Investigation of *Coxiella burnetii* infection in cat uterus

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Abstract

Q fever, caused by the obligate intracellular bacterium *Coxiella burnetii* (*C. burnetii*), is an important zoonotic disease with a worldwide distribution. While ruminants are the main reservoir of *C. burnetii*, which are the primary source of human infection, human cases have also been demonstrated following contact with domestic dogs and cats. The present study investigates *C. burnetii* infection in domestic cats referred to veterinary clinics and hospitals in Tabriz and Tehran cities (Iran) through molecular (Real time PCR) and histopathological methods. For this purpose, samples were collected from 50 cat uteri that underwent hysterectomy surgery. Each sample was divided into two parts; one part was fixed in a 10% formalin buffer for histopathological

examination, while the other part was stored at -70 °C, which used for quantitative PCR assay. After genomic DNA extraction using commercial kits, a real-time-PCR reaction was performed with specific primers and probes for detection of *C. burnetii* genome. For histopathological examination, tissue sections were processed routinely and stained with hematoxylin and eosin. In the present study, all samples showed the negative results for detection of *C. burnetii* genome by real-time-PCR assay. However, at pathological evaluations, the tissue sections showed various degrees of edema, hyperemia, hemorrhage, inflammation, necrosis, fibrosis, cysts, and endometrial hyperplasia, ranging from mild to severe. Generally, it seems that *C. burnetii* infection is not common in the reproductive tissues or vaginal discharge. In conclusion, based on the present findings and considering the zoonotic aspect of *C. burnetii* infection, it appears that *C. burnetii* infection is not common in domestic cats in Tehran and Tabriz. Although further research on other samples is recommended.

Keywords: Q fever, Cat, PCR, Uterus, Zoonotic aspect

1. Introduction

Coxiella burnetii (*C. burnetii)* is one of the potential agents of Q fever affecting humans and many animal species (1). Known as a Gram-negative and obligate intracellular bacterium, its virulence lies in its stable structure that can endure the roughest environmental conditions and can survive outside for prolonged periods (2). Q fever has been reported in several countries worldwide, including the Netherlands (3), Spain (4), and Cyprus (5) in different samples from various hosts. The disease can be zoonotic not only for humans but also for domestic animals like cows, sheep, goats, dogs, and cats (2). Most of the contaminations to humans from it mainly happen with the inhalation of aerosols contaminated with particles from the birth products (6), urine, feces, and milk of sick animals (2). In humans, this pathogen can cause both acute and chronic forms of

infections.. Acute Q fever typically presents as a flu-like syndrome, with fever, myalgia, and headache, but can progress to more serious complications, such as pneumonia, hepatitis, and pericarditis (6). Chronic Q fever is less common, but much more serious resulting in endocarditis, vascular infections, and chronic fatigue syndrome complicated in individuals with suppressed immunity. Human Q fever has been associated with several reservoirs including farms, slaughterhouses, and even domestic cats (7). Outbreaks of Q fever have been associated with exposure to cats in labor, an important reservoir of this bacterium for humans (1). This infection is transmitted in cats through ingestion of ruminant placenta or milk from infected ruminants, consumption of raw contaminated meat, inhalation of environmental contaminants, ingestion of infected prey, and tick bites (6). Cats are usually silent carriers of the infection, but experimental vaccination has resulted in fever, anorexia and depression. (8). Methods were reported to isolate *C. burnetii* from uterine and vaginal swabs of healthy cats, showing the influence of the pathogen on reproduction functions (1). This make it difficult to diagnose based on symptoms because the infection is subclinical. Serology and molecular techniques (PCR) are used to diagnose *C. burnetii* infection in cats. The detection of antibodies against *C. burnetii* in the serum are indicative of exposure to infection, but these antibodies do not differentiate past from current exposure of the host, that is why PCR tests on tissue samples are preferable for a precise diagnosis (8). Given the occurrence of *C. burnetii* in cats and their circulatory function, it is crucial to have tools for fast and precise as well as effective diagnosis of the zoonotic risk associated with this organism. The present study aimed to investigate *C. burnetii* infection in cats referred to veterinary clinics for ovariohysterectomy surgery in the cities of Tabriz and Tehran (Iran) using molecular and histopathological methods.

2. Materials and Methods

2.1. Study area

This study was conducted in two cities, including Tabriz and Tehran, located in two different provinces of Iran (Figure 1). Tabriz is the capital of East Azerbaijan province in northwest Iran (38.0792° N, 46.2887° E, 1351 meters above sea level). Also, it has a tropical and subtropical steppe climate (Köppen-Geiger classification BSk) with a yearly rainfall of ca. 360 mm. Tehran is the capital of Tehran Province in northern Iran (35.6892° N, 51.3890° E, 1,191 meters above sea level). It has a cold semi-arid climate (Köppen-Geiger classification BSk), with an average yearly rainfall of about 250 mm.

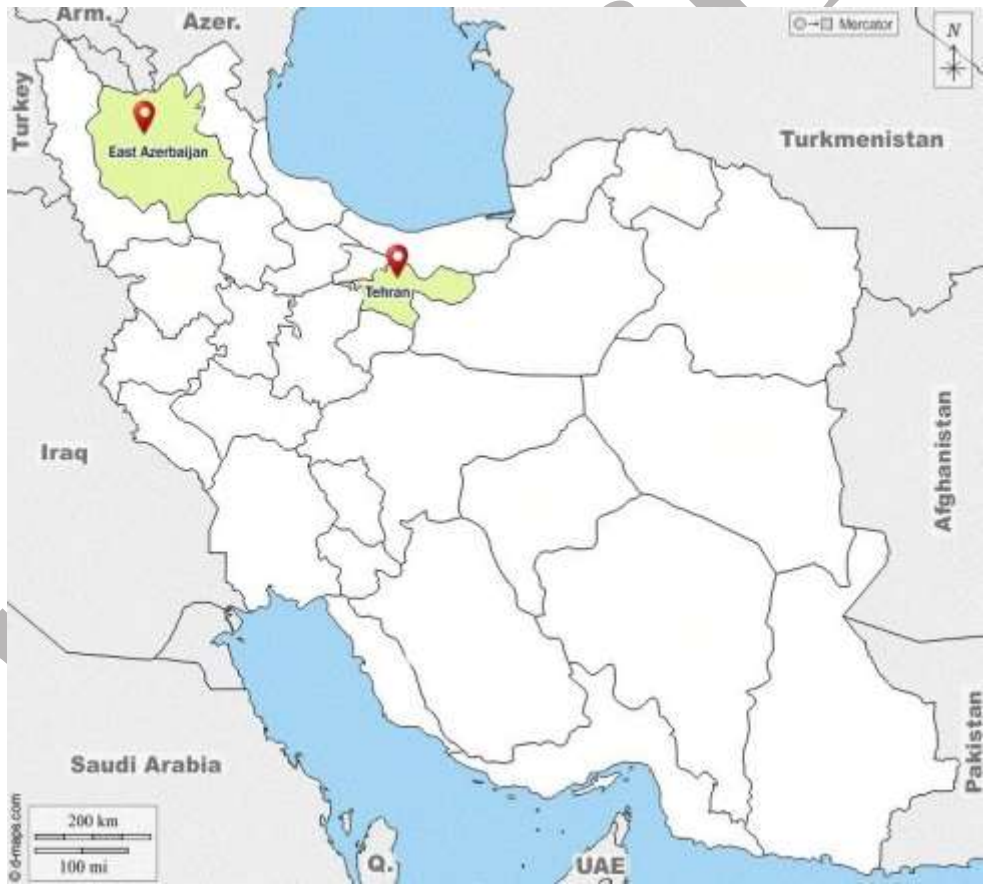

Fig. 1. Study area, URL: https://www.4maps.com/map/iran

2.2.Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals including the protocol approved by the Animal Research Ethics Committee of the University of Tabriz (ID: IR.TABRIZU.REC.1403.065;2024/07/07) were followed.

2.3. Sample collections

In this study, fifty cat uterine tissue samples were collected from veterinary clinics in the cities of Tabriz and Tehran using open ovariohysterectomy (OVH) and abdominal hysterectomy procedures, employing general anesthesia for both methods. This approach ensured that adequate pain management and surgical precision were prioritized, as general anesthesia is commonly administered in such procedures. The uterine tissue samples were collected and divided into two parts: 50 mg was stored in at -70°C for further molecular studies, while the other part was placed in 10% formalin for histopathological studies.

2.4.Molecular studies (DNA extraction and Quantitative-Real-Time PCR assay)

To extract the genomic DNA, commercial DNA extraction kits (Sinaclon, Iran) were used. The IS1111 region of *C. burnetii* was amplified with specific probes and primers using the Quantitative-Real-Time PCR method. The q-RT-PCR had a final volume of 20 µl and included 10 µl of 2x Master Mix, 900 nM forward primer (AAAACGGATAAAAAAGAGTCTGTGGTT), 900 nM reverse primer (CCACACAAGCGCGATTCT), 200 nM probe (6-FAM-AAAGCACTCATTGAGCGCCGCG-TAMRA) (Ampliqon Company), 4 µl of extracted DNA, and 5 µl of double-distilled water, which was performed by a RT-PCR system (Bio Molecular Systems, Australia).

2.5. Histopathological study

After ovariohysterectomy and assessment of macroscopic lesions, uterine tissue samples were placed in 10% buffered formalin. After 24 hours, the routine tissue preparation was carried out

using a tissue processor (DS2080/H, Didsabz, Iran), followed by impregnation and embedding in paraffin. Then, the sections with 5 µm thick were prepared using a rotary microtome (DS4055, Didsabz, Iran), which staining was performed with the common hematoxylin and eosin (Hematoxylin Cryst and Eosin Y, Merck Millipore, Germany). Microscopic studies were conducted using a light microscope (Olympus-CH-3, Japan) to evaluate pathological lesions such as inflammation, necrosis, vascular disorders (edema, hyperemia, and hemorrhage), tissue cysts, hyperplasia, and the probable presence of *C. burnetii* in macrophages.

2.6.Statistical analyses

It is not applicable in the present study.

3. Results

3.1.Molecular findings

In this study, none of the fifty uterine samples collected from cats referred to veterinary clinics in Tabriz and Tehran tested positive for the *C. burnetii* genome.

3.2.Histopathological findings

The results of histopathological studies are summarized in Table 1 and Figure 2. The observed lesions included hyperemia, hemorrhage, inflammation, necrosis, endometrial hyperplasia, fibrosis, and tissue cysts. Of note, the most common histopathological lesions were hemorrhage (78%), endometrial hyperplasia (72%), and hyperemia (48%) with various severity.

Table 1- The histopathological lesions observed in uterine samples (n = 50)

	Histopathological Lesions (Frequency) (%)						
Severity	**cysts**	**fibrosis**	**hyperplasia**	**necrosis**	**inflammation**	**hemorrhage**	**hyperemia**
Normal	49 (98%)	44 (88%)	14 (28%)	33 (66%)	16 (32%)	11 (22%)	2 (4%)

Mild	1 (2%)	5 (10%)	12 (24%)	2 (4%)	22 (44%)	11 (22%)	24 (48%)
Moderate	0 (0%)	1 (2%)	14 (28%)	4 (8%)	9 (18%)	11 (22%)	20 (40%)
Severe	0 (0%)	0 (0%)	10 (20%)	1 (2%)	3 (6%)	17 (34%)	4 (8%)
Total	1 (2%)	6 (12%)	36 (72%)	17 (34%)	34 (68%)	39 (78%)	48 (96%)

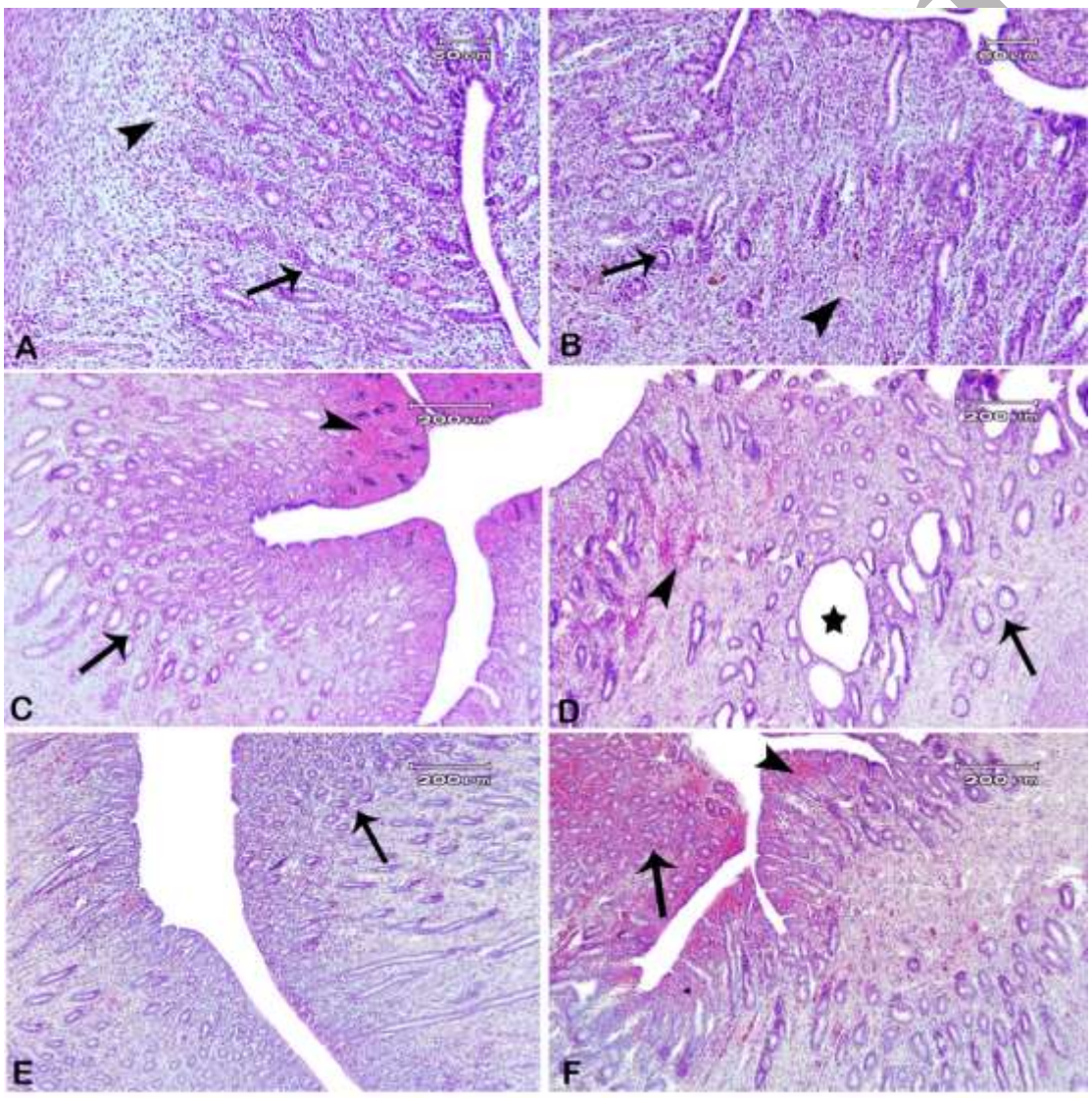

Fig 2. Histopathological results of the present study. Uterus, cat. A and B: Simple hyperplasia of endometrial glands (arrow) with diffuse infiltration of mononuclear cells (arrowhead). C: Simple hyperplasia of endometrial glands (arrow) with hemorrhage (arrowhead). D: Endometrial hyperplasia (arrow) with cyst (star) and hemorrhage (arrowhead). E: Simple hyperplasia of endometrial glands (arrow).

F: Simple hyperplasia of the endometrial glands (arrow) with hemorrhage (arrowhead). Hematoxylin-eosin staining.

4. Discussion

In the present study, no positive sample were detected by q-RT-PCR and no organism was found in the tissue samples by histopathology. However, there were general pathological lesions in the tissue sections. Cats are popular pets in many cultures, but their role in transmitting common diseases, such as Q fever, between humans and animals warrants special attention. . Several studies have already been carried out in different hosts and different sample sources, in Iran. Similar to our study, a previous study was conducted on cats and dogs (blood samples) and indicated that a significant percentage of cats (17.5%) and dogs (11.0%) are carriers of this infection (9). Besides, some studies reported the presence of *Coxiella* infection in other hosts, such as sheep from Kerman province (southeast Iran) (19.40% vaginal samples from aborted animals; using Real-time PCR) (10) and Sistan and Baluchistan province (southeast Iran) (imported and domestic animals 0.97% and 3.23%, respectively, blood sample; using ELISA) (11), Ardabil province (northwest Iran) (blood samples, 33.6%; using ELISA) (12). However, *C. burnetii* was also studied in the camel population of southern Iran (Fars province), but the results indicated that despite the presence of infection (6.19%; using nested PCR), no significant differences in blood were observed between infected and healthy camels (13). Some researchers demonstrated the presence of *C. burnetii* in ticks from sheep (37.5%), cattle (32.14%), and dogs (15%) using nested PCR in Hormozgan province (south of Iran) (14), which indicates the potential transmission of the organism in various hosts. Emerging evidence was conducted regarding the level of milk contamination with *C. burnetii* in Iran, which highlights the food-borne potential of this organism. Importantly, *C. burnetii* was investigated in dairy products and milk with 12.50% of Kope cheese, 13.00% of milk

158 samples using PCR (2), and 16.9% of raw buffalo and cow milk using nested PCR (15) in West
159 Azerbaijan province (northwest Iran). In addition, another study reported the presence of *C.*
160 *burnetii* in unpasteurized milk and dairy products using a touch-down PCR assay (7.14% in cheese
161 samples, 7.69% in yoghurt samples, 34.78% in sheep milk samples, and 3.33% in cow milk
162 samples) in North - East of Iran (16). Also, a previous study detected *C. burnetii* in bulk tank milk
163 samples (14%) from dairy bovine farms using nested-PCR in Qom province (17), Iran. These
164 evidence present the importance of these animals in the spread of Q fever.
165 Studies conducted in various countries highlight the significance of both wild and domestic hosts
166 as reservoirs of Q fever in humans and animals. In this regard, a survey carried out in the natural
167 park of Serranía de Cuenca, Spain, between 2003 and 2013 involving several species, including
168 ruminants and wildcats, the results indicated that a notable percentage of European wild cats
169 (33.3%) and Spanish ibex (23.8%) possess antibodies to this organism, while other animals like
170 sheep (22·5%) and cattle (0·24%) show a lower prevalence (18). Also, a study in North America
171 found that 8.5% of domestic cats in North-central Colorado carry *C. burnetii*, underscoring the
172 need for caution and further research into Q fever (1). In Quebec, Canada, a study was conducted
173 on farm, domestic, and feral cats to investigate the prevalence and risk factors of *C. burnetii*
174 infection. The results indicated that some farm cats were infected with this bacterium, while
175 domestic and wild cats were not, which is in agreement with our findings. Also, those findings
176 suggest that caution should be exercised when keeping cats on farms, although domestic and feral
177 cats pose a lower risk to public health (6). In northern Jordan, the seroprevalence of *C. burnetii*
178 among goats and sheep was assessed using serological tests, revealing infection rates of 27% and
179 43.3% in sheep and goats, respectively. Of note, the presence of cats on farms was linked to an
180 increased prevalence in that study (19). In South Korea, molecular tests indicated that the infection

rate of *C. burnetii* was higher in native goats (22.7%) and cattle (16.4% of the dairy cattle, 15.2% of the beef cattle) compared to horses (5.2%) (20). The prevalence of *C. burnetii* in Estonian ruminants has also been examined, revealing that dairy cows have the highest levels of antibodies (27.16%) in this country (21). In South Africa, a high prevalence of this bacterium has been noted in cattle (24.28%), which correlates with herd size and abortion history (22). In a previous research, this bacterium was identified for the first time in Paraguay, with 45% of sheep serum samples testing positive. It was found that this pathogen is associated with reproductive problems in sheep and poses potential risks to public health (23). Additionally, in Mexico, goats serve as a reservoir for this bacteria, with 82.35% of vaginal samples testing positive (24). The results of investigations conducted in Egypt on serum samples using the ELISA method revealed that the prevalence of antibodies against *C. burnetii* in goats (28%) is higher than in sheep (22.8%). Moreover, a previous study found no evidence of *C. burnetii* in the local Iraqi sheep and goat semen (25). Various factors, including age, gender, and storage conditions, have influenced the spread of the disease. Breeding methods have also significantly impacted the level of contamination; animals raised on larger farms are more susceptible to exposure (26).

in Local Iraqi Sheep and Goats Semen

As previously described, *C. burnetii* infection has a zoonotic potential and public health concern. In Bulgaria, experiments were conducted to assess the prevalence of *C. burnetii* infection among veterinarians and cattle workers, with blood samples tested using ELISA and PCR methods. The results indicated that 37% of the samples contained antibodies suggesting contact with this pathogen. Additionally, the DNA of this bacterium was found in a portion of the samples (20%), highlighting active infections, particularly among older individuals (27). Additionally, another study in Quebec examined the prevalence of *C. burnetii* antibodies among individuals, particularly

focusing on dog owners. This study revealed that occupations associated with domestic animals were more likely to be seropositive, although individuals without occupational exposure also had antibodies. Proximity to ruminant farms did not affect seropositivity (28). Notably, other studies have also highlighted the link between *Coxiella* infection and complications during pregnancy in women (7). In conclusion, Q fever is a zoonotic and food-borne disease that poses health risks to mammals. Given the threats associated with this illness, it is crucial to understand all potential sources of infection and its modes of transmission. Although no positive cases were identified in this study, this disease should be considered, given its public health importance and potential cause of pregnancy disorders in humans.

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Author contributions

Conceptualization: MKh, KN, SE;

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Investigations: MKh, KN, SE, NDJ, BS, MZN;

Writing/preparation of original draft: MKh, NDJ, BS;

Writing, review, and editing: MKh, KN, SE, NDJ, BS, MZN;

Supervision, project administration, and funding acquisition: MKh;

All authors have read and approved the final version of the manuscript.

Ethics

All applicable international, national, and institutional guidelines for the care and use of animals including the protocol approved by the Animal Research Ethics Committee of the University of Tabriz (ID: IR.TABRIZU.REC.1403.065;2024/07/07) were followed.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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