Molecular Detection of *Chlamydia psittaci* in Domestic Pigeons of Kerman City

Fatemeh Hosseinzadeh[1], Elham Mohammadi[2], Hemad shafiei[1*], Mehdi Golchin[2], Mohammadjavad Jahedi[1]

1. **Department of Clinical Science, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran**
2. **Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran**

[*] Corresponding Author: Hemad shafiei

Email: hemad.shafiei@uk.ac.ir

ORCiD:

Hemad shafiei: 0009-0004-9794-7934

Mehdi Golchin: 0000-0002-0969-4921

Mohammadjavad Jahedi: 0009-0006-8640-7670

Abstract

Introduction: Avian chlamydiosis is an important zoonotic disease caused by Chlamydia psittaci, a Gram-negative, obligate intracellular bacterium of the family Chlamydiaceae. The pathogen can be transmitted to humans through contact with contaminated secretions such as urine, feces, and respiratory aerosols from infected birds. Clinical signs in both humans and birds are often non-specific, resembling influenza. In humans, infection can present as mild fever, headache, anorexia, and sore throat, potentially progressing to pneumonia. In birds, disease manifestations range from asymptomatic infection to respiratory distress, sinusitis, mucopurulent nasal discharge, lethargy, diarrhea, anorexia, polyuria, conjunctivitis, reproductive disorders, and reduced egg production. **Objective**: This study aimed to perform molecular detection of C. psittaci in domestic pigeons in Kerman City, Iran, and to evaluate the prevalence, clinical signs, and potential risk factors associated with infection.

Materials and Methods: A total of 100 samples were collected from the choanal clefts of 10 pigeon flocks (10 birds randomly selected per flock) across different areas of Kerman City. DNA extraction was performed using a commercial kit, followed by PCR amplification with specific primers targeting the rPOMP90-3 gene of C. psittaci. Amplified products were analyzed by gel electrophoresis and visualized using a gel documentation system.

Results: PCR analysis revealed that 12% of the pigeons tested positive for C. psittaci. Infections were detected solely in adult birds, often under stressful husbandry conditions, and clinical signs varied among the affected birds.

Conclusion: The observed 12% detection of C. psittaci underscores the significant role of domestic pigeons in the epidemiology of the pathogen in urban Iran. These findings align with national and international studies, emphasizing the need for regular surveillance, public education, and improved management practices to mitigate both veterinary and zoonotic risks. Future research should involve larger sample sizes, multiple avian species, and advanced molecular techniques to further investigate the circulation and genetic diversity of C. psittaci in the region.

Keywords: *Chlamydia psittaci*, Domestic pigeons, Kerman, Molecular detection.

1. Introduction

Avian chlamydiosis is a major zoonotic disease caused primarily by *Chlamydia psittaci*, a Gram-negative, obligate intracellular bacterium of the family Chlamydiaceae. This pathogen infects a wide range of avian hosts and occasionally mammals, including humans, thereby representing a significant public health concern [1]. Historically known as psittacosis or “parrot fever,” the disease was first recognized in parrots and in humans exposed to them, but today it is broadly referred to as avian chlamydiosis in birds and psittacosis (ornithosis) in humans [1]. In birds, *C. psittaci* infections range from asymptomatic carriage to severe systemic illness characterized by respiratory distress, sinusitis, conjunctivitis, lethargy, diarrhea, reproductive disorders, and decreased egg production [1]. In humans, infection is typically acquired by inhaling contaminated aerosols or dust derived from secretions or feces of infected birds, leading to influenza-like symptoms that may progress to pneumonia or systemic complications [1].

The zoonotic importance of *C. psittaci* has been demonstrated repeatedly. The first documented cases of psittacosis were reported in 1879, and the causative agent in humans was identified in 1930 [2]. Severe epidemics occurred between 1929 and 1938 in the United States and Europe, largely linked to the importation of Amazon parrots from Argentina [2,3]. It later became evident that *C. psittaci* was widespread among avian taxa including pigeons, ducks, turkeys, and seabirds [3]. Today, this pathogen has been documented in at least 465 bird species across 30 avian orders, with pigeons and parrots representing key reservoirs [1,2,3].

Chlamydia psittaci exhibits a biphasic developmental cycle involving infectious elementary bodies and metabolically active reticulate bodies replicating within cytoplasmic inclusions [3]. Genotyping based on the outer membrane protein A (ompA) gene has revealed multiple genotypes (A–F, E/B, M56, WC), with genotypes B and E commonly detected in pigeons [1,3]. Such molecular differentiation underscores the importance of genotype-specific surveillance in species living near humans.

The public health importance of *C. psittaci* extends beyond sporadic human cases. Workers in poultry farms, slaughterhouses, and pet bird facilities are at heightened risk of exposure [4]. Although human-to-human transmission is rare, a recent case reported in Sweden underscores the potential for direct transmission [3]. The organism may persist for more than a month when protected by organic material [5]. This wide host range and persistence highlight the need for rigorous surveillance and preventive measures [6].

Traditional diagnostic methods, including culture and serological assays, are limited by the bacterium’s obligate intracellular nature, slow growth, and the absence of detectable antibodies during early infection. Consequently, molecular diagnostic techniques such as polymerase chain reaction (PCR) targeting rPOMP90-3 or ompA genes offer higher sensitivity, specificity, and rapid turnaround [3,7,8].

Given the zoonotic potential of *C. psittaci* and the close association between domestic pigeons and humans in urban environments, pigeons serve as a vital focus for molecular surveillance [9, 10]. In Iran, despite a high density of domestic pigeons, there is a lack of comprehensive molecular data regarding the prevalence of *C. psittaci*. Establishing baseline data is essential for assessing public health risks and informing preventive measures.

Consequently, the present study aimed to conduct molecular detection of *C. psittaci* in domestic pigeons from Kerman City, Iran. Utilizing PCR techniques on samples collected from various flocks, this study sought to estimate the prevalence of *C. psittaci* and underscore the public health implications associated with pigeon-borne infections.

2. Materials and Methods

2.1. Sampling

A total of 100 pigeons were randomly selected from 10 different flocks located in various regions of Kerman city, with 10 pigeons sampled from each flock. Samples were collected from the choanal cleft using sterile swabs. Among the sampled birds, 52 were males (49 adults and 3 juveniles) and 48 were females (45 adults and 3 juveniles). After collection and preparation, the samples were transported to the laboratory on ice for further processing.

2.2. DNA Extraction

Genomic DNA was extracted from the respiratory samples using a commercial extraction kit (SinaClon, Iran) following the manufacturer's protocol. Briefly, the cells were lysed to release nucleic acids, and proteins and other impurities were removed using specific buffers. The purified DNA was eluted in a final volume of 50 µL. DNA quality and concentration were assessed by spectrophotometry and visualization on agarose gel electrophoresis.

2.3. PCR Assay

Amplification of a specific fragment of the *ompA* gene of *Chlamydia psittaci* was performed using specific primers. Each 20 µL reaction mixture contained 10 µL of commercial master mix, 0.5 µL of each primer (10 µM), 2 µL of DNA template, and 7 µL of nuclease-free water. The thermocycling program included an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. Positive controls (standard *Chlamydia* DNA) and negative controls (no DNA template) were included in each PCR run [11] (Table 1).

Table1: Primers used for amplification of the rOMP90-3 gene

Target	**Primer Name**	**Sequence (5'→3')**	**Product Size (bp)**
rOMP90-3	rOMP90-3-F	5′-TTTTCAGGATCCTATTGTCCTCCAGGCA-3′	220
	rOMP90-3-R	5′-GTGAATTCAGCATAAATAGCCCCG-3′	

2.4. Positive and negative controls

To prepare the positive control (accession number ACD10929.1), 2 µL of *Chlamydia psittaci* DNA was added to the reaction mixture. For the negative control, 2 µL of sterile distilled water was used instead of template DNA. Inclusion of both positive and negative controls ensured the accuracy and reliability of the PCR assay [11].

2.5. PCR Product Analysis

PCR products were analyzed by electrophoresis on 1.5% agarose gel containing Green Viewer dye in TBE buffer. Samples and a 100 bp DNA ladder were loaded, and electrophoresis was carried out at 100 V for 50 min. The gels were subsequently visualized under UV illumination using a gel documentation system. The presence of a band at approximately 220 bp was considered a positive result.

3. Results

PCR targeting the ompA gene detected *Chlamydia psittaci* DNA in 12 of 100 sampled pigeons, corresponding to an overall prevalence of 12%. The amplified products appeared as distinct bands of approximately 220 bp, confirming the presence of the bacterium (Figure 1).

Figure1: Examination of PCR products from selected samples for the detection of *Chlamydia psittaci*. M: Marker; S+: Positive sample; C+: Positive control; C−: Negative control; S−: Negative sample.

All positive cases were identified in adult pigeons; no juveniles tested positive. Of the infected birds, seven were males and five were females. The majority showed a moderate body condition index of 3, with only two individuals differing slightly (2.5 and 3.5).

Clinical manifestations varied across infected birds. While several remained asymptomatic, others exhibited signs including lethargy, conjunctivitis with respiratory noise, and digestive disorders. Diarrhea was the most frequently observed clinical sign.

Management and environmental conditions appeared to influence infection status. Most positive pigeons were housed under crowded and unhygienic conditions, often with recent introduction of new birds and previous antibiotic use. In contrast, pigeons kept on rooftops with lower density and better hygiene tested positive less frequently and generally lacked overt clinical signs.

4. Discussion

Avian chlamydiosis, caused by *Chlamydia psittaci*, remains a zoonotic disease of global concern due to its impact on both animal and human health. In the present study, PCR targeting the ompA gene revealed an infection rate of 12% among domestic pigeons in Kerman city. This finding demonstrates the circulation of *C. psittaci* in urban pigeon populations and highlights their role as potential reservoirs for zoonotic transmission.

The prevalence observed in this study aligns with global reports. In Iran, infection rates among pigeons have varied: Mazounieh et al. reported a positivity rate of 52% in Yazd and Chaharmahal-Bakhtiari [12], while Golestani et al. found approximately 50% in Karaj [13]. In contrast, Ghorbanpour et al. detected only 0.7% positivity in Ahvaz [14]. On an international scale, Zschoeck et al. reported positivity rates between 10% and 25% in Germany, de Lima et al [15]. found 16.8% in Brazil [16], and Stenzel et al. documented a rate of 6.8% in Poland [17]. Additionally, Liu et al. recorded 34% positivity in ducks and 10% in pigeons in Taiwan [18], while Ling Hu et al. in China showed widespread infection among poultry and humans [19]. In Egypt, Abdo et al. detected infection rates exceeding 80% in ostriches and pigeons [20]. These variations can be attributed to differences in diagnostic methods, host species, management practices, and geographic conditions.

All positive cases in our study involved adult pigeons, with no juveniles testing positive. This observation may indicate cumulative exposure in older birds. The infected pigeons encompassed both sexes, with a slight predominance of males. Clinical outcomes varied widely, ranging from asymptomatic carriers to those exhibiting overt disease, which included symptoms such as diarrhea, lethargy, conjunctivitis, and respiratory issues consistent with the spectrum reported in previous studies. Environmental and management factors were clearly linked to infection. Most positive pigeons were housed in overcrowded and unsanitary conditions, often accompanied by recent introductions of new birds and prior use of antibiotics. In contrast, pigeons kept on rooftops in less crowded and cleaner environments were less frequently symptomatic. Similar correlations between husbandry stress and infection have been observed in Europe and Asia [21,22].

While this study primarily examined pigeons, it is important to note that *C. psittaci* is known to infect a diverse array of bird species, including ducks, turkeys, parrots, and various wild birds such as seagulls and storks [23]. Ducks frequently exhibit high morbidity and mortality rates, whereas chickens tend to show greater resistance [24]. This host diversity illustrates the pathogen's adaptability and its capacity to circulate within avian populations. The zoonotic implications of these findings are significant. Infected pigeons excrete the pathogen through feces and respiratory secretions, increasing the risk of human exposure, particularly in urban environments. Documented outbreaks of psittacosis linked to pigeons and other birds have occurred in Europe and Asia [25]. Therefore, the 12% detection observed in Kerman pigeons highlights both veterinary and public health concerns, especially for individuals who have close contact with birds.

PCR was employed in this study due to its superior sensitivity and specificity in comparison to culture or serology [9]. However, the use of a single conventional PCR assay that targets a single gene presents a limitation. Employing real-time PCR, sequencing, or multilocus genotyping could enhance accuracy and facilitate strain characterization. The limitations of this study include a relatively small sample size, focus on a single urban area, and a descriptive rather than statistical analysis of risk factors. Nonetheless, the findings provide valuable baseline data for understanding the epidemiology of *C. psittaci* in Iranian pigeons.

This study has certain limitations that should be acknowledged. The primary gaps include the lack of data on both genotypic and phenotypic antimicrobial resistance of the isolated strain. Furthermore, the genotypic virulence factors of the isolate were not characterized. Given the zoonotic nature of this disease and the critical importance of effective treatment in human cases, investigating these specific aspects is crucial. Therefore, it is recommended that future research focuses on elucidating the antimicrobial resistance profiles and key virulence determinants of this pathogen.

5. conclusion

In conclusion, the observed 12% detection in this study underscores the significant role domestic pigeons play in the epidemiology of *C. psittaci* in urban Iran. Infections were detected solely in adult birds, often arising under stressful husbandry conditions, and exhibited a range of clinical outcomes. These findings are consistent with both national and international studies and highlight the necessity for regular surveillance, public education, and enhanced management practices to reduce both veterinary and zoonotic risks. Future research should involve larger sample sizes, include multiple avian species, and utilize advanced molecular techniques to further explore the circulation and genetic diversity of *C. psittaci* in the region.

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Authors' Contribution

1- Study concept and design: Shafiei. H, Mohammadi. L

2- Acquisition of data: Mohammadi. L, Hoseinzadeh, F

3- Analysis and interpretation of data: Shafiei. H, Mohammadi. L, Golchin. M

4- Drafting of the manuscript: Jahedi.M, Hoseinzadeh, F

5- Critical revision of the manuscript for important intellectual content: Shafiei.H

6- Statistical analysis: Shafiei. H, Mohammadi. L, Mohammadi. L, Hoseinzadeh, F

7- Administrative, technical, and material support: Mohammadi. L, Golchin. M

8- Study supervision: Shafiei. H, Mohammadi. L

Conflict of Interest

The authors are responsible for the content of this article and declare that they have no competing interests.

Ethics

All sampling procedures from live birds were performed with priority for animal welfare, in compliance with an approved ethical protocol, using swabs from the choanal Slit.

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Data Availability

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

In this study, DeepSeek AI and Grammarly were used solely for improving the text's phrasing and enhancing its writing quality. No AI tools were employed for generating or shaping the content itself.

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