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Original Article

Molecular diagnosis and genotyping of *Chlamydia psittaci* in captive psittacines and their owners in the middle province of Iran

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Summary

Chlamydia psittaci (*C. psittaci*) is an avian pathogen which its clinical symptoms of the disease may be varies from asymptomatic to several clinical symptoms, which include: conjunctivitis, an inflammation of the lining of the nose (rhinitis), sinusitis, diarrhea (dehydration), respiratory distress, yellow-green urine, loss of appetite, which may cause respiratory disorders in humans. Oropharyngeal(owner and birds) and cloacal (birds) swabs were taken from 54 captive psittacine birds and their owners who attended to veterinary clinics in Isfahan (Totally 108 samples).To study the prevalence of *C. psittaci* in captive birds and their owners using molecular detection assay (PCR),samples were collected during 2014 from a total of 10 various species of parrots. *C. psittaci* was identified in four species of birds (40%). Sequencing was performed to confirm the PCR positive results, demonstrating that all positive samples of *C. psittaci* belonged to genotype A, representing the first report of the presence of this genotype in Iran. The determination of this bacterium in captive psittacine birds presents that there is a potential risk for owners who live or have direct contact with them and that there is a feasibility of infecting other birds and humans.

Keywords: *Chlamydia psittaci*, Psittacine, Birds, Owners, Genotyping, PCR

Introduction

Chlamydia psittaci (*C.psittaci*) is a compatible intracellular gram-negative bacteria which leads to chlamydiosis in birds (Evereltet al.,

1999). The clinical symptoms of the disease may vary from asymptomatic to several clinical symptoms, which include: conjunctivitis, an inflammation of the lining of the nose (rhinitis), sinusitis, diarrhea (dehydration), respiratory distress, yellow-green urine, and loss of appetite (Gerlach, 1994).

Today, *C. psittaci*has been investigated in about 465 bird species spanning 30 bird orders. The disease in birds and humans originally was known psittacosis or parrot fever because it was first recognized in psittacine birds and in humans related with psittacine birds (Kaleta and Taday, 2003).

In humans, the incubation period varies from one to four weeks, and sometimes it causes sudden onset of symptoms of mild fever of 100 to 102 degrees Fahrenheit, which the temperature increase with the progression of the disease and in severe cases, it remains at a high temperature. Loss of appetite, throat pain, photophobia, and severe headache are common symptoms of the disease. In mild cases, a flu-like syndrome appears, and usually, in a week, it returns to the normal body temperature (Fudge, 1996).

These symptoms depend on: 1) strain of pathogenic; 2) species, age, and level of immunity; 3) environment; 4) the presence of secondary pathogens.

The infected birds may get airbags fibrosis, pneumonia, pericarditis, inflammation of the intestines, and enlargement of spleen and liver (Zoghi, 1996).

Maybe all types of psittacine have chronic infection, but they do not show clinical signs of disease. These birds are alternately relieving chlamydia, and they are a significant source of chlamydial infection to humans, birds, and other mammals (Fudge, 1996).

The disease is a common disease with humans and since psittacine species are considered popular pets in terms of public health, they are important.

Most infections are through inhalation of infectious aerosols; therefore, poultry processing plant employees, such as farm workers, veterinarians, and taxidermists are particularly at risk (Dickx et al., 2010; Laroucau et al., 2009; Verminnen et al., 2008). However, from the epidemiology viewpoint and diagnosis, it should be borne in mind that, unfortunately, asymptomatic birds (without symptoms) are also able to excrete the organism and act as a source of infection, and sometimes there may be no bird available for the obvious source (Zoghi, 1996).

The stress factors are; overpopulation, poor levels of hygiene, malnutrition, infections, and changes in temperature along with other in

ambient can stress birds and active the latent infection of chlamydial, which increases the incidence of disease progression and clinical symptoms of the pathogen. These factors can also affect periodically and enhances the original form of chlamydia in the discharge of their hosts (birds in normal cases without clinical symptoms) (Evereltet al., 1999).

Almost 160 species of birds can be carriers of Chlamydia psittaci, which only 25 percent are psittacine (Zoghi, 1996). Most likely young birds develop the disease at the time of contamination or infection (Gerlach, 1994, Harrison, 1989).

Domestic Psittacine are carriers in the 10 - 40 %, but this number in psittacine in touch with other birds is nearly 100%. The purpose of this study is to detect the genotype and frequency of pollution of captive psittacines and their owners in Iran (Isfahan)to Chlamydia psittaci bacterium by using three methods of PCR, genotyping, and direct smear (Giemsa staining).

Materials and methods

In the current study, the samples were collected from psittacines that referred to the clinic and after examination and recording characteristics such as age, sex, history of vaccination, and the observed clinical signs of respiratory infections using a moistened swab with saline, samples were taken from the conjunctiva, and cloaca and at the same time, a swab sample was also taken from the conjunctiva of parrot owner. A total of 54 birds randomly divided into three age groups under two years (21), between 2 and 4 years (21 samples), more than 4 years (12), males (24 cases) and females (30) were collected. Twenty-seven samples of psittacine apparently were healthy (no symptom), 27 samples from psittacine qualified clinical symptoms (clinical symptoms involvement of eyes and respiratory tract).

The owners' samples, regardless of gender, age, and the clinical symptoms and only for common diseases, were examined.

On-site expanded sampling conjunctival swab samples to direct staining (Giemsa method) was prepared and taken swabs at 1% PBS solution with a swab from the cloaca was transferred to the biotechnology laboratory of Veterinary Medicine, Islamic Azad University Shahr-e-Kord Branch and until such molecular tests were stored at -70 ° C.

Polymerase chain reaction (PCR)

PBS solution-treated swab was extracted using a DNA extraction kit made by the Sinagen Company (Iran) according to the DNA kit genomic. The sequence of primers used in the

present study conducted by Sykes et al. (2001), which leads to reproduce a piece with a length of 1094 bp of ompA (Since the outer membrane protein is the main factor in *Chlamydia psittaci* pathogenesis and genotype A is the most dominant pathogenic genotype in humans and dogs, amplification of the outer membrane protein-encoding gene was used to molecular detection of *Chlamydia psittaci* in samples)gene of *Chlamydia psittaci* was chosen to complete the following sequence:

F: ATGAAAAAACTCTTGAAATCGG

R: CAAGATTTTCTAGACTTCATTTTGTT

The PCR reaction in a final volume of 25 µl includes: 2.5 µl of DNA template, 0.2 µM of each primer, 200 µM of dNTP mix (Fermntas Germany), 1.5 mM$MgCl_2$ and 2.5 µl buffer of PCR and 1 unit of the enzyme of Taq DNA Polymerase (Fermntas Germany)

The samples were immediately put in a thermocycler (Eppendorf, Germany), and a temperature program was set as follows:

- A cycle of 95 ° C for 5 minutes
- 30 cycles of repetitive temperature 94 ° C for 1 minute
- 72 ° C for 1 minute
- A final round of 72 degrees for 8 minutes

To conduct the PCR of DNA extracted from the sample indirect testing (Giemsa staining), chlamydial bodies were used for the mass unit as a positive control sample. Distilled water was used as a negative control sample. Eventually, the PCR product of evaluated samples on 1% agarose gel in the presence of marker 1 kb DNA (Fermentas Germany) was observed and recorded in a constant voltage of 90 V for 45 min and a UV light.

Results

Twenty-seven samples (50%) out of 54 samples of parrots in the PCR method of C, had *Chlamydia psittaci* genome.

Psittacine was examined and studied in three age groups under 2 years (21), between 2 and 4 years (19 cases), and more than 4 years (14 samples).The lowest infection rate was determined in psittacine over 4 years with 25% of pollution and the highest prevalence in group-age under 2 years with 39% of the pollution as well as the age group of 2 to 4 years, with 36 percent of pollution. Statistical analysis presented no significant statistical difference between the three age groups (P = 2.396).

Of the 54 samples form psittacine, 24 samples were male and 30 were female. The infection rate in the psittacine population was

determined in 44 percent male, and 56 percent of the female psittacine population, but there was no the significant difference between the pollution and psittacine gender (P = 3.121).

Twenty-two samples of the studied psittacine population were with clinical signs, and 32 samples without clinical signs. After taking the PCR test, 19 positive PCR (3/86 percent) out of 22 samples were with clinical signs, and three cases with negative PCR (7/13) and of 32 cases without clinical signs, eight positive PCR (25%), 24 negative PCR (75%) were recorded. Statistical analysis demonstrated a statistically significant difference between infection with clinical signs of respiratory-ocular *Chlamydia psittaci* with psittacine with no symptoms (healthy) (P = 0.036).

In this study, 10 species of psittacine were studied that was 54 samples, 33 samples of them were African gray, 6 were Cockatiel species, 3 were Love bird species, 2 were Macaw species, 1 was Amazon species, 1 was Cockatoo species, 2 were Ring-necked species, 1 was Conure species, 2 were Alexandrian species, and 3 were Rosella species.

There was a significant difference between *Chlamydia psittaci* infections of the African Gray species with other bird species. (P = 0.066)

The samples taken from the 54 owners, 19 samples (35%) in the PCR method has *Chlamydia psittaci* genome.

The positive samples of owners were all positive samples from genotypes A, and all infected parrot belong to this genotype.

Discussion

Chlamydia psittaci is one of the causes of disease in birds (especially psittacine), various animals, and humans, and it is particularly important because of zoonotic, and high mortality in birds. All species of birds (domestic and wild) are susceptible to this disease also, it can cause other diseases in different animals. For instance, Chlamydia is a major factor in the abortion of ewes and cattle.

Transmissions among multiple hosts are directly, and often, it happens by direct contact of ocular discharge, nasal discharge, respiratory tract secretions, and feces of infected hosts.

Due to the tendency of humans to keep psittacine at their homes, identifying *Chlamydia psittaci* is one of the most important measures in the field of controlling the disease in terms of public health and prevent the spread and transmission of the disease to other hosts.

In this study, by using sensitive and accurate PCR to identify genes OmpA Chlamydia psittaci in psittacine (African gray, lovebirds, cockatiel, Alexandrine, etc.) in Isfahan was discussed. The present results showed that 50 percent of psittacine have positive *Chlamydia psittaci* disease regardless of the presence or absence of clinical signs and all have genome type A. Also, 19 samples of bird owners are eligible for *Chlamydia psittaci* with genotype A.

Few studies have been conducted about the contamination of *Chlamydia psittaci*. Doosti and Arshi in 2011 in a study examined 445 samples of pigeon droppings in Iran, about the prevalence of *Chlamydia psittaci* by PCR method, and the result of their research, the sample was positive for 14.3 percent (Doosti and Arshi, 2011).

Mahzunieh et al. (2013), in a study on pigeons of Charmahal-Bakhtiari and Isfahan Provinces from 220 samples taken from pigeon droppings in bird selling shops and domestic pigeons by using PCR method, the prevalence of *Chlamydia psittaci* was on average 15.9 % (35 samples out of 22) (Mahzunieh et al.,2013). Although previous studies have been conducted on birds have clinical symptoms but, due to the durability and the possibility of transmission through carrier birds, it was necessary to be taken to a separate study, to recognize the status of Asymptomatic birds infected with *Chlamydia psittaci* and their role as a popular pet birds in the spread of bacteria among the human population should be determined, particularly children and the dissemination of bacteria between other species of ornamental birds.

In our study, in Isfahan, 54 samples were collected, 27 samples were positive, and the African gray species had the highest infection rate. According to multiple reports of *Chlamydia psittaci* infection in ornamental birds and especially psittacine species in some parts of the country, it seems that infection with this pathogen among psittacine is serious and important. Extensive studies have been done in other parts of the world in addition to studies in Iran.

In Sheleby-Elias et al. (2013) study, a total number of 117 samples were taken from psittacine of veterinary clinics, 88sample inbird-shops, and 29 samples were collected from wildlife; the samples were from conjunctiva swab and cloacae and have been conducted in Costa Rica to determine the prevalence of *Chlamydia psittaci*. The results showed that 12.4 percent of these samples contained *Chlamydia psittaci*, and most of them were adult parrots (Sheleby-Elias et al., 2013).

In a study of Tania et al. (2002) of 95 healthy psittacines (3 races) samples were collected in Brazil. The cloacae samples were collected for the detection and identification of Chlamydia using the D.I.F method, and the results showed that 16.7 percent of eligible birds had Chlamydia (Tania et al., 2002).

Piasecki et al. (2012) investigated *Chlamydia psittaci* in psittacine without clinical symptoms in Poland, and 156 swab samples were collected from 34 different parrot species for genotyping expansion. The results indicated that 10.3 % of the samples had been taken by two different PCR methods, was positive, which are divided into two genotypes A and B, and generally, one of these two genotypes is not dividable (Piasecki et al., 2012).In a same study, Krawiec et al. (2015) reported 22 positive samples (81.5%) belonged to *C. psittaci*in wild birds in Poland. Interestingly, they also presented all spleens and livers of *Chlamydia*-positive birds showed a positive result in the real-time PCR (Krawiec et al., 2015).

However, our study showed that *Chlamydia psittaci* infection in birds without clinical symptoms is low (14 percent) but psittacine without clinical symptoms can be raised as a *Chlamydia psittaci* resource. Due to detect bacteria in samples were taken in psittacine over 2 years as well as the absence of clinical signs in the birds, it seems that these bacteria can remain in the body for a long time and alter the bird into a biological carrier and over time, the infectious factor is disposed of by feces and secretions. Since these carriers will be considered a potential risk for human infection and psittacine; therefore, their detection, control, treatment will be very important. The importance of identifying these potential risks is the goal of this research.

Given that in the present study, the samples are only taken from the psittacine family and because this family is the original host of *Chlamydia psittaci*, thus the results of this study, in comparison with other researches that have been done in other parts of Iran, has a big difference.

Since the African gray species is one of the popular species among the bird owners, thus the high number of taken samples and also the high percentage of this species in Isfahan is the cause of high infection among this group.

However, the *Chlamydia psittaci* infection reported in psittacine of Isfahan shows the presence and distribution of disease-causing organisms in different psittacine.

The samples of psittacine specimens' owners were taken, regardless of gender, age, and the clinical symptoms and only for the prevalence of this factor among owners of domestic birds.

In this study, in all positive samples of owners, the parrot was also positive, and it reflects the importance of the disease due to the significant prevalence of notable between parrots and owners.

Conclusion

According to the results of this study, the importance of spreading the disease in human societies and also domestic psittacine community, it should be considered in both the public health system concerning the presence of Chlamydia. The main purpose of this study was identifying the prevalence of *Chlamydia psittaci* in Isfahan province and give suggestions to control and prevent the factor due to the zoonotic agent.

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Ethical approval

The experiment was approved by the Research Ethics Committee of Tabriz University of Medical Sciences and performed according to the Helsinki's humanity research declaration**.**

Conflict of interest statement

There is no conflict of interest.

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