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Short Paper

Molecular detection of *Brucella abortus* and *Brucella melitensis* in domestic ruminants and their ticks in selected areas of western Iran

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Abstract

Background: Brucellosis is one of the most important zoonotic diseases caused by Gram-negative bacteria belonging to the genus *Brucella*. Detection of *Brucella* species in different countries is of utmost importance. **Aims:** This study aimed to detect *Brucella abortus* and *Brucella melitensis* in domestic ruminant blood samples and their ticks in western Iran. **Methods:** Sampling was conducted on ruminants from August to September 2020 in four different counties of Kurdistan Province, including Divandareh, Marivan, Baneh, and Sanandaj. Totally, 250 blood samples were collected from 250 small ruminants. There were no ticks on the skin of six (2.4%) ruminants, and 244 ticks were isolated from 244 animals. After genomic DNA extraction from all the collected samples, quantitative polymerase chain reaction (qPCR) was performed to detect IS*711* gene. **Results:** Based on qPCR results, *Brucella* genus was detected in two blood samples (0.8%) from female sheep and four ticks (1.6%) from male sheep, including three *Dermacentor marginatus* (1.22%) and one *Rhipicephalus turanicus* (0.4%). Although *B. melitensis* was not detected in any tick or blood sample, one tick sample (*D. marginatus*) was positive for *B. abortus*. **Conclusion:** Considering the positivity of ticks for brucellosis in this study, there is a possibility of *Brucella* transmission from infected ticks to humans and animals through tick bites, nevertheless, in order to identify the *Brucella* transmission relationship between ticks and animals, serological tests should be used in future studies.

Key words: *B. abortus*, *B. melitensis*, *Dermacentor marginatus*, *Rhipicephalus turanicus*, Ruminant

Introduction

Brucellosis is one of the most important zoonotic diseases with high economic and health impacts (Rossetti *et al*., 2017). The genus *Brucella* is classified into 12 known species, 9 terrestrials, and three marine species (Whatmore *et al*., 2016). *Brucella melitensis* and *Brucella suis* (except for Biovar 2), followed by *Brucella*

abortus and *Brucella canis*, are the most dangerous *Brucella* species (Wakjira and Dilba, 2010). Humans can be infected by contaminated aerosol particles, undercooked meat, unpasteurized milk, and direct contact with infected animals (Ahmed and Ibrahim, 2020; Islam *et al*., 2023). Additionally, human-to-human brucellosis transmission can occur in infected mothers who breastfeed their infants, sexual transmission, and unusual transmission via tissue transplantation or blood transfusions (Xu et al., 2023). In susceptible animals, brucellosis is most commonly transmitted through direct contact with infected animals or through an environment contaminated by discharge from infected animals (Pal *et al*., 2020). Cattle, dogs, sheep, goats, and pigs are major reservoirs of *Brucella* species for human infection. Brucellosis is prevalent in Mediterranean countries, Western Asia, and some parts of Africa and Latin America (Islam *et al*., 2023). Brucellosis is more prevalent in countries without effective disease control programs (Dadar *et al*., 2022). It is an endemic disease in many parts of Iran, especially in areas where people are in close contact with animals. According to the Iranian Ministry of Health, there are 22 cases of brucellosis per 100,000 people in Iran (Delam *et al*., 2022; Zeinali *et al*., 2022). Arthropods, particularly ticks, spread diseases between humans and animals (Cull, 2021). Despite the research on the prevalence of brucellosis in humans and animals, no study is conducted on *Brucella* species in ruminant ticks in Iran. There are many tick genera and species, including *Dermacentor*, *Haemaphysalis*, and *Rhipicephalus*, which carry microbes, including *Brucella*, that transmit tick-borne brucellosis (Wang *et al*., 2018, 2019; Huang *et al*., 2020; Jia *et al*., 2022). Detecting *Brucella* in reservoirs and nonspecific hosts (such as ticks) provides valuable information regarding the pathogenesis of brucellosis and facilitates effective control measures (Wang *et al*., 2019). Due to the higher sensitivity and specificity of molecular-based methods, such as quantitative polymerase chain reaction (qPCR), compared to culture and serological tests, these methods have a critical role in the detection and typing of *Brucella* species particularly *B. abortus* and *B. melitensis* for the control and eradication of brucellosis in ruminants. Despite serological evidence in humans and livestock, no molecular data is available on *Brucella* species in Kurdistan, an endemic region of the disease in western Iran. The aim of this study was to detect *B. abortus* and *B. melitensis* in ruminants and ticks of this region by molecular method.

Materials and Methods

Sample collection

Sampling was conducted from August to September 2020 in four different counties of Kurdistan province, including Divandareh, Marivan, Baneh, and Sanandaj. This study was approved by the Ethics Committee of the Pasteur Institute of Iran (IR.PII.REC.1398.051) and all methods were performed in accordance with relevant guidance and regulations.

Blood and tick samples were collected from two hundred and fifty ruminants including sheep and goats. All samples were collected with previous consent. All animals were clinically healthy, without a history of acaricide or antibiotic therapy. Flocks and animals were randomly selected. The mean age (±SD) of sheep and goats was 3.31 (±1.4) and 4.78 (±1.8), respectively (Table 1). Five ml of blood was collected in Venoject tubes containing an anticoagulant (EDTA). Blood samples were collected in EDTA-containing tubes and stored at 4-8°C until they were transferred to the laboratory and then stored at 4°C until DNA extraction. The skin surfaces of the animals were examined for tick

Table 1: Prevalence of *Brucella* in ticks based on tick species, tick gender, livestock species, and the geographical location of the sample collection

Category and subcategory	No. of ticks (%)	No. of ticks positive for *Brucella* genus (%)	No. of ticks positive for *Brucella abortus* (%)	No. of ticks positive for *Brucella melitensis* (%)
Ticks species				
Dermacentor marginatus	164 (67.21)	3 (1.22)	1 (0.4)	0
Rhipicephalus turanicus	30 (12.30)	1 (0.4)	0	0
Rhipicephalus sanguineus	26 (10.66)	0	0	0
Haemaphysalis concinna	24 (9.83)	0	0	0
Total	244	4 (1.6)	1 (0.4)	0
Ticks gender				
Female	119 (48.8)	0	0	0
Male	125 (51.23)	4 (1.6)	1 (0.4)	0
Total	244 (100)	4 (1.6)	1 (0.4)	0
Livestock's species				
Sheep	229 (93.85)	4 (1.6)	1 (0.4)	0
Goat	15 (6.15)	0		
Total	244 (100)	4 (1.6)	1 (0.4)	0
Geographical location (cities in Kurdistan province)				
Baneh	11 (4.51)	0 (0)	0	0
Divandareh	185 (75.82)	3 (1.22)	1 (0.4)	0
Marivan	46 (18.85)	0	0	0
Sanandaj	2 (0.82)	1 (0.4)	0	0
Total	244 (100)	4 (1.6)	1 (0.4)	0

infestation after blood sampling and animals were included in the study if they were infested. One tick per animal was randomly removed using fine-pointed stainless-steel tweezers. Each tick was placed in a separate 2 ml tube, and 70% ethanol was added. Tick samples were transferred to the parasitology laboratory of the Veterinary Faculty of Islamic Azad University of Sanandaj. The collected ticks were examined separately in this study. A parasitologist determined the genus and species of the collected ticks according to the classification and morphological keys previously described for identifying the ticks (Lah *et al*., 2016; Balinandi *et al*., 2020). All blood and tick samples collected from the animals were transferred to the Pasteur Institute of Iran (National Reference Laboratory for plague, tularemia, and Q fever) for further experiments under cold conditions (Bounaadja *et al*., 2009).

DNA extraction

Before DNA was extracted from the ticks, the salivary glands and sexual organs of the ticks were isolated and transferred to sterile microtubes. The samples were dried and crushed in liquid nitrogen in a clean Chinese pounder. Genomic DNA was extracted using G-spin™ Total DNA Extraction Mini kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. Total DNA was extracted from each tick sample. Genomic DNA was extracted from blood samples using a commercial kit (FavorPrep Blood/Culture Cell Genomic DNA Extraction Mini Kit, Favragen Biotech, Taiwan) according to the manufacturer's recommendations. For quality control, the extracted DNA samples were confirmed by gel electrophoresis and stored at -20°C for further experiments (Bounaadja *et al*., 2009).

Detection of *Brucella* genus using qPCR

The qPCR was performed for the detection of the IS*711* gene in blood and tick samples using the previously described specific probe (FAM-AAG CCA ACA CCC GGC CAT TAT GGT-TAMRA) and primers (F: GCT TGA AGC TTG CGG ACA GT; R: GGC CTA CCG CTG CGA AT) on the Corbett 6000 Rotor-Gene system (Corbett, Victoria, Australia) (Hinić *et al*., 2008). The PCR mixture (final volume, 20 µL contained 10 µL of 2X Real Q Plus Master Mix for Probe (Ampliqon, Denmark), 1 µL of a mixture of probe (200 nM), forward and reverse primers (900 nM), 4 µL of extracted DNA, and 5 µL of double-distilled water (DDW). DDW was used as a negative control, and the *Brucella*-positive sample from the Biobank of the Pasteur Institute of Iran was used as a positive control. The amplification protocol was 10 min at 95°C, followed by 45 cycles of 15 s at 95°C, and 60 s at 60°C. The qPCR results were analyzed using the Rotor-Gene® Q 2.3.5 software (QIAGEN).

Identification of *B. abortus* and *B. melitensis* using qPCR

B. abortus and *B. melitensis* were detected using qPCR (Dadar *et al*., 2019). Primer sequences for the identification of this species are shown in Table 2.

Statistical analysis

Descriptive statistical analysis of the collected data was performed using the statistical SPSS software (version 26).

Results

Sample characteristics

A total of 250 blood samples were collected from small ruminants in four different counties of Kurdistan province: Divandareh (185/250, 74%), Marivan (46/250, 18.4%), Baneh (15/250, 6%), and Sanandaj (4/250, 1.6%). Among 250 ruminants, (233/250, 93.2%) and (17/250, 6.8%) were female and male, respectively. The ruminants selected in this study included 232 sheep (92.8%) and 18 goats (7.2%). There were no ticks on the skin of six ruminants (2.4%), and 244 ticks were isolated from 244 animals. Based on morphological studies, collected ticks included *Dermacentor marginatus* (67.21%), followed by *Rhipicephalus turanicus* (12.30%), *Rhipicephalus sanguineus* (10.66%), and *Haemaphysalis concinna* (9.83%). All the collected ticks were adults, and 51.2% were male (Table 1 and Fig. 1).

The prevalence of *Brucella* genus in ticks and blood samples of small ruminants

The *Brucella* genus was detected in two blood samples (0.8%) of female sheep, and four (1.6%) tick samples of male sheep, including three *D. marginatus* (1.22%), and one *R. turanicus* (0.4%) (Fig. 2).

The prevalence of *B. abortus* and *B. melitensis* in tick and blood samples of small ruminants

B. abortus and *B. melitensis* were not detected in blood samples. Additionally, all tick samples were

Table 2: The probe and primers used for the identification of *B. abortus* and *B. melitensis* using qPCR in the present study

Bacteria	Target sequence		The sequence of primers (5´→3´)	Size fragments (bp)
B. melitensis	*BMEII0466*	Forward	TCGCATCGGCAGTTTCAA	67
		Reverse	CCAGCTTTTGGCCTTTTCC	
		Probe	TEX-CCTCGGCATGGCCCGCAA-BHQ-2	
B. abortus	*BruAb2_0168*	Forward	GCACACTCACCTTCCACAACAA	81
		Reverse	CCCCGTTCTGCACCAGACT	
		Probe	HEX-TGGAACGACCTTTGCAGGCGAGATC-BHQ-1	

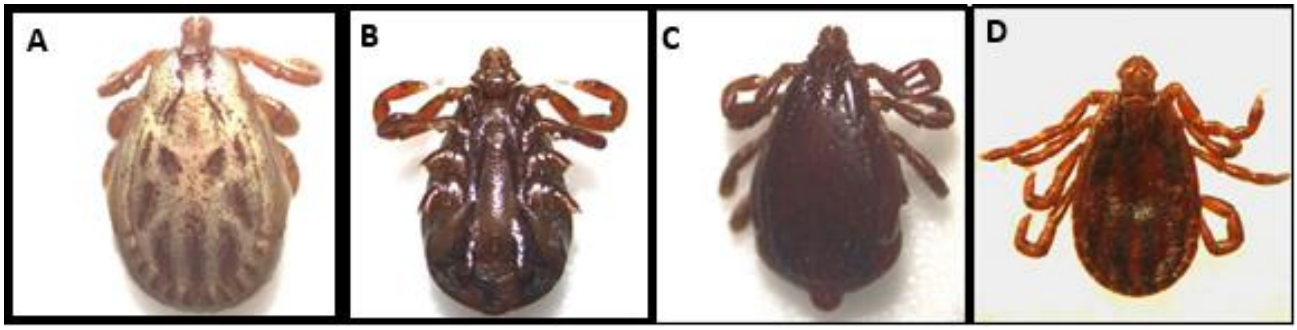

Fig. 1: The collected ticks from small ruminants in Kurdistan province in 2020. **A**: *Dermacentor marginatus*, **B**: *Rhipicephalus turanicus*, **C**: *Rhipicephalus sanguineus*, and **D**: *Haemaphysalis concinna*

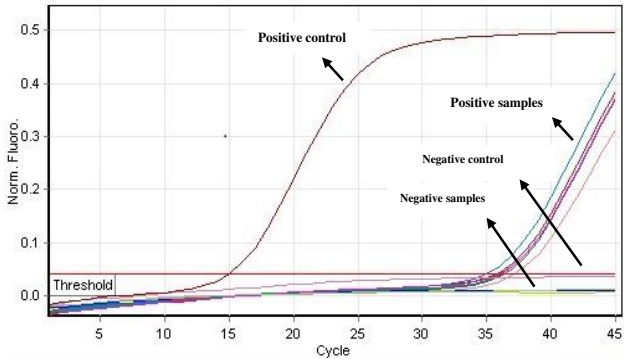

Fig. 2: Detection of *Brucella* genus using qPCR. The amplifiaction curve of positive and negative samples and positive and negative controlos are showed by arrows

negative for *B. melitensis*, and only one *D. marginatus* (0.4%) of male sheep was positive for *B. abortus*. The relationships between positive brucellosis and tick species, tick gender, livestock species, and the geographical location of the sample collection are presented in Table 1.

Discussion

In the present study, *Brucella* genus was detected in 1.6% (4/244) of ticks and 0.8% (2/250) of blood samples from western Iran (Kurdistan province) using qPCR. Although *B. melitensis* was not detected in any tick or blood sample, one tick sample (*D. marginatus*) was positive for *B. abortus*. In animals, after a short period of bacteremia, *Brucella* is localized in specific organs, such as the mammary glands and genital organs of the animal, and in this way, it finds shedding in the environment. Therefore, it is possible to find positive ticks for *Brucella* but negative in host blood. Brucellosis is commonly caused by *B. melitensis* and *B. abortus* in Iran (Golshani and Buozari, 2017). The prevalence of *B. abortus* and *B. melitensis* in ticks from domestic ruminants in Iran has not been studied despite warnings of a potential outbreak (Golshani and Buozari, 2017; Zeinali *et al*., 2022). *B. abortus* is widespread in Latin America, the Middle East, Africa, and Asia which results in significant economic losses and health problems in humans and animals (Franc *et al*., 2018). Lack of strict biosafety measures can result in failure to control the disease.

Recently researchers have investigated the role of ectoparasites in the spreading of *Brucella* species (Saeed *et al*., 2019). Identification and isolation of *Brucella* species are important for brucellosis management and control measures (Mu *et al*., 2022). In addition to *Brucella*, ticks can also be host pathogenic, non-pathogenic, or endosymbiotic organisms (Zeng *et al*., 2022). Changes in weather and land use have contributed to the growth of hard ticks and ectoparasites, which transmit microorganisms. Brucellosis may be transmitted naturally by *Boophilus*, which can be the host of *Brucella* species (Hosseini-Chegeni *et al*., 2017). *Dermacentor* and *Rhipicephalus* ticks have gained attention because of their geographic distribution, vectorial role, and identification as carriers of *B. abortus* (Zhao *et al*., 2021). To assess the significance of *Dermacentor* and *Rhipicephalus* in *Brucella* transmission, qPCR has been conducted in various studies (Zhao *et al*., 2021).

This is the first molecular study to detect of *B. abortus* and *B. melitensis* in domestic ruminant blood samples and their ticks in western Iran. Ticks are thought to transmit *Brucella* pathogenic species such as *B. abortus* and *B. melitensis*, but few studies have explored this issue. In China, a significant *Brucella* infection has been identified in ticks, which can be considered as a potential vector of this pathogen. Among *Dermacentor nuttalli* ticks in different regions of China, infection rates range from 0-87% (Huang *et al*., 2020). Other studies on ticks in China reported *B. abortus* and *B. melitensis* infection rates of 4.6% in *D. marginatus* ticks, 24.26% in *Haemaphysalis longicornis* ticks, and 26.3% in *Haemaphysalis anatolicum* and *D. nuttalli* ticks (Li *et al*., 2020; Zhang *et al*., 2021). In the present study, the *Brucella* genus was detected in two blood samples (0.8%) from female sheep, and four ticks (1.6%) from male sheep, including three *D. marginatus* (1.22%) and one *R. turanicus* (0.4%). All *Brucella* genus positive blood and tick samples were negative for *B. melitensis*, and *B. abortus* was detected in *D. marginatus*. The results of the present study are in agreement with those of Khan *et al*. (2017) who isolated *B. abortus* from ticks in Pakistan. The detection of *Brucella* in livestock blood samples and their ticks suggests the possibility of transmission of brucellosis to livestock and humans through tick bites. Based on these results, ticks may have

a role in spreading *B. abortus* and *B. melitensis* throughout Iran. Additionally, ticks may transmit *Brucella* species horizontally and vertically (Omitola and Taylor-Robinson, 2020). According to a previous research (Egyed *et al*., 2012) tick proximity to infected animals is correlated with the prevalence of tick-borne pathogen infections. Tick control in ruminants may reduce the incidence of the disease, indicating that ticks are brucellosis vectors in domestic animal populations (Miller *et al*., 2016). In a study performed in Iran, it was found that 38.42% of the examined samples were infected with *Brucella*, and the species found in this study belonged to *B. abortus* (Dadar *et al*., 2019). The Brucella can be transmitted to humans by tick biting, however, in vitro studies are needed to fully understand its mechanism. To prevent *Brucella* from spreading through tick bites, animals should be immersed in acaricide (Namgyal *et al*., 2021). Further research is needed to clarify how tick bites spread bacteria. The consistency of transmission of infection from *D. marginatus* to domesticated animals and humans should also be assessed.

Due to the existence of the genus *Brucella* in ticks of Kurdistan province, there is a possibility that infection can be transmitted through tick bites to livestock and humans. In addition, ticks are vectors for numerous pathogens, including *Brucella* species. Thus, tick control is important to prevent the spread of brucellosis. This study shows that *D. marginatus* and *R. turanicus* can carry the genus *Brucella*. In addition, blood samples from animals in which *Brucella*-infected ticks were isolated were negative. Considering the positivity of ticks for brucellosis in this study, there is a possibility of *Brucella* transmission from infected ticks to humans and animals through tick bites, nevertheless, in order to identify the *Brucella* transmission relationship between ticks and animals, serological tests should be used in future studies.

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Conflict of interest

The authors declare no conflict of interest.

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