Seasonal impact on Q fever in sheep, Bosnia and Herzegovina


University of Sarajevo - Veterinary Faculty, Zmaja od Bosne 90, Sarajevo 71000, Bosnia and Herzegovina. nejra.hadzimusic@vfs.unsa.ba

Abstract
This study investigates the prevalence of *Coxiella burnetii* antibodies in sheep and examines the seasonal impact on Q fever distribution. A total of 253 blood samples from sheep in Bosnia and Herzegovina during summer and winter were used, and, the research employed ELISA testing for antibody detection. Findings revealed a significant seasonal variation in seroprevalence, with 41 positive cases identified: 37 in winter and 4 in summer, indicating a higher infection rate during colder months. Statistical analysis suggests a significant association (p<0.05) between season and infection rates; winter conditions, increased indoor density, and lambing activities may elevate transmission risks. These results underscore the importance of considering seasonal factors in Q fever management and surveillance in sheep, contributing to a better understanding of its epidemiology and informing public health strategies. The study highlights the need for further systemic-epidemiological research across different geographies and management practices to elucidate the full impact of seasonality on Q fever prevalence.

Key words: Q fever, *Coxiella burnetii*, sheep, seasonality, seroprevalence, ELISA.

INTRODUCTION
The long-standing proximity between humans and animals has facilitated the transmission of infectious diseases known as zoonoses, which are of increasing concern globally. One notable zoonosis is Q fever, caused by *Coxiella burnetii*, a gram-negative bacterium that can infect multiple host species (Roest et al. 2013). Notorious for its resilience in the environment and low infectious dose, *C. burnetii* is a professional hazard for individuals in animal-related occupations and has been classified as a potential biological weapon by the Center for Disease Control and Prevention (CDC) (Oyston and Davis 2011).

*C. burnetii* is an example of strict intracellular parasitism, as this bacterium has developed survival mechanisms in the phagolysosome, a compartment with...
the most unfavorable conditions for a bacterium’s life. Understanding how *C. burnetti* resists the degrading functions of the vacuole it resides in is a key thesis regarding the pathogenesis of this bacterium (Toman et al. 2012). *C. burnetti* is an organism stable in the environment and has the lowest infectious dose among all bacteria, with less than ten bacteria capable of causing infection. This bacterium infects several host species including humans, ruminants (cattle, sheep, and goats), pets, and in some cases reptiles, birds, and arthropods (Honarmand 2012). It is a professional disease of veterinarians, livestock farmers, butchers, and laboratory staff, making it one of the most common laboratory infections (Winter and Campe 2022). Domestic ruminants are cited as the main reservoirs of this bacterium.

Data on the prevalence of Q fever worldwide vary greatly. According to some reports, the estimated level of seroprevalence is 82% in cattle, while in sheep and goats it is somewhat lower, at 73% (Guattee et al. 2011). Other sources report a prevalence of up to 35% in sheep and goats, and even up to 90% in cattle (Neare et al. 2023). *C. burnetti* is excreted into the external environment as part of milk, blood, urine, feces, nasopharyngeal secretions, and during childbirth as part of the placenta (releasing over $10^9$ bacteria per gram of placenta), vaginal secretions, and amniotic fluid. Once they reach the external environment, the bacteria desiccate and are carried long distances via aerosols (Honarmand 2012).

Q fever is a zoonosis spread throughout the world, except for New Zealand (Cutler et al. 2007). Mammals, birds, and arthropods (mainly ticks) are considered the main reservoirs of *C. burnetti*, with cattle, sheep, and goats cited as the most common sources of infection in humans (Porter et al. 2011). Animals such as dogs and cats can also be reservoirs of *C. burnetti*, which is significant for the epidemiology of Q fever, especially in urban areas where these animals are in close contact with humans. Cases of human infection through contact with infected dogs and cats have been described (Komiya et al. 2003). Ticks are among the more significant reservoirs, and although inhalation is considered the primary route of infection, this pathogen circulates in nature through ticks, which are presumed to be responsible for heterospecific transmission and spatial dispersion among vertebrates (Duron et al. 2015). *C. burnetti* has been isolated from other types of arthropods (fleas, mites, bedbugs) but transovarial transmission or transmission of the pathogen to vertebrates has not been proven in them. In ticks, *C. burnetti* can survive for over 1000 days, and during feeding on an animal, ticks excrete large amounts of *C. burnetti* in feces (from $10^{-3}$ to $10^{10}$/g of feces), in which pathogens can survive up to 635 days (Eldin et al. 2017).

The prevalence of Q fever varies between different geographical areas, depending on epidemiological differences in those areas. Epidemics occur frequently and worldwide, including 415 cases of human epidemics. In Europe, cases of acute Q fever more commonly occur in spring and early summer. Sheep have been identified as the source of most epidemics, but goats are also considered an important reservoir. In most epidemics, confirmation of the identity of *Coxiella* strains present both in the source and in the host, for example by genotyping, is lacking, precisely because of the multitude of potential sources and the transmission of this bacterium over long distances by the wind (Roest et al. 2013). One of the largest epidemics of Q fever occurred in the Netherlands between 2007 and 2010, with more than 4,000 reported cases, and an estimate that the total number of cases was 40,000 (Delsing et al. 2010). In regions with widespread goat farming, the highest infection rate was observed (Roest et al. 2011). The public health strategy for controlling epidemics, developed by Dutch authorities, was implemented in the spring of 2008, mandating compulsory vaccinations for goat and sheep farms (Hogerwerf et al. 2011). As the measures taken proved ineffective, the following year it was decided to cull over 50,000 pregnant goats and sheep, leading to a reduced number of human deaths the following year (Schneeberger et al. 2014).

A hyperendemic area of Q fever is the capital of French Guiana, Cayenne, where *C. burnetti* causes 24% of pneumonic diseases, the highest prevalence reported worldwide (Epelboin et al. 2012). A dramatic increase in the incidence of Q fever was noticed in the 1990s, with the seroprevalence rate varying from 2% in 1992 to 24% in 1996. The incidence continued to rise, reaching up to 150 cases per 100,000 inhabitants in 2005 due to the presence of the most pathogenic strain described so far, present only in that area (Eldin et al. 2017).

Although the occurrence of Q fever is most commonly associated with rural environments and contact with domestic animals, Tozer and colleagues (2011) concluded that the seroprevalence of *C. burnetti* is the same in people in both rural and urban environments.

Seasonal changes and wind play a significant role in the development of Q fever epidemics in certain countries. Examples include Q fever epidemics recorded in southeastern France, where Tissot-Dupont et al. (2004) found a correlation between the incidence of infection, the presence of sheep, and the local mistrail wind in the town of Martigues. Also, in Germany, long-term research on Q fever revealed the impact of seasonal changes on the development of epidemics, where epidemics occur during the transition from winter to spring and from spring to summer as a result of lambing (Hellenbrand et al. 2001).

One of the larger epidemics was also recorded in the vicinity of Zadar, Croatia, where the spread of *C. burnetti* infection was contributed to by the north wind (bora) that disperses microorganisms, and the description of the epidemic noted 14 sick among 101 employees, in a plastic packaging factory located near pastures in a rural area around Zadar (Medić et al. 2005). The study’s primary goal is to determine the presence of *C. burnetti* antibodies in sheep serum and establish the impact of the season on the prevalence of Q fever in sheep.

**MATERIAL AND METHODS**

For the purpose of this retrospective study, a total of 253 blood samples from sheep across Bosnia and Herzegovina (Figure 1) were collected from January to...
March (winter season) and June to September (summer season) during the year 2019. Blood was drawn from the jugular vein into coagulation accelerator tubes (5 mL) using 18-gauge needles. The blood samples were transported at refrigeration temperature (+4°C) to the Veterinary Faculty of the University of Sarajevo, where the samples were centrifuged for 15 minutes at 3,000 revolutions per minute. The serum isolated in this manner was stored in tubes at -20°C. Serum analysis was performed in the Laboratory of Virology and Serology at the Veterinary Faculty of the University of Sarajevo.

For immunoassay testing, an ELISA test (IDEXX-Q Fever C. burnetii-Antibody Test Kit was used. The OD values of the samples were analyzed in relation to positive and negative controls using the following the manufacturer instructions. The results were interpreted by calculating the S/P % = 100 × (S − N)/(P − N) where S, N, and P are the OD (450 nm) values of the test sample, negative control, and positive control, respectively. Samples were considered to be ELISA positive, if S/P % ≥ 40; suspect, if 30 ≤ S/P % < 40; and negative, if S/P % < 30.

Statistical analysis. The research findings were statistically analyzed using the chi-square test of independence (χ² test) to determine the association between the occurrence of infection with C. burnetii and the seasonal trend (winter vs summer) of Q fever. The analysis was conducted using the software package SPSS Statistics by considering 5% level of significance and 95% confidence intervals (95% CI). P values of <0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Our study, conducted on sheep blood serum samples during the year 2019 in Bosnia and Herzegovina, identified 41 of 253 (16%) positive animals for the presence of antibodies to C. burnetii (Table 1).

During the summer season, four individuals were found to have developed an immunological response to C. burnetii, while analysis of samples collected during the winter season identified 37 positive samples (Table 1). Distribution of positive and negative samples in relation to the sampling season is presented in the Table 1.

Table 1. Blood serum analysis results according to the season of sampling

<table>
<thead>
<tr>
<th>Season</th>
<th>Number of Positive Samples</th>
<th>Number of Negative Samples</th>
<th>Total Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>37</td>
<td>24</td>
<td>61</td>
</tr>
<tr>
<td>Summer</td>
<td>4</td>
<td>188</td>
<td>192</td>
</tr>
<tr>
<td>Both seasons</td>
<td>41</td>
<td>212</td>
<td>253</td>
</tr>
</tbody>
</table>

Our research results indicate that the percentage of positive samples in the winter season (61%) is higher than that in the summer season (2%). Besides the fact that the number of individuals that developed an immunological response in the winter period is higher compared to the summer, the mentioned differences between seasons are also statistically significant (p<0.05). The possible reason for increased detection of C. burnetii infection in the winter season is considered to be the increased density of sheep within barns and the longer period of sheltering in the same. Indeed, the aggregation of a larger number of animals in barns during the cold period of the year may contribute to the transmission of infection due to inhalation of C. burnetii-contaminated dust particles (Welsh et al. 1957, Yanase et al. 1997). In the context of intensive farming systems, contact between healthy and infected animals represents a higher risk for C. burnetii infection than extensive grazing systems (Yanase et al. 1997). The increased number of positive samples in the winter period, according to reports, is also caused by lambing or abortion, which potentially leads to the excretion of larger quantities of C. burnetii into the environment through milk, as well as vaginal excreta of infected animals (Agerholm 2013, Kargar et al. 2013).

Extensive research on the impact of season on the occurrence of Q fever in sheep is not available in the literature. Available literary sources provide conflicting research results (Wolf et al. 2020, Debeljak et al. 2018). For example, studies conducted in the border region between Serbia and Montenegro, at the end of March and beginning of April 2016, showed a large number of positive sheep in that season (Debeljak et al. 2018). Specifically, to determine the immunological response in sheep, mini farms were tested, and the seroprevalence was found to be 68.8%. High seroprevalence in this period is explained by the authors as a consequence of lambing, sheep shearing, and other outdoor activities (Debeljak et al. 2018). However, a study conducted by Wolf et al. (2020) found that in southern Germany, the predominant sheep breed is Merino Landrace, known for lambing throughout the year, even during the summer months when conditions are optimal for the survival and aerogenic spread of C. burnetii. Conversely, in northern Germany, where sheep exhibit...
seasonal breeding patterns and commonly lamb indoors in spring due to the region’s cold and humid climate, the aerogenic transmission of *C. burnetii* appears to be less probable. The rate of animal loss due to abortion and weak offspring during the lambing season can range from 3% to 80%, and the presence of *C. burnetii* in serums is subject to increasingly frequent serological tests. Within a research project from 2009 (Eibach et al. 2012), analysis of blood serum samples (n=261) using the ELISA test showed antibodies against *C. burnetii* in 47% of individuals, confirming the presence of the infectious agent in humans immediately after the lambing season. The obtained titers confirmed the assumption that the infection had occurred earlier, and therefore likely at the time of lambing.

Literature references (Banazis et al. 2010, Wolf et al. 2020, Probst et al. 2021) on the number of positive samples for *C. burnetii* in sheep vary depending on the degree of sheep manipulation, physiological status, and geographical location. For instance, a study conducted in southwestern Turkey (Kilic et al. 2005) found only 3% positive samples when 100 sheep were sampled during March and April 2002, testing for the presence of IgG antibodies to phase II *C. burnetii*. Explaining the low seroprevalences, the authors highlight the fact that blood serum sampling of the examined sheep was done outside the lambing season. During lambing, higher prevalences of *C. burnetii* infection are expected since pregnancy is a significant factor in the outbreak of Q fever, as there is a considerable increase in microorganisms within the trophoblast of the placenta (Kilic et al. 2005). Contrary to these findings, other authors’ research in eastern Turkey from June to December identified a higher seroprevalence (10.5%), with the authors citing the lambing season and a history of abortion in the examined flocks as reasons for the elevated seroprevalence (Çetinkaya et al. 2000). Higher seroprevalence can also be explained by local ecological factors, conditions and type of breeding (extensive, semi-intensive, and intensive systems), flock size, and differences in laboratory sample testing procedures, as stated in a study conducted in Iran in 2017 (Mobarez et al. 2017).

Results from a study conducted in Egypt in 2019 show that the prevalence of antibodies against *C. burnetii* tends to increase during the summer months (Sobhy et al. 2019), and a greater number of cases of *C. burnetii* infection in the summer season was proven in the study by Vilbic-Cavlek et al. (2012). However, some studies shown the increased detection of *C. burnetii* infection during the winter season; authors link these results to the higher density of animals and prolonged periods of confinement in barns. The close proximity of animals in barns during colder months may facilitate the rapid spread of the infection through the inhalation of dust particles contaminated with *C. burnetii* (Yadav et al. 2021). Furthermore, in intensive farming systems where healthy animals are housed close to infected ones, there is an elevated risk of transmission of *C. burnetii* compared to animals in extensive grazing systems (Yadav et al. 2021).

From the aforementioned, it is clear that in light of the results obtained by our research, further examination of the impact of seasonal changes on *C. burnetii* infection through more extensive systemic-epidemiological studies in different geographical areas, as well as under different zoo-hygienic conditions, is necessary. The heterogeneity of available results in the global literature presented in this paper’s discussion opens the door to new hypotheses regarding the causes of *C. burnetii* infection and the detection of immunological response in sheep during different seasons. In addition to seasonal variations, the geographic location of sampling and zoo-hygienic conditions of sheep keeping, the characterization of infectious material such as placenta and amniotic fluid (after abortion), vaginal fluids, urine, feces, and milk is important for understanding the potential risk of future Q fever epidemics (Oliveira et al. 2017).

The significance of our research is precisely through the role of sheep as a source of infection for humans. Indeed, their role is proven in those cases where other possible sources of infection are excluded in epidemiological investigations, and the presence of infection in sheep is proven. In the study conducted by Saglam and Sahin (2016) on sheep positive for Q fever, the presence of *C. burnetii* in milk samples was proven, indicating a potential risk to the health of humans and animals.

In Bosnia and Herzegovina, the primary sources of animal protein consumed by humans include beef, lamb, and poultry. Lamb is a significant part of the diet, especially in rural areas where sheep farming is common. This fact is particularly relevant in discussions about public health and food safety, given the findings from our study demonstrated presence of *C. burnetii* in blood samples, underscoring a potential health risk to both humans and animals. Thus, it is crucial to consider these factors in any discussion about food safety practices in regions where lamb is a common dietary staple.

Our research results also support data from relevant literature related to the widespread prevalence of Q fever. Especially from the aspect of species, where the role of sheep in the epizootiology and epidemiology of Q fever is gaining importance and is increasingly the subject of research in different countries, both in urban and rural environments (Angelakis and Raoult 2010). When it comes to Q fever in Bosnia and Herzegovina, the results of a descriptive-analytical study record the most common prevalence of this zoonosis in sheep (10.7%), compared to cattle (5.7%) and goats (3.5%) during 2017 (Toman 2020).

The transmission of Q fever is underscored by the fact that in 2012, according to a report by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), the European Union (EU) confirmed 643 cases of Q-fever in humans. Almost all member states that reported found *C. burnetii* in sheep, cattle, and goats, indicating that this bacterium is widespread in the EU (EFSA and ECDC 2014).

Given that Q fever has been identified as a reemerging zoonosis in most parts of Europe and as the second most frequently reported laboratory infection (OIE 2019), a better understanding of the immune system’s reactions to *C. burnetii* infection is needed to more clearly view the processes the bacterium develops for its own adaptation. This would also allow for a more precise interpretation of the ultimate outcome of the infection, whether it is asymptomatic, acute, or chronic (Porter 2011). Most studies conducted to confirm the immunological response
to C. burnetii in sheep, as in our case, used the ELISA immunoassay test. This method is generally the method of choice in veterinary medicine because it is practical to perform, suitable for screening a large number of animals, and has high specificity (Roussel et al. 2007, Niemczuk et al. 2014).

Finally, the significance of our research is reflected through the increasingly relevant One Health concept. This concept involves the systemic integration of biomedical and other sciences, emerging as a justified solution to today’s challenges related to improving the health and welfare of humans and animals. The inseparability of human health, animal health, and environmental quality has been extensively proven, with a concurrent trend in the importance and extent of interaction. This is confirmed by studies on human epidemics of Q fever, which are connected with domestic and wild ruminants.

The obtained results proved that there is an impact of the season on the spread of Q fever in the context of the lambing period, conditions and types of sheep breeding, and flock size. Furthermore, statistical analysis of the obtained results determined a statistically significant (p<0.05) impact of the season on the number of positive individuals.

Conclusion of our study is the significant seasonal variation in the prevalence of Q fever, evidenced by a higher rate of positive samples during the winter season compared to the summer. This finding suggests that the increased density of sheep in barns during colder months, along with conditions associated with lambing or abortion, significantly elevates the risk of C. burnetii transmission.

Furthermore, our research underscores the effectiveness of the ELISA test as a fast, simple, and practical method for routine serological analysis of Q fever in sheep, affirming its utility in veterinary practice. Given the potential risk to human health from C. burnetii, especially through the handling of infected animals or consumption of contaminated animal products, these results are crucial for developing targeted interventions to control the spread of Q fever, particularly in regions with intensive sheep farming.

This study also highlights the need for continuous monitoring and research to understand better the epidemiological patterns of Q fever, advocating for a One Health approach that integrates human, animal, and environmental health strategies to mitigate this zoonosis effectively.

ORCID

Hadžimusić, N.  nejra.hadzimusic@vfs.unsa.ba, https://orcid.org/0000-0001-9278-1876
Velić, L.  lejla.velic@vfs.unsa.ba, https://orcid.org/0000-0001-8764-7023
Cengić, B.  benjamin.cengic@vfs.unsa.ba, https://orcid.org/0000-0001-9020-8435
Livnjak, A.  amela.livnjak@vfs.unsa.ba, https://orcid.org/0000-0002-5318-5147
Šerić-Haračić, S.  sabina.seric-haracic@vfs.unsa.ba, https://orcid.org/0000-0001-9102-3467
Hrković-Porobija, A.  amina.hrkovic@vfs.unsa.ba, https://orcid.org/0000-0002-9966-9411

REFERENCES


Citación recomendada