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Original Article

Coxiella burnetii and *Borrelia* spp. in peripheral blood of dromedary camels in Fars, Iran: molecular characterization, hematological parameters, and acute-phase protein alterations

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Abstract

Background: Dromedary camels (*Camelus dromedarius*) are raised in extremely strict ecological conditions of deserts. Camels are vulnerable to many zoonotic infections. There are limited data on the occurrence of Q fever and borreliosis in camels, in Iran. **Aims:** The current study was focused on the occurrence of *Coxiella burnetii* and *Borrelia* spp. infection in the blood samples of Iranian camels using molecular assays. Effect of the presence of these infections on various hematological factors and some acute-phase proteins (Hp, a1AGP, SAA) were also investigated. **Methods:** Blood samples were collected from 113 clinically healthy camels to investigate the presence of the infections using nested PCR. Moreover, the sequence of positive samples was analyzed phylogenetically. Routine haematological tests were performed and the concentrations of acute-phase proteins were measured in serum using enzyme immunoassay. **Results:** PCR result showed that 6.19% (95% CI: 2.53-12.35%) (7/113) of camels were positive for *C. burnetii*. In addition, sequencing results of the corresponding gene of the outer membrane protein (*com1*) revealed two different genotypes of *C. burnetii* agent in camels from Southern Iran. In the PCR assay, *Borrelia* spp. DNA was not detected in the samples. No significant difference was observed in hematological parameters or acute-phase proteins between positive and negative Q fever camels except for mean corpuscular hemoglobin (MCH) and red cell distribution width (RDW). **Conclusion:** Clinically healthy camels might be very important reservoirs of zoonotic pathogens. Q fever is not considered a notifiable disease in camels of Iran, and clinical cases may scarcely be recognized by the healthcare system. Due to a lack of adequate information, additional studies on the molecular epidemiology and clinical pathology aspects of *C. burnetii* infection in Iran are needed.

Key words: Acute-phase proteins, *Borrelia*, Camel, *Coxiella burnetii*, Nested PCR

Introduction

Dromedary or one-humped camels (*Camelus dromedarius*) are specifically raised by nomads throughout arid areas of Africa, India, and East Asia for their high productive potential (Gagaoua *et al*., 2022). In Iran, camel husbandry is a traditional way of subsistence for people settled in deserts, and camel raising is expanded in recent years (Mohammadpour *et al*., 2020; Hassani, 2021). Given the increasing global temperature and desertification, camel raising is acquiring more attraction since camels are animals that can survive as sustainable livestock to develop animal production and future agriculture. However, there are relatively limited data in terms of behavioral, physiological, and health properties as well as zoonotic diseases (Gagaoua *et al*., 2022) of camels. These animals may be infected by various zoonotic pathogens, such as *Coxiella burnetii* and *Borrelia* spp. that threaten public health (Bahari *et al*., 2021; El Tigani-Asil *et al*., 2021).

Coxiella burnetii, a small obligate intracellular Gram-negative bacterium, causes Query (Q) fever or coxiellosis as an important globally distributed zoonotic disease (Woldehiwet, 2004). Different domestic and wild animals are susceptible to this pathogen. Livestock, comprising cattle, sheep, goats, and camels constitute main reservoirs of the infection (Muema *et al*., 2022). Q fever is transmitted between animals through inhalation of contaminated aerosols from aborted tissues or fluid, urine, feces, semen, consumption of contaminated milk and other dairy products, and tick bites (Nokhodian *et al*., 2018; Holloway *et al*., 2022). There is also report suggesting the potential for sexual transmission through semen (Ruiz-Fons *et al*., 2014).

Compared to other domestic animals such as cattle and sheep, a high seroprevalence rate of Q fever in dromedaries was previously reported. It is suggested that the camel is one of the most susceptible animals to *C. burnetii* (Schelling *et al*., 2003; Gumi *et al*., 2013; Horton *et al*., 2014; Mohammed *et al*., 2014). A previous study has also reported a seroprevalence of up to 90% for Q fever in camels in South East Ethiopian pastoral livestock (Gumi *et al*., 2013). According to a systematic review in Iran, Q fever seropositivity was reported to be about 28.26% in camel (Mohabbati Mobarez *et al*., 2017). The disease almost has an asymptomatic manifestation in animals; while few reproductive abnormalities such as abortion, stillbirth, and delivery of weak birth are reported, elsewhere (Li *et al*., 2018; Hassani *et al*., 2021). Serological and molecular methods have been applied to study the occurrence of *C. burnetii* in camels (Rahimi *et al*., 2010; Abdullah *et al*., 2019; Eckstein *et al*., 2022; Hussain *et al*., 2022).

Lyme borreliosis (LB), a tick-borne relapsing fever (TBRF), is another notable zoonotic disease that may infect camels. The disease is caused by *Borrelia* species, spirochetes, distributed worldwide, including the North-Central United States, Central Asia, and Eastern Europe. The geographical areas involved with the disease are also expanding (CDC, 2022). B*orrelia burgdorferi* sensu lato (Bbsl) is the most important genospecies transmitted by the *Ixodes ricinus* ticks, the principal vector of Bbsl in countries located in the northern hemisphere. Infected tick vectors can spread the disease to domestic and wild animals and humans (Weck *et al*., 2022). Common clinical manifestations in camels comprise fever, weight loss, erythema migrans, lameness and arthritis (Raza *et al*., 2021). Limited studies have been reported for the diagnosis of LB in camels. For example, a serological study reported a prevalence of 47.8% in Egypt (Helmy, 2000), and a molecular study reported 3.6% prevalence in China (Zhai *et al*., 2018).

In a study from Iran, Borreliosis was not detected in the blood samples of camels (Sazmand *et al*., 2019).

Borreliosis is diagnosed by serological (ELISA, IFA) or molecular (PCR) methods. PCR is an accurate and sensitive assay to characterize different species of Bbsl (Usman *et al*., 2022).

When pathogens *C. burnetii* or *Borrelia* spp. enter the body of animals or humans, they affect the immune system or hematological factors. Detection of various acute-phase proteins (APPs) in camels is proposed to be used as a "herd health" marker (Hussain *et al*., 2022).

To the best of our knowledge, limited studies are carried out in Iranian camels in order to detect *C. burnetii* or *Borrelia* spp., and also investigate their effects on hemato-biochemical variables in this host. Most studies have focused on ruminants (Nokhodian *et al*., 2018; Khoobdel *et al*., 2021; Hussain *et al*., 2022). As a result of the presence of Q fever in Iran, the outcomes of the present study can be employed by the official veterinary organizations and healthcare providers to clarify the epidemiology and manage the diseases in camels.

Therefore, this study was conducted to investigate the prevalence of *C. burnetii* and *Borrelia* spp. in blood samples of camels using nested polymerase chain reaction (PCR) assays in Fars, Iran. Moreover, the current study was planned to find out any possible effect of *C. burnetii* and *Borrelia* sp. infections on different hematological parameters and major acute phase proteins i.e., serum amyloid A (SAA), alpha-1-acid glycoprotein (a1AGP), and haptoglobin (Hp).

Materials and Methods

Ethics statement

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Shiraz University of Medical Sciences Ethics Committee approved the research (IACUC No.: 4687/63).

Blood sampling

Altogether 113 blood samples were collected from clinically healthy camels (aged 1-11 years, 16 (14.16%) males and 97 (85.84%) females) in Fars province, Iran.

The following formula was used for estimation the sample size in this research:

$$n = \left(\frac{Z_{1-\frac{\alpha}{2}}}{d}\right)^2 pq$$

n: Sample size
Z: The level of confidence (z=1.96)
P: The expected prevalence
d: Precision (corresponding to effect size, d=0.07)

Fars Province covers 122,400 km^2 of semi-dried areas and is located in an arid and semi-arid region of southern Iran. It encompasses 52,069 square mile area (8.09% of the entire country) at (27°03′) and (31°42′) northern latitude and (50°30′) and (55°36′) eastern longitude. Although the sampling was performed in Fars, the camels were not permanently resided in the sampling area. They were conveyed to the southern provinces, such as Hormozgan and Bushehr, to find fresh pasture in hot and cold seasons. Information about gender and age was also recorded.

Blood samples were drawn from the internal jugular vein of each animal and collected in two sets of tubes: without anticoagulant for quantification of serum acute phase proteins (3 ml) and with ethylene diamine tetra acetic acid (EDTA) for hematological analysis and DNA extraction (2 ml). The samples were carefully transferred to the laboratory under refrigeration conditions. Sera were separated from the blood samples following centrifugation at 2000 × g for 15 min. Sera and whole blood samples were finally stored at -20°C for further use.

DNA extraction

Genomic DNA was extracted from 100 µL of EDTA-preserved whole blood samples using DNeasy Blood and

Tissue kit (Qiagen®, Hilden, Germany), as was recommended by the manufacturer. The extracted DNA samples were eluted in 100-200 µL of Tris-EDTA (TE) buffer and their purities were assessed by NanoDrop spectrophotometer at 260 and 280 nm (ThermoFisher, USA).

Nested-PCR for *Coxiella burnetii* detection

The nested-PCR assay was performed on the extracted DNAs (n=113) to amplify the outer membrane protein gene (*com1*) using two pairs of *C. burnetii*-specific primers, including forward: 5´-AGT AGA AGC ATC CCA AGC ATT G-3´ and reverse: 5´-TGC CTG CTA GCT GTA ACG ATT G-3´. The PCR mixture was prepared in a final volume of 25 µL, comprising *Taq* DNA Polymerase 2x Master Mix (Amplicon, Iran), primers (1 pmol for each), DNA template (10 ng), and DEPC-treated water (8.5 µL); then, amplified in a thermocycler (Bioer Technology, China), as per the following cycling steps: initial denaturation (94°C, 3 min), 35 cycles of denaturation (94°C, 45 s), annealing (65°C, 45 s), and extension (72°C for 45 s), with a final extension (72°C, 5 min).

The first-round PCR products (2 µL) were used as DNA templates for the second round of the nested-PCR, established using assigned primers, comprising forward: 5´-GAA GCG CAA CAA GAA GAA CAC-3´ and reverse: 5´-TTG GAA GTT ATC ACG CAG TTG-3´. The second-round PCR amplification conditions were similar to the first reaction (Abdali *et al*., 2018).

Positive (*C. burnetii* strain ATCC:VR-615) and negative (ultra-pure sterile water) controls were also considered. The amplified products of the first (501 bp) and second (438 bp) rounds were identified on a 1.2% agarose gel containing 0.5 µg/ml safe stain and visualized by a UV transilluminator.

Nested-PCR for *Borrelia* spp. detection

Nested-PCR was performed as per the study established by Dibernardo *et al*. (2014). To identify *Borrelia* species, the *16S-23S rRNA* intergenic spacer (IGS) regions was initially targeted using IGSF1 (5´-GTA TGT TTA GTG AGG GGG GTG-3´) and IGSR1 (5´-GGA TCA TAG CTC AGG TGG TTA G-3´) primers. For the second-round of the PCR amplification primers IGSF2 (5´-AGG GGG GTG AAG TCG TAA CAA G-3´) and IGSR2 (5´-GTC TGA TAA ACC TGA GGT CGG A-3´) were used (Bunikis *et al*., 2004).

Borrelia miyamotoi was also provided from Pasteur Institute, Iran and used as a positive control. As the primer was designed for a wide range of *Borrelia* spp. PCR product was also produced by this species.

DNA sequencing and phylogenetic analysis

The purified positive amplicons (438 bp) were obtained using a PCR product purification kit (Fermentas, Burlington, USA), and then submitted for sequencing bi-directly using the Sanger method (Macrogen, South Korea). The sequences were initially analyzed using Geneious Prime 2020.2.5 software, then subjected to the National Center for Biotechnology Information (NCBI), and compared with the associated sequences using BLAST. MEGA X software was used to draw phylogenetic tree and identity percentages using maximum likelihood and maximum parsimony methods (Ghaemi *et al*., 2019). Also, the collected sequences were aligned using the alignment tools through the CLC Main Workbench version 7 (CLC Aarhus, Denmark) and the obtained results were used to perform a pairwise comparison to calculate the genetic diversity and similarity indices.

Hematology

A complete blood count (CBC) was carried out using an automatic blood cell counter (Exigo, Stockholm, Sweden). Differential counting of white blood cells (WBC) was also performed following Giemsa staining of blood films (Sigma-Aidrich, CA, USA) under a light microscope.

Acute-phase proteins measurement

Commercial camel-specific kits (Shanghai Crystal Day Biotech, Shanghai, China) were used to measure the amounts of serum amyloid A, haptoglobin, and alpha-1 acid glycoprotein in the sera using a quantitative sandwich enzyme immunoassay.

Statistical analysis

Data were analyzed using IBM SPSS Statistics software (ver. 26). Independent sample t-test and Mann-Whitney test were conducted. The significant relationships between the occurrence of diseases and the age or sex of the infected camels were confirmed by Chi-square test. $P<0.05$ was considered significant.

Results

Detection of *C. burnetii* and *Borrelia* spp. using nested-PCR

Of the 113 blood samples, 7 (6.19%, 95% CI: 2.53-12.35%) were found positive, with a band size of 438 bp, for *C. burnetii* using nested PCR assay. From the infected camels (n=7), 6 aged lower than 2 years, out of which 3 were lower than 1 year old and 3 were between 1 to 2 years old. Furthermore, among the *C. burnetii* positive camels (n=7), 2 (28.57%) and 5 (71.43%) were male and female, respectively. The statistical analysis did not show any significant difference ($P<0.05$) between the occurrence of *C. burnetii* infection and age or sex of camels.

Also, the result of PCR for *Borrelia* spp. detection showed that all blood samples were negative.

Phylogenetic analysis

The obtained sequences of PCR products (438 bp) showed two different genotypes based on the partial sequences of *Com1* gene (MH373681-MH373682). These Iranian sequences and other related *Com1* genes existing in the GenBank database with different sources

were used for the phylogenetic analysis (Fig. 1). According to the drawn phylogenetic tree, both genotypes were classified in a single cluster. The phylogenetic tree also showed the Iranian strains positioned into a different major group compared to the *C. burnetti* strains reported from the steller sea lion (*Eumetopias jubatus*) in USA (GU797241) (Fig. 1). The obtained sequence from strain Fars-GH4 was 100% similar to those of *C. burnetti* reported from China (CP035112 and MW226880: *Haemaphysalis flava*), Greece (CP014561: *Homo sapiens*), Japan (AB004711), USA (CP040059: *Dermacentor andersoni*) and Italy

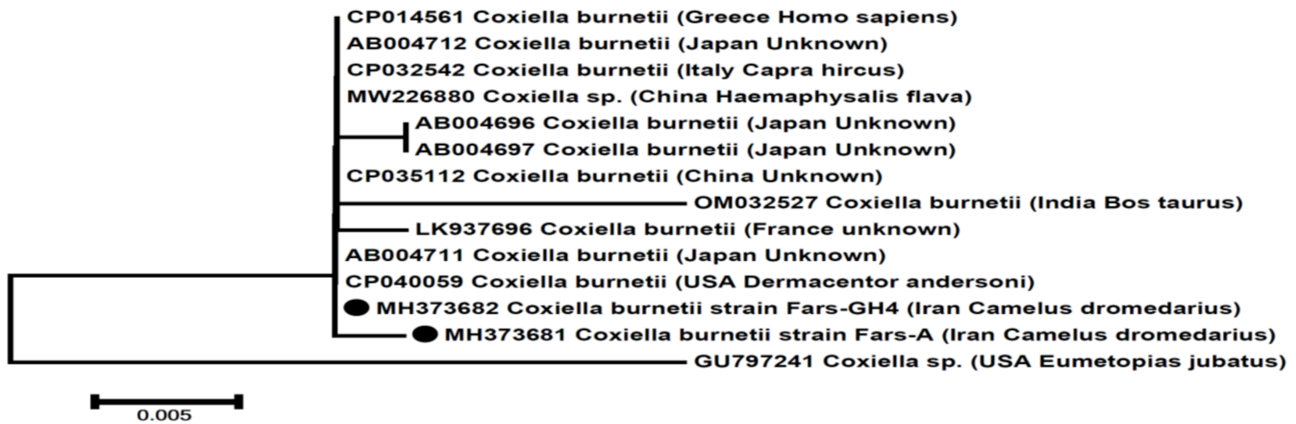

Fig. 1: Phylogenetic tree was made based on the partial outer membrane protein gene (*com1*) sequence obtained from Iranian strains in the present study, with other homologue sequences belonged to this parasite existing in the NCBI database and a total alignment of 438 bp length. The maximum likelihood (ML) method through MEGA X Software was used to provide phylogenetic tree. The sequence from the present study is shown with a dark circle

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
MH373681 Coxiella burnetii strain Fars-A (Iran Camelus dromedarius)	1		1	1	1	1	2	1	1	1	2	2	19	24	33
MH373682 Coxiella burnetii strain Fars-GH4 (Iran Camelus dromedarius)	2	99.77		0	0	0	1	0	0	0	1	1	18	23	32
CP040059 Coxiella burnetii (USA Dermacentor andersoni)	3	99.77	100.00		0	0	1	0	0	0	1	1	18	23	32
AB004711 Coxiella burnetii (Japan Unknown)	4	99.77	100.00	100.00		0	1	0	0	0	1	1	18	23	32
CP035112 Coxiella burnetii (China Unknown)	5	99.77	100.00	100.00	100.00		1	0	0	0	1	1	18	23	32
LK937696 Coxiella burnetii (France unknown)	6	99.54	99.77	99.77	99.77	99.77		1	1	1	2	2	19	24	33
MW226880 Coxiella sp. (China Haemaphysalis flava)	7	99.77	100.00	100.00	100.00	100.00	99.77		0	0	1	1	18	23	32
CP014561 Coxiella burnetii (Greece Homo sapiens)	8	99.77	100.00	100.00	100.00	100.00	99.77	100.00		0	1	1	18	23	32
CP032542 Coxiella burnetii (Italy Capra hircus)	9	99.77	100.00	100.00	100.00	100.00	99.77	100.00	100.00		1	1	18	23	32
AB004696 Coxiella burnetii (Japan Unknown)	10	99.54	99.77	99.77	99.77	99.77	99.54	99.77	99.77	99.77		0	19	24	33
AB004697 Coxiella burnetii (Japan Unknown)	11	99.54	99.77	99.77	99.77	99.77	99.54	99.77	99.77	99.77	100.00		19	24	33
AB004712 Coxiella burnetii (Japan Unknown)	12	95.66	95.89	95.89	95.89	95.89	95.66	95.89	95.89	95.89	95.66	95.66		5	14
OM032527 Coxiella burnetii (India Bos taurus)	13	94.52	94.75	94.75	94.75	94.75	94.52	94.75	94.75	94.75	94.52	94.52	98.81		19
GU797241 Coxiella sp. (USA Eumetopias jubatus)	14	92.47	92.69	92.69	92.69	92.69	92.47	92.69	92.69	92.69	92.47	92.47	96.67	95.48	

Fig. 2: Estimation of partial outer membrane protein gene (*com1*) nucleotide difference (upper right) and genetic similarity (lower left) for *Coxiella burnetii* (data in the parentheses indicate country and isolation source) using the CLC Main Workbench ver. 7 (CLC Aarhus, Denmark)

Table 1: Mean±SE of different hemogram parameters and acute phase proteins in *C. burnetii* positive and negative camels. Statistically significant parameters at P<0.05 level are specified with superscripted stars

Infection status	Number	Hemogram parameters and acute phase proteins									
		HCT (%)	RBC (10^6/µL)	Hb (g/dL)	MCV (fl)	MCH* (pg)	MCHC (g/dL)	RDW* (%)	Hp (mg/dL)	a1AGP (mg/dL)	SAA (µg/ml)
C. burnetii positive	7	27.97 ±2.84	6.01 ±0.71	11.50 ±0.60	41.65 ±1.27	19.95 ±1.62	48.53 ±4.95	21.20 ±1.66	0.290 ±0.00	2677.06 ±512.72	19.09 ±3.43
C. burnetii negative	106	28.48 ±0.52	7.03 ±0.18	10.95 ±0.19	40.90 ±0.66	15.73 ±0.26	38.72 ±0.65	25.97 ±0.54	0.291 ±0.01	2592.98 ±231.91	17.34 ±1.96
P-value		0.866	0.068	0.347	0.699	0.048	0.105	0.005	0.886	0.868	0.665

* Showing significant difference (P<0.05). HCT: Haematocrit, RBC: Red blood cell, Hb: Hemoglobin, MCH: Mean corpuscular hemoglobin, MVHC: Mean corpuscular hemoglobin concentration, RDW: Red cell distribution width, Hp: Haptoglobin, a1AGP: alpha-1-acid glycoprotein, and SAA: Serum amyloid A

Table 2: Mean±SE of total white blood cells (WBC) and differential counts (10^3/µL) in *C. burnetii* positive and negative camels. No significant difference was observed between the two groups

Infection status	Number	Leukogram parameters						
		WBC	Neutrophil total	Lymphocyte total	Monocyte total	Eosinophil total	Platelet	Band neutrophil total
C. burnetii positive	7	16.70 ±2.69	6345 ±501765.44	6116.50 ±1466.16	588.50 ±492.01	1093.17 ±620.524	92.50 ±28.50	223.00 ±178.38
C. burnetii negative	106	12.56 ±0.33	7529.74 ±348.14	4128.17 ±373.44	179.74 ±37.68	538.02 ±118.036	81.72 ±4.33	185.59 ±34.99
P-value		0.186	0.538	0.083	0.445	0.155	0.603	0.743

(CP032542: *Capra hircus*) (Fig. 2).

Hematological findings and APPs measurement

The results of the hematological test, including RBC indices, WBC differential count, and acute-phase proteins are provided in (Tables 1 and 2). No significant difference was observed in hematological parameters or acute-phase proteins between positive and negative Q fever camels except for mean corpuscular hemoglobin (MCH) and red cell distribution width (RDW).

Discussion

This study focused on the occurrence of *C. burnetii* and *Borrelia* spp. in 113 clinically healthy camels in Southwestern Iran using nested-PCR. Our study showed 7 (6.19%) positive cases for *C. burnetii*, while no *Borrelia* spp. DNA (0%) was detected in the blood samples. The potential risk of Q fever in camels is not well established in the Middle East and Iran, while this zoonotic disease has been comprehensively studied in ruminants (Nokhodian *et al*., 2018; Khoobdel *et al*., 2021; Holloway *et al*., 2022). In camels, the diagnosis of coxiellosis is difficult in field conditions since it is mainly asymptomatic or represents nonspecific symptoms. Serological and molecular assays are usually used to detect *C. burnetii* infection. Few studies are available on Q fever identification in dromedary camels in Iran; for instance, the serological prevalence was reported to be 28.7% in the Khorasan province, North East of Iran (Janati Pirouz *et al*., 2015). In camels, limited seroprevalence studies on Q fever have been previously reported from the neighboring countries of Iran, including Pakistan (31.1%) (Hussain *et al*., 2022) and Abu Dhabi, United Arab Emirates (7.9%) (Afzal and Sakkir, 1994).

In other countries, most studies of coxiellosis prevalence are carried out serologically in camel populations, including Saudi Arabia (62% and 51.6%) (Hussein *et al*., 2008; Jarelnabi *et al*., 2018), Jordan (49.6%) (Holloway *et al*., 2022), Tunisia (44.4%) (Selmi *et al*., 2018), Kenya (38.6%) (Muema *et al*., 2022), Egypt (16%) (Ali *et al*., 2016), Algeria (71.2%) (Benaissa *et al*., 2017), Chad (80%) (Schelling *et al*., 2003) and Ethiopia (90%) (Gumi *et al*., 2013). Although serological tests, used routinely as a screening method, emerge an epidemiological history of the infection in camels, they have some limitations; antibodies which can not discriminate between acute and chronic phases of Q fever are not sufficient for the disease treatment, and are not strongly correlated with the bacterium shedding (Zhang *et al*., 1998; Barlozzari *et al*., 2020). Molecular methods such as nested PCR assay have been developed as precious diagnostic methods to assess the prevalence of coxiellosis in animals due to their high sensitivity and specificity, the ability to diagnose the pathogen at the early stage of the infection with a low amount of DNAs in blood samples (Abdullah *et al*., 2019). This study targeted the *com1* gene, encoding outer membrane protein (OMP), to detect *C. burnetii* in the blood samples using nested-PCR. This gene is highly conserved among 21 strains of *C. burnetii* isolated from clinical and environmental sources in Japan (Zhang *et al*., 1998). A variety of molecular coxiellosis prevalence reports in camels are available from different countries in the world; for example, in the Canary Islands, Spain (0%) (Mentaberre *et al*., 2013), and Egypt (16.9%) (Abdullah *et al*., 2019). The molecular prevalence of Q fever in dromedary camels in Iran has been scarcely reported at 2.4% in Khorasan, North East of Iran (Janati *et al*., 2017), and 10.76% in Isfahan in the central region of Iran (Doosti *et al*., 2014). The results of our study are inconsistent with those of Isfahan which neighbors Fars province.

The evidences on the presence of *C. burnetii* in camels' blood elucidate that the pathogen would be shed in the camels' milk, urine, feces, and parturition fluids (Devaux *et al*., 2020; Mohammadpour *et al*., 2020). Fecal shedding is proposed as a preferred way in camels, leading to the spreading of the pathogen in a herd (Hussein *et al*., 2015). Due to close contact between ranchers and infected camels and the consumption of their infected milk and meats, the risk of Q fever zoonotic disease would rise in humans (Mohammadpour *et al*., 2020). Moreover, since the disease is asymptomatic in infected camels, illegal transportation of camels between neighboring countries may extensively spread coxiellosis among animals and humans (Mohammadpour *et al*., 2020). A study in Chad investigated the presence of Q fever in pastoralists and their livestock and considered the disease as an occupational hazard. More research is needed to assess the role of camels in *C. burnetii* transmission to other animals and humans.

Studies in Iran have revealed a significant

seroprevalence of Q fever in humans and animals, with a high prevalence of *C. burnetii* in milk and abortion samples of domestic animals (Heydari *et al*., 2021). Chronic Q fever is a rare condition that occurs in around 5% of patients after acute Q fever. It can manifest as endocarditis, vasculitis, joint infection, or lymphadenitis. Endocarditis is the most common form, affecting 60-78% of cases of chronic Q fever worldwide (Moradnejad *et al*., 2019). However, the exact prevalence of Q fever endocarditis in Iran is not well-established and requires further extensive studies. Q fever is considered an important cause of culture-negative endocarditis in Iran, demanding increased attention from healthcare professionals and the healthcare system (Heydari *et al*., 2021). Additionally, a study in the Khorasan Razavi province reported a 7.4% prevalence of acute Q fever in cases of undifferentiated febrile illnesses using PCR (Khalili *et al*., 2016).

The phylogenetic analysis of Iranian *C. burnetii* isolates indicated 92.69-100% identity with previously published sequences worldwide from different host animal and human sources.

The hematological findings showed no significant increase between positive and negative Q fever camels except in MCH and RDW. This may be due to the intracellular characteristic of the pathogen that invades monocytes and macrophages and escapes from host defense. Further researches using experimental studies are needed to elucidate the hematological and acute phase protein changes during *C. burnetii* infection in camel.

This study also investigated the presence of *Borrelia* DNA in the camels' blood samples using PCR in Fars province, Southern Iran. PCR is an alternative method to prevent and control Lyme disease (Zhai *et al*., 2018). No positive sample was found in the present study. This probably could be due to the very low bacteria remaining in the blood after the infection, or low *Borrelia* prevalence in this region. Further serological studies are also recommended for Lyme disease in Fars province. Our result is similar to previous studies carried out on camels' blood samples in the Aegean region (Turkey) and Riyadh Province (Saudi Arabia) (Alanazi *et al*., 2018; Erbas *et al*., 2018). However, other countries have reported seropositivities in camels, including Pakistan (2.47%) (Raza *et al*., 2021) and China (3.6%) (Zhai *et al*., 2018). These results suggest that camels are reservoirs for Lyme disease. In our study, although *Borrelia* DNA was not found, more studies are recommended to investigate the antibodies in camels' blood.

Both *C. burnetii* and *Borrelia* spp. are considered tick-borne pathogens in domestic animals. Many studies have been conducted on ticks of camels with low to high prevalence of coxiellosis and borreliosis in the world (Sazmand *et al*., 2019). In this study, ticks associated with camels were not considered and further surveys are suggested as complementary data for our results in this region.

In Iran, the population of camels is rising in recent years. Transporting the camels to the neighboring countries, such as Pakistan, Afghanistan, and the United Arab Emirates (UAE), has not been specifically restricted and thus might be problematic. This study may help healthcare providers and veterinary organizations to devise surveillance long term strategies in this region.

Our study revealed that the camels in Fars province are relatively infected with *C. burnetii* in the molecular assay. This result highlights the role of camels as a reservoir for transmission of the zoonotic disease to humans. Since Iran is in the neighborhood of countries with a high prevalence of Q fever and there are no specific restrictions on animal transportation, the disease may be outspread in the region. Besides, appropriate measures are recommended by healthcare providers to reduce the expansion of the pathogen. Serological assays are also suggested for screening coxiellosis prevalence in Fars province, Iran.

Also, *Borrelia* DNA was not detected using the PCR test. Further serological investigations are also recommended for detection of the borreliosis in dromedary camels.

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Conflict of interest

The authors declare that they have no conflict of interest.

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