboratory of Abant İzzet Baysal University, Faculty of Medicine. The blood samples were taken to the laboratory in 4-°C iced-cooled boxes. Blood samples were centrifuged and the sera were separated and stored at –20 °C until analysis.

The presence of IgG antibodies against *C. burnetii* phase II antigens was measured using a commercially available indirect immunofluorescence antibody (IFA) (Vircell, Spain), according to the manufacturers' recommendations. A titer of at least 1/64 was considered positive.

Statistical analysis

Statistical analysis was performed using Epi Info v.6.0 (CDC, Atlanta, GA, USA). Explanatory variables were tested for their associations with the serological status using the χ^2 test, or, when appropriate, Fisher's exact test.

Results

In all, 311 serum samples were collected from the 9 districts of Bolu; however, 18 samples were excluded because of hemolysis and sample tube breakage. The remaining 293 samples (248 adult and 45 children) were included in the study. Of the 293 subjects, 128 (43.6%) were male and 165 (56.4%) were female. Age range of the participants was 2-82 years.

According to the test results, 61 (20.8%) samples were positive for *C. burnetii* phase II antibodies. Of these 61 positive samples, 59 (23.8%) belonged to participants older than 18 years of age and 2 (4.4%) belonged to participants younger than 18 years ($P < 0.001$). In all, 19.3% of the females and 22.6% of the males tested positive. Exclusion of children from the analysis increased the prevalence rate (22.6% in females vs. 25.2% in males), but did not change the significance of difference between genders ($P > 0.05$).

A history of contact with animal birth products was significantly related to *C. burnetii* seropositivity (Table). Demographic and clinical features of the subjects are presented in the Table.

Discussion

C. burnetii causes highly variable clinical manifestations, ranging from asymptomatic to acute, fatal, or chronic infection; however, about 60% of patients are asymptomatic (7). For this reason the disease is not very well recognized and remains undiagnosed throughout the world. According to studies from Turkey, the seropositivity rate for *C. burnetii* ranges from 7.1% to 39.3% (4-6,8). In the present study 20.8% of a sample population representative of the rural area of Bolu had antibodies against *C. burnetii*, which suggests the endemic nature of this infection in the study region. Our results are in agreement with those obtained in previous studies in Turkey. We know that, according to official sources, no case has been reported in Bolu until this study. According to these results, we think that Q fever is undiagnosed in Bolu, as it is in most regions of developing countries.

Q fever prevalence studies in the pediatric population are limited in Turkey; therefore, we included a pediatric group in the present study. Mean

Table. Demographic and clinical characteristics of the subjects.

Parameters	*Coxiella burnetii* IgG positive n = 61 (%)	*Coxiella burnetii* IgG negative n = 232 (%)	P	OR
Gender (female/male)	32/29	133/99	*	
Mean age (range)	40.8 [5-81]	39.7 [2-82]	*	
Adult patients (age ≥ 18 years, n = 248)	59 (96.7)	189 (81.4)	< 0.01	6.71 (1.53-41.32)
Pediatric patients (age < 18 years, n = 45)	2 (3.3)	43 (18.6)		
Inhalation of stable dust extract	43 (70.5)	156 (67.2)	*	
Headache	26 (42.6)	96 (41.3)	*	
Arthralgia	27 (44.2)	83 (35.7)	*	
Cough	19 (31.1)	42 (18.1)	0.02	2.05 (1.03-4.05)
Fever	7 (11.4)	37 (15.9)	*	
Tick bite	4 (6.6)	21 (9.1)	*	
Milking	32 (52.5)	103 (44.4)	*	
Contact with animal birth products	30 (48.3)	54 (23.2)	< 0.001	6.90 (3.48-13.73)

*P > 0.05.

age of *C. burnetii* seropositive participants was 40.8 years. *C. burnetii* seropositivity increased from 4.4% (2 children [5 and 6 years old] were positive) among those younger than 18 years of age to 23.8% among those older than 18 years of age. This result confirms that exposure to *C. burnetii* occurs mainly after 20 years of age. Similarly, Cardenosa et al. reported that the seroprevalence of *C. burnetii* was 0% among those younger than 14 years of age and 9.1% among those between 15 and 29 years of age (9). Different *C. burnetii* seroprevalence rates were reported in children by seroepidemiological studies (10-12). The differences may be the result of increased exposure to infected animals with age.

It is known that increased exposure to farm animals due to travel to rural areas, outdoor activities, contact with pets, urbanization of rural areas, and windborne spread may contribute to the increased infection rates among urban residents. It is well known that the aerosol route is the primary mode of human *C. burnetii* infection (9). Contamination by *C. burnetii* aerosols may occur directly from parturient fluids of infected animals, which may infect newborn animals and the placenta (3,13). Ergonul et al. reported that *C. burnetii* seropositivity was 7.5% in veterinarians in 2 cities in Turkey and that *C. burnetii* seropositivity was not higher than in the general population (14). Nonetheless, assisting in the birth of animals without the help of a veterinarian is a common practice in rural areas of Turkey. In the present study the seroprevalence rate was 6.9-fold higher (range: 3.48%-13.73%) among subjects that had contact with animal birth products (Table). This finding supports the notion that people in close contact with animal birth products, like farmers, veterinarians, and abattoir workers, have increased risk of infection.

It was reported that contact with raw milk was probably a minor factor in the transmission of *C. burnetii* (15). Although *C. burnetii* was isolated from arthropods, arthropod-borne transmission of Q fever in humans is not likely to be important (3). In the present study we did not find any relationship between tick bites, milking, or inhalation of stable dust extract, and *C. burnetii* seropositivity, and *C. burnetii* seropositivity was not associated with gender.

A significant difference was not observed between such symptoms as headache, arthralgia, and fever, but there was a significant difference between *C. burnetii* seropositive and seronegative groups in terms of cough (Table). The 3 main symptoms of Q fever infection are pneumonia, flu-like syndrome, and hepatitis. *C. burnetii* should be considered in the differential diagnosis of patients with cough and respiratory symptoms in the Bolu region.

The present study has some limitations. First, we did not test cross-reactions among relevant pathogens, for example *Bartonella* spp., *Legionella* spp., or *Chlamidya* spp. Second, according to the literature we used a low cut-off value (titer Ig G ≥ 1/64). If 1/128 had been selected rather than 1/64, then the seroprevalence would have been lower; however, the 1/64 value has been used in other studies (16,17).

Conclusions

C. burnetii seroprevalence was 20.8% in the rural area of Bolu and seroprevalence increased with age. Although the *C. burnetii* seroprevalence rate was high, Q fever was unrecognized and underreported in Bolu. Therefore, physicians and local health departments must consider this important and prevalent infectious agent, and Q fever should be considered in the differential diagnosis of patients that have been in close contact with animals and have a suitable clinical course.

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