FERAL PIGEONS AS RESERVOIRS FOR HAZARDOUS
CHLAMYDOPHILA PSITTACI STRAINS WITH ZOONOTIC
POTENTIAL

S. ASGHARI1, H. STAJI2, H. R. MOHAMMADI3 & I. ASHRAFI TAMAI4

1Graduate of Veterinary Medicine, 2Department of Pathobiology, 3Department of Clinical Sciences; Faculty of Veterinary Medicine, Semnan University, Semnan, Iran; 4Department of Microbiology, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran

Summary

Chlamyphila psittaci is found in pigeons worldwide. The abundance of feral pigeons living in close contact with humans and livestock are considered a significant risk factor for human and farm animal infections. In Iran, little is known about the prevalence of C. psittaci and its genotypes in pigeons. The present cross-sectional study aimed to investigate the prevalence of C. psittaci in feral pigeons and to genotype the detected strains. In total, 384 fresh faecal samples were collected from different areas in Semnan (Iran). Out of all samples, 0.52% were positive for C. psittaci genome in Real Time-PCR. The partial ompA gene sequencing revealed that detected strains were identified as genotypes A and E. This is the first report of C. psittaci genotypes A and E in feral pigeons in Iran. The occurrence of C. psittaci genotypes A and E in the faeces of feral pigeons suggests potential environmental contamination with C. psittaci by pigeons and raise a public health concern.

Key words: Chlamyphila psittaci, feral pigeons, genotypes, Iran

Chlamydia psittaci (C. psittaci), a recognised zoonotic threat, is an obligate intracellular Gram-negative bacterium causing clinical problems in birds and mammals (Knittler & Sachse, 2015; Burnard & Polkinghorne, 2016). Feral pigeons are abundant birds and are considered major reservoirs of important pathogenic bacteria including C. psittaci. They can shed this infectious agent in the environment without any symptoms or when they are clinically diseased (Heddema et al., 2006; Burnard & Polkinghorne, 2016). Pigeons are circulating in urban and suburban regions worldwide and get in close contact with humans in public places and livestock in farms (Magnino et al., 2009; Dickx et al., 2010). Zoonotic transmission of C. psittaci from columbiformes was reported for the first time in 1941 (Meyer, 1941). Since then, many zoonotic cases
Feral pigeons as reservoirs for hazardous Chlamydia psittaci strains with zoonotic potential

Associated with pigeons have been described (Dickx et al., 2010). Based on the sequencing of major outer membrane protein (ompA) gene, C. psittaci strains fall into nine genotypes, namely A to F, E/B, M56, and WC. Genotypes A to F are associated with birds, whilst genotypes M56 and WC represent mammalian strains (Geens et al., 2005; Van Lent et al., 2012). The C. psittaci genotypes cluster with their related host species. Although genotypes A to E and E/B have been found in pigeons, these birds are considered as important reservoirs for genotypes A, B, and E (Dickx et al., 2010; Pannekoek et al., 2010; Van Lent et al., 2012). Genotype B is the most prevalent in pigeons, but other genotypes e.g. A are more virulent (Dickx et al., 2010). However, in Iran, only genotype B has been discovered in pigeons (Madani & Peighambari, 2013; Mina et al., 2019). To the best of our knowledge, there are very few published reports regarding the genotypes of C. psittaci from other avian and mammalian hosts.

In 2013, C. psittaci strains from companion and wild birds were genotyped for the first time in Iran and identified genotypes were as followed: genotype A from African grey parrot and lorikeet, genotype B from a rock dove and canary, a third new genotype named I from African grey parrots, and a fourth new genotype J from a ring-necked parakeet and Alexandrine parakeet (Madani & Peighambadi, 2013). Recently, in another survey, the prevalence of C. psittaci in feral pigeons circulating in silos, animal farms and urban area and genotyping of detected strains by analysis of the sequence of the partial ompA gene and to estimate the potential risk of such bacterium infection in humans and farm animals.

In this cross-sectional study, the sample size was calculated using the following equation:

\[ n = \frac{4 \times P \times Q}{L^2} \]

where \( n \) represents the minimum sample size needed for the prevalence estimation, \( P \): prevalence (assumed prevalence of C. psittaci in pigeons of the region was considered 50% because of unavailability of previous data regarding its prevalence), \( Q: \) 100–P, and \( L: \) allowable error or precision (considered 0.1 in the present study). Then, the minimum target sample size became 100 stool samples from birds.

Between April and July 2019, fifteen farms (livestock and broilers) located in four different districts of Semnan city, and one wheat silo located in the southwest district of the city, were investigated. A total of 384 stool samples from feral pigeons (Columba livia) living in the mentioned areas were collected. About 1 g of stool per sample was collected from the ground using a sterile swab and transferred into sterile 1.5 mL microtubes. The samples were then immediately placed on ice during transport. Then, molecular analyses were started the day after sample collection.

Genomic DNA extraction from 384 faecal samples was performed using the
conventional phenol/chloroform method (Abedi et al., 2018) and extracted genomes were preserved in –20 °C freezer until molecular evaluations. Identification of C. psittaci by real-time polymerase chain reaction (RT-PCR) was carried out using a primer pair VD [forward VD1-f: 5’ ACTACGGAGATTTATGTTTTCGAT CGTGT-3’ and reverse VD2-r: 5’ CGTGC ACCYACGCTCCAAGA- 3’] which amplify a 418 bp fragment existing in variable region of the sequence encoding ompA (outer membrane protein A) in the bacterium (Sachse et al., 2009).

The nucleotide sequence of the amplified fragment was analysed with an ABI 3730XL DNA Analyzer according to an automated Sanger dideoxy fluorescent nucleotide method. Then, the BLAST software was applied to determine the homology of the amplified fragment to DNA sequences existing in GenBank and the phylogenetic tree was constructed by a neighbour-joining model in MEGA-6.

To determine the genotype from ompA sequencing data, the sequence of a sample was added to a multiple ompA sequence alignment of C. psittaci strains previously identified in Iran and some other isolates existing in PubMed-NCBI database from other countries.

During the present investigation, 0.52% (99% CI 0.46–0.58) of faecal swabs from feral pigeons (n = 2/384) were positive by the RT-PCR method targeting the ompA encoding gene. The origin of two detected strains was different: one was detected from pigeons from siloes and the other was detected from pigeons circulating in broiler farms.

Fig. 1. Neighbour-joining phylogenetic tree based on partial ompA gene sequence of C. psittaci. Isolate 1 and isolate 2 are the strains detected in this study.
Partial ompA coding regions were amplified from both positive stool samples of birds and phylogenetic analysis was performed based on an alignment of 418 bp. Both amplified sequences from the present investigation belonged to the same phylogenetic lineage A (Fig. 1) encompassing six C. psittaci strains, obtained from pigeons, Polish wildfowl, a parrot-human outbreak, and an aborted foal (Table 1). The strong genotypic linkage between two of these strains (89_13210 and CLV) was supported by sequence analysis since ompA sequences obtained from our investigation and these hosts were identical (Fig. 1). There was however a lower identity between Iranian strains from pigeons existing in the PubMed database reported before and two detected strains of C. psittaci from the present study.

In the present study, Real-Time PCR detected C. psittaci in 0.52% of the feral pigeon’s faeces which is consistent with previous investigations suggesting that this organism was an existing chlamydial species in pigeons (Magnino et al., 2009). The low Chlamydia prevalence observed in tested birds, compared with studies carried out previously in Iran (Madani et al., 2011; Madani & Peighambari, 2013; Ghorbanpoor et al., 2015) regarding the prevalence of C. psittaci in pigeons (0–18%), could be due to various factors, for instance, the specificity and sensitivity of the detection methods, different geographical and ecological conditions, the number of the monitored birds and bird’s welfare and sanitation.

The primer pairs of the RT-PCR used in this investigation and ompA sequencing have been applied effectively for genotyping of C. psittaci in previous studies and it was stated that these techniques were highly sensitive and specific for phylogenetic purposes (Sachse et al., 2009; Orme-las-Eusebio et al., 2016). Characterisation of detected strains by RT-PCR and subsequent partial ompA sequencing of strains determined them as phylogenetically very close to genotypes E and A (Fig. 1). Historically, it was stated that genotype A was associated with psittacine birds and caused zoonotic disease in humans, while strains related to genotype E have been identified during an outbreak of human pneumonitis that occurred in 1920–1930s.

### Table 1. Reference strains of C. psittaci located in lineage A (Fig. 1) with the strains detected in the present study

<table>
<thead>
<tr>
<th>Reference strain ID</th>
<th>Accession No.</th>
<th>Genotype</th>
<th>Host</th>
<th>Clinical signs in host</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>89_13210</td>
<td>EU682085.1</td>
<td>E</td>
<td>pigeon</td>
<td>healthy</td>
<td>Sachse et al., 2017</td>
</tr>
<tr>
<td>CLV</td>
<td>DQ230096.1</td>
<td>A</td>
<td>parrot and human</td>
<td>outbreak of psittacosis</td>
<td>Heddema et al., 2006</td>
</tr>
<tr>
<td>CR</td>
<td>KF770962.1</td>
<td>B</td>
<td>pigeon</td>
<td>healthy</td>
<td>Dolz et al., 2013</td>
</tr>
<tr>
<td>Qld/H/PI</td>
<td>MG587894.1</td>
<td>not identified</td>
<td>foal</td>
<td>aborted foal</td>
<td>Jelocnik et al., 2018</td>
</tr>
<tr>
<td>nier_A124</td>
<td>KX603697.1</td>
<td>E</td>
<td>domestic pigeon</td>
<td>healthy</td>
<td>Jeong et al., 2017</td>
</tr>
<tr>
<td>15_42d/7</td>
<td>KX424651.1</td>
<td>not identified</td>
<td>Polish wildfowl</td>
<td>healthy</td>
<td>Szymanska-Czerwinska et al., 2016</td>
</tr>
</tbody>
</table>
Subsequently, E strains have been identified from a variety of birds worldwide as well as pigeons (Geens et al., 2005; Van Lent et al., 2012). Phylogenetic analysis of our strains demonstrated that both strains were very close to 89_13210 and CLV type strains of C. psittaci (Fig. 1). The 89_13210 type strain was isolated from a pigeon and its genotyping based on ompA sequencing demonstrated its affiliation to genotype E (Sachse et al., 2009), while the CLV type strain was isolated from an outbreak of psittacosis due to C. psittaci genotype A in a veterinary teaching hospital (Amsterdam, Netherland) infecting parrots, staffs, and students (Heddema et al., 2006). As our knowledge about the genetic diversity of C. psittaci strains increased, evidence is developing that these microorganisms infect a broader range of vertebrate hosts than previously thought (Burnard & Polkinghorne, 2016). In another study conducted by Jelocnik et al. (2018) molecular typing and ompA genotyping of C. psittaci isolate from a case of equine abortion revealed that the infecting equine strains were genetically very close to the C. psittaci detected in pigeons. All found strains belonged to an evolutionary lineage of C. psittaci strains mostly associated with infections of pigeons.

C. psittaci genotype B is shown to be the major genotype existing in pigeons (Wang et al., 2018). There are very few reports demonstrating the genotypes of this bacterium associated with pigeons in Iran. All genotyped strains detected from pigeons in Iranian publications have been identified as genotype B (Madani & Peighambhari, 2013; Mina et al., 2019) but our findings present the first detection of C. psittaci genotypes A and E from this bird species in Iran. Although investigations conducted in different parts of the world have introduced genotype B as the dominant genotype in columbiforms, other genotypes including A, D, E and a new genotype E/B have been identified within C. psittaci strains circulating in these birds (Geens et al., 2005; Heddema et al., 2006; Van Lent et al., 2012; Jeong et al., 2017; Wang et al., 2018). These findings confirm the fact that feral pigeons can serve as an important reservoir for different genotypes of this bacterium and shed the infectious agent to human and animal hosts. Whether the distribution of C. psittaci by feral pigeons in Semnan (Iran) poses a considerable zoonotic hazard for humans remains undetermined. Also, there is the risk of infecting livestock and poultry which are in closer contact with human beings.

In conclusion, our findings highlight the significance of feral pigeons as a potential hazard for humans and farm animals. Besides, two genotypes of C. psittaci (A and E) were identified for the first time in this study in Iranian pigeons using partial ompA sequencing. Thus, it can be concluded that more C. psittaci genotypes should be expected to exist naturally in pigeons. More investigations and monitoring seem to be required to determine the epidemiologic distribution of C. psittaci genotypes in feral pigeons associated with different geographic regions.

ACKNOWLEDGEMENTS

Authors express their thanks to the Head of Faculty of Veterinary Medicine (Semnan University) for providing facilities for the present study and Mrs. Behnaz Raeisian for her cooperation in laboratory experiments.
Feral pigeons as reservoirs for hazardous Chlamydia psittaci strains with zoonotic potential

REFERENCES


Correspondence:

Hamid Staji  
Department of Pathobiology,  
Faculty of Veterinary Medicine,  
Semnan University, Semnan, Iran,  
phone: 0098 (0912) 2164775,  
e-mail: hstaji@semnan.ac.ir