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RESEARCH ARTICLE

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Prevalence of Coxiella burnetii infection and risk factors in aborted sheep and goats in Kerman province, south-east of Iran

Mohaddeseh Soltani Nejad, Mehdi Golchin, Mohammad Khalili, Elham Mohammadi, Mohammad Ali Shamshirgaran

Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

ABSTRACT

Coxiellosis, also known as Q fever, is a zoonotic disease caused by Coxiella burnetti and has become a significant cause of small ruminant abortion globally. Q fever is endemic in Iran, but there is a dearth of epidemiological data regarding the true prevalence of *C. burnetii* in some areas of Iran. Small ruminants, mainly goats and sheep, are considered the primary reservoir for human infection, posing a considerable threat to human health. Theses reservoirs can shed the bacterium into vaginal mucus.The objective of this study is to examine the prevalence of coxiellosis through the detection of the *IS1111* gene of C. burnetii using Real-time PCR and also to identify the related risk factors (such as the location of livestock, age, species, and parity) associated with the disease in the vaginal discharge of small ruminants residing in Kerman province, located in the southeast of Iran. During the winter of 2019 and autumn of 2020, a total of 134 vaginal samples from aborted small ruminants (70 samples from sheep and 64 from goats) were gathered from 32 herds located in different areas of Kerman province. The results showed that 26 samples, comprising 14 from goats (21.88 %) and 12 from sheep (17.14 %), were positive for coxiellosis. According to the findings of our study, the detection of *C. burnetii* showed that coxiellosis is circulating in the studied area. Additionally, our analysis revealed no statistically significant association between the prevalence of Q fever and small ruminants' location, number of parturition, and age that were examined as potential risk factors.

Keywords

Q fever; Coxiellosis; Real-time PCR; Small ruminants; Prevalence; Kerman province

Abbreviations

SCV: small cell variant
LCV: large cell variant
POMP: Polymorphic Outer Membrane Proteins
PBS: phosphate-buffered saline
PCR: Polymerase chain Reaction
CDC: Centers for Disease Control and Prevention

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Corresponding author: *Mehdi Golchin*
Email: *golchin@uk.ac.ir*
Tel: *+98(34)3332-2913*

Introduction

Coxiellosis, also known as Q fever, is a zoonotic disease with a global distribution and is caused by the pleomorphic Gram-negative intracellular bacterium Coxiella burnetti (*C. burnetii*) [1,2]. This pathogen is classified within the Coxiella genus, Coxiellaceae family, and Gammaproteobacteria class based on 16S rRNA sequence analysis [3,4]. C. burnetii can endure environmental conditions for prolonged periods throughout its developmental cycle. This non-replicating form of the disease plays a crucial role in spreading the infection to humans and animals [1,5–7]. Upon entry into host cells, the spore-like form of *C. burnetii*, known as the small cell variant (SCV), transforms into the actively replicating large cell variant (LCV) before ultimately being released from the host cell as SCV once again [7–10]. Due to its capacity for rapid dissemination over long distances in a short period, C. burnetii has been included in the "B" list of potential biological weapons by the Centers for Disease Control and Prevention (CDC) [11]. The zoonotic nature of Q fever has been recognized by its inclusion in the OIE Animal Health Code, with Member Countries required to report any incidence of the disease to the OIE [10]. This pathogen exhibits a broad host range, affecting humans and numerous animal species, such as avian and mammalian species, with ruminants being the principal reservoirs [10–12]. Livestock serves as the primary reservoir of the microorganism, which can lead to Q fever outbreaks in humans [13].

In humans, acute infection typically presents as a self-limiting, influenza-like illness, whereas endocarditis is the predominant symptom in chronic cases. However, untreated acute infections may progress to chronic infections in 1-5% of cases [7,13,14]. Coxiellosis in small ruminants is usually asymptomatic, but chronic infection may result in reproductive disorders, such as stillbirth, weak offspring, infertility, endometritis, milk yield losses, and abortion [10,13,15].

The occurrence of abortion in animals following colonization of the placental macrophages by *C. burnetii* is attributed to the organism's high tropism for reproductive organs. Consequently, vasculitis, diffuse inflammation, necrosis, and calcification in the inter-cotyledonary region may ensue [15]. Notably, the pathogenicity of *C. burnetii* is influenced by virulence factors such as lipopolysaccharide (LPS) and host factors such as pregnancy-related immunosuppression [7,10,15]. In addition, the high affinity of the bacterium for the placenta results in a higher incidence of infection in females than males [16].

Phase I of *C. burnetii*, the virulent form characterized by smooth full-length LPS, undergoes phase variation to Phase II (rough LPS) after multiple passages in cell culture. Phase II is less contagious than Phase I [7,10].

In humans and small ruminants, inhalation of contaminated aerosols is the primary route of infection, with the bacterium being excreted by infected animals via various bodily fluids, such as urine, milk, vaginal discharge, fetal fluids, and abortion products [12,17]. Goats and sheep have been shown to shed the organism in their vaginal mucus for weeks [8,18]. Other transmission modes include consuming contaminated milk and dairy products, handling contaminated tissues, direct contact, and sexual and vertical transmission [13,19,20].

Effective control and prevention of C. burnetii infection are critical due to the organism's high environmental resistance, low infectious dose (less than ten bacteria), multiple transmission routes, presence in different reservoirs, and rapid spread over long distances via wind [7,11,13]. Laboratory tests, including staining, serological, and molecular tests, are more useful than clinical diagnosis for identifying C. burnetii, as no specific clinical signs are associated with this disease. This underscores the importance of appropriate diagnostic measures in the early detection and management of C. burnetii infection [21,22].

C. burnetii is challenging to identify using traditional staining techniques, and serological assays have limitations in detecting the bacterium in shedder animals [5,12,23]. The OIE recommends using Polymerase chain reaction (PCR) as a highly sensitive and specific method for detecting *C. burnetii*. PCR amplifies specific DNA sequences from the bacterium, enabling accurate detection and surveillance of the infection in humans and animals. The PCR-based method has become essential for diagnosing and monitoring C. burnetii infection, especially when traditional diagnostic approaches are inadequate [24].

Studies have demonstrated that molecular identification tests such as PCR and quantitative Real-time PCR (qPCR) are highly effective in detecting *C. burnetii* in abortion materials due to multiple copies of the bacterial genome's insertion sequence and no need for prior isolation or cultivation. They can potentially improve the accuracy and efficiency of disease diagnosis in both veterinary and human medicine [5,12,25].

The PCR-based assay has been shown to have high specificity for detecting *C. burnetii* in vaginal abortion samples without cross-reacting with other bacteria present [26]. However, inadequate epidemio-

Abbreviations-Cont'd

LPS: Lipopolysaccharide
qPCR: quantitative Real-time PCR
ELISA: Enzyme-Linked Immunosorbent Assay.

logical studies and a lack of information about Q fever have resulted in misdiagnosis and the re-emergence of the disease in different regions of Iran [11]. To address these problems this study was conducted to determine the prevalence of *C. burnetii* in small ruminants of Kerman province using real-time PCR. This research aimed to recognize the risk factors linked with coxiellosis to enhance the control and management of Q fever in the targeted area. Using PCR-based assays in disease surveillance and diagnosis can improve the accuracy and efficiency of disease management strategies in veterinary and human medicine.

Result

Our investigation involved collecting 134 samples, comprising 70 from aborted sheep and 64 from aborted goats, of which 26 samples (19.4%) tested positive for coxiellosis using the Real-time PCR method. The prevalence of infection was 21.87% (14 out of 64) and 17.14% (12 out of 70) in goat and sheep samples, although the difference was not statistically significant. We also examined the prevalence of *C. burnetii* infection by analyzing independent variables, including the location of livestock, parity, age, and species of small ruminants, as presented in Table 1. It is also revealed that the prevalence of coxiellosis did not exhibit any significant association with age categories in sheep or goats ($p < 0.05$). No significant correlation was observed between the prevalence of coxiellosis and parity in either goats or sheep ($p < 0.05$). However, we observed a rise in the prevalence of *C. burnetii* infection in sheep as the number of parturitions increased.

Our investigation found a variation in the prevalence of coxiellosis across the studied regions, ranging from 0% in Kerman to 30% in Baft for goats and 0% in Bam to 22% in Kerman for sheep. However, we did not observe a significant correlation between the location and the occurrence of infected animals, as determined by a p-value of less than 0.05 (Table 2).

Table 1.
Prevalence of infection *C. burnetti* by independent variables included the location of livestock, animals, parity and age in sheep and goat.

Variable	Different groups in each variable	No. of animals (%)	Test for C. burnetii (PCR)		Prevalence (%)	Odds Ratio	95 % CI	P-value
			+ve	-ve				
Animals	Sheep	70 (52.24)	12	58	17.14	1.00		0.64
	Goat	64 (47.76)	14	50	21.88	1.35	0.56-3.02	
Parity	<2	59 (44.03)	10	49	16.94	1.00		
	2-4	40 (29.85)	8	32	20	1.22	0.42-3.23	0.90
	>4	35 (26.12)	8	27	22.86	1.45	0.49-3.91	0.66
Age	<2	46 (34.33)	7	39	15.22	1.00		
	2-4	49 (36.57)	12	38	24.49	1.76	0.60-4.55	0.41
	>4	39 (29.10)	7	31	17.95	1.26	0.42-3.73	0.92
Location of livestock	Baft	60 (44.78)	15	45	25	1.00		
	Bam	10 (7.46)	1	9	10	0.33	0.02-2.11	0.52
	Bardsir	25 (18.65)	5	20	20	0.75	0.27-2.15	0.83
	Kerman	14 (10.45)	2	12	14.29	0.5	0.10-2.19	0.61
	Shahr-e-babak	25 (18.65)	3	22	12	0.41	0.12-1.47	0.30

CI=95% confidence interval
P-value for difference of prevalence of groups belonging to each variables
Significant variables based on $p < 0.05$

Table 2.
Relationship between positivity for *C. burnetii* in vaginal samples from aborted small ruminants of small ruminants and main risk factors such as province, number of parturition, and age.

	Category	Goat	Prevalence	CI	P-value	Sheep	Prevalence	CI
Parity	<2	7/31	22.58%			3/28	10.71%	
	2-4	3/14	21.42%	0.23-3.69	0.76	5/26	19.23%	0.43-8.09
	>4	4/19	21.05%	0.26-3.90	0.82	4/16	25%	0.64-12.11
Age	<2	5/20	25%			2/26	7.69%	
	2-4	7/27	25.92%	0.26-3.80	0.79	5/22	22.72%	0.60-18.88
	>4	2/17	11.76%	0.07-2.55	0.55	5/22	22.73%	0.60-18.88
Location of livestock	Baft	8/26	30.76%			7/34	20.58%	
	Bam	1/4	25%	0.05-5.79	0.72	0/6	0%	0.00-2.78
	Bardsir	3/15	20%	0.14-2.75	0.70	2/10	20%	0.17-5.14
	Kerman	0/5	0%	0.00-2.138	0.38	2/9	22.22%	0.20-6.26
	Shahr-e-babak	2/14	14.28%	0.07-1.74	0.44	1/11	9%	0.03-2.97

CI=95% confidence interval
P-value for difference of prevalence of groups belonging to each variables
Significant variables based on $p < 0.05$

Discussion

Q fever is a re-emerging zoonotic disease caused by C. burnetii. In recent outbreaks, C. burnetii has been identified as one of the responsible agents for abortion in small ruminants [3,8,27]. The disease was initially discovered in 1935 among abattoir workers in Australia and has since been reported in various parts of the world [28]. The first case of Q fever in Iran was documented in 1952 [11].

Over the past few years, Q fever has been identified as a significant cause of abortion in small ruminants, resulting in substantial economic losses for affected communities [3,6]. There is also a concern that ruminants infected with C. burnetii may serve as a potential source of human infection, posing a risk to public health [13].

The significance of coxiellosis is being overlooked in Iran [14]. The primary data on the disease in small ruminants predominantly consists of serological information, and the lack of a highly sensitive detection method hinders the conduction of molecular surveys [15,18]. The precise identification of C. burnetii using molecular techniques is a crucial factor in preventing the spread of this pathogen amongst both humans and ruminants, highlighting the importance of developing more accurate detection methods [29].

The most effective methods for diagnosing coxiellosis from vaginal mucus involve directly detecting the bacterium through PCR or the Real-time PCR approach [3,18]. Vaginal mucus is a significant route for shedding C. burnetii by infected ovine and caprine into the environment.

This study aimed to determine the prevalence of coxiellosis in vaginal samples from aborted ovine and caprine from ovine and caprine in Kerman province using the Real-time PCR method, which is a highly sensitive and accurate diagnostic approach. The overall prevalence rate was approximately 20%, with 17% in sheep and 22% in goats. These findings are consistent with previous studies conducted in Iran, which detected C. burnetii DNA in aborted fetuses at rates of 17.95% (20.43% in goats and 15.47% in sheep) using nested and RT-PCR [30], and prevalence rates of 17.3%, 20.8%, and 16.6% in sheep in different regions of Iran [5,31,32].

The frequency of coxiellosis in our study was found to be higher than the rates reported in western Iran (0.9% in sheep) and southern Iran (2% in ruminant fetuses) [25,33]. However, the frequency of coxiellosis in our survey was lower than that reported in other studies conducted in Iran, including those in Ardabil, Mazandaran, the southeast, and northeast regions of Iran [2, 34-36], as well as a serological survey in southeast Iran [37]. A review of coxiellosis cases in Iran between 2000 and 2015 reported 27% and 33% infection rates in sheep and goats, respectively [20].

Variations in the reported prevalence rates of coxiellosis in different surveys may be attributed to several factors, including differences in animal management practices, timing and method of sampling, and type of diagnostic test used to detect the bacterium [6,10,12,17,18,38]. In a recent study, the prevalence

of infection is higher in goats than in sheep, which is consistent with most available data in Iran and other countries [11,16,20,39–44]. The difference in prevalence rates between the two species may be due to differences in their immune response or shedding of the bacterium during two consecutive delivery periods in goats [6,11]. Although our study demonstrated that the prevalence of infection is higher in goats than in sheep, it was not significant. A similar trend has been reported in animals with a history of abortion in Iran [17].

The prevalence of coxiellosis in small ruminants varies across different countries, as reported in several studies [1,6,45–51,9,12,13,19,24,26,38,41]. High rates of infection have been documented in Kosovo (66%) and Ethiopia (64%) [27,52], while lower rates of positivity have been observed in Brazil (8%) based on placental samples [15]. Various factors contribute to the prevalence of coxiellosis in small ruminants, including farm-associated factors (such as livestock location and herd size), animal-associated factors (such as age, breed, and parity), animal management practices, type of sample collected, and diagnostic method used [12,18,26,32,45]. This variability has been reported in studies conducted in different parts of the world, including those in Turkey, Egypt, Brazil, Germany, and Pakistan.

Regarding localities, climate change may play a role in the emergence of vector-borne diseases [10]. Additionally, ticks, which are carriers of C. burnetii in animals, may contribute to the regional prevalence of the infection [11]. Airborne transmission has also been suggested as a significant factor in the regional spread of the bacterium, as reported previously [13,53]. Severe winds and the agent's spread to different regions may also influence the emergence of new diseases [11]. Although some surveys demonstrated a significant relationship between location and the rate of C. burnetti infection [2,34,35,47,50,51,54], our study findings suggest that the geographical region could not be a significant factor in the prevalence of coxiellosis in ovine and caprine. Similarly, a study conducted by Roshan et al. (2018) in Sistan and Baluchestan reported that the location of livestock was not a significant risk factor for the epidemiology of *C. burnetii* in aborted fetuses of sheep.

Previous studies have shown a significant positive association between increasing age and a higher prevalence of coxiellosis in small ruminants, as reported in studies conducted in various countries [2,6,17,32,36,39,42,51,54,55]. However, in the present study, we did not observe a significant correlation between age and the prevalence rate of the infection. Similar findings have shown no significant association between Q fever seropositivity and the age of small ruminants [19,34,47,56].

Our study did not find a significant association between parity and the prevalence of C. burnetii in sheep and goats. Similarly, studies conducted by Kayedi et al. (2017) and Esmaeili et al. (2019) in Iran reported that parity was not a significant risk factor for the epidemiology of C. burnetii in goats and sheep. In contrast to our findings, a study conducted by Roshan *et al.* (2018) in the Sistan region of southeastern Iran reported a decrease in the prevalence rate of coxiellosis with increasing parity in aborted fetuses of ewes. Also, a statistically significant difference between parity and prevalence of infection was reported in goats in Ethiopia [52].

Conclusion

Our findings suggest that *C. burnetii* infection is present in the vaginal discharge from aborted small ruminants of small ruminants in Kerman City, southeast Iran. However, the prevalence rate of the disease varies across different animal species with different ages and parity, as well as across different provinces within Kerman. These results may benefit veterinary organizations and practitioners in controlling and preventing future disease outbreaks. Further research is necessary to gain a comprehensive understanding of the epidemiology of the disease in other regions of Iran and to ensure accurate surveillance of this zoonotic agent. It is important to note that controlling coxiellosis in small ruminants is critical for preventing Q fever in humans.

Materials and Methods

Sample collection

A total of 134 vaginal samples from aborted sheep (n = 70 samples) and aborted goats (n = 64 samples) were collected from 32 herds in Kerman province between the autumn of 2019 and the winter of 2020. The samples were collected 24 hours after the abortion and were dissolved in a phosphate-buffered saline (PBS) solution. The resulting solutions were transported on ice to the Microbiology Laboratory of the Shahid Bahonar University of Kerman-Iran and stored at -20 °C until further DNA extraction. Genomic DNA was extracted from approximately 200 µL of the abortion fluid using the Roche High Pure PCR template preparation kit, following the manufacturer's instructions. The isolated DNA was stored at -20°C for subsequent Real-time PCR analysis.

A questionnaire was created to gather information on several risk factors, including the location of small ruminants, the age of ewes and goats, and parity. According to the classification in a previous study [57], small ruminants were divided into three groups according to age: under two years, two to four years, and more than four years.

Real-time PCR

For detection of C. burnetti via Real-time PCR, the IS1111 insertion sequence gene (the repetitive transposon-like region) was amplified in LightCycler 96® System (Roche, Germany) by forward primer -AAAACGGATAAAAAGAGTCTGTGGTT), and

reverse primer (CCACACAAGCGCGATTCAT).

A reaction mixture with a total volume of 20 µL was prepared to perform real-time PCR. The mixture consisted of 10 µL of 2x Real Q Master Mix (Ampliqon, Denmark), 800 nM of each primer, 3.4 µL of sterile distilled water, and 5 µL of DNA template. The reaction was carried out using the following program: preincubation at 95°C for 15 minutes, followed by 45 cycles of 15 seconds at 95°C, 20 seconds at 54°C, and 20 seconds at 72°C. DNA from the Nine Mile strain (RSA 493) and molecular-grade water were used for positive and negative controls, respectively. If the threshold cycle (Ct) value of the IS1111 gene was less than 29, the sample was considered positive for coxiellosis.

Statistical analysis

The information gathered from the questionnaire and diagnostic results underwent statistical analysis using GraphPad Prism 9.0 software (Graph-Pad Software, San Diego, CA). The data were analyzed using Chi-Square tests with Yates's correction. The aim was to determine risk factors associated with a particular outcome. The analysis involved calculating the odds ratio for a range of factors, such as animal species, individual age, number of parturition, and location of the livestock. The results were presented as odds ratios and 95% confidence intervals, with a P-value of less than 0.05 considered statistically significant.

Authors' Contributions

MS: Conceptualization, Data curation, Investigation, Writing – original draft preparation; MG: Conceptualization, Methodology, Validation, Resources, Project administration, Supervision, Visualization, Writing – review & editing; MK: Conceptualization, Validation; EM: Methodology, Investigation; MAS: Formal analysis, Writing – original draft preparation, Writing – review & editing. All the authors read and approved the final manuscript.

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Competing Interests

The authors declare that there is no conflict of interest.

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