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Are Dogs and Cats Possible Reservoirs for Human Q Fever in Iran?

Mahdieh REZAEI[1], Mohammad KHALILI[2], Mehdi SABERI[1], Ali Reza NAKHEI[3], Farnoush BAKHSHAEE SHAHRBABAKI[3], Shamim ABDOLI NASAB GOROUHI[3], Domenico OTRANTO[4], Marialaura CORRENTE[4]

[1]Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
[3]Graduated student, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran
[4]Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy

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ORCID IDs of the authors: M.R. 0000-0003-0783-4123; M.K. 0000-0001-9390-9392; M.S. 0000-0003-0499-7891; A.R.N. 0000-0002-0873-6915; F.B.S. 0000-0002-0647-8967; S.A.N.G. 0000-0002-3494-1613; D.O. 0000-0002-7518-476X; M.C. 0000-0002-0164-6730.

Abstract

Q fever caused by *Coxiella burnetii* is a zoonosis of public health concern, primarily transmitted by ruminants and other potential animal species. Dogs and cats have been previously implicated in the transmission of the infection; however, their involvement in the epidemiology of Q fever has been poorly investigated. Therefore, in this study, the occurrence of *C. burnetii* was investigated in dogs (n=81) and cats (n=28) subjected to ovariohysterectomy or vaginal swab sampling. *C. burnetii* was detected by nested trans-polymerase chain reaction assay in 5 uterine samples of both dogs and cats (i.e., 12.1% and 17.8%, respectively) as well as in 8 of 40 (20%) vaginal samples of dogs. The presence of *C. burnetii* was determined in 18 of 109 (16.5%) samples. The genital tract of dogs and cats can harbor *C. burnetii*, and these animals may be considered as a source of infection. Therefore, Q fever should be considered a notable public health threat in Iran, and the pet owners should be made aware of the zoonotic potential of *C. burnetii*.

Keywords: *Coxiella burnetii*, Iran, nested trans-polymerase chain reaction, pets, Q fever, reproductive samples

Introduction

Q fever caused by *Coxiella burnetii* is a significant re-emerging zoonosis of public health concern reported worldwide (Greene, 2012). *C. burnetii* is a gram-negative, obligate, intracellular bacterium (Norris et al., 2013) that infects a range of host species, including domestic and wild animals as well as human beings (Agerholm, 2013; Gwida, 2012). Ruminants are well known as the primary reservoir of the infection (Greene, 2012), although transmission of the infection from dogs and cats is also recognized (Knobel et al., 2013). The transmission occurs through inhalation and ingestion of the organisms, and arthropods (e.g., fleas and ticks) have also been implicated in the infection (Porter et al., 2011). The microorganism exhibits a tropism for the reproductive system (Havas and Burkman, 2011), and the infected animals may shed massive numbers of environmentally resistant organisms in body secretions (e.g., vaginal discharges and semen), milk, and afterbirth products (Kilic et al., 2008; Porter et al., 2011). This highly contagious bacterium can survive in the environment for at least several weeks (Mediannikov et al., 2010). Q fever can be acute or chronic, but most infections in animals are asymptomatic. The chronic or persistently focal forms in humans are usually characterized by clinical signs as they often involve serious complications, such as endocarditis, hepatitis, or chronic fatigue syndrome (Cooper et al., 2011; Skerget et al., 2003). Abortions, stillbirths, weak offspring, metritis, and infertility have been reported in animals and humans (Berri et al., 2001; Brouqui et al., 2007; Cantas et al., 2011), although the infection is often asymptomatic and underdiagnosed (Komiya et al., 2003; Porter et al., 2011). Diagnosis is made by serologic surveys, organism isolation, polymerase chain reaction (PCR), and immunohistochemical methods (Greene, 2012).

Address for Correspondence: Mahdieh REZAEI • **E-mail:** mahdiehrrezaei@gmail.com; m.rezaei@uk.ac.ir
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The epidemiological role of ruminants in Q fever has been well documented, but information regarding pet animals has been minimally investigated. There are several reports of human transmission with a history of exposure to breeding queens or bitches and neonates during parturition (Marrie et al., 1988a; Marrie et al., 1988b; Marrie et al., 1989; Norris et al., 2013). The exposure to environmental aerosols or fomites of the infected dogs and cats can infect the people who interact closely with these animals (Greene, 2012). In some areas, pets seem to play a more prominent role than ruminants in transmitting the disease to humans (Cooper et al., 2011). Q fever is endemic in Iran, and most human cases have been related to livestock contact (Ezatkhah et al., 2015), whereas limited data are available about the distribution of this disease in companion animals. In this study, the existence of *C. burnetii* DNA in the reproductive tract specimens from dogs and cats in Kerman (south-eastern Iran) was investigated using nested trans-PCR assay.

Materials and Methods

Sample collection and DNA extraction

From March 2014 to September 2016, specimens (n=109) were collected from animals presenting to the veterinary teaching hospital of Shahid Bahonar University of Kerman for routine procedures. The protocol was approved by the animal care committee of the Veterinary College of Shahid Bahonar University of Kerman (No: 950101). The uterine samples were taken from 41 female dogs, including 27 shelter and 14 pet dogs, and 28 female cats, including 19 stray and 9 client-owned cats, which were admitted for ovariohysterectomy surgery. Vaginal samples were also acquired from 40 referred animals, including 19 stray and 21 pet dogs. For each case, data including age, breed, type of housing (indoor or outdoor), health status, and history of pregnancy or reproductive disorders, were recorded using a detailed questionnaire (Table 1).

Vaginal swab samples were collected and transferred into sterile Eppendorf tubes containing 500 µL sterile phosphate-buffered saline. A full-thickness uterine sample (3 mm × 3 mm) was taken at the ovariohysterectomy surgery from each dog and cat. The uterine samples were also maintained in 70% ethanol. Extraction of DNA was performed using the DNA extraction Mini Kit (GenAll®Exgene TM, Korea) according to the manufacturer's instructions. A negative extraction control was considered between every 3 samples to evaluate the efficiency of nucleic acid extraction. All the specimens were preserved at –20°C for next steps.

Molecular detection of bacterium (nested trans-PCR assay)

In this study, nested trans-PCR assay was performed using the IS*1111* gene target as previously described (Berri et al., 2000; Parisi et al., 2006). Nested trans-PCR amplifications were carried

Table 1. Distribution of samples according to the age, breed, pregnancy, reproductive disorders, and type of housing

Sample	Age (years)			Breed	Pregnancy		Reproductive disorders	Housing		
	≤2	2–7	≥7	Type and number of breeds	Yes	No	Yes	No	Yes	No
Dog (uterine)	15	18	8	Crossbreed: 29, Terrier: 6, Rottweiler: 1, Doberman: 1, German: 2, Dachshund: 1, Spitz: 1	6	35	Total: 11; vaginal prolapse: 1, pyometra: 2, breast tumor: 2, dystocia: 1, metritis: 3, uterine atresia: 1, abortion: 1	30	27	14
Dog (Vagina)	9	22	9	Terrier: 11, Crossbreed: 12, Rottweiler: 1, German:10, Dachshund: 2, Bulldog: 1, Husky: 1, Pitbull: 2	5	35	Total: 12; Stillbirth: 4, vaginal prolapse: 2, pyometra: 3, breast tumor: 1, dystocia/ metritis: 1, mating problem: 1	28	21	19
Cat (Uterine)	22	6	-	DSH: 23, DLH: 3, Persian: 2	10	18	Total: 6; stillbirth: 1, dystocia: 4, abortion: 1	22		

DSH: Domesticated Short Hair cats; DLH: Domesticated Long Hair cats

out via 2 runs of PCR. The volume was 25 µL, including 5 µL of DNA template, 12.5 µL commercial master mix (Hot Start Qiagen Master Mix), 4.5 µL water, and 1.5 µL of each primer at a concentration of 10 mM. Master mixes were prepared according to the instructions of the manufacturer.

MG thermal cycler (Eppendorf, Germany) was used for DNA amplification. The first round of PCR amplification was at 95°C for 2 minutes, followed by 5 cycles at 94°C for 30 seconds, 66°C–61°C (the temperature was decreased by 1°C between consecutive steps) for 1 minute, and at 72°C for 1 minute. These cycles were followed by 35 cycles consisting of 94°C for 30 seconds, 61°C for 30 seconds, and 72°C for 1 minute, and a final extension step at 72°C for 10 minutes.

Thereafter, 5 µL of the first amplification product was used for the second amplification using the nested primers. In the second amplification, the cycling conditions included an initial step at 94°C for 3 minutes, followed by 35 cycles at 94°C for 30 seconds, 50°C for 45 seconds, 72°C for 1 minute, and the final step at 72°C for 10 minutes. The amplicons were analyzed by agarose gel electrophoresis on a 1.5% tris-borate-ethylene diamine tetra-acetate agarose gel, stained with fluoro dye, visualized with an ultraviolet transilluminator, and photographed.

Table 2. Frequency and percentage of detected *C. burnetii*

Samples	Frequency	(%)
Uterine (dog)	5 of 41	12.1
Vaginal (dog)	8 of 40	20
Uterine (cat)	5 of 28	17.8
Total	18 of 109	16.6

DNA (6 ng) extracted from *C. burnetii* reference Nine Mile, phase II, strain (RSA 493, Slovakia) was used as the positive control and sterile distilled water as negative control.

Statistical analysis

Statistical analysis was performed using the IBM Statistical Package for the Social Sciences software version 21) (IBM SPSS Corp., Armonk, NY, USA), and $p<0.05$ was considered statistically significant.

Results

C. burnetii DNA was detected using nested trans-PCR (Figure 1) in the uterine samples from 5 of 41 (12.1%) dogs and 5 of 28

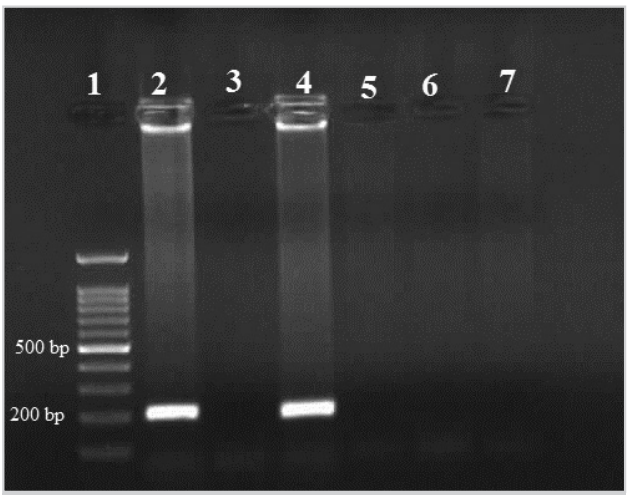

Figure 1. Detection of *Coxiella burnetii* DNA in the reproductive samples collected from dogs by nested trans-polymerase chain reaction. Lane 1: molecular size markers (100-bp DNA ladder); lane 2: positive control; lane 3: negative control; lanes 4 to 7: samples.

Table 3. Association between the detection of *Coxiella burnetii* in the reproductive tract samples of dogs and cat and various factors

Variable/Sample	Uterine (dog)		Vagina (dog)		Uterine (cat)	
Age	**Positive/negative**	**p**	**Positive/ negative**	**p**	**Positive/ negative**	**p**
Under 2 years	2/13	0.8	3/6	0.4	3/19	0.2
Between 2 and 7 years	3/15		3/19		2/4	
Above 7 years	0/8		2/7		0/0	
Pregnancy	**Positive/negative**	**p**	**Positive/negative**	**p**	**Positive/negative**	**p**
Yes	0/6	0.3	1/4	0.74	0/10	0.08
No	5/30		7/28		5/13	
Reproductive disorders	**Positive/negative**	**p**	**Positive/negative**	**p**	**Positive/negative**	**p**
Yes	2/9	0.1	1/11	0.22	1/5	0.7
No	3/27		7/21		4/18	
Housing	**Positive/negative**	**p**	**Positive/negative**	**p**	**Positive/negative**	**p**
Indoor	1/13	0.7	3/18	0.42	2/7	0.1
Outdoor	4/23		5/14		3/16	

(17.8%) cats. The *C. burnetii* DNA was also found in 8 of 40 (20%) vaginal swab samples of dogs (Table 2).

Of the 5 *C. burnetii*-positive uterine samples from dogs, 1 was owned by a client and 4 were stray dogs. All the positive samples belonged to non-pregnant dogs who were less than 7 years old (Table 3). *C. burnetii* DNA was isolated from the vaginal samples of 3 client-owned and 5 stray dogs. Only 1 of these *C. burnetti*-positive dogs was pregnant, and there was only 1 *C. burnetii*-positive dog with a history of reproductive disorders, such as weak offspring, abortion, and stillbirths. However, age, pregnancy, reproductive disorders, and type of housing had no significant effect on *C. burnetii* positivity in the uterine and vaginal samples of dogs (Tables 1 and 3).

The presence of *C. burnetii* DNA was established in the uterine samples of 2 client-owned and 3 stray cats. None of the *C. burnetti*-positive cats were pregnant, and 1 of them had a history of abortion. They were all less than 7 years old. No significant difference was found among all the groups that were categorized according to age, pregnancy, reproductive disorders, and type of housing (Tables 1 and 3).

Discussion

It has been proved that in some parts of the world, small animals have an important role in spreading the Q fever infection (Greene, 2012). Dogs and cats were investigated as potential reservoirs of *C. burnetii* in this study. Our data show that the uterus and vagina of dogs and cats can be infected by *C. burnetii*.

In Japan, cats are one of the most important reservoirs of *C. burnetii* (Komiya et al., 2003). After an outbreak of Q fever in a veterinary hospital in Sydney, the role of companion animals in *C. burnetii* transmission to humans has been emphasized (Maywood and Boyd, 2011). Exposure to parturient queens or bitches and newborn kittens or puppies during or shortly after parturition has been mentioned as a significant risk factor to acquire Q fever (Kosatsky, 1984; Langley et al., 1983; Marrie et al., 1988a; Marrie et al., 1988b; Marrie et al., 1989; Norris et al., 2013). Buhariwalla et al. (1996) reported *C. burnetii* pneumonia in all 3 members of 1 family after exposure to an infected parturient dog, and 3 of 4 puppies died within 24 hours post-parturition.

In other surveys, *C. burnetii* infection was diagnosed using serological tests, and a prevalence range of 0%–35% and 1.5%–42% was reported in dogs and cats, respectively (Cooper et al., 2011; Havas and Burkman, 2011; Kilic et al., 2008; Matthewman et al., 1997; Norris et al., 2013). This bacterium has also been isolated from the reproductive organs of both healthy and ill dogs and cats (Greene, 2012). In our study, 12.1% of the uterine samples collected from dogs were positive for *C. burnetii* DNA, whereas this rate was 17.8% in the feline uterine specimens. *C. burnetii* was also identified in 8 of 40 (20%) vaginal samples of dogs. Comparing our findings with those of a study conducted in Colorado, *C. burnetii* DNA was molecularly detected in 8.5% of the uterine samples taken from clinically healthy, non-parturient client-owned cats, but the bacterial DNA was not amplified from the vaginal samples. The author stated that apparently healthy cats can serve as a source of human *C. burnetii* infection (Cairns et al., 2007). In another study using IS*1111* PCR, the prevalence of *C. burnetii* in cats with or without clinical and histopathological evidence of reproductive problems was 8.1% (Fujishiro et al., 2016). Nagaoka et al. (1998) isolated *C. burnetii* by inoculation into cyclophosphamide-treated mice from 31% of the vaginal swabs collected from feline clients. In another study performed on breeding pets in Netherlands after the 2007–2010 Q fever outbreak, *C. burnetii* was found in 7% of the placenta from dogs using real-time PCR but was not detected in the placenta of cats (Roest et al., 2013).

In contrast, Kopecny et al. (2013) reported strong seropositivity among the queens using complement fixation test (7.4%), indirect fluorescent antibody test (26%), and enzyme-linked immunosorbent assay (41%); however, the reproductive tissue samples were negative by PCR analysis. Currently, there is no golden standard for the detection of *C. burnetii* owing to its slow growth and high number of negative serology cases after sampling only within 2 weeks of illness. Therefore, PCR is considered as the diagnostic method of choice for the identification of *C. burnetii* infection. The PCR method with primers targeting IS*1111* has also been found to be very specific and sensitive for the detection of *C. burnetii* in different clinical samples (Gwida, 2012; Porter et al., 2011). The duration of *C. burnetii* genital tract shedding remains unknown in dogs and cats; nevertheless, it has been mostly determined to take place before, during, and shortly after parturition (Maurin and Raoult, 1999). After an experimental infection in cats, *C. burnetii* was isolated from the uterus of cats for 10 weeks post-parturition. As the shedding of *C. burnetii* in the genital tract is intermittent, the sampling time can affect the presence of this bacterium in the reproductive system (Higgins and Marrie, 1990).

In most cases, the infected animals that excrete the bacteria in the environment are apparently healthy and asymptomatic (Porter et al., 2011). Excretion of the organisms in birth products, such as placenta of the infected animals, can transmit the infection to humans (Greene, 2012). The reproductive disorders can contribute to *C. burnetii* infection (Agerholm, 2013). It has been described that after a chronic infection, *C. burnetii* colonizes the uterus and mammary glands, and over 10^9 bacteria may be shed into the environment during parturition (Gwida, 2012). In dogs, reproductive disorders associated with *C. burnetii* infection have not been reported, and the clinical aspects remain obscure (Agerholm, 2013). *C. burnetii* was detected in

the genital tracts of healthy cats and also in cats with reproductive disorders (Agerholm, 2013; Fujishiro et al., 2016). In this study, *C. burnetii* DNA was detected in the uterine samples of non-pregnant dogs with reproductive failure. Moreover, 1 of the positive vaginal samples belonged to a pregnant animal, and another to a dog who had a history of reproductive disorders, including weak offspring, abortion, and stillbirths. From 5 positive uterine samples of cats, only 1 cat had a history of abortion. Of the 3 positive cats in the study by Fujishiro et al. (2016), 1 had pyometra with a history of abortion and early kitten death, the second one had a history of stillborn kittens and early kitten death despite normal uterine histopathology, and the 3rd one was normal. In another study, 2 of 9 positive vaginal samples belonged to cats that experienced abortion (Nagaoka et al., 1998).

We mostly detected *C. burnetii* in young and middle-aged animals in this study. All the positive feline uterine samples were correlated to stray or client-owned cats that were allowed outdoors. From 5 positive canine samples, 1 belonged to a pet dog and 4 were related to stray dogs. Of the 8 positive vaginal samples, 5 belonged to stray dogs. Similar to our finding, *C. burnetii* was frequently diagnosed in cats less than 5 years old by Nagaoka et al. (1998). In another study by Kilic et al. (2008), cats older than 3 years were more affected than the younger ones. Outdoor housing, which implies contact with farm and wildlife and provides higher opportunities for acquiring infections, is attributed to a higher prevalence of Q fever in dogs and cats (Cooper et al., 2011; Greene, 2012; Hornok et al., 2013). Similar to our findings, a higher prevalence of infection has been reported in stray animals than in domestic animals (Willeberg et al., 1980). In the study of Komiya et al. (2003), 14.2% and 41.7% of pet and stray cats, respectively, were seropositive; therefore, the stray cats were considered as 1 of the main reservoirs of *C. burnetii* in Japan. The prevalence of *C. burnetii* infection depends on the geographic areas, variation in sampling, and study populations, and the techniques used can be largely different. A small sample size and unbalanced distribution could lead to insignificant statistical results between the groups.

In conclusion, there are only few studies about the distribution and incidence of *C. burnetii* infection in Iran. According to the previous studies in south-east Iran, a high seroprevalence of *C. burnetii* was demonstrated in domestic ruminants, particularly those with reproductive problems (Abiri et al., 2019; Esmaeili et al., 2019a). These findings are in agreement with the high prevalence of human Q fever cases reported from this area (Esmaeili et al., 2019b; Nokhodian et al., 2018). According to the results of this study, it is demonstrated that dogs and cats can harbor *C. burnetii* in their reproductive tissues; therefore, the infected pets can be a source of zoonotic infection in people who are in contact with these animals. Early diagnosis and treatment of animal infections is necessary to avoid Q fever outbreaks. Appropriate precautions should be taken when in contact with aborted fetal tissue or parturient pets and their secretions. Further studies are required to evaluate the importance of pets in the epidemiology of *C. burnetii* infections. Moreover, the role of *C burnetii* in reproductive abnormalities should be clarified.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Animal Care Committee of Veterinary College of Shahid Bahonar University of Kerman (No: 950101).

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Author Contributions: M.R. (Supervisor of Thesis) guided experimental design, wrote the manuscript and analyzed the data; M.K. (Supervisor of Thesis) guided experimental design and provided advice for PCR experiments; M.S. was the Advisor of Thesis; A.R.N. was responsible for collecting the vaginal swab samples of dogs and doing PCR for these specimens. F.B.S. was involved in collecting uterine samples of dogs and performed PCR for this part of the experiment. S.A.N.G. collected uterine samples of cats and performed their PCR reaction. D.O. and M.C. edited and revised the manuscript; All authors read and approved the final manuscript.

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