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Research Article

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Serosurvey of H9N2 Avian Influenza Virus in Local Domestic and Feral Pigeons

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Abstract

Background and aim: There are global concerns about members of the Columbidae family, namely pigeons or doves, for their role as the potential interspecies bridge in influenza a viruses ecology. This study was carried out to find the serological status of local domestic and feral pigeons to H9N2 avian influenza virus.
Materials and Methods: For this reason, serological survey was carried out from April to December 2022 in two-hundred blood samples (one-hundred samples from each group) of clinically healthy local domestic (*Columba livia domestica*) and feral pigeons (*Columba livia*) in different locations of Shiraz city, Southwestern Iran, using hemagglutination-inhibition (HI) test. The studied pigeons had no history of vaccination against any disease.
Results: The results showed that both groups of pigeons had antibody titers to varying degrees against H9N2 avian influenza virus. The mean HI titers and seroprevalence against H9N2 were 3.9/16% in local domestic and 4.31/25% in feral pigeons, respectively. According to statistical analysis of results by Student's t test, there was a significant difference ($p<0.05$) between two groups in terms of HI antibody titers and percentage of seropositive results.
Conclusions: Results of this study revealed that both local domestic and feral pigeons had HI antibody titers to some extent against H9N2 AIV. The number of positive results and antibody titers in feral pigeons were higher than local domestic pigeons due to free-flying properties of feral pigeons. Both groups of pigeons in this study can be potential healthy reservoirs of low pathogenic avian influenza virus and play an important role in spreading of AIVs as natural carriers.

Keywords: *Serosurvey, H9N2 Influenza Virus, Pigeon*

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Introduction

Avian influenza is a serious disease of poultry and some mammals caused by certain serotypes of the influenza a virus (AIV), a member of the family Orthomyxoviridae. Ducks and shorebirds are the global natural hosts in which AIVs usually cause sub-clinical infections (Alexander, 2000). Serotypes are classified by the combination of two major antigens on the virion, namely hemagglutinin (H) and neuraminidase (N). Until recently, 16 H-types and 9 N-types were acknowledged, but a 17th and 18th H-type plus a 10th and 11th N-type were recently discovered in bats (Tong *et al*., 2012; Tong *et al*., 2013). Based on their pathogenicity in poultries, they are divided into two pathotypes of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) (Swayne *et al*., 2008). The first avian influenza virus H9N2 subtype was isolated from turkeys in the United States in 1966 (Homme *et al*., 1970). Since then, the H9N2 virus has widely circulated in multiple avian species in Asia, Africa, Europe and the Middle East and caused significant economic losses (Guan *et al*., 1999; Sun & Liu 2015; Lee & Song 2013; Naeem *et al*., 1999; Nagy *et al*., 2017; Lin *et al*., 2017; Arbani *et al*., 2023; Sikht *et al.,* 2022; Jonas *et al.,* 2018; Xia *et al.,* 2023; Sagong *et al.,* 2023; Tan *et al.,* 2023). Although chickens appear to be the primary host for most poultry adapted H9N2 lineages, the virus is also endemic in minor poultry in many regions and appears to have evolved and adapted separately to members of these species, for example: quail, guinea fowl, partridge and pheasants (Zhou *et al*., 2016; Xu *et al*., 2007). Most avian species, such as domestic, pet, and wild birds, are natural and experimental hosts of avian influenza (AI) viruses (Liu *et al.,* 2014). Moreover, aquatic wild birds, such as ducks, are a natural reservoir of AI viruses (Swayne & Suarez, 2000). The ability of AI viruses to mutate, recombine, and adapt to new hosts and the risk they pose to public health make them important hazards (Boon *et al*., 2007). This virus proliferates in gastrointestinal and respiratory tracts and is transmitted by respiratory aerosols or fecal-oral routes (OIE, 2015). Birds from various orders are susceptible to AI; however, susceptibility, severity, and symptoms of the disease are different among them. Terrestrial birds, including pigeons and doves, can freely fly and easily contact with a wide range of domestic, nondomestic, and aquatic birds. Therefore, it can be said that Columbidae plays a substantial role in the ecology and interspecies transmission of LPAI and HPAI viruses (Boon *et al*., 2007; Abolnik, 2014; Kandeil *et al.,* 2017). In the study conducted by Kandeil *et al*., two reassortant H9N2 viruses were isolated from pigeons. This reassortment is the first to be reported in Egyptian poultry (Kandeil *et al*., 2017) According to previous studies, ducks, starlings, gulls, and pigeons are the least susceptible birds to this disease. Moreover, infection with AI in these birds is associated with few or no clinical signs (Perkins & Swayne, 2003). There are global concerns about members of the Columbidae family, namely pigeons and doves, regarding their potential role as the interspecies bridge in the ecology of type A influenza viruses. Feral pigeons and doves naturally associate with environments where food, water and nesting sites are available, leading to close association with humans and poultry in cities and on farms (Abolnik, 2014). Furthermore, they can fly long distances which increases the risk of the transmission of the virus. In some countries, such as Egypt, an increasing number of pigeons are raised in commercial farms as a source of meat for humans (Alquttory *et al*., 2016). Moreover, pigeons are sometimes kept as companions or for the purpose of racing which puts them in close contact with other birds and mammals. Pigeon racing is a popular sport that is growing in East Asia and is regarded as one of the multi-million dollar industries (Zhang *et al*., 2020). Scientific information on pigeon susceptibility to AIV infection under natural conditions is scarce and there is a disagreement amongst researchers over it and its consequences. However, the susceptibility of pigeons and their role in the transmission of the H9N2 influenza virus is still questionable. Therefore, further natural and experimental studies are required to elucidate whether or not Columbidae is involved in the transmission or spread of avian influenza viruses. The major objective of the present study was to evaluate the serosurvey of H9N2 avian influenza virus in local domestic and feral pigeons and potential role of columbids as reservoirs and vectors of H9N2 AIVs.

Materials and Methods

Two-hundred blood samples (one-hundred samples from each group) were taken from local domestic

pigeons (*Columba livia domestica*) and feral pigeons (*Columba livia*). Samples were randomly selected from clinically healthy pigeons in different locations of Shiraz city, Southwestern Iran, from April to December 2022. The studied pigeons had no history of vaccination against any disease. All birds were bled via brachial (wing) venipuncture (1 to 2 ml). Blood samples were maintained at room temperature and allowed to clot, then centrifuged for serum separation. Sera were separated and stored at -20ºC until used (Khawaja *et al*., 2005). Haemagglutination-Inhibition (HI) test was performed based on OIE protocol (OIE, 2015), using antigens with 4HA units (antigens were provided by Razi Vaccine and Serum Research institute). Samples with an antibody titer of > 4 with $\log_2$ were considered as positive samples. Results were analyzed using student's t test at the level of $p<0.05$ as a statistically significant level.

Results

In the present study, local domestic and feral pigeons were monitored and screened for H9N2 avian influenza antibody titers by hemagglutination-inhibition (HI) test. The HI assay is a relatively inexpensive procedure utilizing standard laboratory equipment, is less technical than molecular tests, is more specific and easily completed within several hours. Results revealed that some pigeons in both groups had varying degrees of antibodies against H9N2 avian influenza virus. HI antibody titers in local domestic pigeons were 2 to 5 $\log_2$ (mean 3.9 $\log_2$) and 3 to 7 $\log_2$ (mean 4.31 $\log_2$) in feral pigeons (Table 1). The percentage of seropositive results in local domestic and feral pigeons were 16% and 25%, respectively (Table1). According to the results of the student's t test, there was a significant difference between HI antibody titers and percentage of seropositive results between two groups ($p<0.05$).

HI titers group	Local Domestic Pigeons	Feral Pigeons
2	7	0
3	12	23
4	65	52
5	16	8
6	0	5
7	0	12
Mean titer	3.9	4.31
%Seropositive samples	16	25

Table 1. HI antibody titers, Mean titer and percentage of seropositive samples in Local Domestic and Feral Pigeons.

Discussion

In spite of positive antibody titers in two groups of pigeons, no clinical signs of disease were observed. The absence of clinical signs of influenza in pigeons, in spite of antibody titers in some birds, could be due to persistent exposure and acquired resistance of these birds to influenza virus in the environment, and therefore, these birds may be naturally vaccinated against this virus and play an important role in spreading of AIVs as natural carriers (Rehman *et al.,* 2022; Sikht *et al*., 2022; Xia *et al*., 2023; Sagong *et al*., 2023). Previously, it was reported that the pigeons are resistant or have an innate resistance to AI infection and are not transmission hosts, despite their immune dysfunction (Perkins & Swayne, 2002; Fang *et al*., 2006). In Iran, domestic pigeons are raised in open cages and are free to fly, and they frequently mix with wild pigeons and other wild bird species (Motamed *et al*., 2020), therefore HI antibody titers and seropositivity of this group in our experiment could be related to this. Presence of higher antibody titers in feral pigeons than local domestic pigeons, may be explained to the free-flying properties of feral pigeons which are ubiquitous throughout the world and often occupy a variety of habitats or due to its long period contact with other wild birds, so these properties may contribute to AIV transmission and maintenance in nature (Stallknecht & Shane, 1988; Al-Attar *et al*., 2008; Hadipour, 2010; Hadipour 2011). Lower HI antibody titers and seropositivity results in the present study compared with serological studies about H9N2 AIVs in other avian species such as backyard poultry and free- flying birds (Hadipour, 2010; Hadipour *et al.,* 2011), may be due to the difference in type and distribution pattern of AIV receptors. Expression of different types of sialic acid receptors of the influenza virus in tissue is thought to be one of the major determinants of its host range and tropism (Petersen *et al*., 2012). Franca *et al*., in their

study showed that there was moderate to strong expression of α2, 6 receptors in the respiratory tract of rock pigeon and mourning dove (Franca *et al*., 2013). Moreover, according to another research conducted by Liu et al. SAa2,6 Gal was the major receptor in the airway of pigeons, while in chickens SAa2,3Gal was the main receptor, which may be partly due to inefficient virus attachment and replication, and consequently resistance of pigeons to AIVs (Liu *et al*., 2007). Since there is a direct association between virus replication and disease virulence, the lack of replication fitness involving host specific co-factors and viral proteins would contribute to a decrease of replication efficiency and resistance of pigeons (Perkins & Swayne, 2003). Pigeons that are easily infected, efficiently replicate the virus, and shed the virus in high quantities through the oropharyngeal or fecal routes are considered significant agents in the ecology of the virus (Pantin-Jackwood & Swayne, 2009). From four continents, a total of 2046 apparently healthy pigeons were sampled for antibodies against AIV and only the results of 164 (8.01%) blood sample tests were positive (Abolnik, 2014). According to a study conducted by Fallah Mehrabadi et al. percentage of seropositive pigeon samples was 21% (Fallah Mehrabadi *et al*., 2016). Al-Attar et al. (2008) reported that the percentage of positive serum samples against H9N2 AIVs in wild pigeons was 81.82% and 50% in ELISA and HI tests, but all serum samples of starlings showed negative results (Al-Attar *et al*., 2008). In molecular study of H9N2 AIVs in pigeons, PCR results of all swab and tissue samples were negative (Motamed *et al*., 2020). Ducks and pigeons, unlike chickens, have innate immune systems, such as retinoic acid-inducible gene I (RIG-I), an RNA sensor in the cytoplasm that plays an important role in clearing the influenza infection (Abolnik, 2014). Therefore, it can be concluded that pigeons are susceptible to AI infection, while the virus cannot replicate or extend in this species. Moreover, pigeons are dead-end hosts, which means that despite the replication of the virus in them, it does not result in clinical diseases, shedding, and transmission through contact with other birds. If there is any nasal or cloacal shedding, the duration will be short with lower titer than that in other species (Boon *et al*., 2007; Brown *et al*., 2009; Pantin-Jackwood & Swayne, 2009; Zhang *et al*., 2020).

Conclusions

Members of the Columbidae family are usually kept as companions or pet birds, thereby have direct contact with humans. Besides, wild and domestic pigeons can fly for long distances, move between different regions, and contact with wild birds and poultries, their carcasses are accidentally consumed by other animals, and AI viruses are mechanically transmitted by their foot and feathers. Moreover, pigeon and doves are sold in live bird markets; therefore, pigeons act as a bridge species in the ecology of AIVs due to their potential to transmit viruses between poultry and migratory water fowl.

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بررسی سرولوژی ویروس آنفلوانزای طیور تحت تیپ H9N2 در کبوترهای اهلی و وحشی

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چکیده

زمینه و هدف: پرندگان خانواده کبوترسانان از جمله کبوترهای اهلی و وحشی، نگرانی جدی در ارتباط با انتقال بین گونه ای ویروس های آنفلوانزای تیپ A می باشند. تحقیق حاضر در ارتباط با بررسی سرولوژیک ویروس آنفلوانزای طیور تحت تیپ H9N2 در کبوترهای اهلی و وحشی می باشد.

مواد و روش‌ها: به منظور انجام این تحقیق، در فاصله زمانی اردیبهشت تا دی ماه ۱۴۰۱، تعداد ۲۰۰ نمونه خونی از کبوترهای اهلی و وحشی (از هر گروه تعداد ۱۰۰ نمونه) ظاهرا سالم و بدون علایم بالینی در مناطق مختلف شهر شیراز اخذ گردید و با آزمایش سرولوژی ممانعت از هماگلوتیناسیون (HI) مورد بررسی قرار گرفت. کبوتر های مورد مطالعه تاریخچه واکسیناسیون علیه هیچ بیماری نداشتند.

یافته‌ها: نتایج تحقیق حاضر نشان داد که هر دو گروه از کبوترها سطوح مختلفی از آنتی بادی علیه ویروس آنفلوانزای H9N2 را دارا بودند. میانگین تیتر آنتی بادی در کبوترهای اهلی و وحشی به ترتیب ۳/۹ و ۴/۳۱ و درصد موارد مثبت سرمی به ترتیب ۱۶ و ۲۵ درصد بود. تحلیل نتایج بر اساس آزمون آماری Student's t test، اختلاف آماری معنی داری بین دو گروه از لحاظ تیتر آنتی بادی و درصد موارد مثبت سرمی نشان داد ($p<۰/۰۵$).

نتیجه‌گیری: نتایج تحقیق حاضر نشان داد که هر دو گروه کبوترهای اهلی و وحشی به درجاتی دارای تیتر آنتی بادی علیه ویروس آنفلوانزای H9N2 بودند. تعداد موارد سرمی مثبت و تیتر های آنتی بادی در کبوترهای وحشی به مراتب بالاتر از کبوترهای اهلی بود که این امر می تواند به دلیل طبیعت آزاد زی این پرندگان و طیف پروازی گسترده تر و مواجهه با سایر پرندگان حامل باشد. در پایان می توان گفت که هر دو گروه کبوترهای اهلی و وحشی می توانند مخازن بالقوه و بدون علایم ویروس های کم حدت آنفلوانزا بوده و نقش مهمی در گسترش و پراکندگی این ویروس ها داشته باشند.

واژه‌های کلیدی: *بررسی سرولوژی، ویروس آنفلوانزای H9N2، کبوتر*

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