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The seroprevalence of *Coxiella burnetii* in Erzincan, Turkey: Identification of the risk factors and their relationship with geographical features

Aytekin Cikman[1], Merve Aydin[1], Baris Gulhan[1], Faruk Karakecili[2], Adalet Ozcicek[3], Ozan Arif Kesik[4], Mehmet Parlak[5], Fatih Ozcelik[6] & Bilge Gültepe[7]

[1]*Department of Medical Microbiology;* [2]*Department of Infectious Diseases;* [3]*Department of Internal Medicine, Faculty of Medicine;* [4]*Department of Geography, Faculty of Arts and Sciences, Erzincan University, Erzincan;* [5]*Department of Medical Microbiology, Faculty of Medicine, Yuzuncu Yil University, Van;* [6]*Clinical Biochemistry Laboratory, Erzincan Military Hospital, Erzincan;* [7]*Department of Medical Microbiology, Faculty of Medicine, Bezmi-Alem University, Istanbul, Turkey*

ABSTRACT

Background & objectives: Coxiella burnetii (C. burnetii) bacterium, the causative agent of Q fever has regained importance due to the increasing cases of infections and outbreaks. A cross-sectional descriptive study was conducted to investigate the seroprevalence of *C. burnetii* in human populations of Erzincan province located in the eastern Turkey, identify the risk factors, and to explore the relationship between geographical features.

Methods: A total of 368 people residing in the rural (306) and urban (62) areas of the province were included in the study. Serum samples were analyzed for the presence of *C. burnetii* phase II IgG antibody using ELISA (Virion/Serion, Wurzburg, Germany). Spatial analyses were performed to evaluate correlations between seroprevalence and geographical features.

Results: The overall seroprevalence of *C. burnetii* was found to be 8.7% (32/368). In rural residents it was 8.5% (26/306), while in urban population it was 9.7% (6/62). Cattle breeding and contact with animal afterbirth waste were found to be major risk factors, and were significantly correlated with seropositive cases ($p<0.05$). The seropositive cases were only observed in the areas between 1067 and 1923 masl. Of the total seropositive cases, 65.6% were within 1000 m and 87.5% within 4000 m of rivers and their main tributaries. Around 59.4% cases were observed in areas with a slope of 0 to 5°.

Interpretation & conclusion: The results of the study showed that *C. burnetii* seroprevalence was higher than expected, and significantly differs according to geographical features of a region. Significant risk factors include raising cattle and exposure to infected animals or their birth products/secretions. It is also more frequent in areas with higher number of rivers and streams, higher altitude and lower slope.

Key words *Coxiella burnetii;* geographical features; Q fever; risk factors; seroprevalence

INTRODUCTION

Coxiella burnetii (C. burnetii) is a small, pleomorphic, non-motile, non-encapsulated, gram-negative obligate intracellular bacterium[1]. According to the new classification based on the phylogenetic analysis of the gene sequences, *C. burnetii* species are now included in the gamma (γ) subgroup, order Legionellales of the Proteobacteria family[2]. *Coxiella burnetii* is the causative agent of Q fever, a zoonotic disease in humans[3]. The *C. burnetii* infection in humans can be asymptomatic or symptomatic with three different clinical forms; namely self-limited febrile illness, acute Q fever (atypical pneumonia, hepatitis) and chronic Q fever (endocarditis)[4].

The disease is transmitted to humans mostly by the inhalation of air contaminated with *C. burnetii*[5]. It is also directly transmitted directly through contact with infected sheeps, goats or cattles, or indirectly through contact with their urine, faeces and afterbirth wastes[6]. It has also been reported that *C. burnetii* infections can be acquired from unpasteurized milk products, blood transfusion and unprotected sexual intercourse[7].

The largest reported Q fever outbreak affecting > 3500 people in the Netherlands from 2007 to 2010, attracted its attention and regaining importance among the health agencies[7]. The World Health Organization (WHO) has defined Q fever as an "infection of increasing importance"[2]. The *C. burnetii* is widely present throughout the world, with its seroprevalence differing according to regions[8-12]. Several investigatory studies have been conducted in different regions of Turkey, and reported *C. burnetii* positivity in different percentages[8, 13-17]. Further-

more, despite the higher prevalence reported in the rural areas in the past, the more recent studies have indicated that the rural areas do not actually present a significant risk factor[10, 18]. However, the relationship between the prevalence of *C. burnetii* and geographical features has not been described in earlier studies and still remains unclear. Therefore, this study investigated the seroprevalence of *C. burnetii* in Erzincan province in eastern Turkey, and explored the associated risk factors and its relationship with geographical features of the study area.

MATERIAL & METHODS

Study area

Erzincan province is located in the northwest of the Eastern Anatolia Region in the Upper Euphrates basin between 39° 44' 21.084" N and 39° 29' 24.54" E coordinates. The province comprises of nine districts; Refahiye, Kemah, Kemaliye, Tercan, Cayirli, Ilic, Otlukbeli, Uzumlu and the Erzincan City center. The climate of Erzincan displays a transition between the climates of the Black Sea and the Eastern Anatolia regions. The province has a surface area of 11,623 km^2 and is surrounded by mountains and plateaus. The Karasu River divides the region into east and west, with a dam in the Tercan area. The Kelkit Valley in the province has favourable vegetation and climatic conditions for a large tick population. The study area contains several rivers, streams and wetlands; thus, agriculture and animal husbandry are common practices.

Blood sample collection

This study was based on a descriptive cross-sectional design to determine the seroprevalence of *C. burnetii* in Erzincan. The study comprised of 368 participants; 306 were rural residents and 62 were living in the urban part of the province. The demographic information about the participants, such as their gender, age, education level, occupations (cattle breeding in particular) and residential address were recorded. All participants were asked to complete a short questionnaire to determine their history concerning animal farming, tick bites (within the last five years), contact with ticks, milking and contact with animal afterbirth wastes. A face-to-face interview method was used for the completion of the questionnaire. The participants were divided into three groups according to age; <29, 30–59 and >60 to evaluate the positivity of *C. burnetii*. A 10-ml of venous blood sample was collected from each participant. The samples were centrifuged at 1610 × g for 10 min and serum was separated and stored in different aliquots at –20 °C until assayed.

Ethical approval

The study was approved by the Ethics Committee of Erzincan University (Approval No: 24.02.2014–1/7). Written informed consent was obtained from all participants using the relevant forms.

Antibody detection using ELISA

The serum samples were examined for the presence of *C. burnetii* phase II IgG antibodies using a commercial ELISA kit (Virion/Serion, Wurzburg, Germany), according to the manufacturer's instructions. The serum samples were diluted in antibody dilution buffer (1: 500) and each serum sample was examined twice.

The absorbance values were determined at 450 nm (reference wavelength of 620–690 nm) using an Epoch ELISA reader (BioTek Instruments, Inc., Winooski, VT, USA), on the Gen5^{TM} data analysis software (BioTek Instruments, Inc., Winooski, VT, USA). The cut-off values of all the samples and the optical density of control samples were recorded. Antibody activity was calculated in IU/ml using a standard curve in accordance with the manufacturer's recommendations. The results were quantitatively evaluated (<20 IU/ml–Negative; 20–30 IU/ml–Weak Positive; and >30 IU/ml– Positive). The weak-positive samples were re-tested.

Mapping

ArcGIS 10.1 (ESRI, Redlands, CA) and Google Earth (Google, Mountain View, CA) software packages were used to draw the maps of Erzincan province, districts, rivers and their tributaries for performing spatial analysis. The data-sets were created using the "Open Street Map" service on ArcGIS Basemap. Then, the location of seropositive cases was positioned on Google Earth and exported to ArcMap. The relationship between the number of seropositive cases and the altitude along with the slope gradient of the study area was also explored. The necessary data for this analysis was obtained from the Advanced Spaceborne Thermal Emission and Reflection Radiometer Global Digital Elevation Model (ASTER GDEM), the National Aeronautics and Space Administration (NASA, US), and the Ministry of Economy, Trade and Industry (METI, Japan). In addition, buffer analyses were performed for the rivers and their tributaries (Buffer/Multiple Ring Buffer) for the spatial analysis of the study area.

Statistical analysis

The data obtained from all participants were analyzed using Microsoft Excel (Microsoft Corporation, Redmond,

WA, USA) and SPSS version 15.0 (SPSS, Inc., Chicago, IL, USA). The risk factors for the seroprevalence of *C. burnetii* identified from the questionnaire were assessed using the Mann-Whitney U-test and the Kruskal-Wallis ANOVA test for non-parametric and independent groups, and the unpaired *t*-test for the parametric and independent groups.

RESULTS

Of the 368 participants, 238 (65%) were females and 130 (35%) were males with the average age being 51±16.8 yr. The participants who were *C. burnetii* IgG positive, consisted of 20 (62.5%) female and 12 (37.5%) male, with the average age being 49 ± 15.9 yr. The average age of participants, that were negative for *C. burnetii* IgG was 51 ± 17 yr. No statistically significant difference was found between average age and gender (unpaired *t*-test, $p = 0.5059$). Furthermore, the comparison of age groups in terms of *C. burnetii* seropositivity, revealed no statistically significant difference ($p < 0.05$) (Table 1).

In total, 32 participants were found positive for *C. burnetii* IgG antibodies, with the overall seroprevalence of 8.7%. In rural areas, it was 8.5% (26/306) and in the urban areas, 9.7% (6/62). Four out of six people residing in Erzincan city center reported a history of contact with ticks or tick-bites. Among the *C. burnetii* positive cases, 40.6% (13/32) had a history of tick-bites and 59% (19/32) reported previous contact with ticks. However, no statistically significant relationship was found between the *C. burnetii* IgG positivity and contact with ticks or tick-bites ($p>0.05$) (Table 2). The distribution of *C. burnetii* seroprevalence in Erzincan province varied across the districts with 10% in the City center, 11.1% in Uzumlu, 8.7% in Kemah, 7.4% in Tercan, 6.7% in Cayirli and 3.4% in Refahiye.

Of the *C. burnetii* IgG positive participants, 84.3% (27/32) were engaged in animal farming, 78.1% (25/32) in cattle breeding and 84.3% (27/32) had a history of contact with animal afterbirth waste. Majority of those engaged in animal farming (77%) were cattle breeders. When *C. burnetii* IgG positive and negative participants were compared in terms of gender, age, history of animal farming, milking, contact with ticks or tick-bites and whether residing in the rural or urban area, no statistically significant difference was found ($p > 0.05$); however, the difference between the rural and urban groups was statistically significant with respect to the participants involved

Table 1. Prevalence of *C. burnetii* by age group in Erzincan province

Age groups (yr)	*C. burnetii* IgG$^+$	*C. burnetii* IgG$^-$	Total	% Prevalence	*p*-value*
0–29	3	42	45	6.7	0.4918
30–59	20	173	193	10.4	
60–99	9	121	130	6.9	

*Kruskal-Wallis Test (Non-parametric ANOVA).

Table 2. Prevalence of *C. burnetii* in accordance with risk factors

Risk factors		*C. burnetii* IgG$^+$	*C. burnetii* IgG$^-$	Total	% Prevalence	*p*-value*
Being female	Yes	20	206	226	8.8	0.9111
	No	12	130	142	8.5	
Residing in rural areas	Yes	26	279	305	8.5	0.8653
	No	6	57	63	9.5	
Engaged in animal farming	Yes	27	244	271	10	0.2640
	No	5	92	97	5.2	
History of tick bites	Yes	13	103	116	11.2	0.3460
	No	19	233	252	7.5	
Contact with ticks	Yes	19	152	171	11.1	0.1827
	No	13	184	197	6.6	
Cattle breeding	Yes	25	190	215	11.6	0.0416†
	No	7	146	153	4.6	
History of milking	Yes	23	191	214	10.7	0.1559
	No	9	145	154	5.8	
History of contact with AAW	Yes	27	201	228	11.8	0.0202†
	No	5	135	140	3.6	

*Mann-Whitney U-test; †Values significant; AAW—Animal afterbirth wastes.

in cattle breeding and their history of contact with animal afterbirth waste ($p < 0.05$) (Table 2).

The seropositive cases were also assessed for their association with the geographical features of the study area; such as rivers, altitude and slope grade of the area (Fig. 1). Of all the seropositive cases, 65.6% were found to be within 1000 m and 87.5% within 4000 m of rivers and their main tributaries. The slope in this study area ranged from 0 to 72.4°. About 59.4% of the seropositive cases were observed in areas with a slope of 0 to 5°, and all cases were observed in areas within a slope of 21.6°. The altitude (meter above sea level) ranged between 817 and 3518 masl for the whole study area; however, the seropositive cases were only observed in the areas between 1067 and 1923 masl. Half of the positive cases were found in the areas with an altitude of 1100 to 1200 masl (Table 3).

Table 3. Relationship between the *C. burnetii* positivity and geographical features of the study area

Geographical features	*C. burnetii* IgG$^+$ (n=32)	Rate of positive cases (%)
Distance to rivers (m)		
<1000	21	65.6
1001–2000	2	6.3
2001–3000	4	12.5
3001–4000	1	3.1
4001–5000	–	–
>5000	4	12.5
Slope (°)		
0–5	19	59.4
5.1–10	3	9.4
10.1–15	2	6.3
15.1–20	4	12.5
20.1–25	4	12.5
Altitude (masl)		
1000–1200	16	50
1201–1400	7	21.9
1401–1600	5	15.6
>1601	4	12.5

DISCUSSION

Coxiella burnetii is found in all geographical regions and climatic conditions except New Zealand[8]. Despite being found throughout the world, the seroprevalence of *C. burnetii* significantly differs according to regions and occupations[8-12]. It has been reported as 68% in slaughterhouse workers in Iran[9], 5.3% in the rural environs and 5% in the urban areas of Australia[10], 32% in the Nile Delta in Egypt[11], and under 1% in Chad[12].

Several studies have been conducted in Turkey, to investigate *C. burnetii* seropositivity and the prevalence of Q fever in humans. As in other parts of the world, the results of seropositivity in Turkey also significantly differ in terms of the regions and study groups[8, 13-17]. For example,

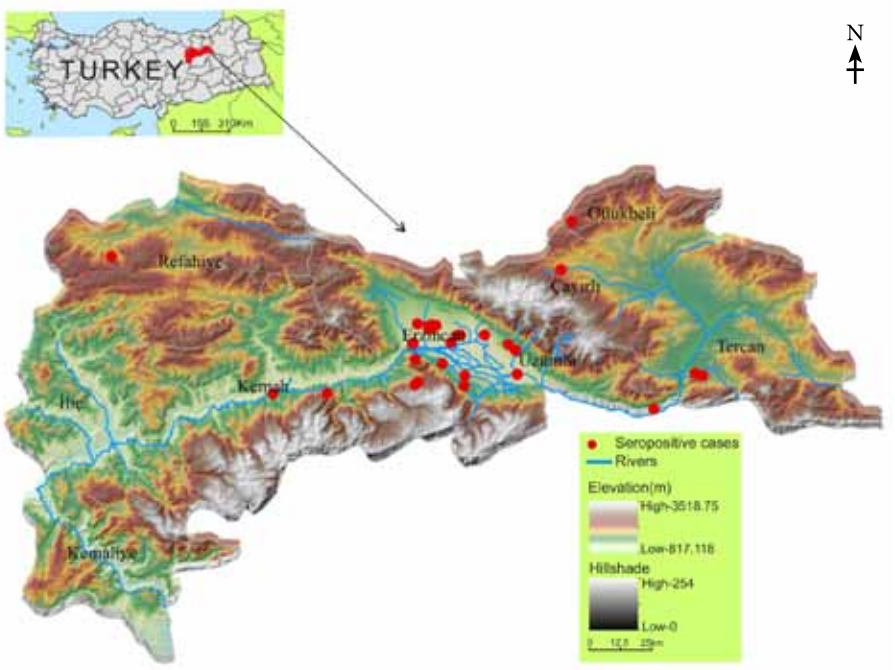

Fig. 1: Distribution of *C. burnetii* IgG positive cases in Erzincan province, Turkey and the relationship between the *C. burnetii* positivity and geographical features. Maps were created with ArcGIS software 10.1 (ESRI, Redlands, CA).

Karabay *et al*[13] reported 20.8% *C. burnetii* IgG positivity in people residing in urban areas of Bolu, while in another study carried out by Kilic *et al*[8] in Ankara, it was 32.3%.

In a series of studies conducted with high risk groups; Berktas *et al*[14] reported an overall seroprevalence of 36.6% (65.9% in slaughterhouse workers, 42.9% in butchers and 32.8 in farmers); Kilic *et al*[15] reported it to be 20.6% (23.3% in slaughterhouse workers, 28.6% in veterinarians and 14% in students attending the university veterinarian faculty; Celebi *et al*[16] found the prevalence of *C. burnetii* to be 30.7% (30.6% in veterinarians, 32% in veterinary technicians, 28.5% in animal lovers and 5% in the control group); and, Ergonul *et al*[17] reported *C. burnetii* IgG positivity as 7 and 8% in veterinarians working in two different regions. Despite these evidences, the number of studies conducted to investigate the seroprevalence of *C. burnetii* in Turkey over the last few years is very limited. Therefore, this study is important in terms of reporting on the current status of the seroprevalence of *C. burnetii, i.e.* 8.7%.

Several types of arthropods are considered to be carriers for *C. burnetii* with the main species being ticks[19]. *Coxiella burnetii* has been detected in several genera of ticks[20]. In Turkey, the common genus is *Hyalomma*[21], which has been shown to carry or act as the reservoir for *C. burnetii*[20]. With the rise in epidemical Crimean-Congo haemorrhagic fever in Turkey[22], *C. burnetii* infections need to be considered, particularly in regions where the tick population is high, since the transmission is often through ticks. In the present study, 40% of the participants who were *C. burnetii* positive had a history of tick bites and 59% had a history of contact with ticks. Similarly, Berberoglu *et al*[23] reported the seroprevalence of *C. burnetii* as 14.3, 10.1 and 1.8 in rural areas and 12, 1.7 and 1.9% in urban areas for the provinces of Antalya, Diyarbakir and Samsun, respectively. The same study found a lower prevalence of IgG in the rural areas of Samsun. Similar to the present study area, Samsun has a high tick population and is endemic for Crimean-Congo haemorrhagic fever. However, Antalya and Diyarbakir have a lower tick population.

In terms of the Districts of Erzincan, *C. burnetii* seropositivity was mostly observed in the City center and Uzumlu, while no seropositivity was found in Ilic, Kemaliye and Otlukbeli. However, this finding cannot be generalized due to the small sample size from these districts.

Many studies have suggested that a history of engaging in animal farming is a significant risk for *C. burnetii* infection[24]. In the current study also, the seroprevalence of *C. burnetii* was found to be higher in those engaged in animal farming. Different risk factors have been reported in terms of animal species. For example, Cetinkaya *et al*[25] reported high *C. burnetii* positivity in sheep and cattle. The highest prevalence was found in sheep and the people having contact with sheep.

Schimmer *et al*[26] found the highest *C. burnetii* positivity in goats and people working in goat farms. Klaasen *et al*[27] reported that people in contact with sheep and goats are at higher risk of getting infection. Contrary to these studies, De-Lange *et al*[28] found that cattle contact is a higher risk factor. Similarly, in this study, the seropositivity of *C. burnetii* was found to be 78.1% (25/32) in cattle breeders (p <0.05). Furthermore, in several studies, contact with infected farm animals, direct contact with the afterbirth wastes of these animals and milking have been reported as significant risk factors[13, 24-25]. This is in agreement with the results of this study, which showed a statistically significant relationship between *C. burnetii* positivity and direct contact with animal wastes. However, contrary to earlier studies, milking was not identified as significant risk factor.

In many studies, no difference has been found between male and female participants in terms of *C. burnetii* positivity[8, 29]. Karabay *et al*[13] found a higher seropositivity in women however, this was not statistically significant. Similarly, despite the higher number of seropositive women (8.8%), no statistically significant difference was found in terms of gender. The higher seroprevalence in women can be attributed to the common practice of domestic farming in Turkey, in which women are more frequently engaged.

In the literature, *C. burnetii* seropositivity has been reported to differ according to age but not significantly in any particular age group[13, 30]. In the current study also, the highest seropositivity was observed in the 30–59 age group (10.4%) with no statistical significance compared to other age groups (Table 1).

The relationship between *C. burnetii* and geographical features is not highlighted in earlier studies. Hence, in this study, the relationship between the number of seropositive cases and geographical features such as rivers, altitude and slope gradient of the area were investigated for the first time. Of the *C. burnetii* IgG positive cases, 65.6% were within 1000 m and 87.5% within 4000 m of rivers and their main tributaries. About 59.4% of the positive cases were observed in areas with a slope of 0 to 5°. Around 50% of the positive cases were found in the areas between 1100– 1200 masl (Table 3). All this indicates that *C. burnetii* occurs more frequently in areas with a high number of rivers and streams, higher altitude and a lower slope gradient. In these areas, the intensity, distribution

and variety of reservoir animals and arthropods might be associated with the seroprevalence of *C. burnetii*. The low number of positive cases and limited geographical features were the main limitations of this study.

CONCLUSION

The study demonstrated the seroprevalence of *C. burnetii* in Erzincan province of Turkey and showed its association with the geographical features of area. The data obtained in the study showed that the seroprevalence of *C. burnetii* is higher than expected and significantly differs according to the geographical features of a region. *Coxiella burnetii* infection is an important zoonotic disease that should be considered particularly in people engaged in animal farming and those with a history of tick-bites or contact with ticks. The significant risk factors are raising cattle and contact with animal afterbirth wastes. The seroprevalence of *C. burnetii* is higher among people living in an area with a higher number of rivers and streams, a higher altitude and lower slope gradient.

Conflict of interest

The authors declare that there is no conflict of interest in this study.

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Correspondence to: Dr Merve Aydin, Department of Medical Microbiology, Faculty of Medicine, Erzincan University, 24030, Erzincan, Turkey.
E-mail: mervegazi@yahoo.com.tr, maydin@erzincan.edu.tr

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