



Molecular Detection of *Chlamydia Psittaci* in Feral Pigeons in The Sulaymaniyah Region, Iraq

Article Info.

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Abstract

Feral pigeons are the main non-domestic host of zoonotic *Chlamydia psittaci*, which causes avian chlamydiosis. Although *C. psittaci* is important, few studies are available in the literature concerning this pathogen as a public health problem in Iraq. It was the objective of this work to use PCR amplification of the *C. psittaci* species-specific gene and detection in organs, cloacal, and pharyngeal swabs from clinically healthy feral pigeons collected from the Sulaymaniyah area. During the period between July and December 2025, one hundred feral pigeons of both sexes, of different age groups from various regions in Sulaymaniyah Governorate, were examined for *C. psittaci*. PCR amplification was performed on DNA extracted from samples targeting the *Chlamydia*-specific *ompA* gene, and the results were blasted to confirm positivity. Of 200 tested samples, 10 (5%) were real-time PCR-positive for *C. psittaci*; 4 were from pharyngeal swabs (2%), and 5 were from cloacal swabs (2.5%). One (0.5%) of the twenty pooled liver and lung samples was also positive for *C. psittaci*. Phylogeny of the *ompA* gene structures showed that's all feral pigeon. The isolates were of genotype B; however, it is interesting that a *C. psittaci* strain from feral pigeons in Argentina in 2024 shared 100% sequence homology with Sulaymaniyah isolates (Sul/1/2025 and Sul/2/2025). This work has provided evidence that the feral pigeon forms a natural reservoir for *C. psittaci* with potential risks to human health.

Keywords: *Chlamydia psittaci*, Feral pigeon, PCR, Iraq, *ompA* gene.

Introduction

Intracellular chlamydiae are obligatory parasitic coccoid Bacteria that are Gram-negative and capable of extracellular transmission as a non-multiplicative (dormant) particle, the elementary body (EB). At this writing, 12 species are assigned to the *Chlamydiaceae* family, which only has one genus, *Chlamydia* [C.] (1). Among these most significant zoonotic organisms is *Chlamydia psittaci*. *C. psittaci* mostly infects pigeons and parrots, resulting in the disease of psittacosis (ornithosis). Although some birds may be subclinical, signs of the disease in affected birds include weight loss, diarrhea, anorexia, labored breathing (dyspnea), hyperthermia or sudden death (2). Feral pigeons are the second primary cause of ornithosis and psittacosis in humans and the most significant reservoir of *C. psittaci* (3). City-dwelling and synanthropic pigeons are also the main source of *C. psittaci* infections in humans in urban areas through occupational and non-occupational exposure risks (4). Feral pigeons frequent many urban habitats, soiling areas such as public squares and church belfries with their excrement (5). The infection is commonly spread by inhaling dust containing respiratory secretions or dried excrement from diseased birds, potentially causing pneumonia or flu-like symptoms in humans (6).

Currently, 17 genotypes of *C. psittaci* have been discovered within the bird reservoir, some of which are known to be harmful to human health. Due to the genetic diversity of *C. psittaci*, its genotypes are categorized into A through F, E/B, M56, and WC based on the sequences of the outer membrane protein A (*ompA*). Each genotype has a primary host preference: genotypes A and F primarily infect parrots, B infects pigeons, C infects ducks and geese, D infects turkeys, and E infects a variety of birds, including pigeons (7;8). Variable genes (*omp2* and *ompA*) and conserved genes (*16S rRNA*, *23S rRNA*) have been utilized to detect *Chlamydia* and examine species or strain-level variations in field studies (9).

This pathogen's intracellular lifestyle makes diagnosis more difficult, plus there are limited, accurate diagnostic tools available (10). Various detection methods for *C. psittaci* exist, such as enzyme-linked immunosorbent assays (ELISA), fluorescent antibody tests (FATs), and rapid diagnostic techniques; however, these methods require laboratory settings to identify proteins or antigens (11). Conventional PCR methods often exhibit higher sensitivity than isolation techniques. PCR is an effective tool due to its high sensitivity and specificity, offering advantages such as speed, ease of standardization, and safety compared to culture methods for processing numerous specimens (12). Current conventional PCR techniques for detecting *C. psittaci* target the *16S–23S rRNA* or *ompA* gene (13).

Given that pigeons live in many urban and rural locations worldwide and often come into close contact with humans, and since avian chlamydiosis is considered one of the neglected zoonotic sicknesses (10), this study aimed to assess the presence of *C. psittaci* in the organs, cloacal, and

pharyngeal swabs of feral pigeons in Sulaymaniyah province through PCR amplification of the species-specific gene.

Materials and Methods

Sample collection

One hundred clinically healthy pigeons of all ages and genders from different parts of Sulaymaniyah were tested for *C. psittaci* between July and December of 2025. Cloacal swabs (n = 80), pharyngeal swabs (n = 80), and pooled liver and lung samples (n = 20) were among the samples collected. The samples taken were placed in labeled plastic containers and transported in a cool box to the University of Sulaimani's College of Veterinary Medicine for study in order to identify *C. psittaci*. Sterile cotton swabs were used to collect two cloacal and pharyngeal samples from each pigeon. After being collected and continuously twisted, each swab was removed from a 1.5 ml sterile microtube containing 0.3 ml of sterile saline solution (3). Before being processed, the samples were kept at -80°C.

DNA extraction

DNA was extracted from the samples using the Add Prep genomic DNA extraction kit (Add Bio, Korea), following the manufacturer's instructions. A Nanodrop spectrophotometer (Blue-Ray Biotech, Taiwan) was employed to measure the DNA concentration (ng/μl) and purity (260/280 ratio). The purified DNA was stored at -20°C before PCR analysis.

Gene amplification

Genus-specific primers were used for distinguishing *C. psittaci*. The primer (omp-F: 5'-ATGAAAAAACTCTTGAAATCGG-3'; omp-R: 5'-CAAGATTTTCTAGACTT CATTGTT-3') for *C. psittaci* was developed based on the order of the *ompA* gene, yielding a product size of 1058 bp (14). The primers were designed by Macrogen® (South Korea) specifically for this study.

DNA-primed PCRA were completed in 0.2 mL PCR tubes with Taq PCR mix (Gene Sand, China). 5 μL of DNA were added to each tube, 10 μL of master mix and 1 μL (10 pmol) forward primer omp-F and reverse primer omp-R. To obtain a final volume of 20μL, 3μL DEPC-treated water was included. Amplifications were performed in a PCR thermocycler (Prime, UK) using an initial denaturation at 95°C for 5 minutes; followed by 40 cycles of: Denaturation (30 s at 95°C), annealing (30 s at 58°C) and extension (45 s at 72°C); and the last extension step was carried out for five minutes at 72°C. Analysis of PCR products (8μL) was performed in 1% agarose gel (Trans gene, China) with 1xTris/Borate/EDTA buffer (TBE; Addbio, Korea). Gel was stained with 5μL gel red dye (Gene Sand, China), and electrophoresis was run for 50 min at 120 volts.

Sequencing of the *ompA* gene and phylogenetic analysis

To ensure nucleotide accuracy, both forward and reverse primers were utilized inside the phylogenetic sequencing of the *ompA* gene. A contiguous section of approximately 1 kb was generated by assembling and overlapping the resulting sequences. The assembled sequences, corresponding to isolates Sul/1/2025 and Sul/2/2025, were submitted to GenBank with accession numbers PX569697 and PX569698.

Gene homology in the identified nucleotide sequences was searched using NCBI BLAST. The sequences were aligned with representative species and genotypes of *Chlamydia* available globally to classify the genotypes of the local isolates. ClustalW was employed for nucleotide sequence alignment, and MEGA 11 (15) was used to conduct phylogenetic analysis utilizing the maximum-likelihood (ML) method based on the Kimura 2-parameter model.

Results

Among 200 samples collected from 100 clinically healthy pigeons, ten (5%) tested positive for *C. psittaci* infection; five came from cloacal swabs (2.5%) and four from pharyngeal swabs (2%), respectively. Additionally, one (0.5%) of the twenty pooled liver and lung samples tested positive for *C. psittaci*. Nine pigeons (9%) yielded positive results, with one pigeon testing positive in both pharyngeal and cloacal swabs. By amplifying the 1058 bp region of the *ompA* gene, the presence of *C. psittaci* in the samples was verified (Figure 1).

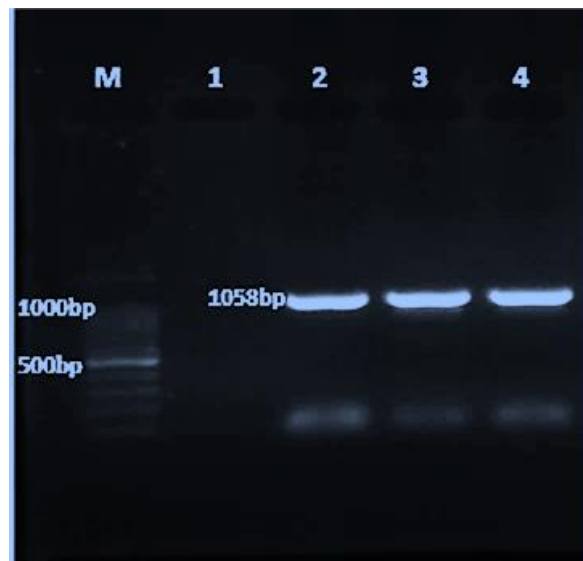


Figure 1: On a 1% (w/v) agarose gel, the partial portion of the *C. psittaci ompA* gene was amplified at 1058 bp, with M indicating a 1500 bp DNA ladder. A negative sample has been displayed in Lane 1, positive control strains of *C. psittaci* (1058 bp) are shown in Lane 2, and positive samples of the *ompA* gene were presented in Lanes 3–4.

Phylogenetic Analysis

The *ompA* gene sequences were used to create a phylogenetic tree. Sul/1/2025 and Sul/2/2025, two isolates from feral pigeons in Sulaymaniyah Province, Iraq, were shown to cluster within the species *C. psittaci*, according to the analysis (Figure 2). *Chlamydia* sequences that were obtained from GenBank were compared with about 1 kb of the *ompA* gene from both local isolates. The genus was clearly divided into eight primary clusters, each of which represented a distinct species of *Chlamydia*, based on the tree of phylogeny. Nine genotypes were created by further separating the *C. psittaci* isolates. The tree's topology showed that the isolates identified in this study shared a common ancestor with genotype B, which is primarily composed of isolates derived from pigeons worldwide. Notably, a *C. psittaci* strain isolated from feral pigeons in Argentina in 2024 (GenBank accession PQ140167) with the Sulaymaniyah isolates had 100% sequence identity.

Discussion

Wild birds, such as feral pigeons, have adapted over many decades to live successfully inside a particular plus constantly shifting city atmosphere, utilizing ideal environments for development and reproduction virtually year-round (16). However, the threat of dangerous natural causes, such as minimum sixty bacteria that could be hazardous to humans, is linked to the presence of feral pigeons (17). They include *Cryptosporidium* spp. (18), *Campylobacter jejuni* (19), *Escherichia coli* (20) and zoonotic yeast (21). Additionally, *C. psittaci*, the pathogen that causes human psittacosis, has a second significant reservoir in pigeons (11; 22). The majority of feral pigeons with *C. psittaci* infections are latent carriers with no symptoms. The pathogen is shed, frequently sporadically, in feces, respiratory secretions, and conjunctival secretions (23).

The prevalence of *C. psittaci* infection now savage doves in the Iraqi region of Sulaymaniyah was examined in this study. PCR amplification of the *ompA* gene was used to determine the 5% prevalence of *C. psittaci* DNA in feral pigeons in the current reading. *Chlamydial* DNA was found in pharyngeal, fecal, genital discharge, and semen samples in a number of studies using PCR-based techniques and polymerase chain reaction (24). Compared to Ahvaz, Iran (0.71%) and Belgium (2%), our PCR-based prevalence (5%) is higher (12; 25). It is, however, less than the 6.3%, 8%, 8.3%, and 16.8% found in Poland, Sweden, Baghdad, Iraq, and Brazil (4; 26; 27; 28). And significantly lower than Egypt's 83.3% and Iran's 25.3% (29;30). The host types (wild or native), functional status (with or without symptoms), preservation (free or in an enclosure), test group, procedure, and geographic location may all contribute to the observed variation in the results.

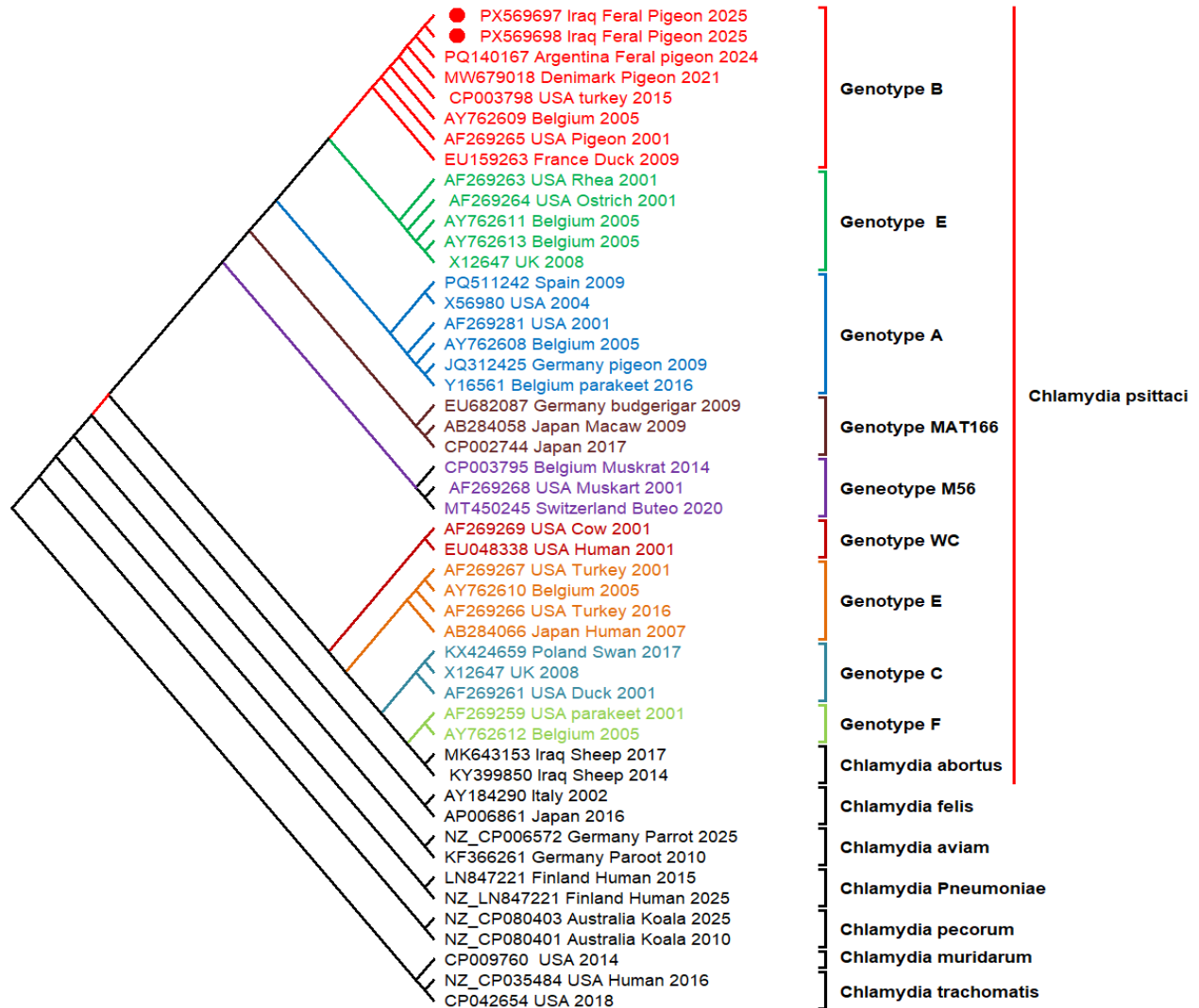


Figure 2: Phylogenetic analysis based on a 1 kb fragment of the *ompA* gene demonstrated clustering of *Chlamydia* isolates into eight distinct species. Within *C. psittaci*, nine genotypes were identified. The Iraqi isolates grouped within *C. psittaci* genotype B, sharing a common ancestor predominantly with *Chlamydia* strains isolated from pigeons.

Pharyngeal, cloacal, and (pooled liver and lung) samples were found at rates of 0.5%, 2%, and 2.5%, respectively, in the present study. Our findings confirm the efficacy of pharyngeal and cloacal swabs in identifying *C. psittaci* in birds without symptoms. The target gene, primer design, DNA extraction technique, and sample type selection all have significant effects on diagnostic sensitivity (13). The genotype and prevalence of *C. psittaci* were identified using AL-sultani in oropharyngeal swab samples taken from domestic pigeons in the Iraqi province of Baghdad. Overall, the prevalence was 8.3% (5/60). All five genotyped PCR-positive samples had genotype B (27).

Ghorbanpoor *et al.* also looked into *C. psittaci* using PCR testing, were found in pharyngeal samples from 280 asymptomatic pigeons in Iran. Two samples (1.4%) were found to contain unique DNA after PCR using *C. psittaci*-specific primers (12). Szymańska-Czerwińska *et al.* found *Chlamydiaceae* in 4.7% of the Columbidae family's cloacal or fecal swab samples; however, only one of the 64 examined pigeons had *C. psittaci* DNA confirmed (31). Lustosa *et al.* used a PCR assay to find the prevalence of *C. psittaci* in cloacal swabs from wild pigeons in a hospital environment in Brazil. 123 pigeon cloacal swabs were used to obtain DNA. In the research area, the pathogen's prevalence was 9.8% (5). In research by Spörndly-Nees *et al.*, pooled liver and lung samples from feral pigeons in Sweden showed 26 cases (8%) of *C. psittaci* infection using real-time PCR tests (26).

Other stressors, like the breeding season, avian diseases, or infestations, may induce and/or increase *C. psittaci* shedding (32). Circovirus (PiCV) is the most prevalent disease affecting the pigeon inhabitants, and is thought to be particularly hazardous for early specimens (YPDS, or New Pigeon Sickness Disorder) (33). Additionally, sporadic *C. psittaci* shedding with bodily secretions or feces might lead to misleading negative results (34). Several factors, such as specimen category (such as cloacae or throat swabs, feces, organs, or tissue), various techniques and lab techniques (such as serological, molecular, and cell culture), the time frame for sampling, the distribution, and the number of birds in urban environments, may contribute to the variation in prevalences across studies (32).

With a success rate of 5% (10/200), *ompA* gene fragments were successfully amplified and sequenced among the *C. psittaci* positive samples found by PCR. The recovered sequences displayed the maximum similarity of 100% with the *C. psittaci* strain isolated from feral pigeons in Argentina in 2024 (GenBank accession PQ140167), with nucleotide identities ranging from 99.6% to 100.0%. The two strains belonged to genotype B, according to NCBI's phylogenetic tree analysis (Figure 2). Although it has been found in some bird species, type B has been determined to be prevalent in pigeons. Pigeons are frequently linked to genotype B, which has zoonotic significance (5). This genotype's existence in the urban pigeon population of Sulaymaniyah highlights public health concerns, particularly for those who have contact with birds for work or pleasure.

Conclusion

In conclusion, we were able to identify and genetically characterize the *C. psittaci* found in feral pigeons during our investigation by using molecular techniques. By employing this technique, the analyzed samples demonstrated the presence of *C. psittaci* in feral pigeons that were clinically normal. Significantly, this research advances our knowledge of *C. psittaci*'s molecular epidemiology in Sulaymaniyah province, Iraq. The transmission of genotype B, a strain frequently linked to pigeons and recognized for its zoonotic potential, was validated by genotyping based on

the *ompA* gene. The most effective way to limit contamination by feral pigeons is an integrated management approach, which includes enforcing feeding bans and placing netting, spikes, or wires on buildings to discourage roosting. Additionally, advise employees to wear protective gear, such as masks and gloves, when cleaning droppings.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethics statement

The ethics committee of Sulaimani University's College of Veterinary Medicine approved the study (Approval No. VMUS.EC.Doc.34-2025 in June).

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الكشف الجزيئي عن الكلاميديا البيغائية في الحمام البري في منطقة السليمانية، العراق

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الخلاصة

تُعدّ الحمام البري المخزن الطبيعي الرئيسي لمرض الكلاميديا البيغائية، وهو مرض حيواني المنشأ يُسبب داء الكلاميديا في الطيور. وعلى الرغم من أهميته، فقد تم تجاهل الكلاميديا البيغائية إلى حد كبير كمشكلة صحية عامة في العديد من المناطق، بما في ذلك العراق. هدفت هذه الدراسة إلى استخدام تقنية تفاعل البوليميراز المتسلسل (PCR) لتضخيم الجين الخاص بالنوع للكلاميديا البيغائية للتحقق من وجودها في أعضاء الحمام البري السليم سريريًا، ومسحات المجمع، والبلعوم من منطقة السليمانية. في الفترة ما بين يوليو وديسمبر 2025، تم فحص مئة حمامة برية من مختلف الأعمار والأجناس من مواقع مختلفة في السليمانية للكشف عن الكلاميديا البيغائية. تم تضخيم الحمض النووي من العينات باستخدام تقنية PCR لاستهداف جين *ompA* الخاص بالكلاميديا، وأجري بحث باستخدام برنامج BLAST لتحديد أي نتائج إيجابية. من بين 200 عينة تم تحليلها، أظهرت عشر عينات (5%) نتائج إيجابية لعدوى الكلاميديا البيغائية، أربع منها من مسحات البلعوم (2%) وخمس من مسحات المجمع (2.5%). بالإضافة إلى ذلك، أظهرت عينة واحدة (0.5%) من بين عشرين عينة مجمعة من الكبد والرئة نتائج إيجابية للكلاميديا البيغائية. وكشف التحليل الجيني لتسلسل جين *ompA* أن جميع عزلات الحمام البري تنتمي إلى النمط الجيني B. والجدير بالذكر أن سلالة من الكلاميديا البيغائية تم تحديدها في الحمام البري في الأرجنتين عام 2024 أظهرت تطابقًا تامًا في التسلسل مع عزلات السليمانية (Sul/1/2025 و Sul/2/2025). وتخلص هذه الدراسة إلى أن الحمام البري في المناطق الحضرية يُعد مخزنًا طبيعيًا للكلاميديا البيغائية، مما يشكل خطرًا لانتقال الأمراض الحيوانية المنشأ.

الكلمات المفتاحية: الكلاميديا البيغائية، حمام بري، تفاعل البوليميراز المتسلسل، العراق، جين *ompA*.