



Q Fever in Dogs; an Emerging Infectious Disease in Iran

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ABSTRACT

Background: Q fever is an important widespread reemerging zoonosis. The presence of *Coxiella burnetii* in 100 tick-infested dogs was assessed in this study.

Methods: The blood samples from 100 referred dogs were acquired and evaluated by nested-PCR.

Results: *C. burnetii* was detected in 11 out of 100 (11%) blood samples. Most of the positive dogs were kept outdoor and fed on raw diet. Based on our findings, Q fever should be considered as an emerging disease in dogs in Iran; so, zoonotic importance of this population must be notified. To better understanding the role and pathogenic importance of dogs in Q fever outbreak and to determine whether this organism can be transmitted directly from dogs to human further in-depth studies are necessary.

Conclusion: It is determined that *C. burnetii* is present in dogs in southeast of Iran and people who are in contact with this population, especially asymptomatic ones are at increased risk of infection.

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Introduction

Query fever (Q fever) is an important widespread re-emerging zoonosis that caused by *Coxiella burnetii*, a small, obligate, intracellular gram negative bacteria (1-3). Wide variety of domestic and wild animals, including cattle, sheep, goats, horses, dogs, birds, rodents, arthropods and also humans can be infected by *C. burnetii* (2). Ruminants have been documented as the most common reservoirs, which retain this infection in the nature (1, 2, 4). Infected animals and humans shed this bacterium in milk, feces, urine, saliva, vaginal secretions, placenta and amniotic fluids (1). Asymptomatic companion animals can transmit this disease to humans; so, they pose a potential risk in the Q fever outbreak (3, 4, 5). Susceptible hosts such as dogs often acquire infection by inhalation or ingestion of pathogens (4, 6). This bacterial infection is usually asymptomatic in humans and animals, although manifests itself as either acute or chronic diseases with no clinical signs to sudden death (2, 4, 5). Diagnosis of Q fever is based on serologic testing and organism isolation (2, 7). DNA-based methods such as PCR is one of the choice methods for *C. burnetii* detection (1, 8).

Throughout the world, Q fever is considered as a public health concern (1, 7). Importance of *C. burnetii* in dogs and transmission and distribution of it remain inconclusive. On the other hand, interest in companionship increases the significance of this challenging disease. So; the current study was designed to determine the presence of *C. burnetii* in the blood samples using a nested-PCR assay in dogs in Kerman city, southeast of Iran.

Material and method

Sample collection

A total of 100 blood samples were randomly obtained from tick-infested house-hold dogs

referred to the Veterinary Teaching Hospital of Shahid Bahonar University of Kerman, regardless of their age, sex and clinical status, from April 2012 to October 2013. The protocol of this study was approved by the Animal Care Committee of Veterinary College of Shahid Bahonar University of Kerman. After clinical examination, 5 mL blood sample was collected in tube containing EDTA anticoagulant via the cephalic vein of each dog. Specimens were refrigerated at -18 °C until further processing. DNA was extracted from blood samples using VeTeK Company, South Korea, according to the manufacturer's instructions. The extracted DNA was stored at -20 °C until PCR analysis.

PCR assay

To detect the presence of *C. burnetii*, nested PCR assay was performed as previously described. The primer sets; Trans1-Trans2 and 261 F - 463 R, were designed based on a repetitive, transposon-like element (PCR) that has proven to be highly specific and sensitive to assess the *C. burnetii* infections, as it detects even very few copies of the target DNA sequence (9). The sequences of the primers were used for this study are listed in Table 1.

Table 1. Detection of *C. burnetii* DNA in blood collected from dogs by Nested Trans-PCR, Lane 1, molecular size markers. (100-bp DNA ladder); Lanes 2, positive control; Lanes 3, negative control; Lanes 4 to 11, samples.

Protocol	Primer	Sequence	Gen	Amplicon	References
Trans-PCR	Trans1	5-TATGTATCCACCGTAGCCAGTC-3	IS1111	687	(10)
	Trans2	5-CCCAACAACACCTCTTATTTC-3			
Nested PCR	261F	5-GAGCGAACCATTGGTATCG-3	IS1111	203	(9)
	463R	5-CTTTAACAGCGCTTGAACGT-3			

Nested PCR amplifications were performed via two runs of PCR using two sets of primers

including Trans1 and Trans1 for first amplification followed by 261F and 463R for second amplification reaction. The volume of the amplification reaction was 25 μ l containing 5 μ l of DNA template, 2 U of *Taq* DNA polymerase (Bioneer, Korea), and final concentrations of 1x PCR buffer; 3 mM MgCl₂; 1 mM deoxynucleoside triphosphate mix, and the primers at the concentration of 10 mM each. DNA amplifications were performed in a MG thermal cycler (Eppendorf, Germany).

The first amplification of PCR was 95 °C for 2 min, followed by five cycles at 94 °C for 30 s, 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 s, 61 °C for 30 s, and 72 °C for 1 min, then a final extension step of 10 min at 72 °C. In the second amplification, the cycling conditions included an initial denaturation of DNA at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, 50 °C for 45 s, 72 °C for 1 min, then a final extension step of 10 min at 72 °C. Amplicons were analyzed by the agarose gel electrophoresis, stained with ethidium bromide (0.5mg/mL concentration), visualized under UV light, and photographed at final.

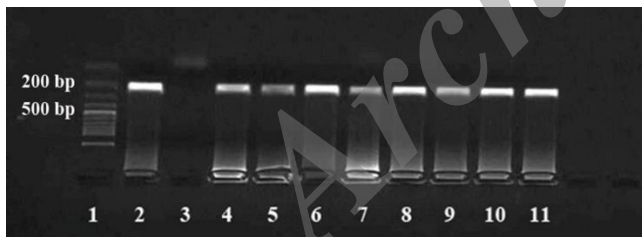


Figure 1. Detection of *C. burnetii* DNA in blood collected from dogs by Nested Trans-PCR, Lane 1, molecular size markers. (100-bp DNA ladder); Lanes 2, positive control; Lanes 3, negative control; Lanes 4 to 11, samples.

Result

In this study, blood samples from 100 referred dogs were acquired and assessed for the presence *C. burnetii*. So, bacterial DNA extraction was done and samples evaluated by nested-PCR. *C. burnetii* was detected in 11 out of 100 (11%) blood samples (Fig. 1). Most of the positive samples of these tick-infested dogs were kept outdoor and fed on raw diet. All collected ticks were identified as *Rhipicephalus sanguineus* regarding to standard characterization criteria including red-brown color, elongated body shape, and hexagonal basis capituli.

Discussion

Q fever is a ubiquitous bacterial zoonosis which is universally considered as an emerging significant public health issue (2). Q fever is endemic in Iran (11), nevertheless, there are no published data regarding *C. burnetii* prevalence in companion animals such as dogs and cats. So, the presence of *C. burnetii* in the blood samples of tick-infested dogs was assessed in this study using the nested-PCR assay. Based on our findings, the disease should be considered as an emerging disease of dogs in Iran; so, zoonotic importance of this population must be notified.

Pets have been documented as *C. burnetii* reservoir throughout the world (2); even so, importance of dogs for Q fever transmission to humans is poorly investigated (1). In accordance with findings of other researchers that determined *C. burnetii* infection in dogs, we detected *C. burnetii* in 11 out of 100 (11%) blood samples using nested-PCR. Compared to our results, other researchers reported different prevalence of *C. burnetii* infection in dogs ranging from 0 to 35% using ELISA or IFA in other countries (3). In Australia, seroprevalence of 21.8% was reported (5). Antibodies against *C. burnetii* were also detected in 5.5% of the feral canines by an immunofluorescent assay test using Phase I and II *C. burnetii* antigen in Iraq (6). Indeed, Hornok et al. (2013) reported 20.3% seropositivity in stray

dogs using ELISA (12); although, only one sample was positive by PCR. *C. burnetii* DNA was also identified in 4 out of 54 (7.4%) canine placentas in Netherlands (13). Dogs can be infected via inhalation, consumption of infected milk, placentas or carcasses and following tick bite. These infected animals can transmit Q fever to humans especially during or post parturition period (3). Buhariwalla et al. (1996) reported three human cases of *C. burnetii* pneumonia following contact by an infected parturient dog (14).

Based on literatures, the possibility of disease transmission to human being by cats is more than dogs but its reason remains unclear (15-16). Cats are considered as one of the main reservoirs of *C. burnetii* in Japan that 14.2% and 41.7% of pet and stray cats were seropositive respectively (15). In addition to seropositive samples, 1.3% of antibody negative cats were positive by PCR assay in the mentioned study. Kopečný et al. (2013) reported strong seropositivity using CFT (7.4%), IFA (26%) and ELISA (41%) between breeding queens (7). DNA of *C. burnetii* was also detected from uterine samples of healthy non-parturient cats in Colorado (16). In contrary, *C. burnetii* DNA was not isolated from placenta (17) and vaginal samples of cats in other studies (16). It seems that differences between *C. burnetii* prevalence in the different reports are related to variation between host factors, geographical and environmental conditions, and identification methods.

In the current study, detection of *C. burnetii* DNA was higher in farm dogs that kept with other animals and; dogs, which fed raw diet. These emphasize that a close attention to these populations can have beneficial effects on controlling and prevention programs. Outdoor housing (contact with farm, wildlife and ticks) and feeding by raw diet may be attributed to higher prevalence of Q fever. Outdoor housing provides higher opportunities of infection from other animals. Susceptibility of feral dogs to acquire the pathogens is higher due to poor diet, improper environment and immune compromised

conditions (5, 12). In this area, commercial or home-made diets are infrequently used for feeding of large breed and outdoor dogs, and most of them fed on raw diet such as carcasses. Domestic ruminants have been considered as the main *C. burnetii* reservoir and exposure to these populations and their products providing higher prevalence (2, 12). At present, few studies have been done regarding distribution and incidence of *C. burnetii* infection in Iran. In southeast of Iran, recent studies showed a high seroprevalence of *Coxiella* in domestic ruminants (18-19) in agreement with the high prevalence of human Q fever cases reported from this area (20-21). NourollahiFard and Khalili (2011) also detected *C. burnetii* in ticks collected from sheep and goats (8). Furthermore, infection may be correlated to dry weather of Kerman and inhalation of dust storms originating from neighbors (11). Sanitary and prophylactic measures like quarantine practice and ectoparasite control should be done in order to prevent of Q fever outbreak (1, 22).

Considering this fact that our research is the first epidemiologic study of dogs in this area, nested-PCR assay was carried out as a screening test; so, confirmatory works such as simultaneous assessment of other biological samples for virological and serological evaluations from these dogs postponed to the future study.

As contact with infected dogs could be a risk factor for human populations, veterinarians should warn owners regarding this zoonotic pathogen (2).

Conclusion

According to the results of this study, it is indicated that *C. burnetii* is present in dogs in southeast of Iran and people who are in contact with this population, especially asymptomatic ones, are at increased risk of infection. To better understanding role and pathogenic importance of dogs in Q fever outbreak and determine whether this organism can be transmitted directly from dogs to human further in-depth studies are necessary.

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Conflict of interest

None declared.

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