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Original Article

Molecular and serological herd-level prevalence of *Coxiella burnetii* in bovine dairy herds in Montenegro

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Abstract

Introduction: Shedding of *Coxiella burnetii* through milk is significant, particularly in dairy cattle, making milk a potential source of infection for humans. The aims of this study were to estimate the individual and herd-level prevalence of *C. burnetii* on dairy cattle, and to assess potential public health risk.
Methodology: The study was conducted as a screening study in 95 randomly selected dairy herds from Montenegro from March to May 2019. No abortions, reproductive disorders, or human diseases were reported in these farms. In order to identify positive farms, anti-C. *burnetii* antibodies and C. *burnetii* DNA were detected in bulk tank milk (BTM) using enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR), respectively. All animals from the positive farms were sampled twice, 2 months apart; the presence of C. *burnetii* DNA in individual milk samples and the presence of anti-C. *burnetii* antibodies in milk and blood serum was detected using qPCR and ELISA.
Results: The overall herd-level prevalence of *C. burnetii* was 9.47% (9/95). Analysis of individual milk samples in the positive farms revealed anti-*C. burnetii* antibodies and *C. burnetii* DNA in 13.48% and 4.49% of the cows, respectively. Antibodies were also detected in 15.73% of the blood samples. No significant differences were observed between the results obtained through serological and molecular examination on the same farm two months later.
Conclusions: Although a low presence was detected in the farms, public health risk cannot be excluded. Further research is needed for unravelling the current epidemiological situation in the country.

Key words: Q fever; milk, cattle; ELISA; PCR.

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Introduction

Q fever is a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii*. Domestic ruminants such as cattle, sheep, and goats are significant reservoirs, and the most common source of infection for humans. The disease mainly causes late-term abortions and reproductive disorders in these animals [1]. Infected animals shed large amounts of the pathogen, particularly in birthing products, but also in urine, feces, and milk [2,3]. Shedding can persist for months, even in females with normal births. Milk is the primary route of shedding in cattle; while feces, urine, and lochia are more significant in small ruminants. *C. burnetii* can be shed in milk for up to 8 days in sheep, and up to 13 months in cows [4]. The pathogen's long-term survival in the environment as well as its low infectious dose for humans underscores its significance. Monitoring Q fever in animals is challenging due to the frequent absence of clinical symptoms of the disease, and reliable diagnosis can only be achieved through laboratory testing [5].

Given its high zoonotic potential, monitoring *C. burnetii* infection in domestic animals may be an important measure to prevent the disease in humans. Antibodies against *C. burnetii* have been detected in cattle milk in numerous countries, with herd prevalence rates ranging from 18% to 94% [6]. The increase in Q fever cases in Europe is likely due to the absence of monitoring and eradication programs in most European countries [7]. Additionally, the significant rise in the trade of live animals may also be a factor contributing to the spread of the disease. The European Food Safety Authority [7] has proposed herd-level diagnostics for Q fever as an important diagnostic procedure to mitigate public health risks. While vaginal swabs and birthing products are used for diagnosing abortion cases, bulk tank milk (BTM) samples are more suitable for assessing the health status of dairy herds and identifying

animals shedding *C. burnetii* [8]. *C. burnetii* shedding through milk raises concerns about the role of milk in human infection, particularly in regions where raw unpasteurized milk is consumed [9]. Active monitoring systems using polymerase chain reaction (PCR) testing of BTM have been suggested to estimate Q fever prevalence in animal populations. Previous reports of Q fever outbreaks in animals and humans in Montenegro indicated the potential role of dairy cows in the epidemiology of Q fever and its transmission to humans in the country; however, there is no wider research so far. Diagnostic laboratory testing of Q fever in domestic animals in Montenegro is carried out in order to detect the source of the disease after the epidemic is reported in humans and persons professionally exposed to infection, or when it is epidemiologically justified. Laboratory testing is also done in the case of clinical suspicion of the presence of the disease and in the case of abortion in cattle, sheep, and goats.

Data on Q fever occurrence in animals in Montenegro are scarce, mostly limited to serological testing of cattle, sheep, and goats, often following abortions. Q fever was serologically confirmed in 243 sheep, 132 goats, and 9 cattle across several municipalities between 2012 and 2016, with cattle cases recorded only in 2013 [10]. In 2018, the occurrence of Q fever in animals was registered in 210 sheep, 5 goats, and 46 cattle in Montenegro [11]. There is only one extensive epidemiological study conducted in sheep and dog populations, revealing seroprevalence rates of 5.03% and 1.16%, respectively [12,13]. The first case of Q fever in humans was etiologically verified in 1995–1996 by the indirect immunofluorescence (IIF) method. This was followed by 126 cases which were detected in humans in the period 1996–2017, and 12 patients treated for Q fever at the Clinic for Infectious Diseases in Podgorica in 2018–2019 [11]. These results highlight the need to carry out further surveys of Q fever in other animal species in this area as well. Although individual serological testing of cattle following abortions in Montenegro in recent years suggests cases of *C. burnetii* infection, comprehensive epidemiological studies in cattle have not been conducted.

The objectives of this study were to detect *C. burnetii* DNA in BTM samples from dairy farms in Montenegro using quantitative PCR (qPCR) and to determine the serological status of dairy cows by enzyme-linked immunosorbent assay (ELISA) of milk and blood serum. Since the serological status does not always correlate with *C. burnetii* shedding into the environment and some animals shedding *C. burnetii* are seronegative, direct pathogen detection is necessary. Thus, this combined approach allows detection of actively shedding animals (PCR) and identification of previous exposure within herds (ELISA), enabling accurate identification of positive farms and further investigation of the infection status through individual sample testing. This study also aimed to assess the potential public health risk posed by Q fever in this Mediterranean country where dairy production is socially and economically significant.

Methodology

Study area

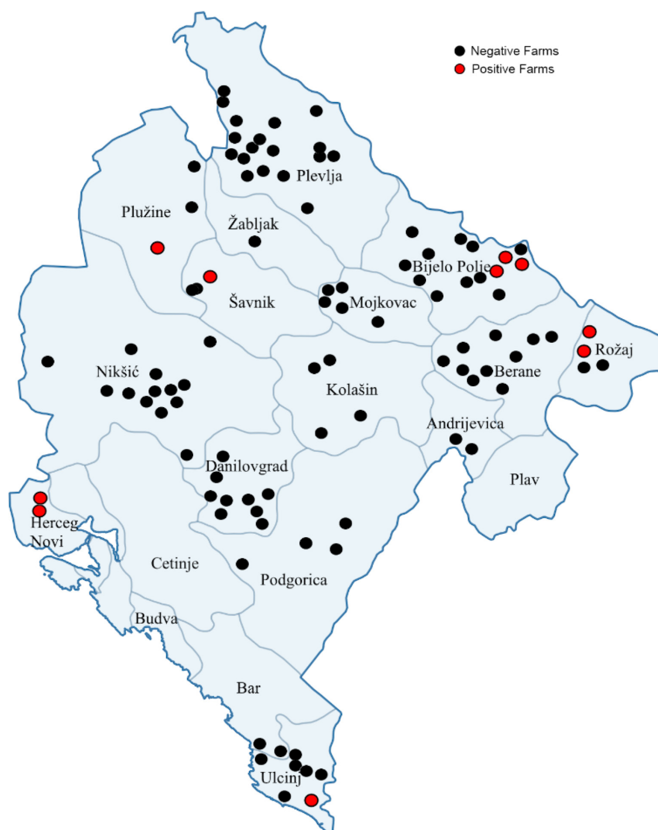
Montenegro is a state in south-eastern Europe and situated on the coast of the Adriatic Sea. Montenegro covers an area of 13,812 km^2 with the geographical coordinates 42°42'15.92" N and 19°23'44.80" E. Its 25 municipalities have a total population of 623,633 inhabitants. The climate in the northern part of Montenegro is continental, characterized by high daily and annual temperature oscillation and low annual precipitation. The climate in the southern part of Montenegro (coastal region) is Mediterranean, with hot and dry summers and relatively mild and rainy winters. The country has 59,469 dairy cows distributed across 21,939 farms, with an average of 2.70 cows per farm [14]. Small-scale farms dominate, while farms with 50 or more dairy cows are rare. Of all farms with dairy cows, 3,262 deliver milk to dairies or are registered for on-farm production of dairy products. The remaining farms produce milk for self-consumption. The total estimated cow milk production in Montenegro is around 168 million liters per year, of which approximately 15% is processed in approved milk processing facilities. The remaining milk quantities are outside the buy-in system for approved milk processing establishments and are used partly on farms for own consumption, while most of it is processed on farms into dairy products (cheese) using traditional methods without prior pasteurization. These products enter the market through retail food outlets (shops, hospitality facilities, farmers' markets), particularly during the summer tourist season, when a significant quantity of these traditionally recognized products is offered to tourists.

Study design and samples

The study was conducted on dairy farms that market milk or dairy products. The required sample size for this study was determined using standard methods for estimating disease prevalence in finite populations, considering a known population of 3,262 registered

dairy farms, 95% confidence level, and 10% error [15]. In the absence of reliable prior data on disease prevalence in the target population, the expected proportion was conservatively set at 0.5. The resulting sample size was adjusted using the finite population correction. The final value was rounded up conservatively to account for potential non-response and to ensure statistical robustness. The final sample size used in the study was 95 farms. Thus, bulk tank milk (BTM) samples were collected from 95 farms (with a total of 553 cows) across 17 municipalities representing different geographical regions of Montenegro (Figure 1) during March–May 2019. The study covered 60 farms with ≤ 5 cows, 23 with > 5 and ≤ 10 cows, and 12 with > 10 cows. The farms were selected using a random sampling method, and after obtaining permission of the owner. The list of identifiers of dairy farms supplying milk to dairies and those registered for on-farm dairy product production (mostly from unpasteurized milk) was obtained from the database of the Administration for Food Safety, Veterinary, and Phytosanitary Affairs (AFSVPA) in December 2018. A 100 mL BTM sample was collected from each farm. The farms where the BTM sample

Figure 1. Geographical position of the tested farms/smallholdings in Montenegro.



tested positive for the presence of antibodies against *C. burnetii* or for the presence of *C. burnetii* DNA were considered positive. Next, 10 mL of milk and 5 mL of blood were individually collected from all cows on those positive farms. The individual milk and blood samples were collected after two months from 89 cows belonging to 9 positive farms. Serological and molecular testing was conducted at the Diagnostic Veterinary Laboratory, Podgorica, Montenegro. The study was carried out in accordance with Montenegro's animal welfare regulations, and with special approval from AFSVPA.

Antibody detection

The bulk milk, individual milk, and blood samples were tested with a commercial ELISA kit for the detection of anti-*C. burnetii* IgG antibodies (IDEXX Q Fever Antibody Test, IDEXX, Switzerland GmbH, Liebefeld-Bern, Switzerland) according to the manufacturer's instructions. The reported sensitivity and specificity of the ELISA kit was approximately 100% as previously reported [16]. Milk serum samples were diluted 1:5, while blood serum samples were diluted 1:400. The optical densities (OD) of the samples were corrected by subtracting the OD of the negative control. The results were expressed as the ratio between OD of the sample (S) and the OD of positive control (P) included in the test kit. Based on the instructions from IDEXX S/P ≥ 40% was considered positive, S/P < 30% was considered negative, and results in the interval 30% ≤ S/P < 40% were considered to be intermediate.

Molecular methods

DNA extraction was performed from 200 µL of each milk sample using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *C. burnetii* DNA was amplified by qPCR to detect the presence of the insertion sequence *IS1111* (transposase gene) using the previously described primers and protocols [17]. The amplification was performed on an AriaMx PCR System (Agilent Technologies, Santa Clara, CA, USA). The QuantiTect Probe PCR Kit (Qiagen, Hilden, Germany) was used for amplification. The thermal protocol included an initial activation step at 95 °C for 15 minutes, followed by 45 cycles of denaturation at 94 °C for 15 seconds and annealing/extension at 60 °C for 60 seconds. The protocol was applied according to the manufacturer's recommendations. Each run included a positive control (DNA of *C. burnetii* Nine-Mile I strain) and a negative contol (RNAse free water). Samples with a Ct value lower than 40 were considered positive.

Table 1. Distribution of dairy farms and positivity percentages, divided by municipalities. The numbers are shown as positive samples/total number of tested holdings/dairy farms.

Municipality	Number of farms tested	ELISA positivity	PCR positivity
Andrijevica	2	negative	negative
Bar	1	negative	negative
Berane	9	negative	negative
Bijelo Polje	14	3 (21.43%)	negative
Danilovgrad	9	negative	negative
Herceg Novi	1	1 (100%)	negative
Kolašin	4	negative	negative
Mojkovac	5	negative	negative
Nikšić	12	negative	negative
Petnjica	2	negative	negative
Pljevlja	17	negative	negative
Plužine	2	1 (50%)	1 (50%)
Podgorica	4	negative	negative
Rožaje	4	negative	2 /50%)
Šavnik	2	1 (50%)	negative
Ulcinj	6	1 (16.67%)	negative
Žabljak	1	negative	negative
TOTAL	95	7 (7.37%)	3 (3.16%)

Holdings or dairy farms from which milk samples tested positive for the presence of antibody against *Coxiella burnetii*, or tested positive for the presence of *C. burnetii* DNA were considered positive. PCR: polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

Statistical analyses

The prevalence was calculated with a 95% confidence interval. Basic descriptive statistical parameters were determined, and a two-tailed paired t-test was applied to evaluate the statistical significance of differences in results obtained on positive farms between day 0 and 2 months later. Pearson's method was used to calculate correlation coefficients between the results. The agreement between serological and molecular methods for 95 BTM samples was assessed using Cohen's Kappa test. An agreement was considered perfect if the κ-value was > 0.81, almost perfect for κ-values between 0.61 and 0.80, substantial for values between 0.41 and 0.60, fair for values between 0.21 and 0.40, none to slight for values < 0.20, and no agreement for a value of 0 [18]. Data was analyzed using GraphPad Prism 6 (GraphPad, San Diego, CA, USA) and Microsoft Office Excel 2010 (Microsoft Corp, Redmond, WA, USA).

Results

The results of the BTM analysis are presented in Table 1. Antibodies against *C. burnetii* were detected in 7 out of 95 samples, while *C. burnetii* DNA was confirmed in 3 out of 95 BTM samples. The concurrent presence of both the antibodies and *C. burnetii* DNA was identified on a single farm. In total, 9 out of 95 farms, located in 5 out of 17 municipalities examined, tested positive (Figure 1).

The results of individual milk (ELISA and PCR) and blood testing (ELISA) are summarized in Table 2. In the case of positive farms, specific antibodies and *C. burnetii* DNA were identified in both milk and blood samples, with slight variations observed after 2 months

Table 2. Laboratory testing results of individual milk and blood samples on farms with confirmed *Coxiella burnetii* infection.

Positive municipalities	Positive farms[1] ID	Number of tested cows	ELISA/blood serum[2]				ELISA/Milk[2]				PCR/Milk[3]			
			Day 0		After 2 months		Day 0		After 2 months		Day 0[4]		After 2 months[5]	
			Positive	%	Positive	%	Positive	%	Positive	%	Positive	%	Positive	%
Bijelo Polje	BP1	4	1	25.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%	0	0.00%
	BP2	2	1	50.00%	1	50.00%	1	50.00%	0	0.00%	0	0.00%	0	0.00%
	BP3	12	4	33.33%	3	25.00%	3	25.00%	3	25.00%	0	0.00%	0	0.00%
Herceg Novi	HN1	9	2	22.22%	2	22.22%	2	22.22%	2	22.22%	0	0.00%	0	0.00%
Plužine	PL1	6	2	33.33%	2	33.33%	2	33.33%	2	33.33%	1	16.67%	0	0.00%
Rožaje	RO1	26	0	0.00%	0	0.00%	0	0.00%	1	3.85%	2	7.69%	1	3.85%
	RO2	8	0	0.00%	0	0.00%	0	0.00%	0	0.00%	1	12.50%	1	12.50%
Šavnik	ŠA1	8	2	25.00%	1	12.50%	2	25.00%	1	12.50%	0	0.00%	0	0.00%
Ulcinj	UL1	14	2	14.29%	2	14.29%	2	14.29%	4	28.57%	0	0.00%	1	7.14%
Total		89	14	15.73%	11	12.36%	12	13.48%	13	14.61%	4	4.49%	3	3.37%
Average		9.89	1.56	22.57%	1.22	17.48%	1.33	18.87%	1.44	13.94%	0.44	4.10%	0.33	2.61%
Maximum		26.00	4.00	50.00%	3.00	50.00%	3.00	50.00%	4.00	33.33%	2.00	16.67%	1.00	12.50%
Minimum		2.00	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%	0.00	0.00%
t value/*p* value[6]			0.1270.907				0.44/0.671				0.50/0.628			

[1] Farms with confirmed *C. burnetii* infection in bulk milk samples (PCR). [2] ELISA: number and percentage of positive serum or milk samples detected by IDEXX Q Fever Ab Test. [3] PCR: number and percentage of positive individual milk samples (real-time PCR). [4] Day 0: sampling immediately after infection confirmation. [5] After 2 months: control sampling two months later. [6] t value/*p* value: paired t-test comparing day 0 and 2-month results; $p < 0.05$ considered significant. PCR: polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay.

of follow-up. A statistically significant positive correlation between seropositive cows on day 0 and after 2 months (Pearson correlation, r = 0.97, $p < 0.001$) suggested that initially positive cows are likely to remain positive even after 2 months, indicating antibody stability in circulation and long-term seroconversion. The initial PCR results from BTM and individual milk samples were positive on two farms; however, negative results were obtained after 2 months, suggesting that shedding may have ceased or was sporadic. One farm was initially ELISA-positive but PCR-negative. *C. burnetii* DNA was detected in 1 cow in this farm after 2 months (Table 2). No statistically significant differences were observed between the results obtained on day 0 and after 2 months on farms with positive findings for antibodies against *C. burnetii* and its genome (Table 2). Comparison of results from serological and molecular methods performed specifically on BTM samples yielded a Kappa agreement coefficient of 0.163 (95% CI: – 0.173 to 0.499), indicating none to slight agreement between the results obtained by the tests.

A lower percentage of serologically positive cows was observed on larger farms (> 10 cows), which may indicate the implementation of better control measures on those farms.

Discussion

This study is the first to evaluate the distribution of *C. burnetii* in dairy cattle herds in Montenegro. Q fever occurs sporadically among all three species of domestic ruminants in Montenegro; however, comprehensive epidemiological studies have not been conducted, except for a 2001 study focusing on sheep [12] which examined 954 sheep using serological methods. In this study, testing was conducted on farms housing dairy cattle by analyzing BTM samples using serological and molecular methods. Collection of BTM represents the best choice for detecting *C. burnetii* infection in infected cows [19]. Sampling BTM has certain advantages—it provides a representative sample of all lactating cows; it is easy to collect, it is non-invasive, and it is cost-effective [20]. Although research often relies solely on serological testing of milk samples, determining the true prevalence of coxiellosis in herds requires additional PCR analyses. PCR and ELISA tests are the most commonly used diagnostic tools for herd-level detection in epidemiological studies conducted worldwide [21]. Serological analysis of BTM samples revealed potential prior exposure of the herd to *C. burnetii.* However, PCR detected *C. burnetii* in milk, thereby identifying current infections [19,22]. Therefore, the DNA-based test appears to be the most sensitive and rapid means for the direct detection of *C. burnetii* and the identification of shedding animals [19,23,24]. The different choices of sampling strategies and diagnostic tools used for diagnosing coxiellosis may explain variations in prevalence rates between countries [25].

The differences observed in the percentage of ELISA and PCR-positive farms demonstrate that the serological status of cattle does not always correlate with the shedding of *C. burnetii* into the environment. Identifying animals shedding *C. burnetii* continues to pose challenges, as the shedding dynamics are not yet fully understood [26,27]. In this study, it was found that two cows that were serologically positive and had *C. burnetii* DNA detected in their milk ceased shedding the pathogen after 2 months; and in the case of one cow that was serologically positive but PCR-negative, *C. burnetii* DNA was detected in its milk only after 2 months. A comparison of the results obtained through serological and molecular testing of milk using Cohen's kappa-based test showed a slight agreement between ELISA and PCR results from testing bovine BTM. This discrepancy could be explained from several perspectives. ELISA-positive and PCR-negative results (6 samples) may indicate prior infection without active *C. burnetii* shedding in cows. Conversely, the presence of PCR-positive and ELISA-negative results (2 samples) might be explained by a recent *C. burnetii* infection in cows that had not yet produced antibodies or that had antibody levels below the detection threshold [20,28,29]. However, comparing results obtained through serological and molecular methods is not entirely appropriate, considering that *C. burnetii* shedding can sometimes occur intermittently in seronegative animals and that animals experiencing abortion or premature delivery may remain seronegative [2]. Shedding can also occur through routes other than milk, such as vaginal mucus and feces [19]. The phenomenon of seronegative animals shedding the pathogen holds particular significance for both animal and public health. These findings highlight the need for direct pathogen detection (e.g., PCR or isolation) in cases of suspected infection or shedding, even in the absence of a serological response [30]. Serological testing of individual cow serum samples on farms revealed a seroprevalence of 15.73%, which decreased to 12.36% after 2 months. Indeed, as antibodies can persist for variable intervals after a pathogen is immunologically or therapeutically eliminated, serology does not confirm active or persistent infection. In this study, a lower number of

seropositive cows were observed on farms with larger herds, consistent with previous findings, although they were limited to PCR testing of BTM samples and did not include individual serological testing [22]. However, most studies have reported that antibody positivity is directly proportional to the herd size [28,31–33], while no correlation between herd size and seropositive animals was found in the study by Astobiza *et al.* [34]. This could have been partially due to variation in the proportion of age classes surveyed within the herd; which, in turn, was caused by differences in the age structure of cattle herds. Also, in this study, where the number of animals per herd was so low, even lower number of positive animals could have led to positive BTM results [34]. Moreover, as previously noted by other authors, BTM serological testing can yield false-negative results in herds with low intra-herd prevalence due to the dilution effect [35]. In this study, this limitation was addressed by performing individual milk sampling from all cows in BTM-positive herds for both PCR and ELISA testing; thereby minimizing the risk of false-negative results and allowing more accurate estimation of within-herd prevalence.

The observed prevalence of Q fever among dairy cattle in Montenegro was 9.47%, which is similar to the herd-level prevalence of 8% reported for Sweden [36], and it is significantly lower than the herd-level prevalence reported in Western or Central European countries such as 83.8% in France [26], 82% in the Netherlands [32], 79% in Denmark [31], 72% in Germany [37], 70% in Spain [34], 66.7% in Hungary [38], and 40% in Poland [39]. Other Northern European countries have reported even lower herd-level prevalence rates in dairy cattle—0% in Norway [40], 0.2% in Finland [41], and 13.2% in Latvia [27], indicating that *C. burnetii* infection in dairy cattle is significantly less prevalent in Northern Europe compared to Western and Central Europe. Unfortunately, there is a lack of comprehensive data on the prevalence of *C. burnetii* infections at the herd level in dairy cattle from Balkan countries surrounding Montenegro—Albania, Kosovo, Serbia, Bosnia and Herzegovina, and Croatia. However, a study investigating the prevalence of *C. burnetii* in dairy herds of various sizes from 6 countries in the Central and Eastern European region (Croatia, Czech Republic, Hungary, Serbia, Slovakia, and Slovenia), conducted between March and October 2019, revealed that the pathogen is widespread in the region, with an overall prevalence of 93.78%, while *C. burnetii* shedding was detected in 44.05% of the samples. A prevalence of 100% was recorded in Croatia, while in Serbia, it was 70.83% [42]. The relatively low prevalence of *C. burnetii* in the dairy cattle population in Montenegro in relation to the countries of Central Europe and neighboring countries is certainly not sufficient evidence to conclude that Q fever is not an endemic disease in the country. Thus, additional investigations, including studies on the population of small ruminants, are necessary. The detected prevalence of antibodies and *C. burnetii* DNA in milk samples (< 10%) indicates active circulation of *C. burnetii* in dairy cattle in the study area. However, the current status could be jeopardized by the practice of purchasing cattle from countries with a high percentage of infected animals. For these reasons, it is essential to adhere to precautionary measures, including quarantine, and determine the *C. burnetii* infection status of newly acquired cattle before introducing them into the herd [27]. Monitoring the status of Q fever in ruminants in neighboring countries is also crucial. In 2016, coxiellosis was confirmed in 10 humans in the border region with Serbia. Testing 30 cattle and sheep herds revealed an overall seroprevalence of 20.6%, concluding that infected animals were the primary sources of infection for humans [43]. In this study, the highest percentage of positive animals was recorded in the municipalities of Bijelo Polje and Plužine, located in border areas with Serbia and Bosnia and Herzegovina.

A significant annual increase in human Q fever cases of 54.63% was observed in Montenegro from 2002 to 2018. However, a notable annual decrease of 92.22% was recorded between 2018 and 2021 [44]. Q fever has re-emerged in the territory of Montenegro after several years of dormancy, specifically among dairy cattle in the municipalities of Danilovgrad and Nikšić. In addition to confirmed cattle infections, human coxiellosis cases have also been reported (unpublished data of the AFSVFA, 2024). Q fever is an underestimated disease in many countries due to inadequate reporting systems or misdiagnosis. As emphasized by the WHO, large outbreaks of Q fever in Europe highlight the potential risk of this disease developing into a significant public health concern.

A limitation of this study is that it was conducted on small-scale farms and individual households whose owners did not permit additional testing of individual samples beyond the specified interval. Consequently, long-term monitoring of seroconversion and pathogen shedding was not conducted. Farm owners were not surveyed regarding tick infestation, presence of rodents on the farm, history of recent abortions, and history of

infertility; nor were risk factors for positive cases among dairy cows investigated. The current findings and situation underscore the persistent need for well-designed studies to enhance understanding of the humoral immune response and pathogen shedding in the three main species of domestic ruminants. Such studies would contribute to an accurate assessment of the true prevalence of *C. burnetii* infection and support public health protection efforts [6]. Although Jado *et al*. reported that genotypes infecting humans are mainly associated with sheep, goats, wild boar, rats, and ticks; and that the only genotype identified in cattle from their study has not been detected in human clinical samples; the possibility of zoonotic transmission of bovine *C. burnetii* strains cannot be excluded and should be further investigated [45].

Conclusions

This is the first study presenting direct evidence of the presence of the *Coxiella burnetii* pathogen in dairy farms in the country. Surveillance must continue, especially considering that Q fever is a zoonotic disease and carries a significant risk of transmission to humans. Although oral transmission of Q fever to humans through the consumption of contaminated milk remains controversial; the risk of infection cannot be dismissed, particularly given the trend of consuming unpasteurized cheese in Montenegro. Furthermore, in order to assess the current epidemiological situation across the entire country, further studies are required in municipalities that were not included in this investigation. Regular cooperation between veterinary and human medicine is necessary in the exchange of information on all detected cases of the disease in order to implement joint disease control measures in a timely manner. In addition, education and incentives for individual household cheese producers to use thermally processed milk as a raw material for the production of dairy products is needed.

Authors contributions

DL methodology, data collection, data Interpretation, writing original draft; BA methodology, manuscript revision; MN manuscript revision; MS statistical analysis, manuscript revision; MS, manuscript preparation, manuscript revision; SO, conceptualisation, writing-review and editing manuscript supervision, validation. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

No conflict of interest is declared.

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