



## Original Article

Investigation of *Coxiella burnetii* Infection in the Uterus of CatsMonireh Khordadmehr<sup>1,2\*</sup>, Niloofar Dokht Jabbari<sup>1</sup>, Katayoon Nofouzi<sup>1,2</sup>, Saber Esmacili<sup>3,4</sup>, Bentolhoda Soleimani<sup>1</sup>, Moein Zehtab Najafi<sup>2</sup>

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## ABSTRACT

**Introduction:** Q fever, caused by the obligate intracellular bacterium *Coxiella burnetii*, is an important zoonotic disease with a worldwide distribution. While ruminants are the main reservoir of *C. burnetii* and the primary source of human infection, human cases have also been reported following contact with domestic dogs and cats.**Materials & Methods:** The present study investigates *C. burnetii* infection in domestic cats referred to veterinary clinics and hospitals in the cities of Tabriz and Tehran (Iran) through molecular (real time polymerase chain reaction [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 10% formalin buffer for histopathological examination, while the other part was stored at -70 °C and 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 (H&E).**Results:** In the present study, all samples showed negative results for the detection of *C. burnetii* genome by real-time PCR assay. However, in 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 reproductive tissues or vaginal discharge.**Conclusion:** 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. However, further research on other samples is recommended.

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## 1. Introduction

**C***oxiella 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, which can endure harsh environmental conditions and 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 is zoonotic not only to humans but also to domestic animals such as cows, sheep, goats, dogs, and cats [2]. Most human infections occur through the inhalation of aerosols contaminated with particles from birth products [6], urine, feces, and milk of infected animals [2]. In humans, this pathogen can cause both acute and chronic forms of infection. 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, particularly 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 have been reported to isolate *C. burnetii* from uterine and vaginal swabs of healthy cats, showing the influence of the pathogen on reproductive functions [1]. This makes it difficult to diagnose based on symptoms because the infection is sub-clinical. Serology and molecular techniques (polymerase chain reaction [PCR]) are used to diagnose *C. burnetii* infection in cats. The detection of antibodies against *C. burnetii* in serum is 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 role in circulation of the pathogen, it is crucial to have tools for fast, precise, and 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 ovariectomy (OVH) 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 —Tabriz and Tehran —located in different provinces of Iran (Figure 1). Tabriz, the capital of East Azerbaijan Province in northwest Iran (38.0792° N, 46.2887° E, 1351 meters above sea level), has a tropical and subtropical steppe climate (Köppen-Geiger classification BSk) with an average annual rainfall of approximately 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 annual rainfall of approximately 250 mm.

### 2.2. Sample collections

In this study, 50 uterine tissue samples from domestic cats were collected from veterinary clinics in the cities of Tabriz and Tehran using open OVH and abdominal hysterectomy procedures, with general anesthesia administered in both methods. This approach ensured adequate pain management, as general anesthesia is routinely used in such procedures. The uterine tissue samples were collected and divided into two parts: 50 mg was stored at -70 °C for molecular studies, while the other part was placed in 10% buffered formalin for histopathological examination.

### 2.3. Molecular studies (DNA extraction and quantitative-real-time PCR (qRT-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 qRT-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-TAM-RA) (Ampliqon Company), 4 µL of extracted DNA, and 5 µL of double-distilled water. The reaction was performed by a RT-PCR system (Bio Molecular Systems, Australia).

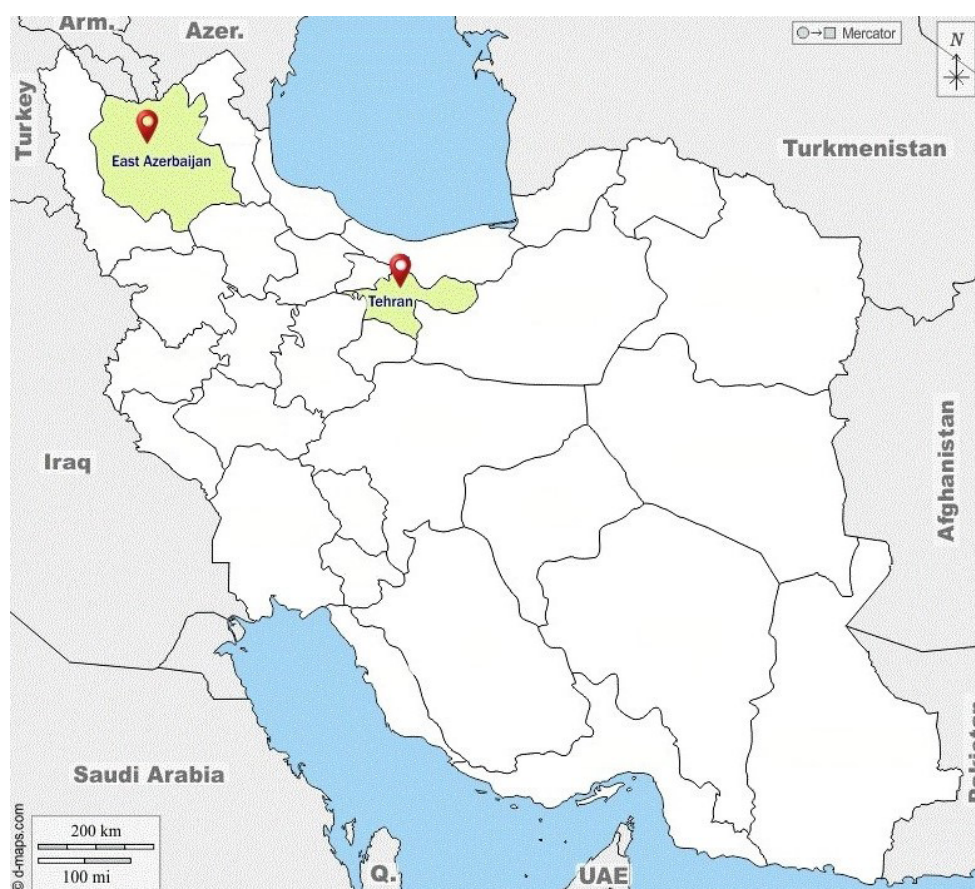


Figure 1. Study area [29]

## 2.4. Histopathological study

After OVH and assessment of macroscopic lesions, uterine tissue samples were placed in 10% buffered formalin. After 24 hours, routine tissue preparation was carried out using a tissue processor (DS2080/H, Didsabz, Iran), followed by impregnation and embedding in paraffin. Then, 5  $\mu$ m thick sections were prepared using a rotary microtome (DS4055, Didsabz, Iran), and staining was performed with common hematoxylin and eosin (H&E) (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.

## 3. Results

### 3.1. Molecular findings

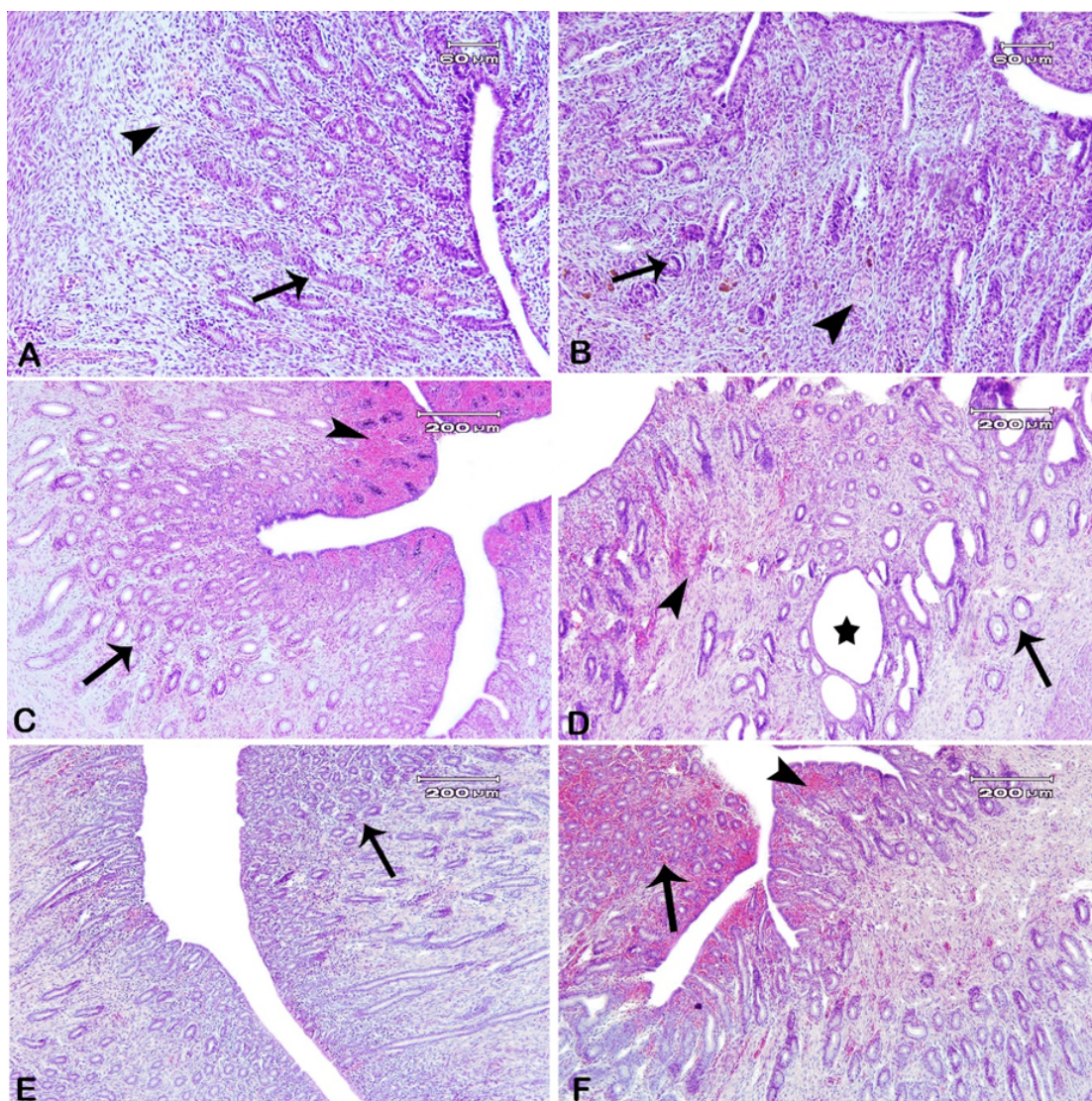
In this study, none of the 50 uterine samples collected from cats referred to veterinary clinics in Tabriz and Tehran tested positive for the *C. burnetii* genome.

### 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%) of varying severity.

## 4. Discussion

In the present study, no positive samples were detected by q-RT-PCR, and no organism was found in the tissue samples by histopathology. However, general pathological lesions were observed in the tissue sections. Cats are



**Figure 2.** Histopathological results of 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 endometrial glands (arrow) with hemorrhage (arrowhead). H&E staining.

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 conducted on cats and dogs (blood samples) 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 samples; using enzyme-linked immuno\_sorbent assay (ELISA) [11], as well as 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

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

| Severity | No. (%)                   |          |             |          |              |            |           |
|----------|---------------------------|----------|-------------|----------|--------------|------------|-----------|
|          | Histopathological Lesions |          |             |          |              |            |           |
|          | 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)    |

of the organism in various hosts. Emerging evidence has been reported regarding the level of milk contamination with *C. burnetii* in Iran, highlighting the food-borne potential of this organism. Importantly, *C. burnetii* was investigated in dairy products and milk, with 12.50% of Kope cheese and 13.00% of milk samples testing positive using PCR [2], and 16.9% of raw buffalo and cow milk using nested PCR [15] in West Azerbaijan Province (northwest Iran). In addition, another study reported the presence of *C. burnetii* in unpasteurized milk and dairy products using a touch-down PCR assay (7.14% in cheese samples, 7.69% in yoghurt samples, 34.78% in sheep milk samples, and 3.33% in cow milk samples) in northeastern Iran [16]. Also, a previous study detected *C. burnetii* in bulk tank milk samples (14%) from dairy bovine farms using nested-PCR in Qom Province, Iran [17]. These findings highlights the importance of these animals in the spread of Q fever.

Studies conducted in various countries highlight the significance of both wild and domestic animals as reservoirs of Q fever in humans and animals. In this regard, a survey carried out in the natural park of Serranía de Cuenca, Spain, between 2003 and 2013, involving several species including ruminants and wildcats, indicated that a notable percentage of European wildcats (33.3%) and Spanish ibex (23.8%) possessed antibodies to this organism, while other animals like sheep (22.5%) and cattle (0.24%) showed a lower prevalence [18]. Also, a study in North America found that 8.5% of domestic cats in north-central Colorado carry *C. burnetii*, underscoring the need for caution and further research into Q fever [1]. In Quebec, Canada, a study was conducted on farm, domestic, and feral cats to investigate the prevalence and risk factors of *C. burnetii* infection. The results indicated that some farm cats were infected with this bacterium, while domestic and feral cats were not, which is in agreement with our findings. Also, those findings

suggest that caution should be exercised when keeping cats on farms, although domestic and feral cats pose a lower risk to public health [6]. In northern Jordan, the seroprevalence of *C. burnetii* among sheep and goats was assessed using serological tests, revealing infection rates of 27% and 43.3% in sheep and goats, respectively. Of note, the presence of cats on farms was linked to an 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% in dairy cattle, 15.2% in beef cattle) compared to horses (5.2%) [20]. The prevalence of *C. burnetii* in Estonian ruminants has also been examined, revealing that dairy cows had 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 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 bacterium, 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%) was higher than in sheep (22.8%). Moreover, a previous study found no evidence of *C. burnetii* in the semen of local Iraqi sheep and goats [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].

As previously described, *C. burnetii* infection has zoonotic potential and is a 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]. Furthermore, 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 foodborne 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 modes of transmission. Although no positive cases were identified in this study, the disease should be considered, given its public health importance and potential to cause pregnancy disorders in humans.

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### Compliance with ethical guidelines

All applicable international, national, and institutional guidelines for the care and use of animals were followed, including the protocol approved by the Animal Research Ethics Committee of the [University of Tabriz](#), Tabriz, Iran (Code: IR.TABRIZU.REC.1403.065).

### Data availability

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

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### Authors' contributions

Conceptualization: Monireh Khordadmehr, Katayoon Nofouzi, and Saber Esmaeili; Methodology, investigations, review, editing, and final approval: All authors; Writing the original draft: Monireh Khordadmehr, Niloofar Dokht Jabbari, and Bentolhoda Soleimani; Supervision, project administration, and funding acquisition: Monireh Khordadmehr.

### Conflict of interest

The authors declared no conflict of interest.

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