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ORIGINAL ARTICLE

Investigation of *Coxiella burnetii* in Iranian camels

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Abstract *Coxiella burnetii* is a zoonotic, obligate intracellular bacterium that caused Q fever. Antibodies to this organism have been reported in a wide range of animals including mammals, reptiles, amphibians, and birds. This study is aimed to detect *C. burnetii* in camel by polymerase chain reaction (PCR). Blood samples from 130 camels were collected between August and September 2011 then examined in laboratory conditions. Detection of the presence of *C. burnetii* DNA was carried out using a PCR assay with specific primers (Coc-F and Coc-R) targeting the *16S ribosomal RNA* gene (242 bp). In this study, a total of 14 (10.76 %) camel blood samples were found PCR positive for *C. burnetii*. This result proves that camels are an important reservoir of *C. burnetii* infection. This study showed relatively high positivity of *C. burnetii* in Iranians camels, and accordingly, it seems necessary to evaluate the prevalence for this microorganism in Iran.

Keywords *Coxiella burnetii* · PCR · Camel · Iran

Abbreviations

C. burnetii	*Coxiella burnetii*
PCR	Polymerase chain reaction

Introduction

Coxiella burnetii is an obligate intracellular microorganism that causes Q fever in humans and animals (Marrie 1995). The genome size of *C. burnetii* was determined by the method of DNA renaturation to be approximately 1.04×109 Da (Myers et al. 1980). This is comparable to the genome size of several other rickettsial species and is well within the range of several free-living bacteria, including *Mycoplasma* spp. (5×108 Da) (Baca and Paretsky 1983). Several reports present electron microscopic evidence suggesting that *C. burnetii* exhibits two morphological forms, a large and small form, distinct from the phase variation phenomenon (Wachter et al. 1975). The etiological agent of Q fever is highly pleomorphic, coccobacillary in shape with approximate dimensions of 0.3×1.0 μm, bounded by an envelope similar to those found in gram-negative bacteria (Burton et al. 1975). *C. burnetii* is currently considered a potential warfare agent and is classified as a category B biological agent by the Center for Diseases Control and Prevention (Kirkan et al. 2008).

C. burnetii is highly infectious; only one organism is required to produce infection under experimental conditions (Ormsbee et al. 1978). *C. burnetii* infections have been reported in humans, farm animals, pets, wild animals, and arthropods (Norlander 2000). Animals are often naturally infected but usually do not show typical symptoms of this infection (Maurin and Raoult 1999). Coxiellosis in livestock is associated with the occurrence of human Q fever; both illnesses are caused by *C. burnetii*. There is a high prevalence of the antibody to *C. burnetii* in veterinarians and meat-processing workers, who come into close contact with livestock (Htwe et al. 1993). Cattle, sheep, camel, and goats are the main sources of this infection. Infected animals shed highly stable bacteria in urine, feces, milk, and through placental and birth fluids (Kirkan et al. 2008). Humans are infected mainly by inhalation of contaminated aerosols or by ingestion of milk or fresh dairy products. *C. burnetii* infection usually is asymptomatic or manifests as a mild disease with spontaneous recovery (Giovanna et al. 2004).

Infection in animals is mainly subclinical but has been associated with late abortions, stillbirth, delivery of weak offspring, and also infertility (Moore et al. 1991). In humans, Q fever is most often asymptomatic, but acute disease (mainly a limited flu-like illness, pneumonia, or hepatitis) or chronic disease (chronic fatigue syndrome or endocarditis) can occur (Norlander 2000). Q fever is a severe disease that requires prolonged antibiotic therapy

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because the infection can result in endocarditis (Raoult and Marrie 1995) or granulomatous hepatitis (Weir et al. 1984). In addition, the *C. burnetii* infection can lead to abortions, stillbirth, or premature deliveries in pregnant women (Raoult and Marrie 1995).

Marmion (1967) and Kazar et al. (1982) reviewed the status of Q fever vaccines. Experimental vaccines included killed and attenuated *C. burnetii* and extracts of the agent. Unfortunately, most, if not all, of these vaccines induced severe local skin reactions (Baca and Paretsky 1983).

Several methods have been described for the detection of *C. burnetii*, but polymerase chain reaction (PCR) is a safe and useful method for detection and diagnosis; in contrast, isolation of *C. burnetii* is hazardous, difficult, and time-consuming (Stein and Raoult 1992). The nonradioactive PCR–enzyme-linked immunosorbent assay (PCR–ELISA) method is reported to be sensitive and specific for the detection of the targeted DNA (Zambardi et al. 1995). The purpose of present study was to investigate *C. burnetii* infection in camel using PCR method in Iran.

Materials and methods

Sampling and DNA extraction

One hundred thirty camel blood samples were collected into tubes that contained EDTA from slaughterhouses located in Isfahan Province. Genomic DNA was extracted using DNA extraction kit (Qiagen, Germany), according to manufacturer's instructions, and assayed on 2 % agarose gel electrophoresis and measured at 260-nm optical density according to the method described by Sambrook and Russell (2001).

Gene amplification

The primer sequences for the *16S rRNA* gene were: Coc-F: 5′-GTA ATA TCC TTG GGC GTT GAC G-3′ and Coc-R: 5′-ATC TAC GCA TTT CAC CGC TAC AC-3′. Primers were designed according to the published sequence for 16S rRNA gene of *C. burnetii* (accession number: D89799). The amplification was done using a thermal cycler (Mastercycler Gradient, Eppendorf, Germany), in a final reaction volume of 25 μl. The PCR mixture consisted of 50 ng of DNA sample, 1 μM of each primer, 200 μM $MgCl_2$, 200 μM dNTPs, 2.5 μl of 10× PCR buffer, and 1 U of *Taq* DNA polymerase (Fermentas, Germany). The following conditions were applied: initial denaturation at 95 °C for 5 min, followed by 30 cycles, denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, elongation at 72 °C for 1 min. The program was followed by final elongation at 72 °C for 5 min.

Analysis of PCR products

PCR products were analyzed on 2 % agarose gel electrophoresis. The electrode buffer was TBE (Tris-base 10.8 g 89 mM, boric acid 5.5 g 2 mM, EDTA (pH 8.0) 4 ml of 0.5 M EDTA (pH 8.0), combine all components in sufficient H_2O and stir to dissolve). Gels were stained with ethidium bromide. Aliquots of 10 μl of PCR products were applied to the gel. Constant voltage of 80 V for 30 min was used for product separation. After electrophoresis, images were obtained over UV light gel documentation systems (UK).

Statistical analysis

The prevalence of isolation of *C. burnetii* from the blood was analyzed by the chi-square test using the SPSS 17 (SPSS Inc. Chicago, IL, USA) software. The probability level for significance was $P \leq 0.05$.

Results

The quality of the extracted DNA from samples was examined by electrophoretic analysis through a 2 % agarose gel. PCR products of 16S rRNA gene on agarose gel showed a fragment of about 242 bp, which was in agreement with the expected size for identification as *C. burnetii*. A total of 14 (10.76 %) samples were found PCR positive for *C. burnetii* obtained from the blood of 130 camels. Some PCR amplification products are shown in Fig. 1. Furthermore, positive control DNA and water samples were included in all amplifications. In this research, *C. burnetii* DNA (serial number, 3154; Genekam Biotechnology AG, Duisburg, Germany) was used as positive control.

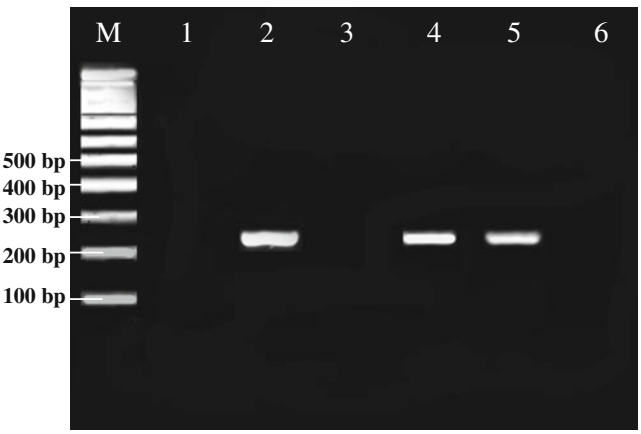

Fig. 1 The 1 % agarose gel electrophoresis of PCR products using oligonucleotide primers for detection of *C. burnetii* (*lane M* is a 100-bp DNA ladder (Fermentas, Germany), *lane 1* is blank (negative control), *lane 2* is positive control for 16s rRNA genes, *lanes 3* and *6* are negative specimens, *lanes 4* and *5* are positive samples)

Discussion

Q fever disease, caused by *C. burnetii*, is important zoonoses found worldwide (Maurin and Raoult 1999). Forty-five years after Derrick and Burnet described Q fever and its rickettsial etiological agent, the disease remains relatively obscure to the general lay and scientific audiences, lacking the dramatic appeals of the classic rickettsioses. Even for workers with Q fever, the aspects of the immunology and pathobiology of the infection, the biology of *C. burnetii*, even the taxonomic position of the organism, and the molecular basis of the parasite–host interrelationships remain inadequately answered questions (Baca and Paretsky 1983). In animals, *C. burnetii* is found in the reproductive system, both in uterus and mammary glands (Kirkan et al. 2008). Also, abortion is the clinical manifestation of a widespread occurrence of *C. burnetii* in the animal population and is mainly concentrated during the reproductive season of small ruminants. In humans, it causes a variety of diseases such as acute flu-like illness, pneumonia, and hepatitis (Parisi et al. 2006). Also, endocarditis can occur as a cause of Q fever in humans, frequently in latent or chronic cases, often with attendant hepatomegaly and splenomegaly and occasionally with cardiomegaly, glomerulonephritis, myocarditis, pericarditis, and cardiovascular lesions (Rosman et al. 1977). Q fever in humans has been shown by the recovery of *C. burnetii* from placentas of women who had experienced Q fever 3 years earlier and who had recovered and were apparently normal. Q fever is endemic among farmers, abattoir workers, dairymen, and others who work with livestock. It should be considered in the diagnosis of pericarditis (Baca and Paretsky 1983).

Q fever in camel has been reported from many countries, including the Sudan, Egypt, Tunisia, India, Kenya, Central Africa, Ethiopia, Nigeria, United Arab Emirates, and Chad (Mansour et al. 2008). Q fever is endemic worldwide except in New Zealand (Hilbink et al. 1993). In Europe, acute Q fever cases in humans are more frequently reported in spring and early summer. They may occur at all ages, but they are more frequent in men than in women. Q fever is usually benign, but mortality occurs in 1 to 11 % of patients with chronic Q fever (Raoult 1990). In southern France, 5 to 8 % of cases of endocarditis are due to *C. burnetii*, and the prevalence of acute Q fever is 50 cases per 100,000 inhabitants (Tissot-Dupont et al. 1992). Large outbreaks of Q fever have also been reported in the Basque Region in Spain (Aguirre Errasti et al. 1984) and also in Great Britain (Guigno et al. 1992). The results of these studies confirmed the findings of our research.

In the USA, the animal studies show wide variation in seroprevalence, with goats having a significantly higher average seroprevalence (41.6 %) compared to sheep (16.5 %) or cattle (3.4 %). Evidence of antibody to *C. burnetii* was reported among various wild animal species, including coyotes, foxes, rodents, skunks, raccoons, rabbits, deer, and birds. This investigation suggests that *C. burnetii* is enzootic among ruminants and wild animals throughout much of the USA and that there is widespread human exposure to this pathogen (McQuiston and Childs 2002).

In present study, PCR technique was used to investigate *C. burnetii* in camels in Iran. Out of 130 camel blood samples, 14 (10.76 %) were found PCR positive for *C. burnetii*. There are many studies which described the detection and prevalence of *Coxiella*. Muramatsu showed that the PCR–ELISA detected coxiellas with a sensitivity tenfold higher than that of conventional PCR. Techniques such as gel electrophoresis combined with analyses of restriction fragment length polymorphisms and Southern blotting are commonly used for detecting and identifying PCR products (Muramatsu et al. 1997). In a study, Khalili and Sakhaee show serologic survey of Q fever in domestic animals in Iran. They used from ELISA kit to identify specific antibodies against *C. burnetii* in goats and cattle. The results showed that 35.5 % of all sera were positive. Goats had a significantly higher average seroprevalence (65.78 %) than cattle (10.75 %) (Khalili and Sakhaee 2009).

In another study, Soliman et al. reported results which showed that the competitive enzyme immunoassay (CEIA) detected 71 % (n=34) of the camel serum samples positive for *C. burnetii* antibody, while 65 % (n=31) were detected by the enzyme immunoassay with protein A conjugate (EIA-PA), with 60 % agreement. Of 40 % with discordant results, 11 camel serum specimens were positive by CEIA and negative by EIA-PA. They also detected *C. burnetii* antibody in sheep (n=40) and goat (n=96) serum samples. Among sheep serum samples, CEIA detected 63 % antibody positive, while EIA and immunofluorescence assay (IFA) detected 50 and 38 % antibody positive, respectively. Of the 96 goat serum samples, 50, 35, and 34 % were positive by CEIA, EIA, and IFA, respectively (Soliman et al. 1992). Rahimi et al. in 2009 detected *C. burnetii* by nested PCR in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran and showed that 13 of 210 (6.2 %) bovine milk samples were positive; the positive samples originated from 5 of 28 (17.9 %) commercial dairy herds. All 110 ovine bulk milk samples from 31 sheep breeding farms were negative and only 1 of 56 (1.8 %) caprine bulk milk samples from 20 goat breeding farms was positive for *C. burnetii* (Rahimi et al. 2009). Our results indicate that, although prevalence of Q fever was 10.76 % in camel, the *C. burnetii* has a relatively high role in Iranian camel.

Conclusion

According to result, this study confirms that camels are important reservoirs of *C. burnetii* and contributed further insights into this important disease in Iran. The prevalence of *C. burnetii* in Iranian camel is widely spread (10.76 %), suggesting that camels might have an important role in the epidemiology of endemic infections among human population in Iran. We also reported that PCR assay improved the detection of *C. burnetii* in camel blood samples. PCR is an effective assay for rapid detection and isolation of various kinds of bacteria from animals, including camel, sheep, cattle, etc., and human.

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