Kafkas Univ Vet Fak Derg
21 (2): 265-269, 2015
DOI: 10.9775/kvfd.2014.12239

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

# Molecular Detection and Prevalence of *Chlamydophila psittaci* in the Blood, Liver and Muscle Tissue of Urban Pigeons *(Columba livia domestica)* in Iran

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**KVFD-2014-12239 Received: 03.09.2014 Accepted: 09.01.2015 Published Online: 11.01.2015**

## Abstract

*Chlamydophila psittaci (C. psittaci)* is a widespread pathogenic bacterium in pigeons. These animals are mostly infected without any clinical signs. Pigeons are probably the most commonly reported chlamydia-infected avian species. Shedding of *Chlamydia* from infected birds has been widely reported. This study was conducted to detect and to determine the prevalence of *C. psittaci* in the blood, liver and muscle tissue of urban pigeons in Iran using conventional polymerase chain reaction. In this study, authors used 90 pigeons from different retail shops across Iran. The study was including 26 female and 64 male pigeons with suspected Chlamydiosis based on clinical signs. During examination of the corpses we took 270 samples in total, including blood, liver and muscle tissue from each animal. *C. psittaci* was detected in 16 (17.78%) blood samples, 14 (15.56%) liver samples and 5 (5.56%) samples of muscle tissue. This study supports the fact that pigeons serve as carriers of *C. psittaci*. Therefore, continuous surveillance of this bacterium will go along way in understanding the distribution and risks associated with *Chlamydia* infected pigeons. This will be beneficial in prevention and control risks of infection in humans.

***Keywords:*** *Chlamydophila psittaci, Molecular detection, Prevalence, Urban pigeons, Iran*

# İran'da Şehir Güvercinlerinde *(Columba livia domestica)* Kan, Karaciğer ve Kas Dokularında *Chlamydophila psittaci*'nin Moleküler Metotlarla Belirlenmesi ve Prevalansı

## Özet

*Chlamydophila psittaci (C. psittaci)* güvercinlerde yaygın olarak bulunan patojen bir bakteridir. Bu hayvanlar çoğunlukla klinik belirti göstermeksizin enfektedirler. Güvercinler muhtemelen en çok Klamidya ile enfekte olan kanatlı türleridir. Enfekte kuşlardan Klamidyaların yayılımı sıklıkla rapor edilmiştir. Bu çalışma ile konvensiyonel polimeraz zincir reaksiyonu kullanılarak İran'da şehir güvercinlerinin kan, karaciğer ve kas dokularında *C. psittaci*'nin belirlenerek prevalansının ortaya konulması amaçlanmaktadır. Çalışmada İran'da değişik satış yerlerinden elde edilen 90 güvercin kullanıldı. Klinik belirtilere dayanarak klamidiyozis şüpheli olan bu güvercinlerin 26'sı dişi ve 64'ü erkekti. Her hayvanın kan, karaciğer ve kas dokularını içeren toplam 270 örnek hayvanlardan elde edildi. *C. psittaci* 16 (%17.78) kan örneğinde, 14 (%15.56) karaciğer örneğinde ve 5 (%5.56) kas dokusunda tespit edildi. Bu çalışma güvercinlerin *C. psittaci* için taşıyıcı olarak görev yaptığı bilgisini desteklemektedir. Bu nedenle sürekli takip ve kontrolün yapılması Klamidya ile enfekte güvercinlerin yaygınlığı ve buna ilişkin risklerin anlaşılması için uzun süreli devam ettirilmelidir. Böyle bir uygulama aynı zamanda insanlara enfeksiyonun yayılmasını kontrol altına almada da yararlı olacaktır.

***Anahtar sözcükler:*** *Chlamydophila psittaci, Moleküler tespit, Prevalans, Şehir güvercini, İran*

## INTRODUCTION

*Chlamydophila psittaci* (*C. psittaci*) is a Gram-negative and obligate intracellular bacterium, with nine (A to F, E/B, M56, and WC) known genotypes [1,2]. *C. psittaci* has been identified in 465 different bird species [3], but the highest rate of infection was found in parrots *(Psittacidae)* and pigeons *(Columbiformes)* [4,5]. The family *Chlamydiaceae*

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is divided into two genera: Genus *Chlamydia* with the species *C. muridarum*, *C. suis* and *C. trachomatis*, and genus *Chlamydophila* with the species *C. abortus*, *C. caviae*, *C. felis*, *C. pecorum*, *C. pneumoniae* and *C. psittaci* [6]. Genotypes are distinguished by sequencing of the outer membrane protein A (*ompA*) gene [7]. These bacteria are obligate intracellular organisms that are transmitted by biologically inactive particles called elementary bodies (EBs) [8]. *C. psittaci* is a bacterium that can be transmitted from pet birds to humans [9] and pigeons, but other bird species can be infected by same bacteria as well [10].

Urban or street pigeons are known to be reservoirs of *Chlamydia* and their zoonotic potentials have already been reported for decades [11]. Pigeons *(Columba livia domestica)* which are mostly located in towns and cities, especially of tourist attractions, are commonly infected with this bacterium. In human medicine this is the causative agent of psittacosis (also known as ornithosis) [12].

Many people especially children derived much pleasure in feeding pigeons during their leisure in city parks. Sometimes, these birds are kept as pets and are also housed within the living rooms, childcare facilities, garden centers and rest homes, which brings about a close interaction with humans [12,13].

Today, the increased pigeon population in major cities of the world is not only a major concern on environmental hygiene due to fecal droppings and fouling odor of buildings and monuments, but also associated risk of transmission infection from animals to humans. The most important pathogenic organism transmissible from feral pigeons to humans is *C. psittaci*, with 101 cases of disease reported in the literatures [10,14].

Exposure to *C. psittaci*-contaminated dust, pigeon feeding, and direct contact with pigeons to a lesser extent have been identified as risk of exposures in many of the human cases [15]. The principal route of human infection with *C. psittaci* is via the respiratory system, by inhaling infected aerosols of dried feces or respiratory secretions from infected birds. Other possible route of infection have been identified including direct contact with the feathers, tissue or secretions of infected birds, mouth-to-beak contact, or by bite wounds and the other open skin wounds, as well [16,17]. Person-to-person transmission is also possible [18] but it is thought to be rare.

Most infected pigeons are asymptomatic and they shed the organism occurs in feces as well as in respiratoric and conjunctival secretions. The clinical signs are often viewed after triggers like a stress, so this asymptomatic flow makes it difficult to assess the risk of bacteria transmission to other animals and humans [19,20].

Up until the 1990s, most epidemiological *C. psittaci* studies were based on serology. However, the significance in terms of worldwide dissemination of the agent is unclear [14]. The use of molecular techniques has enabled researchers in understanding the epidemiology of this pathogen in the past years [14]. There are several studies describing the *C. psittaci* carrier status of urban pigeon populations, especially from fecal droppings have been reported recently [10,21,22]. In Iran, there are available reports on molecular detection of *C. psittaci* in feces of pigeons [23-25]. However, in this study, we examined urban pigeons for detection of *C. psittaci* from the blood, liver and muscle tissue using molecular techniques in Iran.

## MATERIAL and METHODS

All experiments were carried out under the ethical guidelines of the Islamic Azad University of Shahrekord Branch (92/910, in 2013).

### *Sample Collection*

The pigeons were bought from different pigeon retail shops across Iran, where they were sold for food. Experiment criteria include pigeons those show clinical signs such as lethargy, anorexia, ruffled feathers, nasal discharge, diarrhea, and excretion of green to yellow-green feces. A total of 90 birds comprising of 26 female and 64 male pigeons were sampled between December 2013 and February 2014. The total number of samples were 270, including 90 blood, 90 muscle tissue and 90 liver samples and these were aseptically collected into well labeled sample bottles for detection of *ompA* gene of *C. psittaci* using PCR.

### *DNA Extraction*

Genomic DNA was extracted from each sample with DNA extraction kit (CinnaGen, Iran), according to the manufacturer's instructions. The quality and quantity of extracted DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell [26]. The extracted DNA of each sample was kept frozen at -20°C until used. *C. psittaci* strain ATCC VR-125 (Genekam Biotechnology AG, Germany) was used as positive control and a negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water.

### *Gene Amplification*

The *ompA* region was amplified by PCR using primers CPsitt-F (5′-GCTACGGGTTCCGCTCT-3′) and CPsitt-R (5′-TTTG TTGATYTGAATCGAAGC-3′) as described by Heddema for *ompA* region (accession number AB512087.1) [10]. Primers were analyzed at the NCBI using the experimental GENINFO BLAST Network Service to assess degree of homology between these primers and other reported sequences. The samples were placed in a thermal cycler (Mastercycler gradient, Eppendrof, Germany) with an initial denaturation step for 5 min at 95°C, then amplified for 30 cycles of denaturation for 1 min at 94°C, alignment for 1 min at 57°C,

extension for 1 min at 72°C and, final extension step for 7 min at 72°C. PCR products were separated by 2% agarose gel electrophoresis stained with solution of Ethidium Bromide and examined under Ultra Violet illumination (Uvitec, UK). The DNA molecular weight marker was used as a size marker.

***Analysis***

The prevalence analysis was computed in percentage and presented using simple frequency.

# RESULTS

From a total of 90 urban pigeons *C. psittaci* was detected in 16 (17.78%) blood samples, 14 (15.56%) liver samples and 5 (5.56%) samples of muscle tissue *(Table 1)*. Higher prevalence was observed in the blood while lowest detection was recorded in the muscle tissue. The rate of detection was higher in the male compared to the female pigeons for all samples *(Fig. 1)*. The expected size of amplicons for *C. psittaci* is 1041 bp *(Fig. 2)*.

# DISCUSSION

*C. psittaci* is a lethal intracellular bacterial species that causes avian Chlamydiosis, epizootic outbreaks in mammals and respiratory psittacosis in humans. The surveillance and its detection is essential in understanding the epidemiology of this bacteria and associated risks to humans. The detection of *C. psittaci* from pigeons in Iran as observed in this study further reinforce the fact that pigeon served as reservoir of infection and sometimes without clinical signs. To the best of our knowledge, this is the first study in Iran that detected *C. psittaci* from sample sources other than fecal droppings in pigeons. Available reports such as Doosti et al.[27], Doosti and Arshi [23] and Madani et al.[24], have all worked on the detection from cloacal swabs and fecal droppings. The prevalence of *C. psittaci* observed in this study (5.56-17.78%) were closely similar to that reported by Hedemma et al.[14], Doosti et al.[27] and Doosti and Arshi [23] but, lower than 23.5% reported by Madani et al.[24] in Iran. The reason for higher prevalence of *C. psittaci* in male pigeons from all the samples more than female pigeons is not clear. However, this may suggest that infection with *C. psittaci* in pigeons is sex dependent and this may incriminates sex as a risk factor of infection among pigeons. This may also suggest the increase risk of exposure to *C. psittaci* in humans who keep male pigeons as pet or come in contact with male pigeons frequently.

Aerosol transmission has been considered as the primary way of bacteria entry [28] causing respiratory disease in both mammals and birds [29]. Exposure to infected birds' feces, nasal discharges, and aerosol droplets are important transmission way as well. The detection of *C. psittaci* from muscle tissue and liver, as observed in this study, may suggest ingestion or food borne route as another means of exposure especially among animals who preyed on pigeons or human who eat pigeon meat (squab) as delicacies. The possibilities of occupational

***Table 1.*** *Prevalence of C.psittaci in samples determined by PCR*
***Tablo 1.*** *PCR ile doku örneklerinde belirlenen C. psittaci prevalansı*

| Sex | Number of Samples | Prevalence N (%) | | |
|---|---|---|---|---|
| | | Muscle Tissue | Liver | Blood |
| Female | 26 | 1 (3.85) | 4 (15.39) | 3 (11.54) |
| Male | 64 | 4 (6.25) | 10 (15.63) | 13 (20.31) |
| Total | 90 | 5 (5.56) | 14(15.56) | 16 (17.78) |

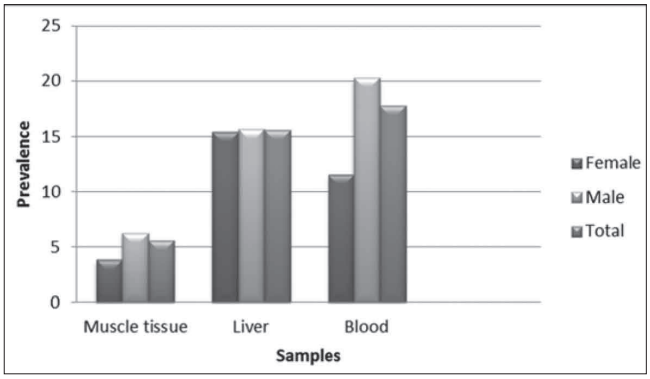


**Fig 1.** Distribution of *C. psittaci* in pigeons in the different samples and different sexes

**Şekil 1.** Güvercinlerin değişik örneklerinde ve cinsiyete göre *C. psittaci*'nin yaygınlığı

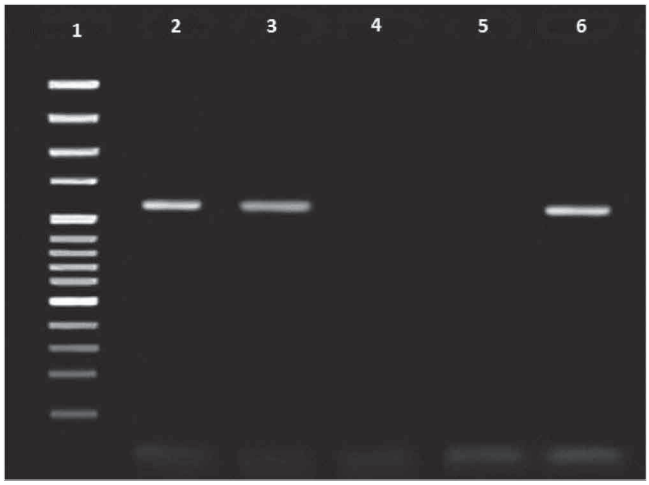


**Fig 2.** Ethidium bromide-stained agarose gel electrophoresis of PCR products (1041 bp) for detection of *ompA* gene in *Chlamydophila psittaci* in pigeon samples. Lane 1: DNA ladder (100 bp Ladders, Fermentas, Germany); lanes 2 and 3 (1041 bp): positive samples; lanes 4 and 5: negative samples and negative control. And lanes 6: positive control (1041 bp)

**Şekil 2.** Güvercin doku örneklerinde etidyum bromür ile boyanarak agaroz jel elektroforez ile belirlenen *Chlamydophila psittaci* ompA genini gösteren PCR ürünleri (1041 bp). 1. sütun: DNA merdiveni (100 bp merdiven, Fermentas, Almanya); 2. ve 3. sütunlar (1041 bp): pozitif örnekler; 4. ve 5. sütunlar sırasıyla negatif örnek ve negatif kontrol. 6. sütun: pozitif kontrol (1041 bp)

exposure during processing of pigeons for human consumption need to be considered as highest prevalence of *C. psittaci* spread. Dickx et al.[21] has reported detection of *C. psittaci* among the employees, chicken and turkey in a slaughterhouse in Belgium, and this further reinforce *C. psittaci* as an occupational hazard.

The detection of *C. psittaci* in the blood, liver and muscle tissue of pigeons may be very important in the pathogenesis of *C. psittaci* in pigeons. Page [30], in his work on experimental infection of turkey with *C. psittaci*, reported that *Chlamydia* were present in the blood, liver, spleen and kidney 48 h post inoculation and 72 h post inoculation in muscles, testes and ovaries. Later, *Chlamydia* was found in large number in cloaca and nasal turbinate. Furthermore, Vanrompay et al.[31], from their experiment on pathogenesis of *C. psittaci* in turkey, reported that Chlamydaemia was observed in these turkey before chlamydial replication could be detected in the digestive tracts, 3-5 days post infection. The higher detection of *Chlamydia* in blood in this study supported the possibility of early detection of *Chlamydia* in birds before detection from feces or cloacal swabs.

The prevalence of chlamydial infections in pigeons has been reported worldwide and is consistently high. The actual risk to humans of the infection from these birds is difficult to quantify. From this study, we concluded that pigeons serve as a reservoir of *C. psittaci* for other animals and humans. Also, male pigeons had higher prevalence of *C. psittaci* and possibly higher risk of infection to humans than female pigeons. Continuous surveillance of this bacterium will go along way in understanding the distribution and risks associated with *Chlamydia* infected pigeons. This will be beneficial in prevention and control of the infection in humans.

### Acknowledgement

The authors would like to express their deep sense of gratitude and sincere thanks to the staff of the Biotechnology Research Center of Islamic Azad University of Shahrekord Branch in Iran. Also the authors would like to acknowledge the valuable contribution of Dr. Ismar Lutvikadic.

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