Determination of the Prevalence of *Chlamydia psittaci* by PCR in Iranian Pigeons

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Abstract

Many areas in Iran such as parks and gardens can be highly contaminated with pigeon feces. *Chlamydia psittaci* is a lethal bacterial that causes endemic avian *chlamydiosis*, epizootic outbreaks in mammals, and respiratory psittacosis in humans. *Chlamydia psittaci* strains in birds infect mucosal epithelial cells and macrophages of the respiratory tract. The aim of this study was to determination of prevalence of *Chlamydia psittaci* in feces of pigeons in Iran using PCR assay. DNA was extracted from 445 fecal samples of pigeons. The prevalence of this pathogen was 14.3% in region of this study. These results indicate that pigeon feces are a source of several zoonotic agents for humans, bird and animals. We suggested that continuous surveys can estimate, and thus help to minimize the risk of humans contracting diseases from pigeons.

Keywords: *Chlamydia psittaci*, Pigeon, PCR

1. Introduction

Chlamydophila psittaci (*C. psittaci*), an obligate intracellular, gram negative bacterium, has 7 known genotypes (A-F and E/B) (Geens *et al.,* 2005). All genotypes can be transmitted to humans and cause psittacosis or parrot fever. Genotypes are distinguished by sequencing of the outer membrane protein A (*ompA*) gene (Vanrompay *et al.,* 1997). *C. psittaci* can infect 465 avian species in 30 avian orders, with at least 153 species in the order Psittaciformes (Vanrompay *et al.,* 2007). It is known that pigeons, like many other bird species, can harbor *C. psittaci* (Heddema *et al.,* 2006). *C. psittaci* is a bacterium that can be transmitted from pet birds to humans (Johnston *et al*., 2000). The genus Chlamydia consists of three species: *C. psittaci*, *C. trachomatis*, and *C. pneumoniae*. Recently, a fourth species, *C. pecorum*, has been proposed (Fukushi & Hirai, 1992). *C. trachomatis* is primarily a human pathogen and contains three biovars and 15 serovars. *C. pneumoniae* is also a human pathogen (Vanrompay *et al.,* 1993). These bacteria are obligate intracellular organisms that are transmitted as metabolically inactive particles called elementary bodies (EBs) (Binet & Maurelli, 2007). *C. psittaci* has the ability to remain infectious in the environment for months, presenting a variety of public health issues, including economically devastating outbreaks in poultry farms and occasionally severe pneumonia in humans (Kaltenboeck *et al.,* 1991). Transmission of this atypical respiratory pathogen can occur through direct contact with infected birds, bird feces, nasal discharges, and aerosols, causing respiratory disease in both mammals and birds (Smith *et al.,* 2005). In birds that have avian chlamydiosis, cloudy air sacs and an enlarged liver and spleen usually are observed, but no specific gross lesion is pathognomonic. The chromatic or immunologic staining of tissue-impression smears can be used to identify organisms (Johnston *et al.,* 2000). Persons at risk include those exposed to pet birds, pigeons, and poultry and in specific occupations such as laboratory and wildlife workers. Human infection can result from even brief exposure to the contaminated excretions or secretions of infected birds (Smith *et al.,* 2005). In contrast to veterinary medicine, psittacosis in humans is not a notifiable disease anymore as the regulation was changed some years ago (Zweifel *et al.,* 2009). From 1941 to 2003, 78 cases in

humans were reported (Haag-Wackernagel & Moch, 2004), due to contact with feral pigeons. Transmission is mainly by inhalation of nasal and fecal secretions. The aim of this study was to determination of the prevalence of *C. psittaci* in pigeon population in Iran by PCR method.

2. Materials and methods

2.1 Sampling and DNA extraction

In present study, 445 samples of fresh feces pigeons without contamination in different areas of Iran were isolates during three months and analyzed by PCR for detection of *ompA* gene of *C. psittaci*. Genomic DNA was extracted from 100 ng fecal samples with DNA extraction kit (Qiagen, Germany), 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 (Sambrook and Russell, 2001). 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.

2.2 Gene amplification

The *ompA* region was amplified by PCR using primers CPsitt-F (5′-GCTACGGGTTCCGCTCT-3′) and CPsitt-R (5′-TTTGTTGATYTGAATCGAAGC-3′) with accession number AB512087.1 as described by Heddema for *ompA* region (Heddema *et al.,* 2006). Primers performed 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 and visualised by ethidium bromide staining. The DNA molecular weight marker was used as a size marker.

3. Results

Out of 445 feces samples 64 (14.3%) were positive for *C. psittaci* infection using PCR. Analysis of PCR products for presence of *ompA* gene of *C. psittaci* on agarose gel revealed a 1041 bp fragment (Figure 1).

The positive control showed the excepted amplification product specific for *C. psittaci* (1041 bp). To investigate the source of infection, PCR on feces revealed positive results for *C. psittaci* although these pigeons did not show any symptoms of *chlamydial* infections. In our study the pigeons were asymptomatically carriers for *C. psittaci* in their body excretions as feces or respiratory discharges. These results suggest that *C. psittaci* infection of pigeons is rare or arises only as a result of very close contact, for example, in nests where the risk of infection with various fecal pathogens increases.

4. Discussion

Chlamydia psittaci is a causative agent of psittacosis, systemic diseases inpsittacine birds which can be of acute, protracted, chronic, or subclinical manifestation and which represents the most important animal chlamydiosis of zoonotic character (Goellner *et al.,* 2006). The *Chlamydiaceae* are etiological agents of many important human and animal diseases. Formerly called *Chlamydia*, with only two recognized species 25 years ago (*C. trachomatis* and *C. psittaci*), the family now contains nine species divided into two genera, *Chlamydia* and *Chlamydophila* (Bush & Everett, 2001). From 2005 to 2009, 66 human cases of psittacosis were reported to the Centers for Disease Control and Prevention (CDC) (Smith *et al.,* 2005). *C. psittaci* is currently grouped into seven avian genotypes (A through F and a newly identified genotype, E/B) and two non avian genotypes (M56 and WC). Recent reclassification of *C. psittaci* has resulted in the separation of *C. abortus* and *C. caviae* into distinct species, although these species are genetically closely related (Van Loock *et al.,* 2003).

The presence of *C. psittaci* in current study was 14.3% (64 of 445). Tanaka et al. found *C. psittaci* in 106 of 463 fecal samples obtained from feral pigeons (Tanaka *et al.,* 2005). Salinas et al. reported the prevalence of *C. psittaci* in feral pigeons was found by culture in 18% of fecal samples (Heddema *et al.,* 2006). Heddema et al. determined the prevalence and genotype of *C. psittaci* in fresh fecal samples from feral pigeons in Amsterdam, the Netherlands. The prevalence was 7.9% overall (26/331). Ten genotyped PCR-positive samples were all genotype *B* (Heddema *et al.,* 2006).

In conclusion, feces of pigeons can spread several zoonotic diseases such as chlamydiosis in Iran. According to these findings it's suggested a vaccine and information on sensible use of antimicrobial drugs in Psittaciformes for prevent to psittacosis in humans and development of drug-resistant bacterial strains.

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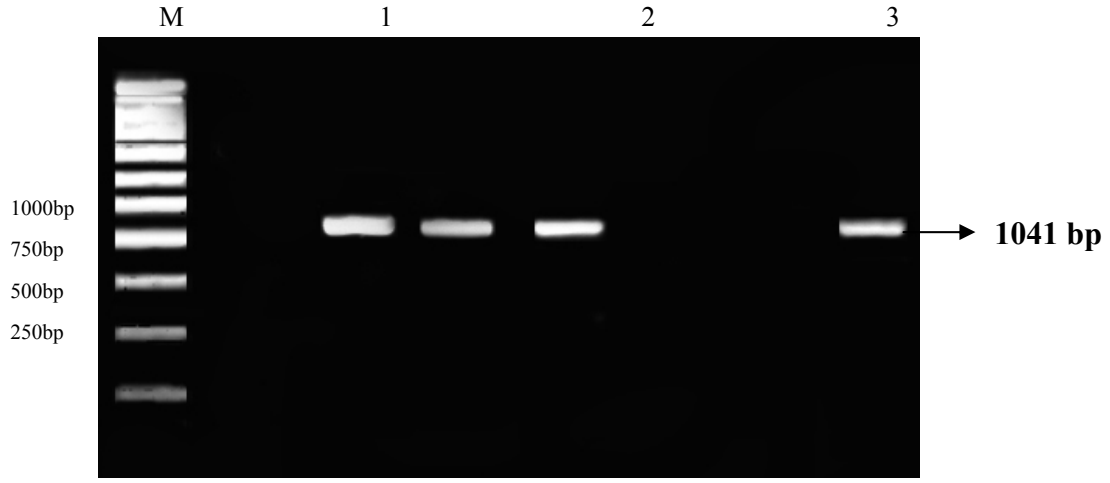

Figure 1. An agarose gel stained with ethidium bromide, for detection of *ompA* gene in *Chlamydia psittaci* (Line M: 1 kb DNA ladder (Fermentas, Germany), lines 1 and 2: negative and positive controls, respectively, lines 3, 4 and 7: positive tests and line 5 and 6: negative tests)

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