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**Prevalence of *Coxiella burnetii* antibodies among febrile patients
in Croatia, 2008-2010**

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Key words: Q fever, seroprevalence, Croatia

Running title: Q fever in Croatia

Abstract

Despite the widespread distribution of Q fever, the prevalence in humans is not accurately known because many infected people seroconvert without symptoms or with a mild febrile disease. The aim of this study was to determine the seroprevalence of Q fever in different regions of Croatia. During a two-year period (2008-2010), serum samples from 552 febrile patients with prolonged cough aged 1-88 were tested for the presence of *C. burnetii* antibodies using indirect immunofluorescent assay. Sera from 27.5% patients showed IgG antibodies. Serological evidence of *C. burnetii* infection was found in patients from all parts of Croatia. Seroprevalence rates differed significantly among regions from 21.5% to 41.2% ($p=0.001$). Males were more often seropositive (31.6%) than females (22.2%; $p=0.016$). According to age, a progressive increase in the IgG seropositivity rates was observed ranged from 6.7% in children less than 10 years of age to 39.2% in patients aged 40-49 ($p=0.001$). Above the age of 50 the IgG seroprevalence remained stable. Patients from rural areas were more often seropositive than patients from urban areas (40.8% vs. 19%), $p<0.001$). Acute Q fever was confirmed in 5.8% patients. Cases occurred throughout the year. A majority of cases were reported during summer months.

Key words: *C. burnetii*, seroprevalence, geographical distribution, Croatia

Introduction

Q fever is a worldwide zoonosis caused by obligate intracellular bacterium *Coxiella (C.) burnetii* (Tissot-Dupont and Raoult 2008). Many animal species are reservoirs of *C. burnetii* in nature. Sheep, goats and cattle are the main reservoirs and the most important source of infection for humans (Angelakis and Raoult 2010). Infected animals are generally asymptomatic, but shed the bacteria in their urine, feces, milk, and in very high concentrations in amniotic fluid during birth or abortion (Arricau-Bouvery and Rodolakis 2005). Transmission to humans occurs primarily through inhalation of contaminated aerosol and, to a lesser extent, ingestion of contaminated dairy products. In humans, Q fever is usually an asymptomatic or nonspecific, flu-like disease with spontaneous recovery. Atypical pneumonia or hepatitis may be observed in more severe cases (Lukšić et al. 2006). In a small proportion of patients the disease may become chronic leading to severe, often fatal endocarditis (Tissot-Dupont and Raoult 2008).

Human infections with *C. burnetii* have been reported in many European countries (Pascual-Velasco et al. 1998, Cisak et al. 2003, Cardenosa et al. 2006, Dorko et al. 2008, McCaughey et al. 2008, Hamzic et al. 2008, Monno et al. 2009), including Croatia. In Croatia, Q fever was first described in 1950 (Mihaljevic 1950). Since then, sporadic cases as well as minor or major outbreaks have continuously been reported (Galinovic-Weisglass et al. 1983, Spiranec et al. 1986, Janic and Golubic 1986, Milotic et al. 2001, Vilibic-Cavlek et al. 2004, Punda-Polic et al. 2007). However, data on the seroprevalence of Q fever are lacking. The aim of this study was to determine the prevalence and distribution of Q fever among febrile patients residing in urban and rural parts of Croatia.

Materials and Methods

Study population

During a two-year period (from August 2008 to July 2010), a total of 552 serum samples from febrile patients with prolonged cough (two weeks or more) aged 1-88 residing in urban, suburban and rural mainland and coastal Croatian regions was collected and tested for *C. burnetii* antibodies. According to the presence or absence of antibodies different subgroups of the study population were defined: seronegative (neither IgM nor IgG antibodies), acute Q fever (positive IgM and IgG antibodies) and past infection (negative IgM and positive IgG antibodies).

The study was approved by the Ethic Committee of the Croatian National Institute of Public Health.

Geographical features

Based on the geographic characteristics of the area as well as the economic activities of the population, five different regions were defined: Zagreb macroregion, Slavonia, Lika and Gorski Kotar, and the Adriatic area: Istria and Dalmatia. Zagreb area is an urban zone while surrounding areas (Kordun, Banovina, Moslavina, Prigorje) are formed of hills. The many villages and small towns spread across the hillside. Slavonia is a geographical region in eastern Croatia. It is generally known as lowland. Mountains higher than 500 m are rare and of insular character. Most of this area is being used for agricultural activities and livestock breeding. Pigs and cattle are the predominant livestock. Inner mountainous region (Lika and Gorski Kotar) separates the continental mainland from the coast. It consists of mountains as high as 1500 m. Their inhabitants work in farming activities, including breeding stock, mainly sheep. The Adriatic area includes the narrow coastal belt and islands. Istria is the largest peninsula located in the north coastal region. About 30% of Istrian population lives in rural zones where farmers bred mostly sheep and goats. Dalmatia is a region located in the south of Croatia. It consists of an urban zone

(towns with their suburbs located in the coast), islands and the rural mainland (Zagora). Zagora is a mountainous area with valleys surrounding small towns and villages. Their inhabitants are involved in agriculture and stock breeding (sheep and goats).

Serologic testing

Specific IgM and IgG antibodies to *C. burnetii* were detected by indirect immunofluorescence assay (IFA) using commercial slides containing *C. burnetii* phase II antigen obtained from culture on Vero cells (Coxiella burnetii-Spot IF; Biomerieux, Marcy l'Etoile, France) and fluorescein-isothiocyanate labeled anti-human IgM/IgG immunoglobulins (Fluoline M/G; Biomerieux, Marcy l'Etoile, France). For IgM detection, sera were absorbed with anti-IgG (RF absorbent; Biomerieux, Marcy l'Etoile, France). IgM titer ≥ 40 and IgG titer ≥ 80 were considered positive.

Statistical analysis

Man-Whitney U and Fisher's exact tests were used to compare differences between groups of ordinal and nominal variables, respectively. Assumption of binomial distribution was used for confidence intervals calculation. For statistical analysis, STATA/IC version 11.1 software was used. $P < 0.05$ was considered to be statistically significant.

Results

Serum samples from 152 of 552 tested patients or 27.5% (95% CI=23.8-31.5) had IgG antibodies and 32 or 5.8% (95% CI=4.0-8.1) had IgM antibodies to *C. burnetii*. Of all seropositive subjects, 120/152 or 78.9% (95% CI=71.6-85.1) showed serologic evidence of past infection and 32/152 or 21.1% (95% CI=14.9-28.4) acute Q fever. The seroprevalence rates differed ($p=0.001$) from one region to another (Figure 1): Zagreb macroregion 35.1%, Slavonia 41.0%, Lika and Gorski Kotar 41.2%, Istria 21.5% and Dalmatia 30.8%.

The prevalence of *C. burnetii* IgG antibodies according to characteristic of participants is shown in Table 1. There was a significant difference in IgG seroprevalence ($p=0.016$) between males (31.6%; 95% CI=26.5-37.1%) and females (22.2%; 95% CI=17.1-28.0).

A progressive increase in the IgG seropositivity rates according to age was observed ($p=0.001$) ranged from 6.7% (95% CI=0.1-22.1) in children less than 10 years of age to 39.2% (95% CI=28.4-50.9) in patients aged 40-49. Above the age of 50 IgG seroprevalence remained stable.

Patients who reside in rural areas were more often seropositive ($p<0.001$) than those who reside in urban areas (40.8%; 95% CI=34.2%-47.8% vs. 19%; 95% CI=14.9-23.6).

The seasonal distribution of acute Q fever cases (positive IgM antibodies) is presented in Figure 2. Cases occurred throughout the year with the highest incidence (from 20.7% to 33.3%) during summer and autumn months.

Discussion

Q fever continues to be a public health problem in many European countries. The prevalence of Q fever in humans is not accurately known. Because many infected people (50-60%) seroconvert without symptoms or with a mild febrile disease, Q fever is probably underestimated (Arricau-Bouvery and Rodolakis 2005). Reported seroprevalence rates in European countries vary greatly ranging from 1% to 60% (Hellenbrand et al. 2001, Cardenosa et al. 2006, Coulombier 2010). In certain risk groups, such as farmers and veterinarians, the prevalences are higher, up to 84% (McCaughey et al. 2008, Monno et al. 2009).

There are few published studies on the prevalence of *C. burnetii* infections in Croatia. *C. burnetii* was documented as etiological agent in 12%, 5.9% and 3% patients presented with atypical pneumonia in 1992 (Mlinaric-Galinovic et al. 1995), 1998-2002 and 2002, respectively (Vilibic-Cavlek et al. 2004, Vilibic-Cavlek et al. 2006). Although some parts of Croatia are well known endemic areas for Q fever (Galinovic-Weisglass et al. 1983, Milotic et al. 2001, Luksic et al.

2006, Punda-Polic et al. 2007), there are very few data available on the seroprevalence of *C. burnetii* antibodies.

In this study, 27.5% of patients showed evidence of exposure to *C. burnetii*. The IgG seropositivity rates varied significantly ($p=0.001$) among regions from 21.2% to 41.2%. These differences can be attributed to the variations in the proportion of population involved in farming activities.

Similarly with other studies published worldwide (Coulombier 2010), gender distribution in this study indicated a male predominance (31.6% vs. 22.2%, $p=0.016$), probably due to a greater occupational exposure to *C. burnetii* in males (Luksic et al. 2006).

Like other studies, the present results showed that the prevalence of *C. burnetii* IgG antibodies tends to increase with age. Higher prevalences at a more advanced age could be explained by longer exposure of older people (Cardenosa et al. 2006).

Risk factors for Q fever include staying in rural areas and contact with livestock (Lukšić et al. 2006). Although patients from rural areas showed a significantly higher IgG seropositivity (40.8%), a high proportion of seropositive patients was found in urban areas (19.0%). *C. burnetii* can survive for months in the environment in the form of spores, which allows it to be transported by wind far away from the original source. This could explain the appearance of Q fever in urban areas, where majority of patients report no direct contact with animals (Arricau-Bouvery and Rodolakis 2005). Contact with farm animals while travelling to rural areas and contact with pets could be another reason for *C. burnetii* infection among urban residents (Hellenbrand et al. 2001, Tissont-Dupont and Raoult 2004, Arricau-Bouvery and Rodolakis 2005).

Acute Q fever was documented in 5.8% febrile patients. In contrast to most previous studies in Croatia, the cases occurred throughout the year. A majority of cases were recorded in summer and autumn. However, recent data from the European Union have shown a seasonal pattern of Q fever with more cases reported during the summer months (Coulombier 2010).

The modern sheep production technology demands well-organized and systematic sheep breeding which implies lambing every eight months (Pavicic, 2010), which may affect the transmission of Q fever throughout the year.

The results of this study suggest that *C. burnetii* is widespread in several areas of Croatia. About one third of participants (27.5%) showed evidence of exposure to *C. burnetii* and 5.8% had acute Q fever. Active surveillance may be warranted in order to identify animal sources and control the disease in humans. In endemic regions, the diagnosis of Q fever must be considered in the case of unexplained fever.

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Disclosure Statement

No competing financial interests exist.

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Figure 1. Prevalence of *Coxiella burnetii* IgG antibodies in Croatia

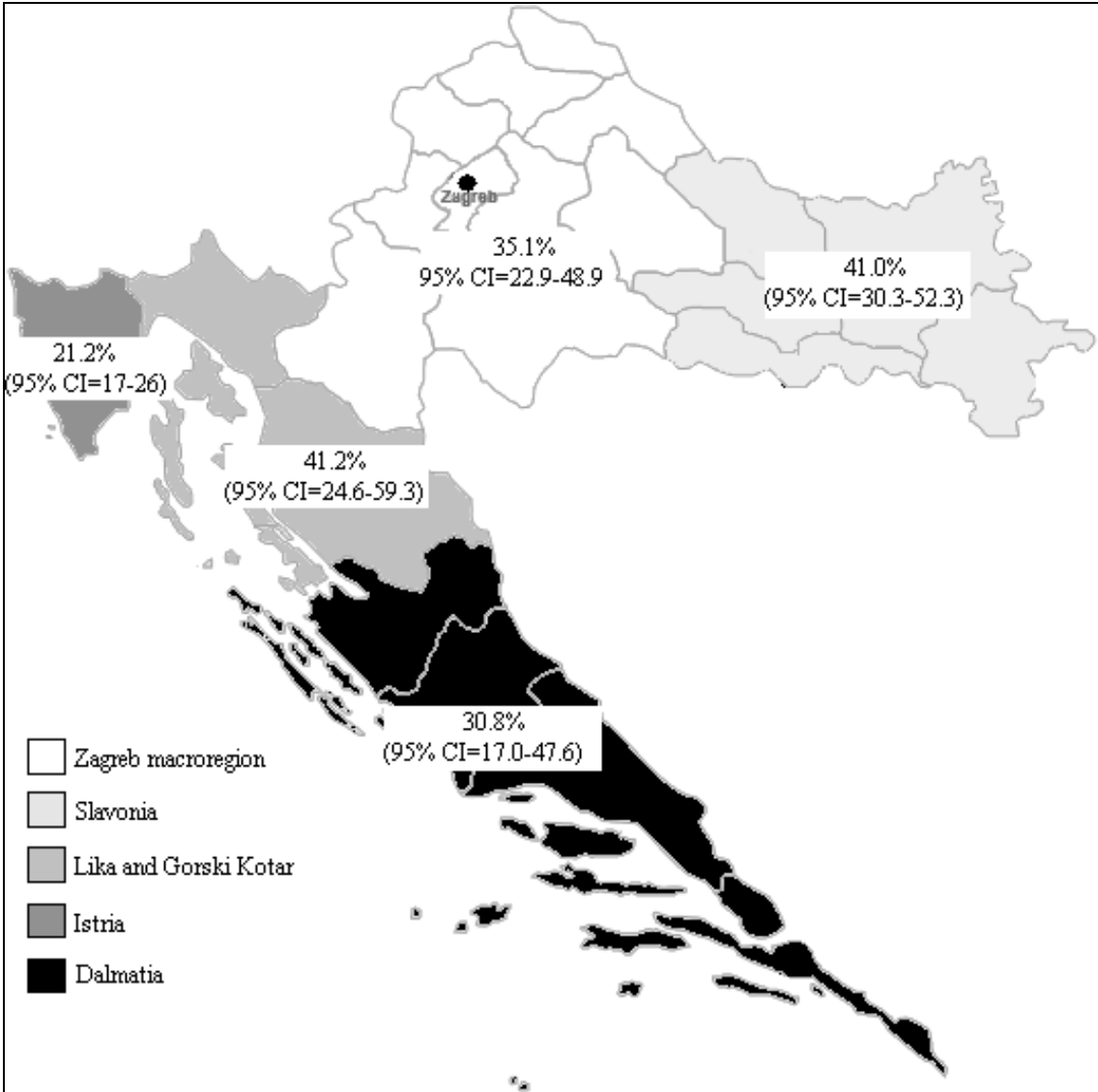


Figure 2. Seasonal distribution of acute Q fever cases

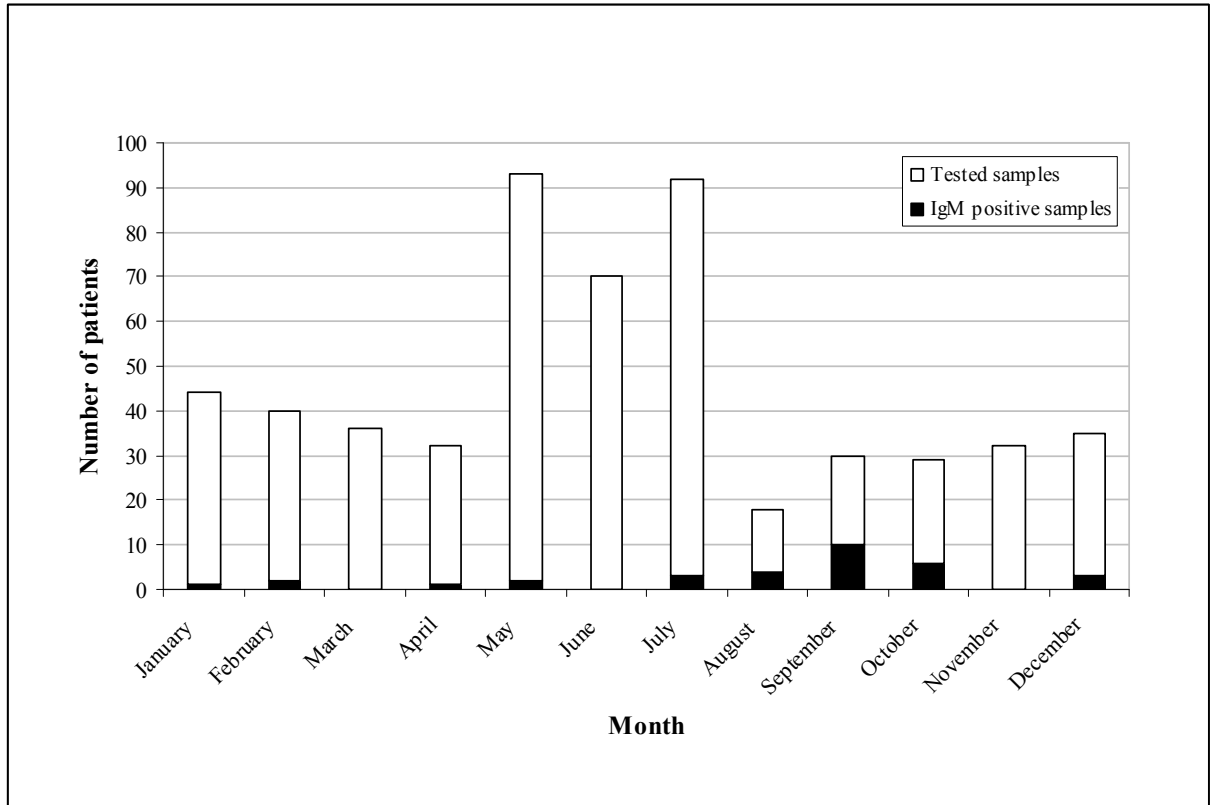


Table 1. Prevalence of *Coxiella burnetii* IgG antibodies according to characteristics of participants

Characteristic	Tested (%)	Positive (%)	95% CI	p value
Gender				0.016
Male	313 (56.7)	99 (31.6)	26.5 - 37.1	
Female	239 (43.3)	53 (22.2)	17.1 - 28.0	
Age group (years)				0.001
< 10	30 (5.4)	2 (6.7)	0.1 - 22.1	
10-19	60 (10.9)	10 (16.7)	8.3 - 28.5	
20-29	74 (13.4)	14 (18.9)	10.7 - 29.7	
30-39	87 (15.8)	25 (28.7)	19.5 - 39.4	
40-49	79 (14.3)	31 (39.2)	28.4 - 50.9	
50-59	83 (15.0)	28 (33.7)	23.7 - 44.9	
60-69	63 (11.4)	17 (27.0)	16.6 - 39.7	
70 +	76 (13.8)	25 (32.9)	22.5 - 44.6	
Place of residence				<0.001
Urban	337 (61.1)	64 (19.0)	14.9 - 23.6	
Rural	213 (38.6)	87 (40.8)	34.2 - 47.8	