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PCR Detection of *Coxiella burnetii* in Milk Samples of Ruminants, Iran

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ABSTRACT

Background: The importance of common diseases between humans and animals (zoonosis), and whose health impacts on the community individuals are obvious to everyone. One of these diseases is human infection with *Coxiella burnetii* and Q fever. Due to the importance of this subject and lack of published documents regarding the presence and distribution of this bacterium in Lorestan province, we aimed to determine the prevalence of *C. burnetii* in this area.

Methods*:* A cross-sectional study was conducted from July 2013 to March 2014. A total of 500 raw milk samples was collected from 130 cattle, 200 sheep, and 170 goats in both warm and cold seasons from 20 selected villages in Khorramabad and Nourabad regions, Lorestan province, Iran. The presence of *C. burnetii* in collected specimens was tested by polymerase chain reaction (PCR).

Results*:* A total of 9 (1.8%) milk specimens were positive for the presence of *C. burnetii* (3 sheep milk samples and 6 goat milk samples). All positive milk samples were obtained from 2 villages (Daraei and Ghalebi) around of the Khorramabad region during the winter season. However, all collected samples from Nourabad were negative for presence of *C. burnett*.

***Conclusion*:** The presence of *C. burnetii* in raw milk of ruminants in the cold seasons could be related to climate changes. Therefore, further investigations via various methods seems to be needed.

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Introduction

Coxiella burnetii is an obligate intracellular bacterium belonging to the family Rickettsiaceae and is the responsible for the Q fever diseases. Q fever is a zoonotic disease caused by *Coxiella burnetii*, a broad range of the hosts of this bacteria are widespread. The bacterium has been found in a wide spectrum of animals, including wild animals, pets and birds. Domestic ruminants seem to be the major reservoirs of human infections (1, 2, 6). Human infection usually occurs via inhalation of aerosols contaminated with *C. burnetii* from the urine, feces, and milk of infected animals (3-6). It has been indicated that ticks may have a role in the transmission of *C. burnetii* among wild vertebrates as a vector (7). During the last 60 years, the previous studies have indicated that Q fever is a nationwide disease in various regions of Iran (8, 9). *C. burnetii* has been detected in most of the milk bulk tanks. Unpasteurized milk appears another source of disease in humans in Iran (minor cause), (3). Serological methods used to detect *C. burnetii* is indirect immunofluorescence, complement fixation, or enzyme-linked immunosorbent assays (ELISA) (10, 11). A drawback of these techniques is that diagnosis is delayed, because *C. burnetii*-specific serum antibodies slowly raised appear several weeks after infection and also can be detectable months after an infection. Therefore, Serological, less sensitive for screening and source-finding studies for *C. burnetii* infection (10). Several PCR-based diagnostic assays have been developed for the detection of *C. burnetii* DNA. The trans-PCR (transposonlike element) by using primers targeting IS1111 has been reported to be highly specific and sensitive for *C. burnetii* detection; because of the existence of at least 19 copies within the genome, thereby enhancing the sensitivity of detection (12, 13). The current study was aimed to detect *C. burnetii* in milk specimens of cattle, sheep, and goats in both warm and cold seasons in the villages of Khorramabad and Nourabad cities, Lorestan, Iran.

Material and methods

Study Area

Lorestan Province is located in the south west of Iran in the Zagros Mountains. The total area of Lorestan covered 28, 392 km .Temperatures vary widely with the seasons and between day and night. At Khorramabad, summer temperatures typically range from a minimum of 12 °C to 32 °C, while they range from a minimum of -2 °C to 8 °C in winter.

Sample collection

This cross-sectional study was conducted from July 2013 to March 2014. A total of 500 raw milk samples were collected from 130 cattle, 200 sheep, and 170 goats in both warm and cold seasons from twenty villages in Khorramabad and Nourabad regions, Lorestan Province, Iran. The samples were collected from heard with a recent history of abortion. In the present study, 15 ml milk samples were taken from villages of each region and twenty villages were included from the abovementioned regions. Moreover, the milk samples showed normal physical characteristics. The samples were immediately transported to the laboratory in a cooler with ice packs and were processed within an hour of collection.

PCR detection

After centrifuging and removing cream and milk layers, *C. burnetii* DNA was isolated using a DNA extraction kit (Sinagene, Iran), according to the manufacturer's instructions. DNA samples were stored in a freezer at -20 °C until use. The presence of *C. burnetii* was tested by polymerase chain reaction (Trans-PCR). Sequences of the primers

are shown in Table 1. Trans-PCR program was carried out as previously described by Vaidya et al (13).

The PCR was conducted in a total volume of 25 µL containing 2.5 µL of 10×PCR buffer (100 mmol/L Tris-HCl buffer, pH 8.3, 500 mmol/L KCl, 15 mmol/L MgCl2, and 0.01% gelatin), 200 µmol/L deoxynucleoside triphosphate mix, 2 µmol/L of each primers, 0.3 IU of Taq DNA polymerase, 5 µL of template DNA, and sterilized water to make up the reaction mixture volume. The DNA amplification reaction was performed in an MG thermocycler (Eppendorf, Germany). The cycling conditions for PCR included an initial denaturation of DNA at 95 °C for 2 min, followed by five cycles at 94 °C for 30 seconds, 66 to 61 °C (the temperature was decreased by 1 °C between consecutive steps) for 1 min, and 72 °C for 1 min. These cycles were followed by 35 cycles consisting of 94 °C for 30 seconds, 61 °C for 30 seconds, and 72 °C for 1 min, then a final extension step of 10 min at 72 °C. Next, the PCR products were electrophoresed (1.5% agarose gel) and stained with ethidium bromide at a final concentration of 0.5 mg/ml. Then, agarose gel was evaluated under UV Illumination, photos were taken and documented for further evaluation. Accuracy of results was approved by using genomic DNA of a *C. burnetii* standard strain (Genekam biotechnology AG) positive control.

Results

As shown in figure 1, the presence of *C. burnetii* in raw milk samples of goat, cattle, and sheep was evaluated by the Trans-PCR method in the autumn and winter seasons. Our results indicated that a total of 9 milk specimens (1.8%) were positive for the presence of *C. burnetii* (3 sheep milk samples and 6 goat milk samples). All positive milk samples were collected from 2 villages (Daraei and Ghalebi) located a-round the Khorramabad during the winter. As a matter of fact, our results demonstrated that cattle milk samples were not positive for *C. burnetii*. Interestingly, all collected samples were negative for *C. burnetii* in Nourabad city in both summer and winter.

Table 1. The PCR primers and standard strain.

Primer	Sequence	size
Primer F	5´-TATGTATCCAACGTAGCCAGTC-3´	687 bp
Primer R	5´-CCCAACAACACCTCCTTATTC-3´	

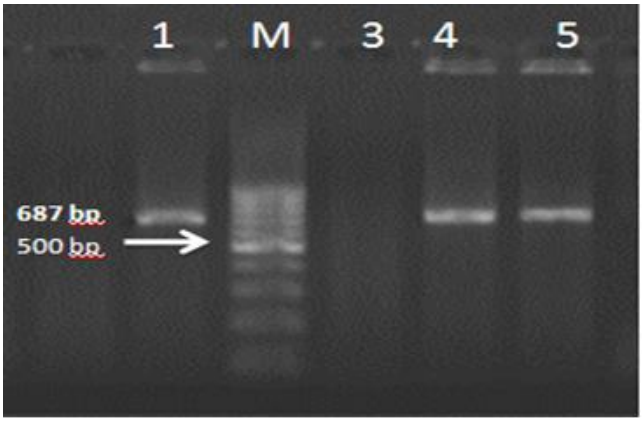

Figure 1. Agarose gel electrophoresis of PCR products for detection of *C. burnetii* in milk samples. Lane 1, genomic DNA of a *C. burnetii* strain (Genekam biotechnology AG) as positive control; lanes 4 and 5, two different positive samples; lane 3 a negative sample; lane M, 100-bp DNA size marker (Fermentas, Lithonia).

Discussion

C. burnetii may be widely prevalent but undiagnosed in livestock in Iran. It is noteworthy that the control of- Q- fever in animal and human is important for management of disease, as well as early and accurate detection of *C. burnetii* are required (6).

In the present study, our findings suggested that 9 milk samples (1.8%) were positive for the presence of *C. burnetii* (6 goats' milk, [1.2%] and 3 sheep milk samples [0.6 %]), while all cattle milk samples were negative for *C. burnetii*.

Previous studies indicated that all of caprine bulk tank milk samples from goat breeding farms in Ghom and Kerman were negative for *C. burnetii* (14). Fretz et al. in Switzerland reported that none of the dairy goat bulk tank milk samples were PCR positive (15). These findings are in agreement with our findings in summer season. On the other hand, the highest prevalence of *C. burnetii* infection in sheep was previously detected during the winter in Germany (16). It has been reported that the contamination rate of *C. burnetii* in milk samples was higher during the winter as compare to other seasons (15). In Turkey, 3.5% of sheep milk samples were positive for *C. burnetii* by PCR assay (17). The absence of the *C. burnetii* could be correlated with the shedding route of bacteria in different ruminants. As a result, examination of milk sample can be associated with misclassifying the status of the animal (15-17). In addition, *C. burnetii* may not persistently shed by animals and sampling shortly after lambing might have led to higher prevalence (18). The absence of milk contamination in the summer may be due to a reduction of the lambing and the milking period (15). In another study conducted by PCR methods in Shiraz, Ahvaz and Yazd, *C. burnetii* was detected in 18.2%, 4.2%, and 5.5% of the herds samples, respectively (19).

The results of different studies in various regions are difficult to compare, because of various assessing methods and different epidemiological circumstances (2). The Trans-PCR was reported to be more sensitive, reliable, and faster method for the detection of *C. burnetii* in milk samples. The findings of previous studies showed that unpasteurized milk can be a potential source of disease in humans in Iran.

Conclusion

The results of this study indicate the presence of *C. burnetii* in Khorramabad villages could be related partly to climate changes and may be associated with the migration of different herd which moved toward neighbour province. Therefore, further comprehensive investigations are required to clarify epidemiological and ecological circumstance of *C. burnetii*. Furthermore, *C. burnetii* causes infection in the uterus and milk glands. Therefore, different specimens such as vagina, urine, placenta fetal fluids can be used to determine *C. burnetii* infection. Furthermore, molecular and serological tests are recommended for *C. burnetii* detection.

Conflict of interest

None declared.

Financial disclosure

The authors declared no financial disclosures.

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