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Short Communication

Molecular detection of *Coxiella burnetii* in goat bulk milk samples in some provinces of Iran

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Q fever is a zoonotic disease caused by the Rickettsial organism *Coxiella burnetii*. The objective of this study was to determine the prevalence rate of *C. burnetii* in bulk milk samples from dairy goat herds in Fars, Ghom, Kerman, Khuzestan and Yazd provinces, Iran. In this study, 296 bulk milk samples from 89 dairy goat herds were tested for *C. burnetii* using a nested polymerase chain reaction (PCR) assay. The animals which their milk samples were collected for this study were clinically healthy. In total, six out of 296 (2.0%) goat milk samples were positive; the positive samples were 4 out of 22 (18.2%) dairy herds in Fars, 1 out of 24 (4.2%) dairy herds in Khuzestan and 1 out of 18 (5.5%) dairy herds in Yazd. All the 76 goat bulk milk samples from 25 goat breeding farms collected in Ghom and Kerman provinces were negative. Although, no extensive prevalence study was undertaken, the results of this study indicate that those clinically healthy dairy goats were important sources of *C. burnetii* infection in Iran.

Key words: *Coxiella burnetii*, polymerase chain reaction (PCR), milk, goat, Iran.

INTRODUCTION

Coxiella burnetii is the causative agent of Q fever in human and animals, and ticks are considered to be the natural primary reservoirs of *C. burnetii* and are responsible for the spread of the infection in wild animals and for transmission to domestic animals (Norlander, 2000; Pluta et al., 2010). Cattle, sheep and goats are the main sources of human infection. Infected animals shed highly stable bacteria in urine, faces, milk and through placental and birth fluids. Infection via inhalation of aerosolized organisms or ingestion of raw milk or fresh dairy products has been reported in humans and animals (Tissot-Dupont and Raoult, 1992).

Infection in animals is mainly sub clinical but has been associated with late abortions, stillbirth, delivery of weak offspring and also infertility (Aitken, 1989). Abortions during coxiellosis epizootics have been described in goats and sheep but abortion in dairy cows is rare, although reproductive disorders and mastitis can occur (To et al., 1998).

In human beings, symptoms are highly variable and about 60% of infections are asymptomatic sero-conversion patients. However, Q fever may lead to serious complications and even death in patients with acute disease. Predominant clinical manifestations are fever, pneumonia and granulomatous hepatitis for acute cases and endocarditis for chronic cases (Arricau-Bouvery and Rodolakis, 2005; Maurin and Raoult, 1999; Zhang et al., 1998).

Serological methods have been used to detect antibodies to *C. burnetii* (Addo, 1980; Rodolakis et al., 2007; Soliman et al., 1992). These assays may not be useful for the diagnosis of acute infection due to the delay in antibody development. Furthermore, it is difficult to discriminate between current and past infection because antibodies often persist after the organisms disappear from the blood (Zhang et al., 1998). Polymerase chain reaction (PCR) assay has become a useful tool for the detection of *C. burnetii* in clinical samples because of the low detection limit and high sensitivity (Berri et al., 2003; Fretz et al., 2007; Guatteo et al., 2007; Öngör et al., 2004; Zhang et al., 1998). This assay has been described

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Table 1. Prevalence of *C. burnetii* in bulk milk samples from dairy goat herds in Fars, Ghom, Kerman, Khuzestan and Yazd provinces of Iran.

Province	Number of herd studied	Number of sample per herd	Number of milk sample	Number (%) of *C. burnetii* positive sample
Fars	22	2-4	60	4 (6.7)
Ghom	10	2-3	36	-
Kerman	15	2-4	50	-
Khuzestan	24	2-4	90	1 (1.1)
Yazd	18	2-4	60	1 (1.7)
Total	99	2-4	296	6 (2.0)

as the most sensitive and rapid means to identify shedder animals (Arricau-Bouvery and Rodolakis, 2005).

The objective of this study was to determine the prevalence rate of *C. burnetii* in bulk milk samples from dairy goat herds in five different provinces in Iran using a nested PCR assay.

MATERIALS AND METHODS

Collection of samples

From January to May 2010, a total of 296 goat bulk milk samples were collected from 89 goat breeding farms in Fars, Ghom, Kerman, Khuzestan and Yazd Provinces of Iran (Table 1). The animals which their milk samples were 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.

PCR detection of *C. burnetii*

C. burnetii was isolated from milk samples by centrifuging and removing cream and milk layers as described previously by Berri et al. (2003). Purification of DNA was achieved using a Genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instruction and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001).

All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). The nested PCR assay used to screen for *C. burnetii* was designed from the nucleotide sequence of the *com1* gene encoding a 27-KD outer membrane protein (OMP) as previously described (Zhang et al., 1998) and the amplification was done according to the method described elsewhere (Fretz et al., 2007). For the nested PCR assay with primers OMP1-OMP2 and OMP3-OMP4, the first amplification was performed in a total volume of 25 µl containing 5 µl of DNA sample, 0.5 mM $MgCl_2$, 0.2 mM (each) dNTPs, 1 µM primer OMP1, 1 µM primer OMP2 and 0.5 U/reaction of Taq DNA polymerase (Roche Applied Science, Germany).

The PCR assay was performed at 94°C for 4 min and then for 30 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min in a DNA thermal cycler (Eppendrof Mastercycler 5330, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany). In the second amplification, the reaction was performed in a total volume of 25 µl containing 2 µl of DNA sample, 0.5 mM $MgCl_2$, 0.2 mM (each) dNTPs, 0.8 µM primers OMP3, 0.8 µM primers OMP4 and 0.5 U/reaction of Taq DNA polymerase. The PCR assay was performed at 95°C for 4 min and then for 30 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min. The PCR-amplified products (OMP1-OMP2: 501 bp; OMP3-OMP4: 438 bp) were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide, and examined under UV illumination. In this study, DNA sample from *C. burnetii* (D0010, Serial Number: 3154; Genekam Biotechnology AG, Germany) and DNase free water were used as the positive and negative controls, respectively.

RESULTS AND DISCUSSION

In this study, a total of 296 bulk milk samples from 89 dairy goat herds in Fars, Ghom, Kerman, Khuzestan and Yazd provinces of Iran were tested for *C. burnetii* using a nested PCR assay. In total, six out of 296 (2.0%) goat milk samples were positive (Table 1). The positive samples were four out of 22 (18.2%) dairy herds in Fars, one out of 24 (4.2%) dairy herds in Khuzestan and one out of 18 (5.5%) dairy herds in Yazd. All 76 caprine bulk milk samples from 25 goat breeding farms collected in Ghom and Kerman were negative.

In a recent study in Switzerland, all 81 ovine and 39 goat bulk milk samples were negative for *C. burnetii* using a nested PCR assay (Fretz et al., 2007). In another study conducted in Chaharmahal va Bakhtiari province in Iran, 1.8% of goat bulk milk samples was positive for *C. burnetii* (Rahimi et al., 2010).

Testing animal based on only bulk milk sample can lead to misclassification of the status of the animal because *C. burnetii* may be shed by other routes such as vaginal mucus, feces, urine, placenta or birth fluids, (Guatteo et al., 2006). It seems that goat excrete *C. burnetii* in their vaginal discharges, feces and milk (Rodolakis et al., 2007). Shedding of *C. burnetii* by infected animals occurs mainly during parturition and lactation. Therefore, detection of *C. burnetii* in bulk tank milk greatly depends on the sampling time. The use of repeated sampling can reduce the likelihood of falsely classifying a herd as *C. burnetii* negative (Guatteo et al., 2007).

Although prevalence of *C. burnetii* was 2.0% in goat bulk milk samples, our data indicate that clinically healthy dairy goats are important sources of *C. burnetii* infection

in Iran. Therefore, in order to prevent the spread of infection in animal and human populations, control goat coxiellosis should be instituted. Although, governmental regulation of milk pasteurization and sanitation in dairy processing plants has been established in Iran for many years, direct sale of unpasteurized milk and dairy products by producers to the consumer is not uncommon in many regions including Fars, Ghom, Kerman, Khuzestan and Yazd provinces. In fact, the consumption of fresh, unpasteurized milk from goat is a traditional practice in some rural areas.

The results also suggest that testing bulk tank milk as an easy and inexpensive method could be used to assess the efficiency of control schemes aimed at controlling and/or preventing *C. burnetii* infection in dairy herds. Further work is required to characterize the epidemiology of the infection more thoroughly. Although, no extensive prevalence study was undertaken, the results of this study indicate that the clinically healthy dairy goats are important sources of *C. burnetii* infection in Iran.

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