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PCR Detection of *Coxiella Burnetii* in Bovine Bulk Tank Milk Samples in Shiraz, Southern Iran

Samane Rahmdel[1], Maryam Sadat Moezzi[2], Negar Azimzadeh[2], Saeid Hosseinzadeh[3*]

1. Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
2. Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
3. Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

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**Corresponding author:*
Saeid Hosseinzadeh,
Department of Food Hygiene and Public Health,
School of Veterinary Medicine,
Shiraz University,
Shiraz, Iran
Tel: +98-71-37251001
Email: hosseinzadeh@shirazu.ac.ir
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ABSTRACT

Background: Q fever is a zoonosis caused by *Coxiella burnetii*, an obligate intracellular organism. In Iran, healthy cattle are the main reservoirs of these microorganisms. The consumption of milk and non-pasteurized dairy products is considered as a common way for transmission of infection from livestock to humans. Therefore, the present study aimed to determine the prevalence of *C. burnetii* in bovine bulk milk samples from dairy herds in Shiraz, southern Iran.

Methods: A total of 100 bulk milk samples were collected from 20 traditional and 80 industrial dairy herds in Shiraz, southern Iran. The samples were then evaluated for the presence of the gene IS1111 using polymerase chain reaction (PCR) method.

Results: Three out of 100 raw milk samples (3%) were contaminated with *C. burnetii*. The prevalence rate in traditional and industrial dairy herds was 10 % (two samples) and 1.2 % (one sample), respectively.

Conclusion: The bovine raw milk can be a potential source of *C. burnetii* in Shiraz, southern Iran. Implementation of good hygienic practices on dairy farms, as well as the avoidance of consumption of raw milk and non-pasteurized dairy products is crucial to reduce the risk of infection transmission.

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Introduction

Q fever is a zoonosis caused by an intracellular *Rickettsia*, *Coxiella burnetii*. The disease has non-specific clinical signs; however, in severe cases, it may be accompanied by hepatitis and pneumonia. Moreover, in a small percentage of infected people, it can lead to chronic infection and endocarditis (1). In recent years, the Q fever has widely spread in many countries, including Iran, as a recurrent illness (2). *C. burnetii*, with a worldwide distribution, is able to survive under extreme environmental conditions and is resistant to high temperatures, drying, and many disinfectants. These features together with the ability for airborne transmission, low infectious dose, and continuous exposure to reservoirs have made the organism a potential bioterrorism agent (3).

C. burnetii can infect a wide range of domestic and wild animals, including ruminants, dogs, cats, birds, reptiles, amphibians, fish, and mites. Among the domestic animals, cattle, sheep and goats, are the major reservoirs of this pathogen and the main sources of transmission of infection to human (3, 4). Although human infection is often caused by the

inhalation of bacterial aerosols from the urine and stools of infected animals (5), the oral transmission may occur due to the consumption of raw milk and non-pasteurized dairy products (2). The duration of bacterial shedding in milk varies from 8 days in cattle to 13 months in the ewe, depending on the animal species (3).

Although Q fever is primarily an occupational disease, raw milk and non-pasteurized dairy products play an important role in the spread of the disease in the general population. Few information is available on the main human infection routes of Q fever and the potential role of raw milk in spreading the disease in south of Iran (4). Therefore, the present study aimed to determine the prevalence of *C. burnetii* in bovine bulk milk samples from dairy herds in Shiraz, southern Iran.

Materials and Methods

For sample collection, 100 bulk milk samples were aseptically collected from 20 traditional and 80 industrial dairy herds during three months. The samples were then transported on ice to the laboratory. To undertake DNA extraction, the milk samples were first centrifuged to remove fat [6000 rpm, 5 min], and the pellet was suspended in a solution containing 250 µL Tris-EDTA buffer [100 mM Tris, 10 mM EDTA, pH=8.0] and 250 µL lysis buffer [0.2 M NaOH, 1% SDS, pH=8.0]. A same volume of phenol and chloroform was then added to the mixture. Following 5 min centrifugation at 6000 rpm, the supernatant was washed twice with phenol and chloroform. After addition of 0.1 volume of 3.0 M sodium acetate [pH=5.2], the DNA was precipitated using ethanol, dried, and resuspended in TE buffer [10 mM Tris, 1 mM EDTA, pH=8.0].

The PCR test was performed using Trans-F and Trans-R primers targeting the IS*1111* repetitive transposon-like region of *C. burnetii* (Trans-F GTCTTAAGGTGGGCTGCGTG, Trans-R CCCGAATCTCATTGATCAGC, 295 bp) (6). The PCR mixture was prepared in a final volume of 25 µL with 1× PCR buffer [Sigma-Aldrich, St. Louis, MO, USA], 3 mM $MgCl_2$ [Sigma-Aldrich], 1.2 µM of each primer, 120 µM dNTP [Sigma-Aldrich], 0.6 U of Taq DNA polymerase [Sigma-Aldrich], and 2 µL DNA template. The thermal profile consisted of initial denaturing at 94°C for 3 min, followed by 35 cycles of 45 s at 94°C, 45 s at 55°C, and 90 s at 72°C and a final extension step of 72°C for 10 min. The PCR products were separated by electrophoresis in a 1.2% [wt/vol] agarose gel containing 0.5 µg/mL ethidium bromide. *C. burnetii* Nine Mile strain (RSA 493) was used as positive control strain.

Results

Three out of 100 bulk tank milk samples analyzed by PCR method (3%) were contaminated with *C. burnetii*. Two positive samples belonged to the traditional dairy herds, while only one sample from industrial dairy herds gave positive result (Figure 1). The contamination rate in the traditional and industrial dairy herds was 10% and 1.2%, respectively.

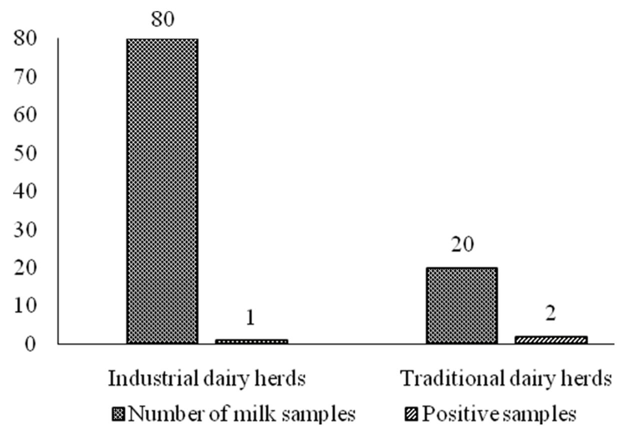

Figure 1: The prevalence of *C. burnetii* in bulk milk samples collected from traditional and industrial dairy herds in Shiraz, Iran

Discussion

Q fever appears to be an endemic infection in Iran. However, difficulties in screening of subclinical cases and in differentiation of Q fever from brucellosis and influenza may have led to underestimate of the health consequences of the disease (2). Serological assessments have indicated a higher prevalence of Q fever in consumers of raw milk. Raw milk is, therefore, considered as a potential source of infection transmission to human beings (7). Reports from different geographic regions indicate that healthy cattle are the major reservoirs for human pathogen, *C. burnetii*, in Iran (8).

The infection in dairy cattle (Coxiellosis) is frequently subclinical despite the shedding of *C. burnetii* in milk (9). Because of the intracellular nature of *C. burnetii*, it cannot be cultured using standard culturing methods; on the other hand, antibody production against the bacterium may last for a couple of weeks which is a significant drawback to the use of serological tests for diagnosis of acute infection (10). As a consequence, the molecular techniques including PCR have been proposed as rapid and sensitive methods to determine *C. burnetii* infection in the majority of ruminants (11).

In the present study, three percent of samples were positive for *C. burnetii*. The reported rates of raw cow milk contamination with *C. burnetii* in Iran range from 0% in Khorramabad (5) to 25% in

AjabShir (12). The prevalence level in the samples from Shahrekord, Bonab, Tehran, Isfahan, and Chaharmahal and Bakhtiari has been reported to be 32%, 22%, 12%, 9%, and 6.2%, respectively (2, 3, 8, 10, 13). However, little information is available on the contamination of raw milk in Fars Province, Iran. In the study conducted by Kargar et al. (14), 11 out of 100 bovine bulk tank milk samples were contaminated with *C. burnetii* in Jahrom, Fars Province. In another study (4), the prevalence of *C. burnetii* in raw milk samples at retail level in Shiraz was 27%. The level of contamination in raw cow milk samples from Colombia, United States, Italy, France, Portugal, Turkey, and Switzerland have been 45%, 43%, 41%, 24%, 20%, 10%, and 5%, respectively (1, 9, 15-19).

The discrepancies in the prevalence rates found in different studies can be partly attributed to differences in geographic conditions, detection procedure (PCR, Nested-PCR, Real Time- PCR), sample type (individual milk samples or bulk tank milk), sample size, and sampling season (14).

In the current study, samples from traditional dairy herds showed a higher prevalence rate which may be due to sub-standard farm construction (e.g. ventilation and waste management system), and unhygienic practices (e.g. poor husbandry and rearing conditions). Similar results have been reported by Nokhodian et al. (8).

Conclusion

The contamination of raw milk samples with *C. burnetii*, albeit at low levels, reinforces the need to increase public awareness of potential risks associated with consumption of raw milk and non-pasteurized dairy products. Furthermore, much more attention should be paid to the good hygienic and production practices in dairy farms. The use of Real Time-PCR method is recommended for future studies to make a more precise evaluation of contamination.

Acknowledgement

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Conflict of Interest

None declared.

References

1 Loftis AD, Priestley RA, Massung RF. Detection of Coxiella burnetii in commercially available raw milk from the United States. *Foodborne Pathog Dis*. 2010;7:1453-1456. DOI:1089/fpd.2010.0579. PMID:20704507.

2 Khademi P, Mahzounieh M, Kotahmer ME. Genomic detection of Coxiella burnetii in cattle milk samples by Nested PCR method in Bonab, Iran. *Arak Med Uni J*. 2015;18:49-57. (Persian)

3 Karimian A, Mahzounieh M, Ebrahimi kA. Genomic detection of Coxiella burnetii in bulk tank milk samples by Nested-PCR method in Shahrekord, Iran. *Pejouhandeh*. 2016;21:52-57. (Persian)

4 Abdali F, Hosseinzadeh S, Berizi E, et al. Prevalence of Coxiella burnetii in unpasteurized dairy products using nested PCR assay. *Iran J Microbiol*. 2018;10:220-226.

5 Norouzian H, Diali HG, Azadpour M, et al. PCR detection of Coxiella burnetii in milk samples of ruminants, Iran. *J Med Bacteriol*. 2018;7:31-35.

6 Khalili M, Nourollahifard SR, Abiri Z, et al. Detection of Coxiella burnetii as one of the causes of infectious abortions in small ruminants by PCR in the Hamedan Province. *J Veterin Microbiol*. 2015;11:129-134. (Persian)

7 Rahimi E. Detection of Coxiella burnetii in ovine bulk milk samples in Fars, Khuzestan, Ghom and Kerman provinces using PCR assay. *Veterin J*. 2014;27:2-7. (Persian)

8 Nokhodian Z, Feizi A, Moradi A, et al. Detection and risk factors of Coxiella burnetii infection in dairy cattle based on bulk tank milk samples in center of Iran. *Prev Vet Med*. 2016;134:139-144. DOI:1016/j.prevetmed.2016.10.003. PMID:27836035.

9 Contreras V, Máttar S, González M, et al. Coxiella burnetii in bulk tank milk and antibodies in farm workers at Montería, Colombia. *Revista Colombiana de Ciencias Pecuarias*. 2015;28:181-187. DOI:17533/udea.rccp.v28n2a07.

10 Ahmadizadeh C, Jamshidian M, Moosakhani F. Phylogenetic analysis of Coxiella burnetii isolates from dairy farms in Tehran. *J Comparat Pathobiol*. 2016;12:1669-1676. (Persian)

11 Gyuranecz M, Dénes B, Hornok S, et al. Prevalence of Coxiella burnetii in Hungary: screening of dairy cows, sheep, commercial milk samples, and ticks. *Vector Born Zoonotic Dis*. 2012;12:650-653. DOI:1089/vbz.2011.0953. PMID:22651386.

12 Khademi MP. Genomic detection of Coxiella burnetii in cattle milk samples by Nested-PCR method, Iran. *Iran J Med Microbiol*. 2015;9:69-72.

13 Rahimi E, Doosti A, Ameri M, et al. Detection of Coxiella burnetii by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. *Zoonoses Public Health*. 2010;57:e38-e41. DOI:1111/j.1863-2378.2009.01289.x. PMID:19968851.

14 Kargar M, Rashidi A, Doosti A, et al. Prevalence of Coxiella burnetii in bovine bulk milk samples in southern Iran. *Comparat Clin Pathol*. 2012;22:331-334. DOI:1007/s00580-012-1406-9.
15 Anastácio S, Carolino N, Sidi-Boumedine K, et al. Q fever dairy herd status determination based on serological and molecular analysis of bulk tank milk. *Transbound Emerg Dis*. 2016;63:e293-300. DOI:1111/tbed.12275. PMID:25208655.
16 Fretz R, Schaeren W, Tanner M, et al. Screening of various foodstuffs for occurrence of Coxiella burnetii in Switzerland. *Int J Food Microbiol*. 2007;116:414-418. DOI:1016/j.ijfoodmicro.2007.03.001. PMID:17434220.
17 Guidi F, Petruzzelli A, Ciarrocchi F, et al. Prevalence of Coxiella burnetii in cows' and ewes' bulk tank milk samples from selected dairy farms of Central Italy. *Italian Ani Sci*. 2017;16:673-676. DOI:1080/1828051x.2017.1321474.
18 Rodolakis A, Berri M, Hechard C, et al. Comparison of Coxiella burnetii shedding in milk of dairy bovine, caprine, and ovine herds. *J Dairy Sci*. 2007;90:5352-5360. DOI:3168/jds.2006-815. PMID:18024725
19 Yesim Can H, Elmal M, Karagöz A. Detection of Coxiella burnetii in cows', goats', and ewes' bulk milk samples using polymerase chain reaction (PCR). *Mljekarstvo*. 2015;65:26-31. DOI:15567/mljekarstvo.2015.0104.