



RESEARCH ARTICLE

Prevalence of *Chlamydophila psittaci* infection in pigeons and paraquets by a real time polymerase chain reaction

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Konya bölgesinde güvercin ve muhabbet kuşlarında *Chlamydophila psittaci* enfeksiyon prevalansının real time pcr ile belirlenmesi

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Öz

Amaç: Kanatlı klamidiozisi evcil ve yabani kanatlılarda *Chlamydophila psittaci*'nin neden olduğu, sistemik infeksiyöz, zoonotik, bazen de ölümcül seyreden bir hastalıktır. *C. psittaci* kanatlı hayvanlarda her zaman ciddi hastalığa neden olmasa da özellikle immunsupresif insanlarda ciddi hastalık tablosuna neden olabilmektedir. Bu çalışmanın amacı, Konya ilinde güvercin ve muhabbet kuşlarına ait dışkı örneklerinde psittakozu neden olan zoonotik bir hastalık etkeni olan *C. psittaci*'nin prevalansını belirlemektir.

Gereç ve Yöntem: 24 farklı yetiştiriciye ait toplam 600 güvercinden alınan 50 adet ve 30 farklı yetiştiriciye ait 632 muhabbet kuşundan alınan 52 adet dışkı örneği bu çalışmanın materyalini oluşturmuştur. Bu örneklerde *C. psittaci* varlığı, ompA geninin varlığına dayalı olarak gerçekleştirilen Real Time PCR yöntemi ile araştırıldı.

Bulgular: Konya yöresinde güvercin ve muhabbet kuşlarının dışkı örneklerinde *C. psittaci* ompA gen varlığı real time PCR ile sırasıyla %2 ve %1 olarak belirlendi.

Öneri: Hastalığın zoonoz karakterli olması ve insanların kümes hayvanları özellikle de güvercin ve kafes kuşları ile yakın teması, hastalığın halk sağlığı açısından önemini her geçen gün artırmaktadır.

Anahtar kelimeler: *Chlamydophila psittaci*, güvercin, muhabbet kuşu, real time pcr

Abstract

Aim: Avian chlamydiosis is a systemic, zoonotic, sometimes fatal disease caused by *Chlamydophila psittaci* in domestic and wild poultry. Although *C. psittaci* does not always cause serious disease in poultry, it can cause serious disease especially in immunosuppressive humans. The aim of this study was to determine the prevalence of *C. psittaci*, in stool samples of pigeons and paraquets, in Konya province.

Materials and Methods: Fifty pigeon feces samples taken from a total of 600 pigeons belonging to 24 different breeders and 52 fecal samples taken from 632 paraquets from 30 different breeders were the samples in this study. The presence of *C. psittaci* was investigated by Real Time PCR based on the presence of ompA gene in these samples.

Results: The presence of *C. psittaci* ompA gene was determined as 2% and 1%, respectively, by real time PCR in stool samples of pigeons and paraquet in Konya region.

Conclusion: The fact that the disease is zoonotic and people's close contact with poultry, especially pigeons and caged birds is increased the importance of the disease in terms of public health day by day.

Keywords: *Chlamydophila psittaci*, pigeon, paraquet, real time pcr





Introduction

Psittocosis, caused by *Chlamydophila psittaci*; is a zoonotic disease seen in many wild and domestic poultry and birds (such as paraquets, canaries, doves, pheasants, sea and shore birds) as well as mammals and humans (Borel et al 2018, Lenny et al 2020). It also causes various infections that are frequently latent but also cause clinical symptoms in mammals (Ang et al 2011, Chu et al 2022). *C.psittaci* can be transmitted to humans through close contact with some infected mammals such as birds, cattle, pigs, sheep, goats, cats and horses (Shaw et al 2019, Abd El-Ghany 2020). In humans, the disease has caused significant death and infection, along with multiple organ and severe respiratory system problems (Bommana and Polkinghorne 2019, Yunfeng al al 2021). Transmission of the disease among humans is rare. It has been reported that there were 78 human cases caused by wild pigeon contact, the source of transmission were nasal secretions and inhalation from feces, and clinical findings ranging from flu-like symptoms to pneumonia were encountered in humans in Switzerland between 1941-2003 (Haag-Wackernagel 2006).

Respiratory distress, mucopurulent nasal discharge, diarrhea, blindness, keratoconjunctivitis, polyuria, dehydration, sinusitis, serous or mucopurulent oculo nasal discharge, tangles in feathers, hepatomegaly, central nervous system disorders are observed, in avian chlamydiosis depending on the genotype of the causative agent and the bird species affected (Arda 2008). *C.psittaci* can be transmitted to the environment through nasal, mouth, respiratory tract discharges and feces. Despite treatment or care, sometimes the agents in the stool can be activated as a result of stress factors such as crowded environment and travel (Arda 2008, OIE 2021).

Especially, paraquets and cockatiels carry the agent asymptotically, and they spread it to the environment with their feathers and body secretions in stress situations for a long time (Sareyyuboglu et al 2007, Borel et al 2018). Transmission can be triggered especially during times of stress such as co-infections, nutritional deficiencies, overcrowding and spawning. In addition, the agents can be spread mechanically by the bite of flies, lice and mites (OIE 2021). In a molecular study, conducted in cage birds with or without clinical findings, *C.psittaci* was determined in 43 (97.5%) of 47 stool samples which method pcr (Sareyyuboglu et al 2007). In a study conducted in Brazil, cloacal swap samples taken from 95 healthy parrots were found to 35% positive by ELISA (Raso et al 2002). In the Netherlands, 7% of stool samples of 331 pigeons were positive for *C. psittaci* by PCR method based on ompA gene amplification (Hedemma et al 2006). In 106 of 463 fecal samples of wild pigeons (22%) in Japan, *Chlamydophila* spp. was detected which method pcr (Tanaka et al 2005). In Croatia, seropositivity for *C.psittaci*

was detected in 174 of 182 adult pigeons (95%) (Prucner-Radovic et al 2005). In Japan, *C. psittaci* were detected 5.9% in 1147 samples taken from 113 bird species which method pcr (Chahota et al 2006).

The aim of this study was to determine the prevalence of *C. psittaci* infection, in stool samples from pigeons and paraquets by a real time pcr, in Konya province.

Material and Methods

Fifty fecal samples were collected from a total of 600 pigeons in 24 different breeders, and 52 stool samples were taken from 632 paraquets in 30 different breeders in the center and neighborhoods of Konya. Clinical signs such as respiratory distress (6/50), weakening (5/50), diarrhea (4/50) and anorexia (1/50) in pigeons and respiratory distress (5/52), diarrhea (3/52), moulting (3/52), weight loss (1/52) in paraquets were observed. Fresh stool samples were taken separately into sterile eppendorf tubes, brought to the laboratory under cold chain, and stored at -20 °C by using.

DNA extraction

Genomic DNA from stool samples was obtained using a commercial DNA extraction kit (nzytech, Cat No: MD03261-Portugal) and kept at -20 °C until real time pcr analysis.

PCR analysis

The presence of *C. psittaci* was determined by using commercial TaqMan Real Time PCR kit (Microsynth, Canada), which contains *C.psittaci* specific primer/probe CPSIT_0607 gene spesific, *C.psittaci* control template, internal extraction control DNA primer/probe, internal extraction control DNA, endogenous control primer probe. Positive control sample and RNase/DNase-free water were used as a positive and negative control in each PCR run.

Real time pcr analysis was performed using Light Cycler 480 probe master mix (Lyo NZYSupreme-Portugal). The presence of *C. psittaci* was detected by making some modifications to the method which was optimized with a positive control sample, reported by Hedemma et al. (2006)

Primers and probs used in this study:

F_5'-CGCTCTCTCCTTACAAGCC-3'

R_5'-AGCACCTTCCCACATAGTG-3'

5'-6FAM-AGGGAACCCAGCTGAACCAAGTTT-3'

IC Probe HEX-5'-TCGAGACAGTGCAACGTAAGCCTA-3'



Table 1. Location information of stool samples and clinical findings of the animals

Sample	Breed	District	Number of Animals	Clinical Findings
2	Pigeon	Sancak/Selcuklu	24	Diarrhea, weakening
1	Pigeon	Sancak/Selcuklu	12	-
1	Pigeon	Sancak/Selcuklu	18	Diarrhea, weakening
1	Pigeon	Doganlar/Karatay	14	Diarrhea, weakening
1	Pigeon	Doganlar/Karatay	8	Dyspnea, weakening
1	Pigeon	Doganlar/Karatay	6	-
4	Pigeon*	Saracoglu/Karatay	60	Dyspnea, weakening
2	Pigeon	Saracoglu/Karatay	24	Diarrhea
2	Pigeon	Saracoglu/Karatay	32	-
1	Pigeon	B. Aymanas/Meram	11	-
3	Pigeon	Tatlıcak/Karatay	36	-
6	Pigeon*	Tatlıcak/Karatay	68	Dyspnea
4	Pigeon	Cimenlik/Karatay	40	-
3	Pigeon	Cimenlik/Karatay	38	Anorexia
2	Pigeon	Cimenlik/Karatay	26	-
1	Pigeon	Mengene/Karatay	16	-
1	Pigeon	Mengene/Karatay	14	-
3	Pigeon	Mengene/Karatay	30	Dyspnea
1	Pigeon	Mengene/Karatay	8	-
2	Pigeon	Cumhuriyet /Selcuklu	21	-
1	Pigeon	B. Kumkopru/Karatay	13	Dyspnea
4	Pigeon	Kececiler/Karatay	47	-
2	Pigeon	Kececiler/Karatay	26	-
1	Pigeon	Kececiler/Karatay	8	Dyspnea
50			600	
Sample	Breed	District	Number of Animals	Clinical Findings
1	Paraquet	Beyhekim /Selcuklu	18	Diarrhea, weakening
1	Paraquet	Beyhekim /Selcuklu	14	-
2	Paraquet	Beyhekim /Selcuklu	27	Diarrhea
4	Paraquet	Yaka/Meram	42	-
3	Paraquet	Yaka/Meram	34	-
1	Paraquet	Yaka/Meram	14	Dyspnea
1	Paraquet	Yazır/Selcuklu	15	-
1	Paraquet	Yazır/Selcuklu	15	Dyspnea
1	Paraquet	Kosava/Selcuklu	15	Molt
2	Paraquet	Kosava/Selcuklu	24	-
2	Paraquet	Kosava/Selcuklu	28	Diarrhea
3	Paraquet	Bosna Hersek/Selcuklu	36	-
3	Paraquet	Bosna Hersek/Selcuklu	34	-
2	Paraquet*	Bosna Hersek/Selcuklu	22	Dyspnea
2	Paraquet	Isıklar/Selcuklu	20	-
1	Paraquet	Isıklar/Selcuklu	14	Molt
3	Paraquet	Isıklar/Selcuklu	32	Molt
1	Paraquet	B. Aymanas/Meram	12	Dyspnea
2	Paraquet	Havzan/Meram	22	-
1	Paraquet	Havzan/Meram	17	-
1	Paraquet	Askan /Meram	8	Dyspnea
2	Paraquet	Askan /Meram	18	-
1	Paraquet	Askan/Meram	13	-
2	Paraquet	İhsaniye/Selcuklu	24	Anorexia
1	Paraquet	İhsaniye/Selcuklu	18	-
1	Paraquet	Cumhuriyet/Selcuklu	14	Dyspnea
1	Paraquet	Cumhuriyet/Selcuklu	16	Dyspnea
1	Paraquet	Cumhuriyet/Selcuklu	10	-
2	Paraquet	B. Kumkopru/Karatay	24	Molt
3	Paraquet	Sakarya/Selcuklu	32	-
52			632	

**C.psittaci ompA* gene was found positive by real time pcr.

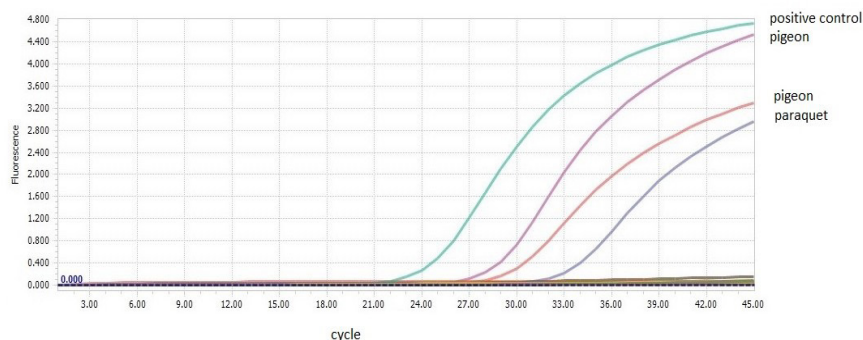


Figure 1. PCR amplification curves of *C. psittaci* positive stool samples of pigeons and paraquet

Total reaction mixture of 20 μ L; It consisted of 10 μ L master mix, 1 μ L forward primer, 1 μ L reverse primer, 1 μ L probe, 1 μ L internal control, 5 μ L genomic DNA and 1 μ L dH₂O. The reaction mixture was prepared in the plate and the presence of *C. psittaci* was investigated using the Light Cycler 480 (Roche Diagnostic, GmBh, Germany) device. Real-time pcr steps; 10 minutes at 50 °C, 10 minutes at 95 °C, 49 cycles 10 seconds at 95 °C, 5 seconds at 62 °C and 10 seconds at 72 °C, finally 30 seconds at 30 °C.

Results

It was noteworthy that respiratory problems was detected in all animals from which positive samples were taken. The clinical findings determined in the neighborhoods and businesses where the materials of the study were taken are given in Table 1. The PCR amplification graph obtained using real time pcr is shown in Figure 1.

C. psittaci was detected from feces of pigeons and paraquets at the rates of 2% and 1%, respectively.

Discussion

Psittocosis is an important zoonotic disease. In recent years, it has been reported that *C. psittaci* is detected at high rates in different bird species in many studies using different diagnostic methods (Raso et al 2002, Tanaka et al 2005, Vanrompay et al 2007). In this study, it was aimed to investigate the *C. psittaci* ompA gene in 102 stool samples with real time pcr method.

In studies conducted in various countries for the determination of *C. psittaci* from stool samples; *C. psittaci* was detected 35% of parrots in 2002, 22% in 2005, 19.2% of swabs taken from wild birds in 2007 respectively in Brazil, Japan and Netherlands (Raso et al 2002, Tanaka et al 2005, Vanrompay et al 2007). The reason why the results obtained from these studies (35%, 22%, 19.2%) have higher positive

values compared to the results of our study (3%) thought may be due to the fact that selected animals are wild animals and therefore the breeding difference between them (such as domestic or wild animals, care in a cage), different animal species, living and care conditions, material selection, region, year and season.

There are limited studies on the prevalence of the disease in poultry in our country. *C. psittaci* was determined by pcr in 33 (34.4%) of 96 stool samples of pet birds (Celebi and Ak 2006). In another study conducted in our country, samples of 140 waterfowl including ducks, geese, swans and pelicans in zoos were examined by ELISA method and positivity for *C. psittaci* were determined in 65.7% of the birds (Karakuzulu 2003).

The presence of *C. psittaci* (ompA gene) was found to be between 30-34% with pcr method in swap samples taken from wild pigeons in studies conducted in European countries such as England (Beeckman and Vanrompay 2009), Sweden (Harkinezhad et al 2009) and Belgium (Sachse et al 2015). In the another study conducted with real time pcr of 177 fecal samples taken from wild birds in the Temporary Wildlife Reception Centre Bogota/Colombia, 29.9% positivity for *C. psittaci* was determined (Ruiz Laiton 2022) Compared to our study, a very high rate of positivity were determined in these studies.

It was reported that the prevalence of *C. psittaci* in wild pigeons in Utrecht and Haarlem was determined as 2% by real time pcr (Herrmann et al 2006). There are different studies reporting that PCR is an effective method for detecting Chlamydiaceae (Karakuzulu 2003, Celebi and Ak 2006, Vanrompay et al 2007, Sara et al 2018, Tatari et al 2016). PCR analyzes have advantages over traditional serological methods such as being sensitive, reproducible and resulting in a short time. In addition, it has advantages such as being simple, fast, easy to standardize, working with many samples at the same time, being more convenient and





reliable than the conventional culture method (Hedemma et al 2006). Living organisms are not required in the pcr method and it has less risk for laboratory workers than other methods (Cetinkaya and Ayhan 2012). Current traditional PCR protocols use single copy genes such as the ompA gene or ribosomal RNA genes (16S-23S), which are amplification targets for the determination of avian *Chlamydophila* species (Herrmann et al 2006, Vanrompay et al 2007, Altintas et al 2020). It was reported by Celebi and Ak (2006) that *C.psittaci* DNA (ompA gene) was determined with the PCR method in 3.4% of stool samples taken from wild pigeons. In another study on domestic birds made by Sareyyupoglu et al. (2007); It has been reported that the DNA of the agents were determined by PCR method (ompA gene) in 91.5% of stool swap samples. These results contain a very high rate of positivity when compared to the results of our study. The reason for this; It is thought that it may be caused by the differences in the breeding, shelter, race, region and age of the animals from which the samples were taken. In this study the samples were domestic pigeons bred in cages rather than in the natural environment and they were bred in a more controlled way may have affected our results.

Conclusion

Chlamydiosis is a zoonotic disease and that it progresses as epidemics in animals has increased its importance in pet breeding. For this reason, knowing the epidemiology, pathogenesis of the disease, methods of prevention and treatment, and taking the proposed measures against the disease has become a necessity in terms of both economic and human health. In this study; *C. psittacii* ompA gene was determined by pcr method at a rate of 3% in dry feces samples taken from domestic pigeons and paraquets bred for hobby purposes in Konya. The importance of studies on public health both in the world and in our country is increasing day by day. It is thought that the data obtained as a result of this study will be a source for similar studies and will especially contribute to training projects aimed at informing our breeders.

Conflict of Interest

The authors did not report any conflict of interest.

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Ethical Approval

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