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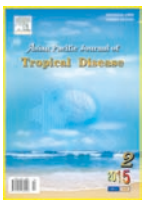

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Detection of *Coxiella burnetii* by PCR in bulk tank milk samples from dairy caprine herds in southeast of Iran

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PEER REVIEW

Peer reviewer

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Comments

This is a good study in which the authors evaluated *C. burnetii* in BTM samples collected from dairy caprine herds by PCR. This study indicates that clinically healthy dairy goats are important sources of *C. burnetii* infection in southeast of Iran.
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ABSTRACT

Objective: To use PCR for the detection of *Coxiella burnetii* (*C. burnetii*) in bulk tank milk samples collected from dairy caprine herds in southeast Iran.
Methods: In the present study, 31 goat bulk milk from 31 dairy goat herds were tested for *C. burnetii* using trans-PCR assay. The animals which their milk samples collected for this study were clinically healthy.
Results: In total, 5 of 31 (16.12%) goat milk samples were positive.
Conclusions: The results of this study indicate clinically healthy dairy goats are important sources of *C. burnetii* infection in this area.

KEYWORDS

Coxiella burnetii, PCR, Milk, Iran

1. Introduction

Q fever is a zoonotic disease, caused by the obligate intracellular bacterium *Coxiella burnetii* (*C. burnetii*) and infection may cause Q fever in man and in animal species. The bacterium has been detected in a large number of animal species and with cattle, sheep and goats as being the most common reservoirs. The reservoirs are extensive but partially known and include mammals, birds and arthropods, mainly ticks.

Infected animals, especially livestock, are considered the most important source of transmission to humans and are more frequently related to outbreaks of human Q fever than other animal species[1,2]. Whereas animals in general show no clinical signs of infection except occasional abortions and other problems with reproduction, *C. burnetii* can cause serious illness in humans. The main sources of *C. burnetii* shedding into the environment are manure, urine, milk, and especially birth materials like amnion fluid and placenta material[3,4]. Q fever is essentially an airborne disease. Infections is transmitted to humans most often via aerosols generated by infected animals or animal products[5-7].

However, available data on *C. burnetii* in aerosols and other environmental matrices are sparse. While infection from commercial milk is unlikely because of the pasteurization process, raw milk or dairy products that are produced by unpasteurized milk (cheese) may contain

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virulent *C. burnetii*.

Drinking milk contaminated with *C. burnetii* has caused seroconversion in human volunteers, without clinical disease inhuman volunteers[8]. The clinical presentation may be acute with influenza-like illness, hepatitis or pneumonia, or chronic with mainly endocarditis. Diagnosis still remains challenging and relies mainly on serology, mostly performed by indirect immunofluorescence assay. However, with serology, it is difficult to make an early diagnosis, especially in acute settings because it may occur during an epidemic. Predominant clinical manifestations are fever, pneumonia and granulomatous hepatitis for acute cases and endocarditis for chronic cases[8].

The isolation of the pathogen is a reliable diagnostic method, but it remains time consuming and hazardous and requires biosafety level BL3 practices. Therefore, the diagnosis of *C. burnetii* infection is usually done by polymerase chain reaction (PCR) or serological examination. The PCR assay with primers targeting IS1111 based on a repetitive, transposon-like element (trans-PCR), has been proved to be very specific and sensitive for the detection of *C. burnetii*[9].

The aim of our study was to use PCR for the detection of *C. burnetii* in bulk tank milk (BTM) samples collected from dairy caprine herds in southeast Iran.

2. Materials and methods

2.1. Collection of samples

From January to September 2012, a total of 31 goat bulk milk were collected from 31 goat breeding farms in Kerman Provinces, Iran. The animals which their BTM collected for this study were clinically healthy and 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.

2.2. Standard strain of C. burnetii

Phenol-killed, purified, and lyophilized cells of the *C. burnetii* Nine Mile, phase I, strain (RSA 493) were used for this study.

2.3. DNA extraction

C. burnetii was isolated from milk samples by boiling 100 mL of each sample for 10 min then, the solution was centrifuged at 13 000 r/min for 5 min. The supernatant was used to test the sensitivity and specificity of the PCR assay or kept at −20 °C until use[10].

2.4. Trans-PCR

A PCR assay targeting IS1111 fragment, a transposon-like repetitive region, was used to detect *C. burnetii* in clinical samples. In this study, trans-1 and trans-2 primers were used from the published data sequence of a transposon-like repetitive region of the *C. burnetii* genome[11]. The primers trans-1 (5′-TAT GTA TCC ACC GTA GCC AGT C-3′) and trans-2 (5′-CCC AAC AAC ACC TCC TTA TTC-3′) were synthesized by Copenhagen (Denmark). The trans-1 and trans-2 primers were designed to amplify a 687-bp fragment of the transposon-like repetitive element. The trans-PCR assay was performed as described previously[9]. The PCR mixture (25 μL) included 2.5 μL of 10×PCR buffer (100 mmol/L Tris-HCl buffer, pH 8.3, 500 mmol/L KCl, 15 mmol/L $MgCl_2$, and 0.01% gelatin), 200 μmol/L deoxynucleoside triphosphate mix, 2 μmol/L of each primers, 0.3 IU of *Taq* DNA polymerase, 3 μL of template DNA, and sterilized water to make up the reaction mixture volume. The DNA amplification reaction was performed in a 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 and then a final extension step of 10 min at 72 °C. Amplicons were visualized by agarose gel electrophoresis, stained with ethidium bromide at a final concentration of 0.5 mg/mL, and photo documented.

3. Results

In the present study, a total of 31 milk samples from 31 dairy goat herds in Kerman Provinces of Iran were tested for *C. burnetii* using a trans-PCR assay. In total, 5 of 31 (16.12%) goat bulk milk samples were positive (Figure 1).

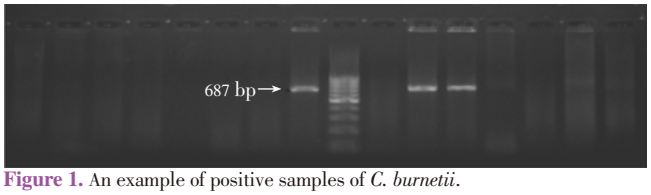

Figure 1. An example of positive samples of *C. burnetii*.

4. Discussion

Trans-PCR assay was demonstrated to be more sensitive and specific. In fact, this element exists in at least 19 copies in *C. burnetii* Nine Mile I genome[11]. And PCR based upon this gene is very sensitive and useful for the direct detection of *Coxiella* DNA in clinical samples.

In this study, 5 of 31 (16.12%) caprine BTM samples were positive. In a previous study conducted in Fars, Ghom, Kerman, Khuzestan and Yazd provinces of Iran, in total 18.2% of dairy herds in Fars, 4.2% of dairy herds in Khuzestan, 5.5% of dairy herds in Yazd were positive. However, all of caprine BTM from 25 goat breeding farms collected in Ghom and Kerman were negative[12]. Our result is similar to a mentioned report in Fars (18.2%) in comparison with 4.2% of dairy herds in Khuzestan, 5.5% of dairy herds in Yazdin goat BTM samples using a nested PCR assay. However, our result is in contrast with above mentioned report in Ghom and Kerman in Iran. In another study conducted in Chaharmahal va Bakhtiari Province of Iran, 1.8% (1 of 56 BTM samples) of goat BTM samples were positive for *C. burnetii*[13]. In addition, Fretz *et al*. in a study in Switzerland reported that none of the dairy goat BTM samples was PCR positive for *C. burnetii*[14]. This was also found in sheep of The Netherlands[15]. In the south eastern provinces of The Netherlands, significantly more dairy goat BTM samples (50.2%) were PCR positive compared with the remaining provinces (15.7%)[15]. However, results from different countries are difficult to compare, both as a result of different test protocols and different epidemiological circumstances[16].

The absence of the bacterium in previous studies could also be that the bacterium resided in other matrices than milk[17,18]. Moreover, the differences prevalence of *C. burnetii* in caprine BTM samples in this study in comparison with other studies may also because of the different routes of shedding *C. burnetii* such as vaginal mucus, feces, urine, placenta or birth fluids in these animals[19]. It seems goat excrete *C. burnetii* in their vaginal discharges, feces and milk[17,20]. Moreover, the infected animals may not persistently shed *C. burnetii*.

Shedding of *C. burnetii* by infected animals occurs mainly during parturition by birth products and lactation. Therefore, detection of *C. burnetii* in BTM samples greatly depends on the sampling time. The use of repeated sampling can reduce the likelihood of falsely classifying a herd as *C. burnetii* negative[21]. Also, sampling shortly after lambing might have led to higher prevalence[17,22]. Therefore, PCR testing of BTM samples has some limitations: a single BTM PCR test result only gives information about shedding in milk at one particular moment. A positive BTM PCR can be caused by only a few shedding animals and shedding via other routes is not determined in this way[17].

Our data indicate clinically healthy dairy goats are important sources of *C. burnetii* infection in southeast of Iran. Although, governmental regulation of milk pasteurization and sanitation in dairy processing plants has been established in Iran for many years but the consumption of fresh, unpasteurized milk from goat is a traditional practice in some rural areas.

Further work is now required to characterize the epidemiology of the infection more thoroughly.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Q fever is a zoonosis caused by *C. burnetii*, which is an aerobic, obligate intracellular, Gram-negative, highly resistant bacterium that may infect mammals, birds, arthropods and man. In domestic ruminants, the primary animal reservoir of *C. burnetii*, the main clinical sign of Q fever is abortion. *C. burnetii* is mainly shed after parturition or abortion in birth products, but shedding also occurs in urine, faeces and milk. Drinking milk contaminated with *C. burnetii* has caused seroconversion in human volunteers, without clinical disease in human volunteers. The clinical presentation may be acute with influenza-like illness, hepatitis or pneumonia, or chronic with mainly endocarditis.

Research frontiers

Studies are being performed for the detection of *C. burnetii* in BTM samples collected from dairy caprine herds and sheep herds. Such studies and pervious studies described the Q fever prevalence on farm level by testing BTM samples using this enzyme-linked immuno sorbent assay and RT-PCR. BTM testing is a proper tool for Q fever monitoring purposes in dairy goats.

Related reports

In a previous study conducted in Fars, Ghom, Kerman, Khuzestan and Yazd provinces of Iran, in total 18.2% of dairy herds in Fars, 4.2% of dairy herds in Khuzestan, 5.5% of dairy herds in Yazd were positive. Another study that was conducted by van den Brom *et al*. (2012), they concluded the higher percentage of BTM positive farms in the area where the human Q fever outbreak started, supports the suspected relation between human cases and infected dairy goat farms. However, results from different countries are difficult to

compare, both as a result of different test protocols and different epidemiological circumstances.

Innovations & breakthroughs

There are few data about occurrence of *C. burnetii* in BTM from different parts of Iran. Moreover, there are few studies in human population in some parts of Iran. Therefore, we can get information about distribution of *C. burnetii* in study area.

Applications

BTM testing is a proper tool for Q fever monitoring purposes in dairy goats. The PCR assay with primers targeting IS1111 based on a repetitive, transposon-like element (trans-PCR), has been proved to be very specific and sensitive for the detection of *C. burnetii*.

Peer review

This is a good study in which the authors evaluated *C. burnetii* in BTM samples collected from dairy caprine herds by PCR. This study indicates clinically healthy dairy goats are important sources of *C. burnetii* infection in southeast of Iran.

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