Original Article

Molecular Detection of *Coxiella burnetii* in Ticks Isolated from Domestic Animals in Slaughterhouses and Farms, Shahr-E-Rey, Tehran, Iran

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Abstract

Background: *Coxiella burnetii* causes Q fever, a zoonotic and vector-borne disease. Ticks serve as vectors for this bacterium. This study aimed to determine the prevalence of *C. burnetii* infection in ticks in Shahr-e-Rey County, Tehran Province.

Methods: From December 2016 to November 2017, 179 ticks were collected on sheep at animal husbandry facilities and slaughterhouses located in Shahr-e-Rey, Tehran Province. Tick samples were morphologically identified and evaluated for the presence of the *C. burnetii* IS1111 gene using real-time PCR.

Results: Ticks were classified into four genera: *Hyalomma* (66.48%), *Rhipicepalus* (23.47%), *Dermacentor* (7.26%), and *Ornithodoros* (2.79%). Furthermore, 35.20% of the ticks were *Hyalomma* nymphs.

All 77 ticks were pooled by species, and *C. burnetii* was found in 22.08% (n= 17). *Ornithodoros lahorensis* was the most prevalent tick infected with *C. burnetii*.

Conclusion: The distribution of *C. burnetii* and reports of Q fever from various regions of the country strongly suggest that the monitoring system should give this disease more attention.

Keywords: Q fever; Ixodidae; Argasidae; *Coxiella burnetii*; Real-time PCR

Introduction

Ticks are ectoparasites that transmit diseases caused by bacteria, viruses, and parasites. *Coxiella burnetii*, the causative agent of Q fever, has been isolated from more than forty species of ticks (1, 2). *Coxiella burnetii* is a Proteobacteria, Legionella, and Coxilaceae gram-negative intracellular Coccobacillus. The bacterium imitates eukaryotic cells and is composed of 32 distinct isolates that are classified into six categories (3). Though the life cycle of this bacterium is unknown, electron microscopy may be used to detect metabolically active small cell variants (SCVs) and large cell variants (LCVs) (4, 5). Cattle, sheep, and goats are regarded as the primary reservoirs of domestic animals.

Q fever is typically transmitted to humans through the inhalation of contaminated dust or aerosols that contain amniotic fluid, placental material, or excreta from infected animals (6). This disease exhibits a wide spectrum of clinical signs and symptoms in humans, including

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asymptomatic infection and acute illness (often presenting as a self-limiting febrile illness, pneumonia, or hepatitis), as well as chronic (primarily endocarditis), specifically impacting individuals with preexisting valvulopathy and individuals who have impaired immune systems. Infections commonly appear in reproductive system abnormalities, such as abortions in sheep. It has been associated with late abortions, stillbirths, suboptimal offspring, and infertility in humans (7). It is a newly identified contagious disease that is rapidly spreading globally, with varying rates of occurrence in different regions (8–10). Q fever has been reported in humans and animals in Iran's border countries, including Turkey, Pakistan, and Iraq, and might be regarded as a major issue in cross-border infection transmission (11). The disease is considered a major zoonotic disease in Iran. The initial occurrence of acute human Q fever was recorded in 1952 in southwest Iran, and subsequent instances were documented throughout the following two decades (12, 13). Research conducted on both domestic and wild animals as well as humans has demonstrated that the disease is widespread across Iran and poses a growing danger to healthcare (14, 15). *Coxiella burnetii* has been reported across Iran's regions, with incidence rates ranging from 10 % to 17% (16–18). The most widely distributed genus of the hard ticks (Parasitiformes: Ixodidae) in the country is *Ixodes*, followed by *Amblyomma*, *Boophilus*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, and *Rhipicephalus*. *Coxiella burnetii* is naturally found in about 40 tick species, including the genera *Ixodes*, *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus*. The most prevalent species infected with *C. burnetii* in Iran are *Dermacentor marginatus*, *Haemaphysalis concinna*, *Hyalomma anatolicum*, and *Rhipicephalus sanguineus* (19, 20). This study aimed to identify *C. burnetii* infection and prevalence in tick-infested animals in Shahr-e-Rey farms and slaughterhouses.

Materials and Methods

Study area

Shahr-e-Ray County is a residential suburb in south Tehran, Iran's capital, with a population of almost 300,000 people (Fig. 1). It has industrialized as a consequence of an assortment of conditions, including its close proximity to Tehran. Numerous industrial and traditional animal husbandry farms, slaughterhouses, and meat-processing facilities may be found throughout this area.

Samples

Over the course of a year, we collected a total of 179 ticks from sheep husbandry households and slaughterhouses in Shahr-e-Ray. The ticks were collected by fine-tipped angled forceps from the ear, mammary glands, under the tail, and the rest of the body of the appropriate host, following the standard method (21), and then transferred to the Vector Biology Laboratory at the School of Public Health, Tehran University of Medical Sciences. Ticks were identified using the identification keys of Hoogstraal (22) and Walker (23).

Extraction of DNA

Tick samples were identified and sent to the Pasteur Institute of Iran and the National Reference Laboratory of Plague, Tularemia, and Q fever for molecular analysis. The ticks were maintained and cultivated for one to two weeks before the molecular analysis. Male and female ticks were pooled by species, crushed, and homogenized. To each sample lysate, 500 µL of lysis solution [0.1 M Tris-HCL (pH 8.25), 0.05 M EDTA, 0.2 M sucrose, and 0.5% SDS] containing proteinase K (10 mg/mL) were added. Suspensions were incubated for one hour at 56 degrees Celsius. Next, 120 µL of 5M potassium acetate was added. They were incubated on ice for ten minutes. The supernatants were recovered following a 10-minute centrifugation at 12,000 g. Extracted DNA was preserved at -20 degrees Celsius until molecular tests were performed (24).

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Detection of *Coxiella burnetii*

Real-time PCR (Corbett Research QIAGEN Cycler Rotor-Gene 6000, Victoria, Australia) with a final volume of 20 µL for each reaction was used to target the IS1111 gene of *C. burnetii* using specific primers and probe sequences (25). Real-time PCR reactions were carried out using the following reaction mixture: Use 10 µL of 2x RealQ Plus Master Mix for Probe (Ampliqon, Denmark), 900 nM forward primer (5′-AAAACGGATAAAAAGAGTCTGGTT-3′), 900 nM reverse primer (5′-CCACACAAGCG ATTCAT-3′), 200 nM probe 6-FAM (5′-AAG CACTCATTGAGCGCCGCG-3′) TAMRA, and 4 µL of DNA template. The PCR amplification protocol included ten minutes at 95 degrees Celsius, followed by 45 cycles of fifteen seconds at 94 degrees Celsius and sixty seconds at 60 degrees Celsius (26).

Results

A total of 179 ticks from 289 sheep matched the taxonomy: *Hyalomma* (51.48%), *Hyalomma* sp. nymphs (23.20%), *Rhipicephalus* (15.46%), *Dermacentor* (7.26%), and *Ornithodoros* (2.60 %). The most common tick species were *Hyalomma anatolicum* (18.99%) and *Rhipicephalus bursa* (12.85%) (Table 1). The real-time PCR detected *C. burnetii* in 22.08% (n= 17) of the 77 male and female ticks. *Ornithodoros lahorensis* showed the highest infection rates. *Coxiella burnetii* was not found in *Hyalomma asiaticum* and *Rhipicephalus sanguineus* (Table 1). *Coxiella burnetii* was found in the majority of ticks collected in the fall (25.0%) and winter (44.44%), respectively (Table 2). Figure 2 indicates that positive samples ascend before cycle 40 and negative samples ascend after cycle 40.

Using real-time PCR (Corbett research RG-6000). Output graph displays positive samples ascend before cycle 40 and negative samples ascend after cycle 40.

Table 1. Ticks collected from infested hosts in Shahr-e-Ray County, Iran, between 2016 and 2017

Species	Stage	Number of Ticks (%)	Number of pool	Positive Pool	Infection to *Coxiella* (%)
Ornithodoros lahorensis	Adult	5 (2.79)	4	4	100
Argas persicus	Adult	26(6)	26	0	0
Dermacentor marginatus	Adult	13 (7.26)	13	3	23.07
Hyalomma anatolicum	Adult	34 (18.99)	15	2	13.33
Hyalomma asiaticum	Adult	3 (1.68)	2	0	0
Hyalomma excavatum	Adult	1(0.2)	1	0	0
Hyalomma marginatum	Adult	11 (6.15)	7	1	14.28
***Hyalomma* sp.**	Nymph	63 (35.20)	13	4	30.77
	Adult	8 (4.47)	4	1	25.00
Rhipicephalus bursa	Adult	23 (12.85)	12	2	16.66
Rhipicephalus sanguineus	Adult	19 (10.61)	7	0	0
Total		179 (100)	77	17	22.07

Table 2. Prevalence of *C. burnetii* infection in ticks collected during several seasons in Shahr-e-Ray County, Iran, from 2016 to 2017

	Spring	Summer	Autumn	Winter	Total
Number of Ticks	99	56	11	13	179
Number of pools tested	36	28	4	9	77
Number of positive pools (%)	7 (19.44)	5(17.86)	1 (25.00)	4 (44.44)	17 (22.08)

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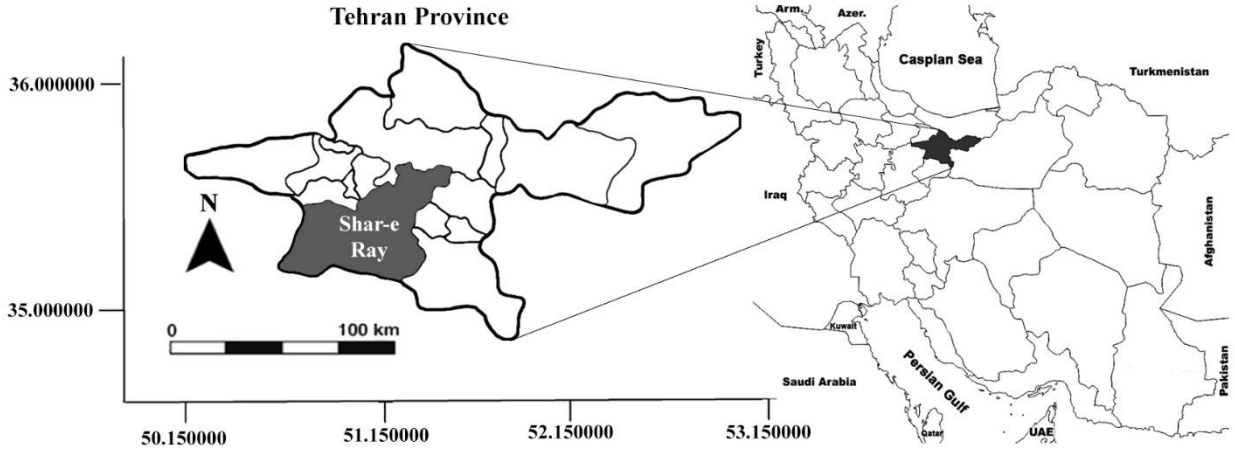

Fig. 1. A map of Shahr-e-Ray in Tehran Province, Iran, showing the study area where the ticks were collected is located at 35°29'45.0"N 51°26'49.6"E.

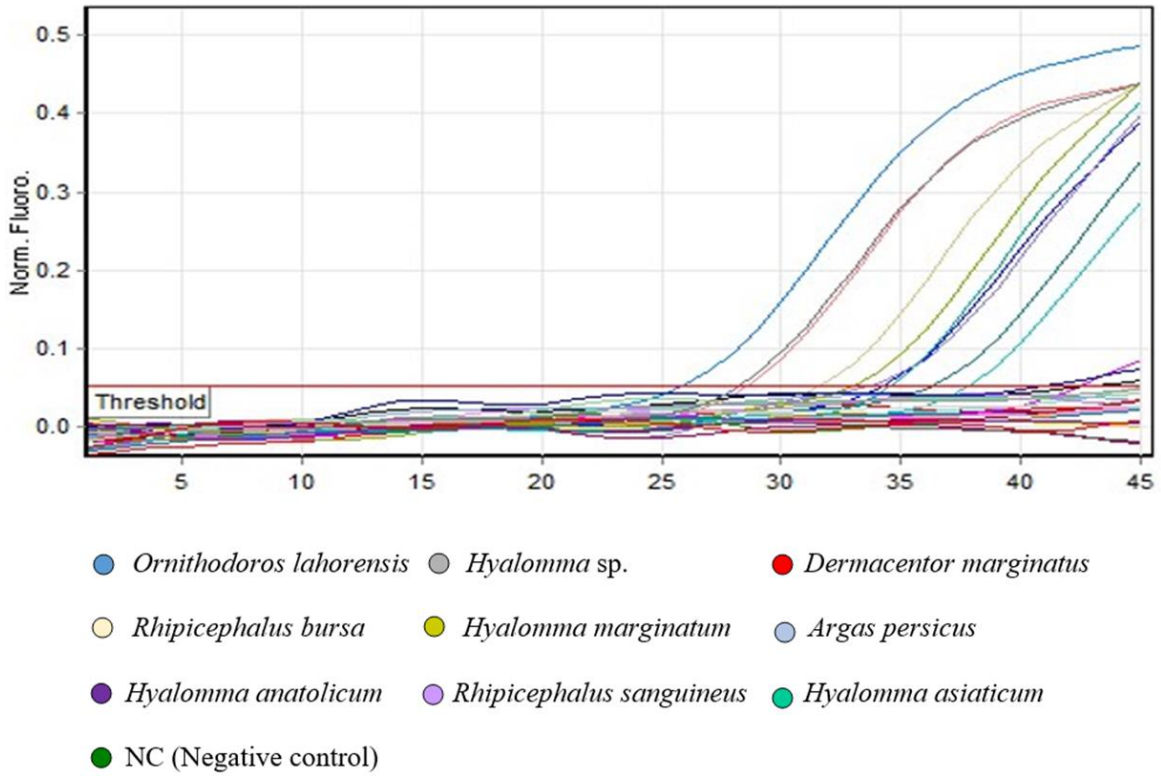

Fig. 2. *Coxiella burnetii* detection in ticks collected from Shahr-e-Ray County, Iran, 2016-2017

Discussion

During a year-long tick infestation evaluation, 289 sheep in Shahr-e-Ray County, Iran, were tested for tick infestation, and their potential contributions to the spread of *C. burnetii* were investigated. Approximately 72% of Iranian provinces reported high tick infestation rates, which might be attributed to climate variances, the diversity of animals farmed, animal

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cleanliness standards, and tick control strategies (which could include the use of insecticides and acaricides) (27–30). This study identified ticks from four genera and eight species, including *D. marginatus*, *Hy. anatolicum*, and *Hy. asiaticum*. The species acquired included *Hy. marginatum*, *Hyalomma* sp., *A. lahorensis*, *Rh. bursa*, and *Rh. sanguineus*. Previous surveys across the country revealed *Hyalomma* to be the most commonly observed tick genus (31, 32). According to Bakhshaei et al. (33), the most common tick species in Kerman Province's Jiroft and Kahnooj Counties are *Hyalomma* and *Rhipicephalus*. *Coxiella burnetii* infections were detected in 18.4% of the 103 tick pools tested. Champour et al. (34) identified *Hyalomma* as the most prevalent genus in Khorasan Province, eastern Iran. In a study by Mohabati Mobarez, positive *C. burnetii* samples from Iranian domestic ruminants were confirmed using a quantitative polymerase chain reaction targeting the IS1111 gene. The *C. burnetii* genome sequencing revealed the presence of 20 copies of the IS1111 transposase (35). The results align with the findings of our investigation, which indicate the presence of *C. burnetii* in ticks obtained from animal husbandry households. Esmaeilnejad et al. conducted a study to identify the tick species found in goats in the Meshkin-Shahr region of Ardabil Province, Iran (36). Ticks were collected, identified, and screened for *C. burnetii* infection using molecular techniques. The findings were comparable to our own, with a predominant presence of *Hyalomma* genus infestation observed in the majority of the investigated animals. Esmailnejad's study found that *Rh. sanguineus* has the highest infection rate compared to *Hyalomma*. Therefore, our investigation did not find any evidence of infection among the collected population of *Rh. sanguineus*. This can illustrate the influence of regional variations in the study on the disparities in bacterial infection among various species and genera. Our investigation revealed a substantial prevalence of infection in nymphs belonging to the genus *Hyalomma*, but Esmailnejad's study did not report such findings. Mohabati Mobarez et al. (37) conducted a study to ascertain the occurrence rate of *C. burnetii* in samples from sheep and cattle abortions using real-time PCR, specifically targeting the IS1111 element of *C. burnetii*, between 2017 and 2018, in nine regions including Tehran, Mazandaran, West-Azarbaijan, East-Azarbaijan, Ardabil, North Khorasan, Razavi Khorasan, Hamadan, and Alborz. Mohabati Mobarez's research indicates that Tehran Province has the highest incidence of *C. burnetii*. Our investigation in one of the regions of Tehran Province indicates a consistent presence of ticks throughout the year. These instances emphasize the significance of being attentive to this illness and carrying out thorough surveillance of the methods through which it is transmitted in Tehran Province. In a study to analyze the prevalence rate of *C. burnetii* in cattle, sheep, and goat milk samples in Mazandaran Province, which is next to Tehran Province, Kazemini et al. (38) used the polymerase chain reaction technique with two distinct types of primers. The occurrence of positive *C. burnetii* varies according to the season, with autumn and winter exhibiting a greater frequency compared to the other seasons. The continued prevalence of bacterial dominance during these two seasons, along with the growing prevalence of bacteria-infected ticks during the autumn and winter seasons, highlight the need for healthcare services in Shahr-e-Ray. Furthermore, additional research is needed to investigate the epidemiology and impact of infected vectors, reservoirs, unpasteurized milk, and dairy products on the occurrence of Q fever. Future research should focus on *Hy. anatolicum* due to its higher prevalence of *C. burnetii* positives compared to other species in our study and previous investigations (39).

Conclusion

The current study provides evidence for the presence of *C. burnetii* in ticks obtained

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from Shahr-e-Ray County, Tehran Province. Moreover, previous studies demonstrate the importance of raising awareness regarding Q fever in Iran. Given the significant presence of animal husbandry in Iran, additional research, preventive measures, and control strategies are necessary.

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Ethical considerations

The study was approved by the Ethics Committee of Tehran University of Medical Sciences (ID: IR.TUMS.VCR.REC.1396.3974).

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. All authors contributed to different parts of the research.

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