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Short communication

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

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Summary

Asghari, S., H. Staji, H. R. Mohammadi & I. Ashrafi Tamai, 2023. Feral pigeons as reservoirs for hazardous *Chlamydophila psittaci* strains with zoonotic potential. *Bulg. J. Vet. Med.,* **26**, No 1, 108–114.

Chlamydophila 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: *Chlamydophila 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 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 & Peighambari, 2013). Recently, in another survey, the prevalence of *C. psittaci* genotypes in asymptomatic and symptomatic birds in northeast Iran has been determined by nested PCR assay and the bacterium was detected in 37 (18.5%) of 200 birds (18 symptomatic and 19 asymptomatic). Also, genotyping of detected *C. psittaci* strains demonstrated genotype A in cockatiels, ring-necked parakeet and African gray parrot, and genotype B was observed in pigeons and a provisional genotype named I was detected in one symptomatic cockatiel (Mina *et al.*, 2019).

Therefore, the present study aimed to describe 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:

$$n = 4\ PQ/L^2,$$

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′ ACTACGGAGATTATGTTTTCGAT 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 circu-

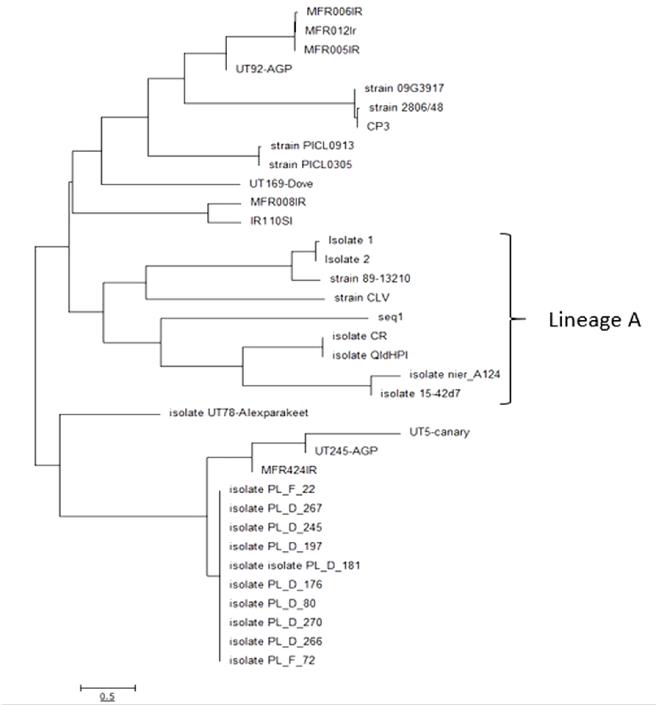

Fig. 1. Neighbour-joining phylogenetic tree based on partial *ompA* gene sequence of *C. psittaci.* Isolate 1 and isolate 2 are the strains detected in this study.

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

Reference strain ID	Accession No.	Genotype	Host	Clinical signs in host	Reference
89_13210	EU682085.1	E	pigeon	healthy	Sachse *et al.*, 2017
CLV	DQ230096.1	A	parrot and human	outbreak of psittacosis	Heddema *et al.*, 2006
CR	KF770962.1	B	pigeon	healthy	Dolz *et al.*, 2013
Qld/H/PI	MG587894.1	not identified	foal	aborted foal	Jelocnik *et al.*, 2018
nier_A124	KX603697.1	E	domestic pigeon	healthy	Jeong *et al.*, 2017
15_42d/7	KX424651.1	not identified	Polish wildfowl	healthy	Szymanska-Czerwinska *et al.*, 2016

lating in broiler farms.

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 parrots-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; Ornelas-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. 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 affilitation 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 & Peighambari, 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.

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