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Histopathological and Molecular Investigation of *Chlamydia felis* Infection in Cat Uterus Underwent Ovariohysterectomy Surgery

Monireh Khordadmehr ✉[1], Faeghehossadat Mousavi[1], Katayoon Nofouzi[1], Karine Laroucau[2], Farinaz Jigari Asl[1], Moein Zehtab Najafi[1], Seyed Hossein Jarolmasjed[3]

[1] Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran. [2] Agency for Food, Environmental and Occupational Health and Safety, Maisons-Alfort, France. [3] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

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ABSTRACT

Veterinary surgeons frequently encounter zoonotic diseases and the associated risks of transmission during the surgical treatment of animals. *Chlamydia (C.) felis*, a bacterium with zoonotic potential commonly found in cats, has been associated with reproductive disorders; however, its effects on uterine health remain poorly characterized. The aim of this study was to investigate *C. felis* infection in cats referred to veterinary clinics in Tabriz and Tehran using molecular and histopathological techniques. Uterine samples were collected from 50 cats undergoing hysterectomy and divided into two parts: one was fixed in 10% buffered formalin for histopathological analysis, and the other was processed for DNA extraction and polymerase chain reaction (PCR). A conventional PCR assay using C. felis-specific primers was used, and tissue sections were stained with hematoxylin and eosin for microscopic evaluation. PCR analysis revealed *C. felis* infection in 3 of 50 samples (6%). Non-specific histopathological findings included varying degrees of edema, hyperemia, hemorrhage, inflammation, necrosis, fibrosis, cyst formation and endometrial hyperplasia, with lesions ranging from mild to severe. These results highlight the potential role of *C. felis* in uterine pathology and underline the need for surveillance of this zoonotic agent in domestic cats in Iran.

Introduction

The family *Chlamydiaceae*, a group of obligate intracellular bacteria, includes several species of importance to both veterinary and human health. The family belongs to the order *Chlamydiales* and is divided into two genera, *Chlamydia* and *Chlamydiifrater*.[1] Among these species, *Chlamydia felis* is a notable pathogen that primarily affects domestic cats.[2] It is associated with a variety of clinical manifestations, including respiratory and ocular infections, and may pose a zoonotic risk to humans.

Chlamydia felis was initially identified as the causative agent of feline pneumonia. However, conjunctivitis has since become the most widely recognized clinical sign of this infection.[3] The bacterium is primarily transmitted through direct contact between infected and susceptible cats, with ocular secretions being the predominant route of transmission.[4] In addition, *C. felis* has been detected in the vaginal discharges of infected cats,[5] raising concerns about the potential for reproductive system involvement and possible venereal transmission, although the latter has not been definitively established.

Uterine infections, such as endometritis, pyometra, and metritis, are major health problems in domestic cats and can lead to severe inflammatory conditions.[6] In recent years, *C. felis* has been suggested as a potential contributor to reproductive health issues in cats,[7] but its role in uterine infections remains underexplored. Given the zoonotic potential of *C. felis* and its impact

✉ Corresponding author. Email: khordadmehr@tabrizu.ac.ir

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on feline health, this study aims to investigate the presence of *C. felis* in the uterine tissues of domestic cats using histopathological and molecular methods.

Materials and Methods

Ethical Approval

This study was conducted in strict accordance with international, national, and institutional guidelines for the care and use of animals. The research protocol was reviewed and approved by the Research Ethics Committee of the University of Tabriz (ID: IR.TABRIZU.REC.1403.065; 2024/07/07).

Study Area and Sample Collection

In this study, 50 uterine tissue samples were collected from cats undergoing ovariohysterectomy at veterinary clinics in Tabriz (n = 25) and Tehran (n = 25), Iran. Surgery was performed as a routine procedure.[8] Briefly, anaesthesia was induced with a combination of ketamine (5-10 mg/kg) and midazolam (0.2-0.4 mg/kg), followed by maintenance with inhalational anaesthesia using isoflurane (100 ml). Surgery was performed via a ventral midline approach. After removal of the uterus and ovaries, the abdominal incision was closed in three layers: the muscle layer with a single continuous suture, the subcutaneous tissue with a subcuticular suture, and the skin with a single interrupted pattern. Absorbable sutures (Dexon 3-0) were used for internal ligatures and sutures, while nylon 3-0 was used for skin closure. Beside, post operation antibiotic therapy was conducted using penicillin procaine G (800 Units). Tissue samples (~100 mg) were either stored at -70 °C (left uterine horn associated with left part of the uterine body) for molecular analysis or preserved in 10% buffered formalin (right uterine horn associated with right part of the uterine body) for histopathological examination.

DNA Extraction

Genomic DNA was extracted from uterine tissue samples using the PsPure Genomic DNA Extraction Kit (Pishgam Sanjesh, Iran; Cat No.: PSEX01) according to the manufacturer's instructions. The quality and quantity of extracted DNA was assessed using the NanoPhotometer NP80 (IMPLEN, Germany). DNA samples were stored at -70 °C until further analysis by PCR.

PCR Assay

A conventional PCR assay was performed using Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) and 3 µl of DNA in a 25 µl reaction volume. Amplification was performed on a T100 thermal cycler (Bio-Rad, USA) using primers targeting the *C. felis pmp*1 gene, which encodes a polymorphic membrane protein.[9] The primer sequences were Cfpmp1c (5´-GGC GAT CCC TAT GTT GAG AA-3´) and Cfpmp1d (5'-CCA CCG AAA CAC CCT GTA GT-3´), which amplified a 155 bp fragment. PCR conditions with initial denaturation at 95 °C and 5 min, daenaturation at 95 °C and 30 sec, annealing at temperature 55 °C with 35 cycles and 25 sec, extension at 72 °C and 15 sec, and final extension at 72 °C and 5 min were used. Amplified products were visualized on 1.5% agarose gels stained with a safe DNA stain (SinaClon, Iran).

Pathological Examination

Tissue samples were fixed in 10% neutral buffered formalin for at least 48 h. The samples were then processed using a tissue processor (DS2080/H, Didsabz, Iran) and embedded in paraffin blocks. Sections of 5 µm thickness were cut using a rotary microtome (DS4055, Didsabz, Iran) and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope (Olympus, CH-30, Japan) to identify pathological lesions, including inflammation, necrosis, vascular disorders (edema, hyperemia, and hemorrhage), tissue cysts, endometrial hyperplasia, and possible presence of *C. felis* within macrophages.

Statistical Analyses (Risk Factor Determination)

In the present study, Chi-Square test was used to assess possible correlation of practical risk factors including age categories (under 12 months old, between 12-24 months old, and over 24 months old), previous infections, vaccination, and pregnancy or parturition history were considered in the statistical analyses. Analyses were performed using SPSS version 22 software for Windows 10 (SPSS Inc., Chicago, IL, USA), and p values < 0.05 were considered statistically significant.

Results

Molecular Findings

Conventional PCR analysis detected the presence of *C. felis* in three samples (15B and 42B (Tabriz), and 26T (Tehran)) out of fifty (6%) uterine samples.

Histopathological Findings

Macroscopic findings frequently included hyperemia, focal hemorrhage, and endometrial hyperplasia (Figure 1). Microscopic examination of the 50 uterine samples revealed a variety of pathological lesions (Figure 2), including hyperemia (96%), hemorrhage (88%), inflammation (endometritis and metritis) (68%), necrosis (34%), endometrial hyperplasia (72%), fibrosis (56%), and uterine cysts (4%).

In PCR-positive samples, inflammatory changes in the uterine tissue were characterized by diffuse lymphocytic

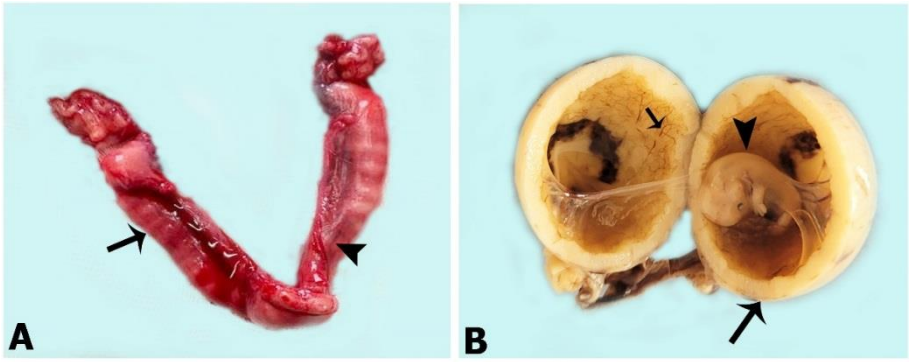

Figure 1. Uterine horns and body, domestic cat. A: There is diffuse hyperemia (arrow) associated with focal hemorrhage (arrow head). B: There is endometrial hyperplasia (long arrow) associated with hyperemia (short arrow) in the endometrium. Arrowhead shows a fetus.

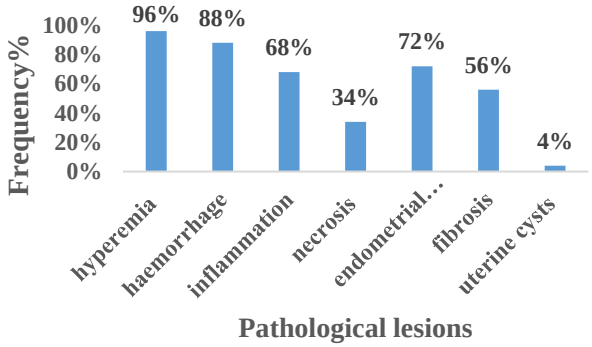

Figure 2. Frequency of the pathological lesions.

infiltration in the mucosa and submucosa. In particular, focal infiltration of neutrophils was observed, especially around blood vessels and certain uterine glands. Epithelial hyperplasia was often associated with simple endometrial hyperplasia, involving hyperplasia of the endometrial glands. In addition, focal necrosis with mild hemorrhage and hemosiderin deposition (indicative of old hemorrhage) were observed (Figure 3). We could not found *C. felis* organism within macrophages.

Risk Factor Analyses

Herein, there were no significant differences ($p > 0.05$) in the statistical analyses of the practical risk factors i.e. age (three categories), previous infections (two categories), vaccination (two categories), and pregnancy or parturition history (two categories) with the *Chlamydia* infection (Table 1).

Table 1. Evaluated risk factors in the present study.

Parameters	Categories	Numbers	*p-value*
Age group	12 months > 12-24 months 24 months >	26 15 9	> 0.05
Previous infections	Yes/No	8/42	> 0.05
Vaccination	Yes/No	26/24	> 0.05
Pregnancy/ parturition	Yes/No	11/39	> 0.05

Discussion

This study identified *C. felis* infection in domestic cats by molecular and histopathological examination. A total of 6% of cats from Tabriz and Tehran tested positive for *C. felis* by conventional PCR. Previous studies of *Chlamydia* infection in cats have reported variable prevalence rates, with molecular and serological tests detecting infection rates ranging from 0% to 47.4%, using real-time PCR on vaginal swabs in Sweden[6] and conventional PCR on conjunctival swabs in Romania.[10]

Histopathological findings such as hyperemia, hemorrhage, endometrial hyperplasia, inflammation, fibrosis, necrosis, and uterine cysts may indicate *Chlamydia* infection, especially in its subclinical form, and these lesions have been observed in other studies.[11,12]

Several studies in Iran have also investigated *Chlamydia* infection in cats. For example, a study conducted in Ahvaz (southwestern Iran) reported a molecular prevalence of *C. felis* using conventional PCR in conjunctival and oropharyngeal swabs from 152 cats, with an infection rate of 23%.[13] Another study in Tehran (northern Iran) found that 20% of cats were positive for *C. felis* using a multiplex real-time PCR method.[14] A similar study in Tehran and Isfahan (central Iran) using conjunctival swabs from 224 cats reported an infection rate of 18% using conventional PCR.[15] Overall, the reported prevalence of *C. felis* in Iranian cats ranges from 18% to 23%.[13,15] In our study, we reported a lower infection rate in uterine samples, which is consistent with expectations given the potential role of the reproductive system in infection and the generally lower prevalence of *C. felis* in reproductive tissues.

Studies from other regions have also documented *Chlamydia* infection in cats. In China, a study of 1,141 cats from northeastern and eastern regions reported a 10%-seroprevalence of *C. felis*, which may reflect subclinical infections.[16] Another Chinese study found an 18% prevalence of *C. felis* in conjunctival, nasal and oropharyngeal swabs from 117 feral cats with clinical signs using real-time PCR.[17] In Korea, recent studies

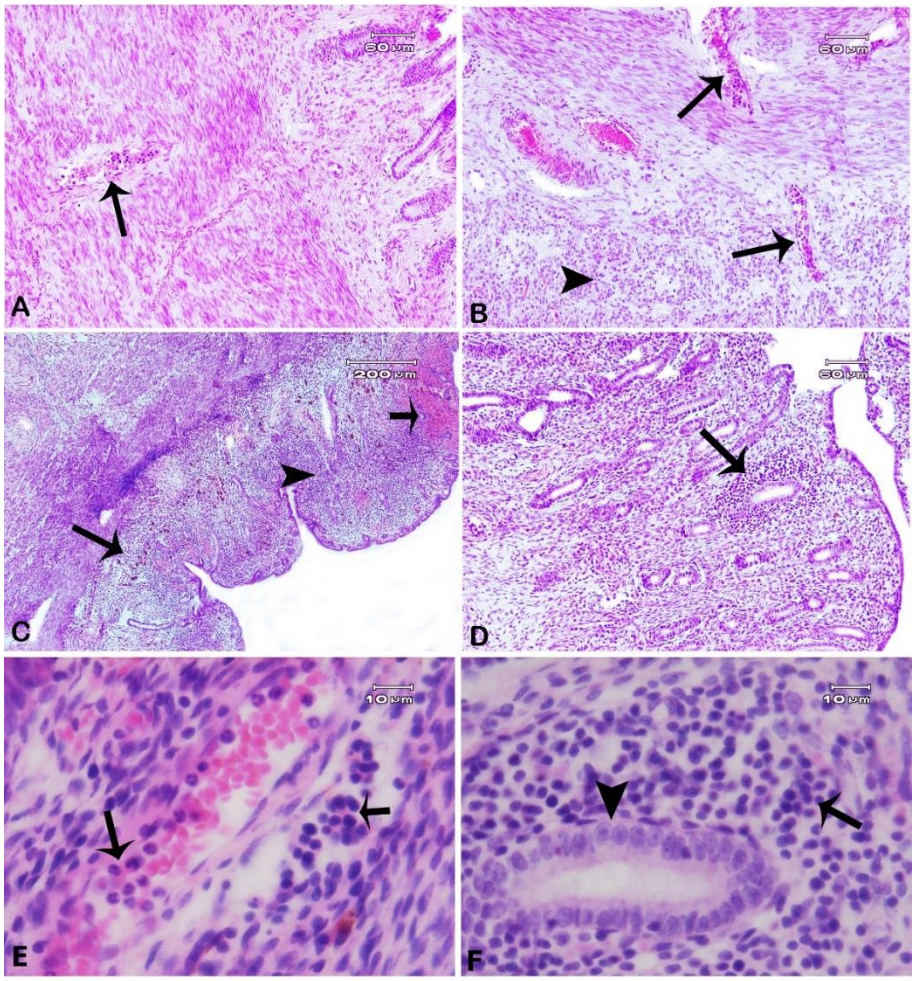

Figure 3. Histopathological findings in the uterus of cats with positive PCR results. A and B (sample 15B): Inflammatory cells including neutrophils (arrows) observed in and around blood vessels, with diffuse lymphocyte infiltration (arrowhead) in the mucosa and submucosa. C (sample 26T): Focal hemorrhage (small arrow) in the uterine mucosa with diffuse mononuclear inflammatory cell infiltration (arrowhead) and hemosiderin pigment deposition (large arrow) in the uterine submucosa. D (sample 42B): Diffuse mixed inflammatory cell infiltration in the uterine submucosa, especially around the uterine glands (arrow), with a predominance of neutrophils (arrow). E (sample 15B): Infiltration of neutrophils (long arrow) and lymphocytes (short arrow) in the uterine submucosa F (sample 42B): Higher magnification of figure D with predominantly lymphocyte infiltration (arrow), particularly around the uterine glands (arrowhead). H&E staining.

detected *C. felis* in 2% of symptomatic cats (n = 100) and reported a prevalence of 6% in nasopharyngeal swabs from 94 symptomatic cats using a real-time PCR assay.[18,19]

European studies have also shown variable prevalence rates. A study in Germany using conjunctival, nasal, oropharyngeal and tongue swabs from 104 symptomatic cats reported a prevalence of *C. felis* of 36% using real-time PCR.[20] In Sweden, serum samples from 214 cats showed a prevalence of 11%, [21] while in Slovakia a study of conjunctival swabs from 93 cats with conjunctivitis showed that 45% had purulent discharge, the most common symptom of *C. felis* infection.[22] In England, a study of 430 cats found a prevalence of only 1% based on oral and buccal swabs, the lowest reported in the region.[23] A study in Romania found a 65% prevalence of *C. felis* in conjunctival swabs from stray cats, with higher detection rates in asymptomatic cats.[10] The overall prevalence in European countries ranged from 1% to 65%, with Romania reporting the highest rate.

In Chile, a recent study reported a 52% seroprevalence of *C. felis* in infected cats (n = 60) using the ImmunoComb ELISA test.[24] In Canada, *C. felis* was found to be the second most common pathogen in cats with respiratory symptoms, [25] while a study in the United States reported a 0% infection rate in symptomatic shelter cats (n = 18) using PCR.[26] The reported prevalence of *C. felis* in North America ranged from 0% to 52%, with the highest rate reported in Chile based on blood samples.

The differences in infection rates observed in different studies may be due to factors such as the type of samples collected, sampling methods, diagnostic techniques, geographical locations and housing conditions of domestic cats. These variables may contribute to the observed variation in prevalence rates and highlight the need for further studies to better understand the epidemiology of *C. felis* infection in different regions.

In conclusion, the present study demonstrated the presence of *C. felis* (6%) in uterine tissue of cats by

conventional PCR, which is lower than that reported in numerous other studies. According to other studies, the highest rate of *Chlamydia* isolation was from conjunctival samples. Thus, examination of vaginal secretions or reproductive tissues appears essential to detect latent or subclinical infections. Given the zoonotic potential of *C. felis*, even at low prevalence, its presence in cat warrants consideration from a public health perspective.

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Conflict of Interest

The authors declare that they have no competing interests.

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