SEROPREVALENCE OF *COXIELLA BURNETII* IN CATTLE IN THE BELGRADE EPIZOOTIOLOGICAL AREA

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Abstract: Q-fever is antropozoonosis which is caused by *Coxiella burnetii,* obligate intracellular pathogen. The most significant characteristics of this pathogen are resistance and stability in the environment, possibility of aerosol dissemination, and very low infective dose. *C. burnetii* can infect domestic and wild animals, rodents, birds and ticks. Q fever in animals is generally asymptomatic, although it can lead to reproductive disorders during pregnancy. The main route of infection in humans is inhalation of contaminated aerosol and dust. Serological studies have shown the presence of antibodies to *C. burnetii* in the serum samples of cattle in Belgrade epizootiological area. Seroprevalence of 18% was found in farm bred cattle, while it was only 1.5% in individual breeding. In farm bred cows that have suffered abortion prevalence was 49%, and only 1.9% in individual breeding. The overall results indicate that the circulation of this pathogen in cattle, in Belgrade epizootiological area, poses a health risk, not only to the cattle, but also to the humans, especially persons working with animals.
Q fever control programs most often recommend serological research and vaccination of animals. Accordingly, it is necessary to define a strategy for the implementation of biosecurity measures and preventive measures against Q fever.

Key words: Q-fever, *Coxiella burnetii*, abortus, serological surveys, antibody, inhalation.

Introduction

Q fever is one of the most well-known anthropozoonoses, present worldwide, with the exception of New Zealand (*Angelakis and Raoult, 2010*). The disease has also been recorded in Serbia, both in humans and in various species of animals. It was established for the first time in humans in 1937, in Australia (*Derrick, 1937*). It was clinically manifested by fever, and due to ignorance about

the nature of the disease, it was called *Q-query fever*. In the same year, the causative agent was isolated from infected people and named *Rickettsia burnetii (Burnet and Freeman, 1937)*. A year later, in the USA, the causative agent was isolated from ticks and named *Rickettsia diaporica (Davis and Cox, 1938)*. Out of gratitude to the researchers, the causative agent was renamed *Coxiella burnetii (C. burnetii).*

        C. burnetii is an obligatory intracellular bacterium, which belongs to the family *Coxiellaceae*, order *Legionellales*, class *Gamaproteobacteria* type *Proteobacteria (Raoult et al., 2005; Shaw and Voth, 2019)*. The species *C. burnetii* is the only member of the genus *Coxiella*. This bacterium is immobile, small, polymorphic, most often cocoid in shape and Gram negative (*Eldin et al., 2017*). The size is 0.2 - 0.4 μm x 0.4 -1 μm (*Abnave et al., 2017*). *C. burnetii* shows a two-phase life cycle that manifests itself in two different morphological forms - as a large cell variant (LCV) and a small cell variant (SCV). The SCV is 0.2 to 0.5 μm in size, while the LCV is larger in size > 0.5 μm (*Maurin and Raoult, 1999; Eldin et al., 2017*). SCV appears in a spore like form, with pronounced resistance to osmotic, physical, chemical and drying factors. This property ensures its long survival in external conditions and gives ecological stability (*Arricau-Bouvery and Rodolakis, 2005; Clark et al., 2018*). The SCV morphotype is metabolically inactive and represents the main infectious stage. The LCV morphotype is metabolically active and allows replication within the vacuole in the host cell (*Maurin and Raoult, 1999; Shaw and Voth, 2019*).

        According to the antigenic structure, *C. burnetii* is divided into two antigenic phases, namely virulent phase I and less virulent phase II (*Arricau-Bouvery and Rodolakis, 2005; Abnave, 2017*). Phases I and II are morphologically identical, but differ in some biochemical properties, including their lipopolysaccharide composition. Strains isolated from infected organisms have complete LPS, express antigen phase I and show high infectivity, while those with antigen phase II are less infectious and have incomplete LPS (*Porter et al, 2011; Gwida et al., 2012*).

        C. burnetii shows exceptional resistance in the environment. The SCV type survives for 7-10 months on wool at room temperature, for more than one month in fresh meat and over 40 months in milk (*Angelakis and Raoult, 2010*). It is resistant to the action of 1% formalin and 1% phenol for 24 hours, but it is inactivated by 0.05% hypochlorite and 1% lysol. Heating at 63°C only partially destroys *C. burnetii*. Pasteurization for 15 seconds at 71.5°C is required for safe destruction in milk *(Arricau-Bouvery and Rodolakis, 2005; Valčić et al., 2014).*

        Due to its pronounced resistance, stability in the environment, aerosol transmission, wind transmission and significantly low dose of infectivity, the Centres for Disease Control (CDC) has classified *C. burnetii* as a group of potential bioterrorism agents of category B *(Eldin et al, 2017; CDC, 2018)*. Also, the stated characteristics of the causative agent indicate the need for strict control when

working in the laboratory in accordance with the biosafety level standard 3 (*OIE, 2018*).

C. burnetii is a highly virulent bacterium (*Shrestha, 2020*). Research has shown that 1–10 viable bacteria (*Sawyer et al., 1987*), and even one bacterium, is sufficient to cause infection (*Waag, 2007*).

Q fever used to be rare and regionally limited. At the beginning of the 21st century, the disease has spread as a re-emergent zoonosis in many European countries *(Gwida et al., 2012; Dijkstra et al., 2012: Pandit et al., 2016)*. It is assumed that the spread of the disease is due to increased virulence of the pathogen, changes in the clinical picture, application of more reliable tests in diagnostics, as well as changes in epidemiological characteristics (*Aricau-Bouvery and Rodolakis, 2005*).

C. burnetii can infect a variety of animal species, including domestic animals such as cattle, sheep, goats, dogs, cats, also rodents, wildlife, reptiles, birds, fish, and ticks (*Angelakis and Raoult, 2010; Gwida et al., 2012*). Ticks play a significant role as reservoirs of pathogens, but also as vectors in the transmission of pathogens, especially from wild to domestic animals (*Cantas et al., 2011*).

In animals, Q fever is mostly asymptomatic, although reproductive disorders such as abortion, stillbirth, placental abruption, and foetal underdevelopment may occur during pregnancy *(Gwida et al., 2012; Pexara et al., 2018)*.

By monitoring of the epidemiological data, it was concluded that humans are most often infected by inhalation of contaminated aerosols and dust particles containing bacteria from infected animals. Most often, this contamination occurs during the birth of infected animals through products such as placenta, amniotic fluid, colostrum, etc. (*Maurin and Raoult, 1999; Vidić et al., 2008; Angelakis and Raoult, 2010*).

Consumption of contaminated raw milk and dairy products is also considered to be a potential source of infection for humans (*Boboš et al., 2013; Radinović et al., 2014; Pexara et al., 2018*). For people who come in contact with animals, such as veterinarians, livestock breeders, slaughterhouse staff, but also laboratory workers, Q fever is considered an occupational disease (*Maurin and Raoul, 1999; OIE, 2018*). According to the data of the City Institute for Public Health of Belgrade, in the period from 1984 to 2018, 78 cases of Q fever in humans were recorded in the area of the city (*https://www.zdravlje.org.rs/*).

The aim of this study was to determine the seroprevalence of *C. burnetii* in cattle in the Belgrade epizootiological area, whether they come from dairy farms or individual households. Based on the obtained research results, it will be possible to assess the epizootiological situation, and accordingly develop Q fever control programs.

Materials and Methods

Serological surveillance is carried out in many countries with the aim of assessing the prevalence of *C. burnetii* in domestic ruminants. The Rulebook on the Program of Animal Health Measures adopted annually by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, Veterinary Directorate, stipulates diagnostic tests in cases of abortion of domestic ruminants to Q fever. In accordance with that, the Scientific Veterinary Institute of Serbia conducts serological tests of cows that have had abortions. In addition, animals that had other reproductive disorders or were in circulation were also examined. The samples originated from commercial farms or individual agricultural households with extensive production, and are located in the epizootiological area of the city of Belgrade.

Blood samples for diagnostic tests were delivered during 2017, 2018 and 2019 to the Scientific Veterinary Institute of Serbia, Belgrade, by the competent veterinary stations. Blood sera were tested for the presence of antibodies against *C. burnetii* using a commercial ELISA test, ID Screen® Q Fever Indirect Multi-species/ ID Vet, Grabels, France. A total of 862 samples were tested, of which 226 originated from aborted cows. Serological tests were performed by stipulated methods performed in accordance with the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (*OIE, 2018*), and the test results were interpreted according to the instructions of the diagnostic kit manufacturer.

Results and Discusion

Our three-year research included the examination of bovine serum for the presence of antibodies against *C. burnetii* in cattle from the epizootiological area of the city of Belgrade. The tested sera originated from cattle reared on three commercial farms and cattle from individual agricultural households. A total of 862 samples were analyzed, of which 145 (16.8%) showed the presence of antibodies to *C. burnetii*. Significantly higher seroprevalence was found in cattle from farms than in cattle from individual agricultural households. The prevalence on farms 1, 2 and 3 was 17.4%, 9.0% and 39.2%, respectively. The antibodies to *C. burnetii* were found in only one sample of a total of 65 tested samples from individual agricultural households. The results (number/percentage) are shown in Table 1.

Table 1. Results of testing for the presence of antibodies to *C. burnetii* in bovine sera

	Year	2017	2018	2019	Total
Farm 1	Examined	412	245	101	758
	Positive/%	25/6	99/40	8/7.9	132/17.4
Farm 2	Examined	4	3	4	11
	Positive/%	0	0	1/25	1/9
Farm 3	Examined	2	25	1	28
	Positive/%	0	10/40	1	11/39.2
Ind. ag. households	Examined	37	12	16	65
	Positive/%	0	1/8.3	0	1/1.5
Total	Examined	455	285	122	862
	Positive/%	25/5.4	110/38.5	10/8.1	145/16.8

226 samples of a total of 862 were from cows after abortion. Of those 226 sera, 175 were from farm animals and 51 were samples of animals from the individual sector. The seroprevalence of Q fever was 38% in cows after abortion. Out of 175 farm animals, 86 (49%) were positive and only 1 sample of 51 cows from individual sector. The results of the analysis (number/percentage) are shown in Table 2.

Table 2. Results of testing the presence of antibodies to *C. burnetii* in the sera of cows after abortion

	Year	2017	2018	2019	Total
Farm 1	Examined	19	117	6	142
	Positive/%	9/47.3	61/52.1	4/66.6	74/52.1
Farm 2	Examined	0	3	4	7
	Positive/%	0	0	1/25	1/14.2
Farm 3	Examined	0	25	1	26
	Positive/%	0	10/40	1	11/42.3
Ind. ag. households	Examined	25	12	14	51
	Positive/%	0	1/8.3	0	1/1.9
Total	Examined	44	157	25	226
	Positive/%	9/20.4	72/45.8	6/24.0	87/38.4

The obtained results show that the highest number of positive animals was established during 2018, as a consequence of the epizootic incidence on Farm 1. We assume that the occurrence of the disease is related to the position of Farm 1, which geographically gravitates to the endemic area of the South Banat district. A milder form of the disease was found on Farm 3, also during 2018, with the remark that fewer blood samples were sampled from this farm. The prevalence of Q fever in the animals with abortion ranged from 47.3% to 66.6% on Farm 1, which was most exposed to the infection. At the same time, only 1 of 51 samples from individual rearing was serologically positive, which indicates a low prevalence, i.e. almost complete absence of Q fever in private sector. Previous research in Serbia in the area of Vojvodina showed that Q fever was found in 9.5% of herds (*Vidić et al., 2008*), while the percentage of infected heads in the population ranged from 5 to 80% (*Radinović et al., 2014*).

Serological surveillance of Q fever revealed significant differences in prevalence, not only between countries but also between individual regions. Thus, 82% of seropositive tests were found in the Netherlands (*van Engelen et al., 2014*), 72% in Germany (*Böttcher et al., 2011*), 48% in the Great Britain (*McCaughey et al., 2010*), 40% in Poland (*Jodełko et al., 2015*), 38% in Italy (*Galluzzo et al., 2019*) and 36% in France (*Gache et al., 2017*). A significantly lower percentage was recorded in northern European countries: 0.24% in Finland (*EVIRA, 2016*) and 8% in Sweden (*Ohlson et al., 2014*). Similarly, 3.4% of seropositive tests were found in the United States (*Angelakis and Raoult, 2010*). There are also differences in the number of infected animals in herds. There were 30% positive cattle in Saudi Arabia *(Jarelnabi et al., 2018)* and 15% in Turkey (*Gulmez and Sahin, 2016*). Lower percentages were found in cattle in Iran 3.23% (*Ghasemi et al., 2018*) and in Italy 5.28% (*Galluzzo et al., 2019*). In Great Britain, 12.5% of positive cows were determined among dairy cows (*McCaughey et al., 2010*), while in China the percentage for the same species was 33 (*El-Mahallawy et al., 2016*). Also, only 2.1% of positive cattle were found in Great Britain (*McCaughey et al., 2010*).

The results of the study indicate a correlation between ruminant abortions with *C. burnetii* infection (*Žutić et al., 2019*). In Cyprus, 35% of aborted cows, 33% of sheep, and 50% of goats were positive for *C. burnetii* (*Cantas et al., 2011*). Lower percentages were found in Italy, where 11.6% of cows and 21.5% of sheep and goats that had aborted were positive for Q fever (*Parisi et al., 2006*). In France, 2,695 cows, 658 sheep, and 105 goats that had aborted, seroprevalence of *C. burnetii* was found in 36%, 55.7%, and 61% of the herds, respectively (*Gache et al., 2017*). In Latvia, seroprevalence was recorded in 13.4% of herds where abortions occurred in dairy cattle (*Boroduske et al., 2017*). In the Netherlands, 3,264 cases of Q fever in humans were reported during the 2007–2010 epidemic (*Dijkstra et al., 2012*). Research has shown that the epidemic appeared in a narrower area where dairy goat farms were located, where abortions occurred in

waves. The cause of the epidemic is believed to have been the airborne transfer of contaminated dust particles from farms to densely populated areas.

Q fever is a very complex disease in both humans and animals, so control and eradication measures require a series of procedures over a long period of time. Q fever control programs most often recommend serological testing and vaccination of animals. Serological examination can identify positive herds and thus determine risk levels on farms and in the regions (*Valčić et al., 2014*). Rodents and ticks as reservoirs of pathogens should be systematically destroyed in natural habitats and on farms (*Vidić et al., 2012; Vidić et al., 2014*). By applying preventive and biosafety control measures, it is possible to reduce environmental contamination, and thus the risk to human and animal health.

Conclusion

The established seroprevalence of Q fever in our research indicates that *C. burnetii* circulates in cattle breeding in the Belgrade epizootiological area. A significantly higher percentage of infection was found in cattle on farms than on individual agricultural households. The presence of this pathogen represents a significant risk to human and animal health, in which the reproductive system is particularly endangered.

The implementation of serological surveillance is one of the most important preventive measures for mass testing and detection of infected animals. The obtained results impose the need for the application of remedial, preventive, biosafety and other animal health measures. But also research has to be continued on a larger number of samples for the future period. Control measures need to be coordinated with those in the region and/or surrounding countries in order to achieve better and geographically broader program to protect animals and humans from Q fever, as a global anthropozoonosis.

Seroprevalencija *Coxiella burnetii* kod goveda na beogradskom epizootiološkom području

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Rezime

Q groznica je antropozoonoza prisutna u celom svetu, izuzimajući Novi Zeland. Uzročnik bolesti je *Coxiella burnetii* (*C. burnetii*), obligatno intracelularna Gram negativna bakterija.

Najznačajnije karakteristike ovog patogena su otpornost i stabilnost u spoljašnjoj sredini, prenošenje putem aerosoli i veoma niske doze infektivnosti. *C. burnetii* može inficirati razne vrste životinja, uključujući domaće životinje, zatim glodare, divlje životinje, ptice i krpelje. Q groznica kod životinja prolazi uglavnom asimptomatski, mada tokom gravidnosti može dovesti do različitih reproduktivnih poremećaja i steriliteta. Ljudi se inficiraju inhalacijom kontaminiranih aerosoli i čestica kontaminirane prašine. Sprovedenim serološkim istraživanjima utvrdili smo prisustvo antitela za *C. burnetii* u serumima goveda sa beogradskog epizootiološkog područja. Ukupno je analizirano 862 seruma, od kojih su 16,8% bili pozitivni na Q groznicu. Znatno viša seroprevalencija, od 18% utvrđena je kod goveda na farmama nego kod onih iz individualnih uzgoja. U svega 1 od 65 seruma goveda iz individualnog uzgoja ustanovljena su antitela za *C. burnetii*. Od ukupno 862 seruma, njih 226 je bilo poreklom od krava koje su pobacile, i to 175 iz farmskog i 51 iz privatnog uzgoja. Seroprevalencija Q groznice iznosila je 49% kod krava poreklom sa farmi, a 1,9% kod krava iz individualnih uzgoja. Rezultati pokazuju da je najveći broj pozitivnih grla ustanovljen tokom 2018. godine kao posledica pojave epizootije na Farmi 1. Dobijeni rezultati ukazuju na cirkulaciju ovog patogena u populaciji goveda beogradskog epizootiološkog područja, što predstavlja rizik, ne samo za zdravlje goveda, već i za zdravlje ljudi, posebno onih koji rade sa životinjama. Programi kontrole Q groznice najčešće preporučuju serološka istraživanja i vakcinaciju životinja. Na osnovu rezultata ispitivanja i epizootiološke procene, potrebno je uraditi program sprovođenja preventivnih i biosigurnosnih mera radi kontrole Q groznice.

Ključne reči: Q groznica, Coxiella burnetii, pobačaj, serološki pregled, antitela, inhalacija.

Acknowledgements

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