" Kerbala Journal of Veterinary Medical Sciences, Vol. 1, Supplement I, 2025 "
"Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Karbala"

Molecular detection and genotyping of *chlamydia psittaci* in domestic pigeons and human contacts in baghdad city

Suhail Wadi Jaafar[1], Atheer abdurazzaq abdulazeez Aldoori[2] , Farah Thamer Samawi[3]

[1]Veterinary department, Ministry of agriculture, Baghdad, Iraq.
[2] University of Baghdad, Ministry of high education and scientific research, Baghdad, Iraq.
[3] AL- Nahrain University, Ministry of high education and scientific research, Baghdad, Iraq.
Corresponding author: sohailalsoltani@gmail.com
atheer@covm.uobaghdad.edu.iq , Farahthamer1978@gmail.com
Received: 20/5/2025 **Accepted:**25/5/2025 **Published:** 3/7/2025

***Abstract*— *Chlamydia psittaci*, the causative agent of avian chlamydiosis, is a well-recognized zoonotic pathogen with pigeons (*Columba livia domestica*) serving as a primary natural reservoir. Despite its significance, *C. psittaci* remains a neglected public health threat in many regions, including Iraq. This study aimed to determine the prevalence and molecular characterization of *C. psittaci* in oropharyngeal samples from domestic pigeons and nasal swabs from their human handlers in Baghdad, Iraq, using a combination of rapid antigen testing and conventional PCR techniques.**

From October 2023 to April 2024, a total of 150 pigeon oropharyngeal swabs and 20 nasal swabs from pigeon breeders were collected. Initial screening for the *C. psittaci* major outer membrane protein (MOMP) antigen using a rapid diagnostic cassette test revealed a 40% positivity rate (60/150) among the pigeon samples. These positive samples were subsequently inoculated into embryonated chicken eggs for pathogen propagation. DNA extracted from the cultured material was subjected to PCR amplification targeting the *16S rRNA* and *ompA* genes specific to *C. psittaci.*

PCR analysis confirmed *C. psittaci* DNA in 8.3% (5/60) of the antigen-positive pigeon samples and in 5% (1/20) of the human samples. Furthermore, all six PCR-positive samples harbored antibiotic resistance-associated genes, including *rpID* (azithromycin resistance) and *rpIV* (spectinomycin resistance), indicating 100% resistance gene detection. Phylogenetic analysis of the *ompA* gene sequences revealed that all avian isolates belonged to genotype B. To our knowledge, this study represents the first molecular detection and genotypic characterization of *C. psittaci* in Iraq.

These findings highlight the zoonotic risk posed by domestic pigeons and underscore the urgent need for targeted surveillance, public education, and biosecurity measures to mitigate the risk of pigeon-to-human transmission of chlamydial pathogens in urban environments.

***Keywords* — *Chlamydia psittaci*; domestic pigeons; zoonosis; PCR; Iraq; *ompA* gene; antibiotic resistance; genotype B.**

INTRODUCTION

Avian chlamydiosis is one of the important neglected diseases with critical zoonotic potential. The causative agent, *Chlamydia psittaci*, affects birds, animals, and humans. The disease is an occupational hazard mainly to custom officers handling exotic birds. Prevalence of the disease in wild birds, pet birds, and poultry (1).

Members of Chlamydiaceae family are obligate intracellular coccoid, Gram-negative bacteria which are transmitted by biologically inactive particles named elementary bodies (Ebs) (2). *C.psittaci* has been reported in at least 467 bird species belonging to 30 different orders. This pathogen causes asymptomatic to severe systemic infections in several bird species, depending on susceptibility of the host species, immune status, infectious dose and virulence of the strain involved (3).

Chlamydia psittaci is the most common chlamydial species which causes infection principally in pigeons and parrots as psittacosis (ornithosis). Affected birds can be asymptomatic; however, common clinical signs are weight loss, diarrhea, anorexia, respiratory signs (dyspnea), conjunctivitis, hyperthermia and sudden death (4).

Currently, 17 genotypes of *C. psittaci* have been identified in the avian reservoir and considered potentially pathogenic for human health . *C. psittaci* is genetically variable, and

genotypes have been designated based on outer membrane protein A (*ompA*) sequences and classified in to A to F, E/B, M56, and WC genotypes, each genotype exhibits strong host preference: A and F are primarily found in parrots, B in pigeons, C in ducks and geese, D in turkeys and E infects a broad range of birds including pigeons (5).

Genetically, variable genes (*omp2* and *ompA*) and conserved genes (*16S rRNA*, *23S rRNA*) are used for both analyzing species/strain level variations in field studies and detection of Chlamydiae (6). Members of the family Chlamydiaceae are characterized by a biphasic developmental cycle of replication in which only one active, developmental form, named the reticulate body (Rb). The second form is the infectious but metabolically inactive elementary body (Eb) that is endocytosed by a susceptible eukaryotic cell and resides within a cytoplasmic vacuole termed the inclusion (7).

In humans, symptoms are generally wide-ranging and influenza-like. Occasionally they may include severe pneumonia, endocarditis, encephalitis and renal disease (8). *C. psittaci* can be transmitted to humans or other mammals and cause psittacosis. This is mostly by direct contact with contaminated aerosol's inhaling, eye secretions, feather dust, or dried faeces from an infected pigeon or environmental contamination with droppings while handling birds (9).

The intracellular lifestyle of this pathogen makes the diagnosis more complicated and there is also lack of accurate diagnostics [1]. Many methods to detect *Chlamydia psittaci* such as rapid diagnosis tests, enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody tests (FATs). Nonetheless, this technique necessitates a laboratory to identify the protein or antigen (10,11).

The sensitivity of conventional PCR assays will usually exceed that of isolation. Current conventional PCR tests for detection of *C. psittaci* target the *16S–23S rRNA* or the *ompA* gene (12). The disease pathology of Chlamydial species is highly similar. The organisms in the order Chlamydiales share > 80% of gene sequences based on 16S and 23S rRNA analysis, so that the researchers did not widely use the proposed subdivision of the genus into *Chlamydia* and *Chlamydophila* based on 16S and 23S rRNA (13). The first-line therapeutic regimens used in the treatment and prophylaxis of chlamydial infections are the tetracyclines and the macrolide azithromycin. Resistance to antibiotics is reported only in some pathogens of the Chlamydiaceae family, but routine screening may assess the actual situation in all pathogens (1).

To the best of our knowledge, there is no study addressing Chlamydiosis in this area; therefore, the purpose of this investigation was to survey and collect baseline data on the prevalence of *C. psittaci* in domestic pigeon in Baghdad, Iraq and helps to diagnose the prevalence of this pathogen for better treatment and prevention.

MATERIALS AND METHODS

Sample Collection

This study was conducted at the Biotechnology Research Center, Al-Nahrain University. A total of 150 oropharyngeal swabs were collected from domestic pigeons of varying ages and both sexes, along with 20 nasal swabs from pigeon breeders. Samples were randomly collected from 40 privately owned pigeon aviaries located throughout Baghdad, Iraq, between October 2023 and April 2024. Each aviary housed at least 50 pigeons. Pigeons displaying classical signs of pneumonia and conjunctivitis were sampled prior to receiving any antimicrobial treatment.

Rapid Antigen Detection

All swabs were initially screened using a Chlamydia rapid cassette test (MOMP antigen). Positive samples were immediately transferred into viral transport medium (VTM) (Liofilchem, USA) under aseptic conditions and transported on ice packs to the laboratory for further analysis.

Inoculation in Embryonated Eggs

Antigen-positive samples were inoculated into the yolk sac of 6–7 day old specific-pathogen-free (SPF) embryonated chicken eggs (0.5 mL per egg). Inoculated eggs were incubated at 39°C in a humidified chamber and monitored daily. Embryonic death typically occurred within 3–10 days. Characteristic vascular congestion of the yolk sac membranes indicated chlamydial infection. Yolk sacs were harvested, homogenized into a 20% (w/v) suspension using SPG buffer, and stored at −20°C for DNA extraction (2).

DNA Extraction and PCR

Samples were vortexed and centrifuged at 5,000 rpm for 5 minutes. DNA was extracted using the G-spin™ Genomic DNA Kit for Bacteria (Intron, Korea), following the manufacturer's protocol. PCR detection of *C. psittaci* was based on amplification of two target genes: first, *16S rRNA* gene, using universal primers for the genus *Chlamydophila* ,second, *ompA* gene specific for *C. psittaci* (3). For antibiotic resistance detection, two additional targets were amplified: first, Azithromycin resistance gene *(rpID*), Second, Spectinomycin resistance gene (rpIV) (4). Primer sequences are listed in Table 1, and their efficacy was validated as per Vilela *et al*. (2019) (15,16). All primers were synthesized by Macrogen Co., Korea.

PCR reactions were prepared in 25 µL volumes, containing: 10 µL GoTaq® G2 Green Master Mix (Promega, USA), 4 µL primer mix (forward and reverse), 4 µL template DNA, 7 µL PCR-grade distilled water. Thermal cycling conditions: Initial denaturation: 94°C for 5 min, 35 cycles of: Denaturation: 94°C for 30 sec, Annealing: 54°C for 30 sec, Extension: 72°C for 1 min and Final extension: 72°C for 5 min. PCR amplicons were resolved on 1.5% agarose gel (Intron, Korea) containing RedSafe nucleic acid stain. A 100 bp Opti-DNA ladder (Safe-Green) was used as a molecular marker. Electrophoresis was conducted at 90 V for 45 minutes. Gels were visualized using a UV transilluminator (Analytik Jena, UK) at 320 nm. The presence of a 418 bp band was considered positive for *C.*

psittaci (15).

Evolutionary analyses

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (17). Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. Evolutionary analyses were conducted in MEGA11 (18).

Table 1. Primers for detection of C. *psittaci* and antibiotics resistance.

Primer		Sequence	Product size	References
16S rRNA gene	F	TCGAGAATCTTTCGCAATGGAC	(222 bp)	Struthers *et al.*, 2021
	R	CGCCCTTTACGCCCAATAAA		
C. psittaci gene	VD1-F	ACTACGGAGATTATGTTTTCGATCGTGT	(418 bp)	Vilela *et al*,. 2019
	VD2-R	CGTGCACCYACGCTCCAAGA		
rpID gene	F	CAGAAGAGGTCCTAATGG	(754 bp)	Binet and Maurelli, 2007
	R	CTTCTCGGTTACATAATGCCG		
rpIV gene	F	GGGTAAGTCTAAAGGAGAC	(390 bp)	
	R	TTGGACATCCTTTCTGACCC		

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study(19).

RESULT & DISCUSSION

Domestic pigeons suspected of *Chlamydia psittaci* infection exhibited classical clinical signs including conjunctivitis, nasal discharge, labored breathing, and audible respiratory sounds (snoring). These clinical manifestations were consistent with avian chlamydiosis and were used as selection criteria for initial screening.

All clinically suspected pigeons (n = 150) were tested using a specific rapid antigen detection cassette for *C. psittaci* (lateral flow immunoassay). Out of these, 60 samples (40%) tested positive for the major outer membrane protein (MOMP) antigen, while 90 samples (60%) yielded negative results (Table 2).

The rapid test employed is a chromatographic lateral flow immunoassay, suitable for on-site screening in pigeon aviaries and diagnostic laboratories, providing a quick preliminary tool to support the diagnosis of *C. psittaci* infection.

All 60 antigen-positive samples were further processed for biological confirmation. Inoculation into embryonated chicken eggs resulted in characteristic pathological changes, including vascular congestion of the yolk sac, which indicated successful replication of the organism. DNA was subsequently extracted from the yolk sac suspensions using a specialized kit. PCR amplification targeting the 16S ribosomal RNA (*16S rRNA*) gene of Chlamydiaceae confirmed the presence of chlamydial DNA in a subset of the samples, as detailed in the subsequent molecular findings section.

A total of 60 oropharyngeal swab samples from pigeons and 20 nasal swab samples from pigeon breeders were subjected to PCR assays targeting the *16S rRNA* and *ompA* genes of *C. psittaci.* Among the pigeon samples, 5 out of 60 (8.3%) tested positive for *C. psittaci* DNA. Additionally, 1 out of 20 (5%) human samples was positive for *C. psittaci*, indicating potential zoonotic transmission (Table 3). Based on age and season, the prevalence of *C. psittaci* infection was high 4/45 (8.9%) in pigeon less than one year old and also high 4/30 (13.3%) in pigeon during the cold season (Table 4).

Amplification of the (222bp) region of the *16S rRNA* gene confirmed the presence of Chlamydiaceae genus (Figure 1-A), while subsequent amplification of the (418bp) region of the *ompA* gene specifically confirmed *C. psittaci* species identity in the same samples (Figure 1-B). This dual-marker approach validated that all six PCR-positive samples belonged to the genus *Chlamydophila* and were specifically *C. psittaci*. The association between seasonal variation, pigeon age, and gender with PCR positivity is summarized in Table 3.

PCR amplification of antibiotic resistance markers revealed that all six *C. psittaci*-positive samples (100%) harbored: The *rpID* gene (azithromycin resistance) with an amplified product of (754bp) (Figure 2-C). The *rpIV* gene (spectinomycin resistance) with a product size of (390bp) (Figure 2-D). These findings indicate that circulating *C. psittaci* strains in Baghdad possess multidrug resistance potential, raising public health concerns about treatment efficacy in both avian and human cases.

The *ompA*-positive PCR products from five of the six *C. psittaci*-positive samples (from pigeons) were successfully sequenced and analyzed for genotype determination. GenBank accession numbers: PV243292.1, PV088367.1, LC863018.1, PV340861.1, and PV400858. The Phylogenetic analysis showed that all positive samples were genetically very close (99–100% identity) to genotypes B reference strains (AY762609.1) as illustrated in Figure 3. These results confirm the presence of genotype B, a known avian-associated genotype of *C. psittaci*. To our knowledge, this is the first report of genotype B in Iraq. All sequences were submitted to GenBank (NCBI), establishing baseline molecular data for the country.

The infection rate of *C. psittaci* in pigeon was significantly higher than that in human contact (P≤0.01). No other statistically significant difference was found between season, age and gender of pigeon.

Table 2: The percentage of *C. Psittaci* infection according to Rapid Test/Cassette

Diagnostic test	+ve samples		-ve samples		Total	
C. psittaci Rapid Test	No.	%	No.	%	No.	%
	60	40%	90	60%	150	100%
Chi-square test –χ^2 (P-value)	6.00 ** (0.010)				--	--

** (P≤0.01).

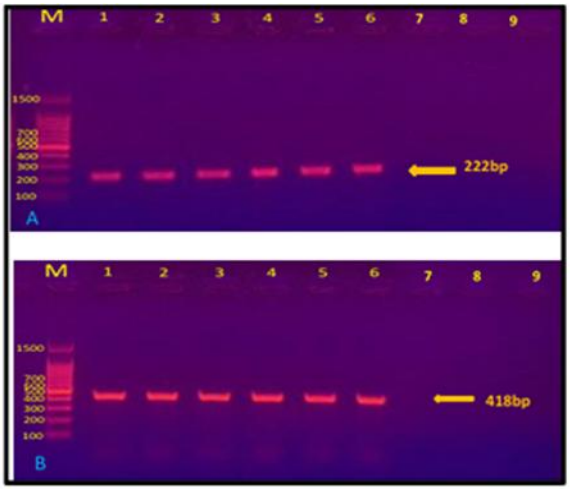

Figure 1. Amplification of partial region of: **A**/ *16S rRNA* gene of *C. psittaci* at 222 bp., **B**/ *ompA* gene of *C. psittaci* at 418 bp. on 1.5% (w/v) agarose gel, M: 1500 bp DNA ladder. Lanes 1 – 5 represent positive samples of pigeon, lane 6 represent positive sample of Human, lanes 7–9 represent negative samples.

Table 3: Prevalence of *C. psittaci* in samples of Domestic pigeon and pigeon breeders (Human) in Baghdad City.

	Domestic pigeon		Human	
The gene	**No. of samples positive**	**Percentage**	**No. of samples positive**	**Percentage**
16S rRNA	5/60	8.3%	1/20	5%
ompA	5/5	100%	1/1	100%
rpID	5/5	100%	1/1	100%
rpIV	5/5	100%	1/1	100%
Chi-square $-\chi^2$ (P-value)	--	12.67 ** 0.0001	--	13.05 ** 0.0001

** (P≤0.01).

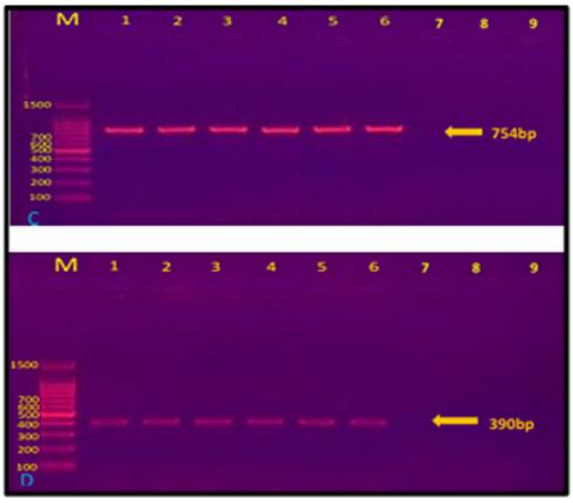

Figure 2. Amplification of partial region of: C/ *rpID* gene of *C. psittaci* at 754 bp., D/ *rpIV* gene of *C. psittaci* at 390 bp. on 1.5% (w/v) agarose gel M: 1500 bp DNA ladder. Lanes 1 – 6 represent positive samples. Lanes 7–9 represent negative samples.

Table 4: Prevalence of *C. psittaci* infection based on gender, age and season in pigeon

PCR		PCR		PCR	
Season	**Positive No.(%)**	**Age**	**Positive No.(%)**	**Gender**	**Positive No.(%)**
Hot	1/30(3.3%)	1year <	4/45 (8.9%)	Male	2/26 (7.7%)
Cold	4/30(13.3%)	1year >	1/15 (6.7%)	Female	3/34 (8.8%)
Total	5/60(8.3%)	Total	5/60 (8.3%)	Total	5/60 (8.3%)
Chi-square $-\chi^2$ (P-value)	1.077 NS (0.208)	--	0.638 NS (0.803)	--	0.594 NS (0.872)

NS: Non-Significant.

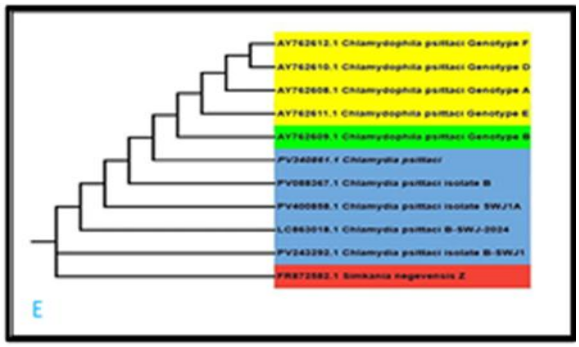

Figure 3. Phylogenetic tree of *C. psittaci* isolates and selected accession numbers from GenBank, based on *ompA* sequence

Chlamydiosis, caused by Chlamydia psittaci, is a significant zoonotic disease that manifests as ornithosis in birds and psittacosis in humans. The global incidence of *C. psittaci* infection has gradually increased in recent years (20). Due to the bacterium's obligate intracellular nature and the biosafety hazard it poses to laboratory personnel, culture-based diagnostics are rarely used. Instead, nucleic acid amplification tests (NAATs), including conventional and real-time PCR, have become the preferred diagnostic tools for *C. psittaci*, offering high sensitivity and specificity (21).

Among the ten genomic targets available for *C. psittaci* detection, the *ompA* gene remains the most commonly used due to its species-specificity and ability to support genotyping (22). Our study represents the first molecular investigation of *C. psittaci* among pigeons and humans in Baghdad, Iraq, and contributes valuable baseline data for the region.

In this study, the prevalence of *C. psittaci* DNA in pigeons was 8.3%, and 5% in human nasal swabs. Detection was based on PCR amplification of both the *16S rRNA* and *ompA* genes, confirming infection at the genus and species level. These findings support the potential for zoonotic transmission, especially among individuals with frequent exposure to birds, such as pigeon breeders.

Previous seroprevalence studies between 1966 and 2005 reported wide variation (12.5%–95.6%) (23). Though serological methods can yield false positives due to cross-reactivity with heat-shock proteins and LPS from other bacteria (24). More recent molecular studies using NAATs have confirmed *C. psittaci* prevalence ranging from 3.4% to 52.6% in feral pigeons [9].

Our PCR-based prevalence (8.3%) is higher than those reported in Ahvaz, Iran (0.71%) and Belgium (2%) (25,23), and comparable to the 5–10% prevalence observed in Amsterdam (26). However, it is lower than the 16.8% observed in Brazil (27), and significantly lower than the 25.3% in Bakhtiari, Iran, where both human (10%) and pigeon infections were confirmed (28). In Germany, prevalence has been reported as high as 46.7–76.7% (4), indicating potential geographic and ecological factors influencing transmission.

Notably, sampling in our study coincided with the avian breeding season, a period associated with stress-induced chlamydial shedding (29), possibly accounting for higher detection rates.

Our results reaffirm that oropharyngeal swabs are effective for detecting *C. psittaci* in symptomatic birds. The choice of sample type, along with DNA extraction method, primer design, and target gene, plays a crucial role in diagnostic sensitivity (22). Human infection was observed at a rate of 5%, lower than the 12.5–30% reported in other occupational exposure studies (30, 28), possibly reflecting differences in bird-human interaction, hygiene practices, or environmental exposure.

Interestingly, infected individuals in our study were asymptomatic, which aligns with findings by Stewardson and Grayson (2010) that *C. psittaci* may exist in a subclinical or nonviable form, especially at low inoculum doses (31). A major finding in our study was the detection of antibiotic resistance genes in 100% of *C. psittaci* isolates. All six PCR-positive samples carried: *rpID* gene, conferring resistance to azithromycin and *rpIV* gene, associated with spectinomycin resistance. That confirms our antibiotic Resistance Concerns.

Although few studies report natural resistance in Chlamydia species, accumulating evidence suggests that mutations in *16S rRNA*, *23S rRNA*, and *rpoB* genes can mediate resistance to aminoglycosides and macrolides (7,32). Binet and Maurelli (2007, 2009) demonstrated that some *C. psittaci* strains retain stable resistance phenotypes and can grow comparably to wild-type strains. While macrolides such as azithromycin remain frontline agents, resistant strains—like 6BC—pose challenges, particularly in cases of treatment failure (14,33).

Macrolides are known to be more effective than fluoroquinolones against *C. psittaci* and offer additional benefits such as modulation of host immune responses, inhibition of oxidant production, and enhanced phagocytosis (32).However, as Andersson and Levin (1999) stated, antibiotic resistance frequency is linked to drug usage intensity and the fitness cost imposed on bacteria—a dynamic that must be monitored in avian and zoonotic reservoirs alike (34).

Among the positive samples of *C. psittaci* detected using PCR, *OmpA* gene fragments were successfully amplified and sequenced, with a success rate of 8.3% (5/60). The sequences obtained shared 99.6%–100.0% nucleotide identity with each other, and had the highest similarities (99.5%–99.9%) with the *C. psittaci* strains obtained from pigeon in Australia and Argentina . According to NCBI, Phylogenetic tree analysis showed that all the 5 strains belonged to genotype B (Fig. 3).

This study provides the first molecular evidence of *C. psittaci* genotype B in Iraqi pigeons. Genotype B is commonly associated with pigeons and has zoonotic relevance. The presence of this genotype in Baghdad's urban pigeon population highlights a public health concern, especially for individuals with occupational or recreational bird exposure.

The detection of antibiotic-resistant *C. psittaci* further underscores the importance of biosecurity, surveillance, and rational antibiotic use in both veterinary and human healthcare settings. Management strategies should include:

- Regular screening of domestic and feral pigeons
- Public education campaigns targeting pigeon breeders and bird owners
- Implementation of protective measures in aviaries and public spaces
- Further research into clinical manifestations in humans and transmission dynamics

Data available to health authorities may not reflect the reality of psittacosis in our country, as it is frequently sub diagnosed. Patients' history of exposure to or contact with birds or other animals is often omitted in the medical consultation. Throughout this study, the use of molecular methods allowed us to detect and genetically characterize the *C. psittaci* present in homing pigeon. Through the use of these strategies, the examined samples revealed the existence of *C. psittaci* in pigeon with clinical symptomatology of the disease, as well as in other clinically normal birds.

CONCLUSION

The present study provides the first molecular evidence of Chlamydia *psittaci* infection in domestic pigeons and their human breeders in Baghdad, Iraq, highlighting a potential zoonotic threat to public health in urban settings. The detection of C. *psittaci* DNA in respiratory secretions of pigeons and in nasal swabs from pigeon breeders suggests that close and prolonged contact with infected birds may facilitate human transmission.

Importantly, this study contributes to the molecular epidemiological understanding of *C. psittaci* in Iraq. Genotyping based on the *ompA* gene confirmed the circulation of genotype B, a strain commonly associated with pigeons and known for its zoonotic potential.

Given the confirmed detection of antibiotic resistance genes (*rpID* and *rpIV*), our findings also raise concern about the emergence of resistant *C. psittaci* strains in avian reservoirs.

REFERENCES

1) Ravichandran, K., Anbazhagan, S., Karthik, K., Angappan, M., & Dhayananth, B. (2021). A comprehensive review on avian chlamydiosis: a neglected zoonotic disease. Tropical animal health and production, 53(4), 414.

2) Andersen A.A. & Vanrompay D. (2008). Chapter 16, Chlamydiosis. In: A Laboratory Manual for the Isolation, Identification and Characterization of Avian Pathogens, Fifth Edition, Dufour-Zavala L., ed. The American Association of Avian Pathologists (AAAP), Jacksonville, Florida, USA.

3) Pospischil, A., Kaiser, C., Hofmann-Lehmann, R., Lutz, H., Hilbe, M., Vaughan, L., & Borel, N. (2012). Evidence for Chlamydia in wild mammals of the Serengeti. Journal of wildlife diseases, 48(4), 1074-1078. https://doi.org/10.7589/2011-10298PMID:23060512

4) Sachse, K., Laroucau, K., & Vanrompay, D. (2015). Avian chlamydiosis. Current Clinical Microbiology Reports, 2, 10-21.

5) Wang, J., Wang, B., Xiao, J., Chen, Y., & Wang, C. (2024). *Chlamydia psittaci*: A zoonotic pathogen causing avian chlamydiosis and psittacosis. Virulence, 15(1), 2428411.

6) Bhardwaj, B., Chahota, R., Gupta, S., Malik, P. and Sharma, M. (2017). Identification of chlamydial strains causing abortions and pneumonia in sheep and goat flocks during trans Himalayan seasonal migration in the northern region of India. Veterinarski arhiv, 87(2), 151-170.

7) Binet, R., & Maurelli, A. T. (2005). Fitness cost due to mutations in the 16S rRNA associated with spectinomycin resistance in *Chlamydia psittaci* 6BC. Antimicrobial agents and chemotherapy, 49(11), 4455-4464.

8) Guzman, D. S. M., Diaz-Figueroa, O., Tully, T., Ciembor, P., Morgan, T., Walden, M., Poston, R.P., Flammer, K., Mitchell, M.A. & Ritchie, B. (2010). Evaluating 21-day doxycycline and azithromycin treatments for experimental *Chlamydophila psittaci* infection in cockatiels (Nymphicus hollandicus). Journal of avian medicine and surgery, 24(1), 35-45.

9) Magnino, S., Haag-Wackernagel, D., Geigenfeind, I., Helmecke, S., Dovc, A., Prukner-Radovcic´, E., Residbegovic´, E., Ilieski, V., Laroucau, K. & other authors (2009). Chlamydial infections in feral pigeons in Europe: review of data and focus on public health implications. Vet Microbiol 135, 54–67.

10) Borel, N., Polkinghorne, A., & Pospischil, A. (2018). A review on chlamydial diseases in animals: still a challenge for pathologists?. Veterinary pathology, 55(3), 374-390

11) Dana, O. I., Mukhtar, R. H., Mohammed, M. O., & Dyary, H. O. (2021). Comparison of a rapid test with bPAG elisa in pregnancy diagnosis in cows. Iraqi Journal of Agricultural Sciences, 52(6), 1475-1481.

12) Sachse, K., Vretou, E., Livingstone, M., Borel, N., Pospischil, A., & Longbottom, D. (2009). Recent developments in the laboratory diagnosis of chlamydial infections. Veterinary microbiology, 135(1-2), 2-21.

13) Everett, K. D., Bush, R. M., & Andersen, A. A. (1999). Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. International Journal of Systematic and Evolutionary Microbiology, 49(2), 415-440.

14) Binet, R., & Maurelli, A. T. (2007). Frequency of development and associated physiological cost of azithromycin resistance in *Chlamydia psittaci* 6BC and C. trachomatis L2. Antimicrobial agents and chemotherapy, 51(12), 4267-4275.

15) Vilela, D. A. R., Marin, S. Y., Resende, M., Coelho, H. L. G., Resende, J. S., Ferreira-Junior, F. C., Ortiz, M.C. Araújo, A.V. Raso, T.F. & Martins, N. R. S. (2019). Phylogenetic analyses of *Chlamydia psittaci* ompA gene sequences from captive blue-fronted Amazon parrots (Amazona aestiva) with hepatic disease in Brazil. Rev. Sci. Tech, 38, 711-719.

16) Struthers, J. D., Lim, A., Ferguson, S., Lee, J. K., Chako, C., Okwumabua, O., Cuneo M., Valle A. M. & Brower, A. (2021). Meningoencephalitis, vasculitis, and abortions caused by *Chlamydia pecorum* in a herd of cattle. Veterinary Pathology, 58(3), 549-557.

17) Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

18) Tamura K., Stecher G., and Kumar S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution https://doi.org/10.1093/molbev/msab120.

19) SAS. 2018. Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

20) Zhu, Z., Wang, X., Zhao, J., Xie, Z., Yang, C., Li, L., & Liu, Y. (2023). Clinical characteristics of six patients with *Chlamydia psittaci* infection diagnosed by metagenomic next-generation sequencing: A case series. Infection and drug resistance, 869-878.

21) Schnee, C., Vanrompay, D., & Laroucau, K. (2018). Avian chlamydiosis. Avian Chlamydiosis. chapter 2.3.1.

22) Soon, X. Q., Gedye, K., Benschop, J., & Gartrell, B. (2025). Molecular detection of *Chlamydia psittaci* in birds: a systematic review. Avian Pathology, 1-20.

23) Geigenfeind, I., Vanrompay, D., & Haag-Wackernagel, D. (2012). Prevalence of *Chlamydia psittaci* in the feral pigeon population of Basel, Switzerland. Journal of medical microbiology, 61(2), 261-265.

24) Yuan, Y., Lyng, K., Zhang, Y. X., Rockey, D. D. & Morrison, R. P. (1992). Monoclonal antibodies define genus-specific, species-specific, and cross-reactive epitopes of the chlamydial 60-kilodalton heat shock protein (hsp60): specific immune detection and

purification of chlamydial hsp60. Infect Immun 60, 2288–2296.

25) Ghorbanpoor, M., Bakhtiari, N. M., Mayahi, M., & Moridveisi, H. (2015). Detection of *Chlamydophila psittaci* from pigeons by polymerase chain reaction in Ahvaz. Iranian journal of microbiology, 7(1), 18.

26) Heddema, E. R., van Hannen, E. J., Duim, B., Vandenbroucke-Grauls, C. M. & Pannekoek, Y. (2006). Genotyping of *Chlamydophila psittaci* in human samples. Emerg Infect Dis 12, 1989–1990.

27) De Lima, V. Y., Langoni, H., Da Silva, A. V., Pezerico, S. B., De Castro, A. P. B., Da Silva, R. C., & Araújo Jr, J. P. (2011). *Chlamydophila psittaci* and Toxoplasma gondii infection in pigeons (Columba livia) from São Paulo State, Brazil. Veterinary Parasitology, 175(1-2), 9-14.doi: 10.1016/j.vetpar.2010.10.006.

28) Mahzounieh, M., Moloudizargari, M., Ghasemi Shams Abadi, M., Baninameh, Z., & Heidari Khoei, H. (2020). Prevalence rate and phylogenetic analysis of *Chlamydia psittaci* in pigeon and house sparrow specimens and the potential human infection risk in chahrmahal-va-bakhtiari, Iran. Arch. Clin. Infect. Dis, 15, e67565.

29) European Commission, 2002. Avian chlamydiosis as a zoonotic disease and risk reduction strategies, Science Communications Animal Health. Animal Welfare, 26.

30) Kalmar, I. D., Dicxk, V., Dossche, L., & Vanrompay, D. (2014). Zoonotic infection with *Chlamydia psittaci* at an avian refuge centre. The Veterinary Journal, 199(2), 300-302. doi: 10.1016/j.tvjl.2013.10.034. [PubMed: 24326117].

31) Stewardson, A.J. and Grayson, M.L. (2010) Psittacosis. Infect Dis Clin North Am; 24(1):7–25. doi: 10.1016/j.idc.2009.10.003.

32) Benamri, I., Azzouzi, M., Sanak, K., Moussa A & Radouani F. (2021). An overview of genes and mutations associated with Chlamydiae species' resistance to antibiotics. Ann Clin Microbiol Antimicrob 20, 59 https://doi.org/10.1186/s12941-021-00465-4.

33) Binet, R., & Maurelli, A. T. (2009). Transformation and isolation of allelic exchange mutants of *Chlamydia psittaci* using recombinant DNA introduced by electroporation. Proceedings of the National Academy of Sciences, 106(1), 292-297. [PubMed: 19104068].

34) Andersson, D. I., and Levin, B. R. (1999). The biological cost of antibiotic resistance. Curr. Opin. Microbiol. 2:489–493.