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Detection of *CHLAMYDIA PSITTACI* in feral pigeons (*COLUMBA LIVIA DOMESTICA*) in Slovakia and their characterisation

Lenka Čechová[1], Monika Halánová[1], Zuzana Kalinová[1], Lýdia Čisláková[1], Miloš Halán[2], Alexandra Valenčáková[3]

[1] *P. J. Šafárik University, Faculty of Medicine, Department of Epidemiology, Košice, Slovak Republic*
[2] *University of Veterinary Medicine and Pharmacy, Department of Epizootology and Parasitology, Košice, Slovak Republic*
[3] *University of Veterinary Medicine and Pharmacy, Department of Biology and Genetics, Košice, Slovak Republic*

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Abstract

Introduction and objectives. *Chlamydia psittaci*, an obligate intracellular bacterium, which is the etiologic agent of avian chlamydiosis in birds and ornithosis/psittacosis in humans, has been reported to be one of the most common pathogens found in feral pigeons worldwide, and thus constitutes a zoonotic risk. The aim of the study was to investigate pigeons in Slovakia living in areas in close proximity to humans for the presence of *C. psittaci*, using pharyngeal and cloacal swabs.

Material and methods. 122 clinically healthy pigeons from different geographical regions of Slovakia were examined for the presence of *C. psittaci*. The adult pigeons of both genders were captured during the summer period in the urban centres of Slovakian towns. Each sample was examined by molecular method PCR, and in the case of positive result the identity of the obtained sequence was examined by a BLAST search.

Results. Of the total number of 244 examined samples, 14 (5.7%) showed positivity for *C. psittaci* infection, 5 of which were from pharyngeal swabs (4.1%) and 9 from cloacal swabs (7.4%). A positive result was detected in 13 pigeons (10.7%). Phylogenetic analysis showed that all the positive samples are genetically very close to genotypes B and genotype E.

Conclusion. Phylogenetic examination of the 14 isolates of *C. psittaci* identified in the presented study, based on 23S rRNA gene sequence, revealed their close relationship with *C. psittaci* genotypes B and E. Both genotypes are predominantly prevalent in pigeons and both can be transmitted to humans. Therefore, it is necessary to perform screening examinations of animals and analyse the epidemiological factors affecting the way of transmission and circulation of pathogen.

Key words

Chlamydia psittaci, pigeons, phylogenetic examination, Slovakia

INTRODUCTION

Chlamydiae are obligate intracellular gram-negative bacteria distributed worldwide, known to cause various forms of disease in animals and humans. According to the most recent taxonomy, the family of *Chlamydiaceae* with the single genus *Chlamydia (C.)* currently contains 12 species [1, 2]. Among them, *Chlamydia psittaci* is one of the most important zoonotic species from the epidemiological point of view, and is the causative agent of avian chlamydiosis in birds and ornithosis/psittacosis in humans.

Based on sequencing and analysis of the ompA gene, *C. psittaci* has 9 known genotypes, 7 avian (A – F, E/B) and 2 mammalian (M56 and WC) all of which can be transmitted to humans [3]. This transmission can occur either through inhalation, ingestion or via direct contact with the infected birds [4]. Some genotypes have the capability to infect more than one type of host [5].

Genotype A is endemic among psittacine birds and causes sporadic zoonotic disease in humans. Genotype B is usually associated with pigeons, but has been isolated also from turkeys and dairy herds. Genotype C isolates have been obtained primarily from duck and geese, whereas genotype D has been isolated mainly from turkeys, seagulls, and budgerigars; genotype E strains isolated from pigeons, genotype F from psittacine birds and turkeys; and genotypes E/B isolated mainly from ducks. The mammalian M56 was isolated during an outbreak in muskrats and hares, and genotype WC isolated in an outbreak of enteritis in cattle [6].

The important source of ornithosis/psittacosis for humans are feral pigeons, which have been ranked as the second major reservoir of *C. psittaci* [7]. Most infected pigeons are asymptomatic and latent carriers. Shedding of the pathogens occurs in faeces as well as in respiratory and conjunctival secretions, often intermittently and without clinical signs [8].

In Europe, the prevalence of chlamydial infections in feral pigeons is consistently high, ranging from 1.6% – 95.6%, depending on the diagnostic method used and sample selection techniques applied [9, 10, 11, 12].

OBJECTIVE

Because pigeons are present in many urban and rural areas worldwide and come into close contact with humans, the aim of this study was to investigate the pigeons in Slovakia living in areas close to humans for the presence of *C. psittaci* from pharyngeal and cloacal swabs, and in the case of positive result to examine the identity of the obtained sequences.

Address for correspondence: Monika Halánová, P. J. Šafárik University, Faculty of Medicine, Department of Epidemiology, Šrobárova 2, 041 80 Košice, Slovak Republic e-mail: monika.halanova@upjs.sk

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MATERIALS AND METHODS

A total of 122 clinically healthy pigeons from different geographical regions of Slovakia were examined for the presence of *C. psittaci.* Adult pigeons of both genders were captured during the summer period in the urban centres of Slovakian towns. Cloacal and pharyngeal swabs, 2 from each pigeon, were collected using sterile cotton swabs. After the collection of samples, each swab was placed in a 1.5 ml sterile microtube filled with 0.3 ml sterile saline solution, turned several times and the swab removed from the microtube. The samples were stored at -80 °C until processed.

Molecular and phylogeny analysis. To identify the *Chlamydia psittaci* or genotypes present, genomic DNA was extracted using the DNA-sorb-AM nucleic acid extraction kit (AmpliSens, Federal State Institution of Science, Moscow, Russia) according to the manufacturer's instructions. Purified DNA was stored at -20 °C prior to being used for PCR.

PCR amplification was performed using the primers U23Fa (5'-GAT GCC TTG GCA TTG ATA GGC GAT GAA GGA-3') and 23SIGR (5'-TGG CTC ATC ATG CAA AAG GCA-3'), [6]. These primers amplified domain I of the 23S rRNA gene. This segment is a signature sequence for chlamydial species, genera and families [6]. U23Fa matches the sequence just after the start of the 23S rRNA gene, and 23SIGR complements the sequence approximately 600 bases downstream.

Reaction mixtures were prepared by mixing 8 µl of Taq polymerase HOT FIREPol Blend Master Mix (Solis BioDyne, Estonia), 10µl of template, 0.4µl of each primer and 1.2µl of purified water.

As a positive control a sample was used in which the presence of *Chlamydia psittaci* DNA was proven in previous examinations and confirmed by sequencing.

All reactions were carried out in BIOMETRA T-Personal Thermocycler. Cycling conditions for PCR were 95 °C for 13 min for one cycle followed by 40 cycles at 95 °C for 20 sec, 65.5 °C for 60 sec, and 72 °C for 60 sec, and a final extension step at 72 °C for 10 min for one cycle. PCR products were analysed by electrophoresis of 10 µl of each 20 µl reaction mixture on a 1.5% agarose gel stained by using GoldView stain (SBS Genetech, China) and visualized fragments were compared with the positive control and the 100 bp DNA ladder.

The identity of the obtained sequences was examined by a BLAST search [13]. DNA sequence alignments and phylogenetic analysis were conducted using the software MEGA5. Phylogenetic trees were created using alignments performed with the BioEditSequence Alignment Editor as a distance method and NJ (neighbor joining) as the tree construction method. All ambiguous positions were removed from each sequence pair. The reliability of branches in trees was assessed using bootstrap analysis with 1,000 pseudoreplicates, with values above 50% being reported. Analysis of sequences for constructing phylogenetic tree also included 8 reference strains, the partial sequence of the 23S rRNA gene of different genotypes of *C. psittaci* from the Gene Bank database, genotype A (AF481052.1), genotype B (U68448.1), genotype C (U68450.1), genotype D (U68419.2), genotype E (U68454.1), genotype F (AF481049.1), genotype WC (U68456.1), genotype M56 (U68452.1) and *Pirellula marina* (AF245367.1) used as an outgroup.

RESULTS

A total of 244 cloacal and pharyngeal swabs from 122 clinically healthy pigeons were examined for the presence of chlamydial infection. Of the total number of 244 examined samples, 14 (5.7%) showed positivity for *C. psittaci* infection, 5 of which were from pharyngeal swabs (4.1%) and 9 from cloacal swabs (7.4%). A positive result was detected in 13 pigeons (10.7%), and in only one pigeon was positive in both samples – cloacal and pharyngeal swabs.

Phylogenetic analysis showed that all positive samples were genetically very close (94–100% identity) to genotypes B (CP003797.1) originally isolated from urban pigeons, and genotype E (CP003792.1), originally isolated from human with low genetic variability (Fig. 1).

DISCUSSION

Natural infections by *C. psittaci* widely occur in many wild and domestic avian species; at present, this number includes approximately 500 avian species from 30 different orders [14]. The disease has zoonotic character, and can be transmit to humans through direct contact or inhalation of an infectious aerosol. Pigeons, especially feral pigeons, are also one of the important reservoirs of *C. psittaci.*

The pathogen was isolated from homing pigeons for the first time in 1940 [15], and in 1941, the first case of transmission of *C. psittaci* from feral pigeons to humans was described [16].

Most infected feral pigeons are asymptomatic and latent carriers of *C. psittaci.* Shedding of the pathogen occurs in faeces, as well as in respiratory and conjunctival secretions, often intermittently [8].

Avian chlamydiosis is widespread in the feral pigeon populations of several European towns and cities. High seropositivity to *C. psittaci* has been detected for several years in most of them. In 51 investigations of feral pigeon populations carried out from 1966 – 2006, a mean seroprevalence rate of 42.3% was found, with a minimum detection rate of 10% and a maximum of 95.6% [17]. More recent data from Europe indicate highly variable PCR positivity rates, for example, 5 – 10 % in Amsterdam, the Netherlands, respectively, during the low-breeding and breeding seasons [18], 0 – 6.3 % in young and breeder pigeons from Belgium [19], 0% in city faecal droppings to 3.2% in cloacal swabs in Basel, Switzerland [20], 52.6% in resuspended cloacal contents in Madrid, Spain [21] and 3.3% in cloacal swabs from urban pigeons in Switzerland [22].

In Slovakia, chlamydiosis in pigeons was serologically confirmed by Řeháček and Brezina (1976), [23], Řeháček et al. (1984), [24], Kocianová et al. (1993), [25], Čisláková et al. (1998), [26], Trávniček et al. (2002), [27], and Sulinová et al. (2011), [28]. The positivity in these studies ranged from 3.92% – 85.1%.

CONCLUSIONS

In the presented study, a total of 244 samples from 122 feral asymptomatic pigeons, as potential source of infection for humans, were examined for the presence of *C. psittaci* by PCR method with 10.7% prevalence. A higher positivity was observed in samples from the cloaca compared to

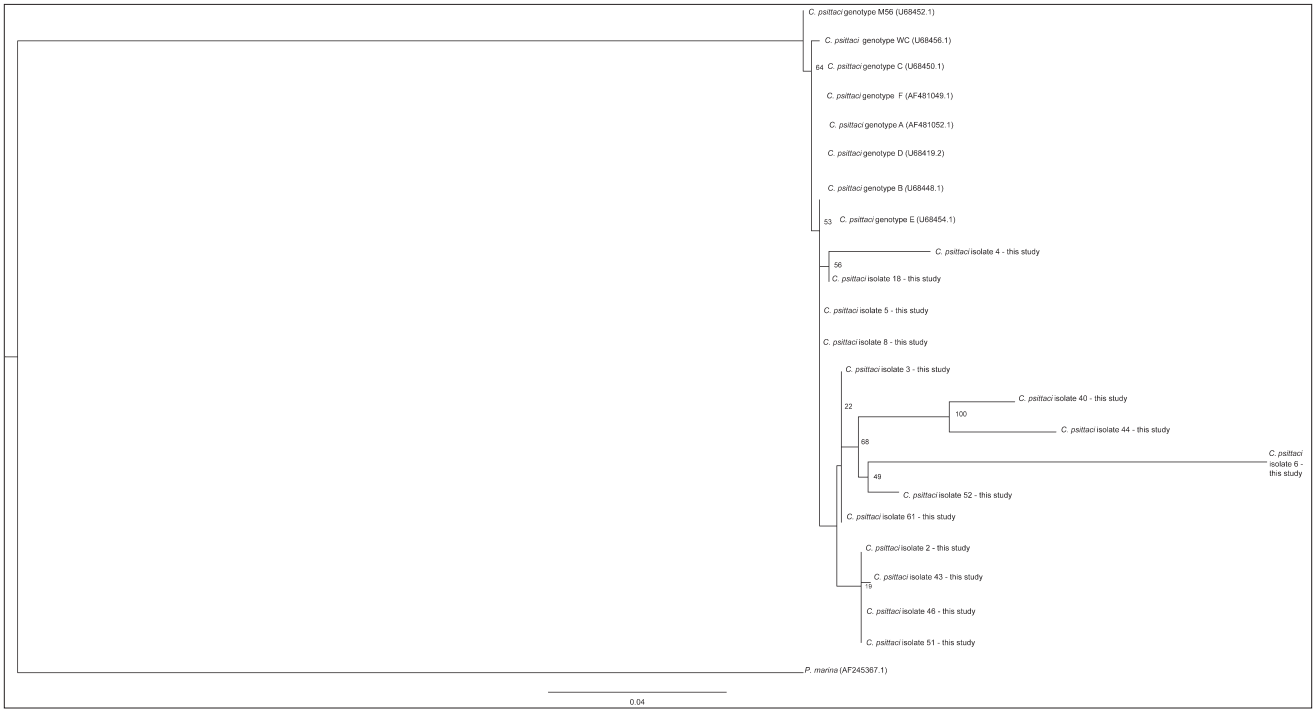

Figure 1. Phylogenetic tree of *C. psittaci* isolates from the presented study and selected accessions from GenBank, based on 23S rRNA gene fragment sequences

samples from pharynx (7.4% vs. 4.1%). After sequencing, all positive isolates were identified as *C. psittaci* (94 – 100% homology).

Phylogenetic examination of the 14 isolates of *C. psittaci* identified in the present study, based on 23S rRNA gene sequence, revealed their close relationship with *C. psittaci* genotypes B and E. Both genotypes are predominantly prevalent in pigeons and both can be transmitted to the humans. The disease in humans may vary from a mild flu-like symptoms to severe atypical pneumonia, and systemic disease with extra pulmonary manifestation. The epidemiological situation in the occurrence of ornithosis/psittacosis in Slovakia is relatively favourable, and during the last 10 years only 22 cases in humans were officially reported. In 10 of these cases, there was in epidemiological anamnesis indicated contact with pigeons or their excreta [29]. Because urban pigeon populations still represent risk to public health, it is necessary to perform screening examinations of animals and analyse the epidemiological factors affecting the way of transmission and circulation of the pathogen with the aim of reducing or halting the spread of this infection, not only between animals but also in pigeon-sensitive persons.

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