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Original Article

Clinical, epidemiological and epizootic features of a Q fever outbreak in the border region between Serbia and Montenegro

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Abstract

Introduction: Q fever is a zoonosis which commonly manifests as an acute febrile disease accompanied by pneumonia or hepatitis. The aim of this study was to reveal the reservoirs, sources and routes of infection relevant for the Q fever outbreak that occurred in the border region between Serbia and Montenegro.

Methodology: A prospective study was conducted from 3rd to 23rd March, 2016 in Brodarevo, village near the Serbian-Montenegro border. The EU case definition for Q fever was applied and serological evidence of IgM and/or IgG antibody for phase II antigen *Coxiella burnetii* used for laboratory confirmation. Animal infection was proven by detection of specific biomarkers for Q fever by ELISA and Real-Time PCR.

Results: In total, ten patients were registered with Q fever, giving an attack rate of 0.5% in the village. A severe form of disease with atypical pneumonia ended up with hospitalization of eight patients. Serological surveillance was conducted in 30 herds of the receptive animals in the outbreak area. Overall the anti-Coxiella antibody seroprevalence was 20.6%. Positive molecular findings (68.4%) accompanied with high seroprevalence (63.2%) were identified in a mini-farm of sheep and cattle in the nearby Orasac, these were considered to be active sources of infection. The most probable route of *C. burnetii* transmission was the inhalation of contaminated aerosols originating from infected animals.

Conclusion: The main reservoirs for human Q fever at the border region between Serbia and Montenegro are infected cattle and ruminants. Adoption of a comprehensive strategy for disease prevention and control at the intergovernmental level is urgent.

Key words: Q fever; *Coxiella burnetii;* outbreak investigation; serosurvey; livestock.

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Introduction

Q fever is a widespread zoonosis caused by *Coxiella burnetii* (*C. burnetii*) [1]. In humans, it is usually manifested as influenza-like disease in conjunction with pneumonia or hepatitis [2]. In less than 5% of cases disease progresses in the chronic form [3]. The majority of *C. burnetii* infections are asymptomatic [3,4]. Q fever is mostly an occupational disease among professionals who are in prolonged contact with animals during work [2]. Infected animals usually do not show signs of disease although pregnant carriers of *C. burnetii* may abort following an infection [3]. Humans are commonly infected by inhalation of contaminated aerosols generated from animal placentas or body fluids of infected cattle, ruminants and other domestic, or wild, animals [5].

The incidence of Q fever, between 2010 and 2014, in the European Union (EU) ranged between 0.1 and 0.3 cases per 100,000 populations but significantly declined following outbreaks in the Netherlands and Hungary [6,7]. The first description of Q fever in Southeastern Europe, so called Balkan grippe, dates from the World War II [8] while first records about disease in the Republic of Serbia originate from 1950 [9,10]. Serbia is a South-eastern European country of about 7,000.000 inhabitants, with increasing incidence of human Q fever; between 0.1 and 1.4 cases per 100,000 populations (2006-2015) [11]. The peak incidence was registered in 2012/2013, when two major epidemics of Q fever occurred [11]. Most cases (78.8%) were registered in the North of the country (Autonomous province of Vojvodina) with the average incidence 3.5 times higher than the rest of the country

(1.4 and 0.4 cases per 100.000 populations, respectively) [11,12].

The maintaining of Q fever in Serbia is influenced by an unfavorable situation in neighboring countries including Republic of Montenegro to the South. Between 2007 and 2016 Q fever incidence in Montenegro ranged between 0-1.1/100.000 of population [8,13,14]. In 2013 and 2014, Q fever was established in cattle and sheep in municipalities on both sides of the state border [15]. Given the long-standing unfavourable epizootiological situation (Figure 1) and mostly asymptomatic disease course, epidemiology of human Q fever in the region is largely unknown. In spring of 2016, for the first time, an outbreak of Q fever emerged in the village Brodarevo situated at the Serbian side of the border with Montenegro.

The aim of this study was to describe the clinical, epidemiological and epizootiological features of Q fever outbreak and to reveal the reservoirs, sources and routes of the of *C. burnetii* transmission. We expect that this study will contribute to the epidemiology of Q fever of both Serbia and Montenegro.

Methodology

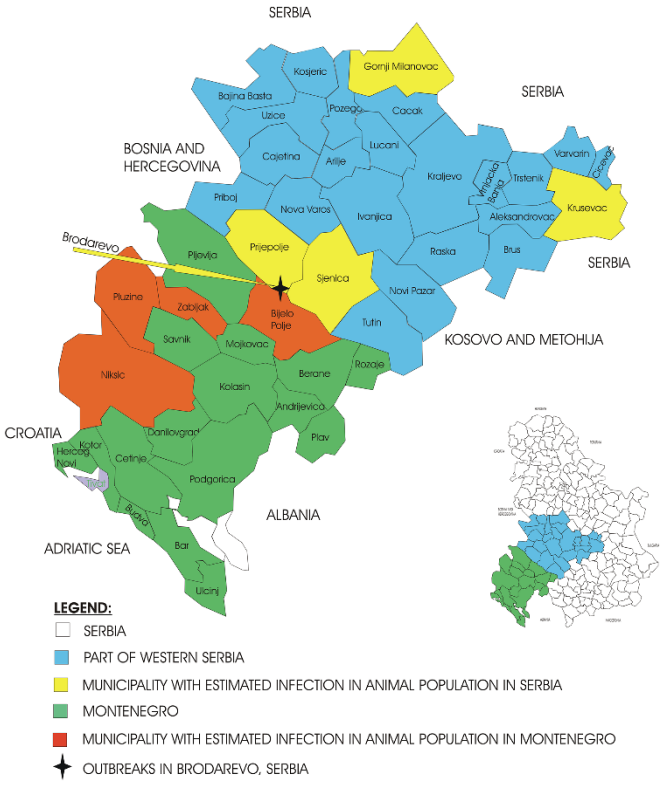
Outbreak case definition and area

Adapted EU case definition for confirmed case of Q fever was applied [16]. Since the source of the ongoing outbreak was unknown at the time of investigation, probable case definition was not applicable. Therefore, Q fever case was defined as any person with laboratory confirmation of Q fever, who either lived or visited Brodarevo and outbreak area in radius of 10 km, between 12 February and 2 March and was presented with at least one of the following symptoms: fever, pneumonia or hepatitis, with onset of disease in the period from March 3-23 and no other likely cause of disease. The risk period for exposure is estimated considering an average incubation period of 20 days and time distribution of cases. Laboratory criteria for confirmation included presence of specific antibody (Ab) response to *C. burnetii* in the patient sera using Enzyme-linked immune-sorbent assay (ELISA) tests. Brodarevo is a village in Prijepolje municipality, with 1,845 inhabitants [17], situated only 14,5 km away from the state border with Montenegro. The whole region is known as the cattle area.

Laboratory confirmation of human and animal Q fever cases

Considering flu-like disease presented in all patients, nasopharyngeal swabs were colelcted from all patients. Sample collection for laboratory diagnosis was

Figure 1. *Q fever* - Epizootiological situation of Q fever in the Southwest of Serbia and Montenegro between January 2012 and January 2016.



part of standard patient management and required only oral informed patient consent. Testing by real-time polymerase chain reaction (Real Time PCR) for the presence of Influenza A and B viruses was conducted at the Institute of Virology, Vaccines and Sera Torlak, Belgrade, Serbia. Simultaneously, sera samples were tested using ELISA tests at the reference laboratory for Q fever, Centre for microbiology of the Institute of Public Health (IPH) of Zrenjanin, Serbia. Serologic evidence of a positive IgM and/or IgG Ab result to phase II antigen *C. burnetii* was used for laboratory confirmation of Q fever. Where the result of the first test was equivocal or negative, for definitive laboratory confirmation of Q fever, paired serum samples were requested at least two weeks apart. The results were interpreted in line with the manufacturer's guidance (VIRION, SERION, QED, Bioscience Inc. San Diego, USA): presence of IgM Ab to phase II of > 1, 1 IU/mL and/or IgG Ab to phase II of > 30 IU/mL were considered positive. Commercial LSIVet Ruminant Q Fever ELISA kit (LSI, Lissleu, France)was used for detection of Ab against *C. burnetii* in blood samples of cattle, sheep and goat [18]. For the detection of *Coxiella* genes a Real Time PCR assay was used [19]. Extraction of DNA from various samples (sheep milk, sheep and

cattle vaginal swabs, preputial swabs of rams) was performed using MAGVET universal DNA/RNA extraction kit (LSI, Ansbach, Germany). Amplification in AriaMx qPCR instrument was performed (Agilent, Santa Clara, USA, Agilent Technology, Singapore) using Brilliant III Ultra-Fast qPCR mix (Agilent, Cedar Creek, USA). Animal samples from Serbia were tested in the Veterinary Specialized Institute (VSI) Kraljevo. In Montenegro, laboratory tests were performed by Diagnostic Veterinary Laboratory in Podgorica.

Epidemiological and veterinary data collection

The Centre for Disease Control and Prevention of IPH Uzice conducted epidemiological investigation including patient interviews, medical records review and evaluation of the potential exposure to *C. burnetii* through contacts with animals, tick bites, movements. The investigation of the Q fever cases was done in the frame of national public health surveillance. Veterinary records were obtained by VSI Kraljevo, field Veterinary Station and Republic veterinary inspectors (RVI). Data about Q fever in Montenegro was submitted from IPH of Montenegro and Veterinary Service of Montenegro, Podgorica. All collected information originated from national Q fever surveillance and monitoring results. Proposed measures for the outbreak control and suppression was undertaken by joint action of IPH Uzice, RVI and VSI Kraljevo.

Data analysis

A prospective study was conducted from 3-23 March 2016 in Brodarevo. Data were analyzed chronologically, demographically and topographically. Number of patients with disease signs and symptoms or abnormal laboratory results were presented. Attack rate of Q fever was calculated as a percentage by dividing the number of the outbreak cases by the number of inhabitants in the village, taking into account that all of them were exposed to the source of infection. Age-specific incidence per 100.000 population was determined. The seroprevalence of anti *C. burnetii* Ab in animal population was established. Data analysis was performed using the Microsoft Excel program (version 2016).

Results

Clinical presentations and laboratory results

In the period from 3-23 March 2016, ten patients with acute Q fever occured in Brodarevo. All patients appeared at the local physician with the flu-like disease. Besides high fever (≥38C), they had malaise (eight patients), cough (four patients) and throat pain (three patients) while one patient had dyspnea and/or diarrhea and/or muscle ache. Eight patients were admitted to the general hospital in Prijepolje, 2-10 days after the symptom onset. Increased values of sedimentation, leukocytes, fibrinogen, alanine-transaminase and aspartate-transaminase were obtained in the blood. Results of the molecular testing on influenza viruses were negative in all hospitalized patients. Serum samples on *C. burnetii* were positive for all patients: specific IgM Ab for phase II were positive in 10 patients; specific IgG Ab for phase II were positive in 8 and inconclusive in 2 patients. Specific IgG Ab for phase I were negative in all patients and in pared samples. Atypical pneumonia was confirmed by chest X-ray in all hospitalized patients. The mean duration of hospitalization was 8.1 (range 7–20) days. All patients were discharged from the hospital as cured. None of the patients had chronic disease or underlying disease, which is why laboratory follow up for chronic Q fever was not undertaken. The age-specific incidence of Q fever ranged from 778.2/100.000 in the age group from 45-54 years up to the 1195.2/100.000 in the age group from 25-34 years. Male to female ratio in patients was 9:1.

Epidemiological investigation

Patients with Q fever shared only the same place of residence and the approximate time of disease onset. Everyone lived in the populated area and confirmed presence in the village between 12 February and 2 March. They all denied contact with livestock. In the previous few weeks, four patients (farmers) fertilized crop plants and raspberry with "organic" garbage of animal origin on the slopes around village. Another four patients consumed uncooked cow milk. The origin of milk products in two patients was cattle mini-farm in the nearby village Orasac but remain unknown for the rest of the cases. The maximum attack rate of Q fever in the village was 0,5% taking into account that all inhabitants were exposed during maximal incubation period. The history of the recent tick bites or occupational exposure was negative. During 2016, five human cases of Q fever were detected in Montenegro; none of them was unveiled in the municipalities with border with Serbia.

Veterinary investigation and public health measures

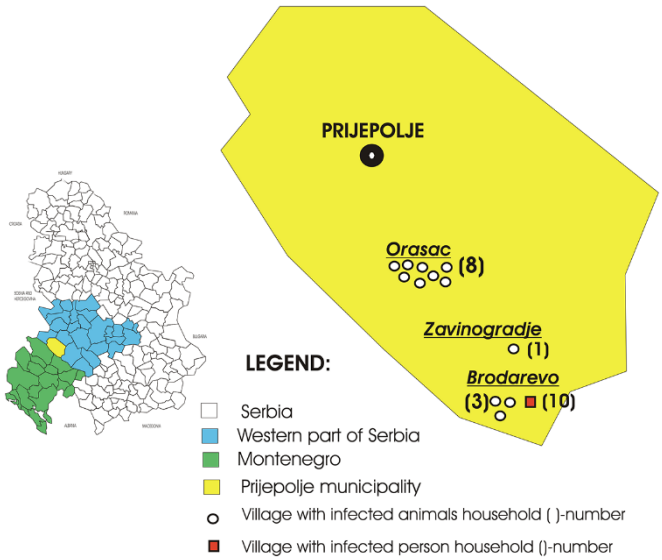
A thorough veterinary inquiry was performed in animal populations in order to identify the possible outbreak source. Mini-farms in the radius of 10 km around Brodarevo were investigated. The survey was

driven by data obtained through patient's interviews and the vicinity of the dwelling and the nearest mini-farms. Overall 30 mini-farms (herds), with 610 ruminants and livestock were involved in the survey. The highest *C. burnetii* Ab seroprevalence was established in goats (26.7%) and sheep (23.5%) (Table 1). Seropositive animals were disclosed in 12 (40%) herds located in Brodarevo and two in neighborhood villages situated in Prijepolje municipality. The majority of seropositive herds (8) were detected in Orasac (Figure 2) including one mini-farm consisting of four goats, 45 sheep and eight dairy cattle. Extremely high overall *C. burnetii* Ab seroprevalence in this mini-farm was detected (n = 36, 63.2%) particularly in sheep (68,8%) but also in goats (100%) and cattle (13%). An additional survey, using Real Time PCR confirmed the presence of *Coxiella* genes and active shedding of bacteria in 39 (68.4%) animals. Positive results were found in the vaginal swabs of one dairy cattle and 25 sheep, milk of 12 sheep and in preputial swab of ram. Primary reservoirs of infection and further transmission within the herd were not established. According to the farm owner's statement, there were no previous abortions in the herds, nor flu-like symptom among residents. The presumptive transmission route of *C. burnetii* was inhalation of contaminated aerosols that originated from body fluids and probably unreported abortion content or birth products of infected livestock in this farm. Contaminated dust was probably dispersed by the wind.

Outbreak control measures

Outbreak control measures included official reporting of cases to the authorities; informing of local physicians to ensure timely notification of cases; updating of news though media to reduce public fear; sharing of data with the veterinary services; exclusion of blood donors residing in the affected region; health promotion campaigns in the households with special emphasis on hygiene practices; monitoring of hand hygiene compliance and usage of personal protective equipment in the general hospital in Prijepolje. All animals with positive results of molecular testing were euthanized. Improvement of the hygiene practice and elimination of manure followed by disinfection of the farm (using disinfectant with peroxide effect of pentacalium bis (peroxymonosulfate) and bis (sulphate) was introduced [20].

Figure 2. Topographic distribution of households with Q-fever infected animals in the outbreak area.



Discussion

Q fever outbreaks are regularly reported throughout Europe, commonly in the vicinity of the mini-farms with infected livestock [1]. In the former Yugoslav Republics Q fever remained one of the leading zoonoses [21,22]. The epidemiology of Q fever is well recognized in Serbia, particularly in the northern province of Vojvodina [23] but is largely unknown in the rest of the country. This study was conducted in order to highlight main drivers of animal and human Q fever in the South of Serbia particularly in the border region with Montenegro.

An outbreak with 10 human cases of acute Q fever emerged in the village Brodarevo in early spring of 2016. An unusually high hospitalisation rate and large proportion of severe cases in this outbreak was striking. Mostly young and middle aged males were affected. All patients denied contact with animals. After thorough

Table 1. Seroprevalence of anti *C. burnetii* (IgG) antibodies in animal population in the outbreak area.

Village	Cattle		Sheep		Goats		Total	
	No of tested samples	Seropositive %	No of tested samples	Seropositive %	No of tested samples	Seropositive %	No of tested samples	Seropositive %
Orasac	42	14.2	243	43.2	7	100	292	**40.4**
Brodarevo	26	0	179	1.7	23	4.3	228	**1.7**
Zavinogradje	52	7.7	38	0	0	0	90	**4.4**
Total	120	**8.3**	460	**23.5**	30	**26.7**	610	**20.6**

epidemiological and veterinary investigation we concluded that the main presumptive route of *C. burentii* spread was the inhalation of contaminated aerosols originating from infected sheep and goats from the mini-farm in Orasac.

In this outbreak eight out of ten patients were hospitalized due to the Q fever and atypical pneumonia. Reported hospitalization rate of the acute Q fever is ≤5% but may reach up to 50% in the outbreaks [2,24]. Pneumonia is present in ≤2% of patients but may be multiplied in outbreaks due to raised attention among physicians [2,3]. Given the concomitant influenza season and common flu-like clinical presentation of Q fever, the possibility that there have been more Q fever cases, in the outbreak area cannot be ruled out.

We established the highest age-specific incidence of Q fever in the age group from 25-34 years. Q fever is rarely reported in children due to the milder clinical presentation in the younger age [25,26]. Q fever is predominantly reported in males. This may be related to the hypothetical protective role of estradiol on the host response to *C. burnetii* infection and more frequent occupational exposure in man [27]. The favorable outcome of Q fever might be explained by absence of underlying disease in patients [1,3].

In Serbia, serological testing of domestic animals for the presence of *C. burentii* IgG Ab is related to reports of human illness or abortion in the livestock [28]. In this outbreak, abortions among domestic animals have not been reported. Although mandatory, the significant level of underreporting of domestic animal abortions was observed [29,30].

The seasonal character of Q fever is related to the more frequent contact with animals due to the lambing, sheep shearing and other outdoor activities. In some animals, *C. burnetii* can be excreted during normal parturition [31,32]. The practice of fertilizing crop plants with manure of animal origin, noticed in this outbreak, is common and was proved earlier as a possible source of Q fever [32,33].

The presumable route of *C. burentii* spread in this outbreak was the inhalation of contaminated aerosols originating from infected animals in the Orasac which is only 10 km away from Brodarevo. Weather conditions in the outbreak area were in favour for this presumption as there were more windy days than average: the wind speed was higher (ranged up to 40 km/h with a maximum speed of 30 to 40 km/h) between 3-6th March 2016 [34,35]. For herds located in areas with high wind speed, open landscape, high animal densities and high temperature, the risk of being infected reached very high values. Increased risk of animal infection with *C. burnetii* and high wind speed, open landscape, high herd densities and high temperature was described earlier [36].

The existence of other sources of infection particularly among seropositive flocks could not be ruled out. A positive serology only indicates past exposure while positive molecular findings qualify current *C. burnetii* shedding [37]. The origin of the *C. burnetii* infection in animals was not clearly established. The most likely way of the infection spread was the introduction of new animals (primarily breeding rams). Further spread of infection probably happened on the joint grazing and further exchange of breeding rams. In this area, milk products (cheese and cream) are commonly preparing from uncooked milk. Considering data obtained through epidemiological investigation and the low number of human cases, alimentary pathway was not considered as a presumptive route of *C. burnetii* infection.

In 2006 the serological survey conducted in sheep near the border with Montenegro revealed the seroprevalence of IgG Ab result to phase I *C. burnetii* in the range of 1.4-7.1% (unpublished data). Monitoring of Q fever between 2012 and 2016 in Montenegro revealed 385 infected livestock [14]. The summer grazing of the livestock at Pester's plateau allowed mixing of uninfected with infected herds from both sides of border. Low awareness of farmers regarding the use of protective equipment and handling of birth products, manure including inadequate disposal of animal waste probably contributed to the long-term maintenance of Q fever in the region [29].

Limitations of this study are related to the financial constraints that dictated a narrow range of indications for molecular testing of human and environmental samples which might provide more evidence to support the link of the human cases with possible source of infection [38]. Additionally, source-finding investigations are often biased by the selection of farms. Therefore, the possibility that number of positive animals was underestimated by this survey cannot be excluded.

Conclusions

Systematic animal monitoring accompanied with the serological surveys in the human population would enable disease control in the region. Our results indicate that the Southwest of Serbia is still endemic for Q fever. Sources of *C. burnetii* infection are primarily cattle and ruminants. This outbreak highlights the necessity of urgent adoption of comprehensive strategy in order to

combat the spread of Q fever at the international as well as interstate level.

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Laboratory testing of animal samples was conducted in Department for laboratory diagnostic of Veterinary Specialized Institute Kraljevo, Serbia

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