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Serological survey of high and low pathogenic avian influenza viruses in migratory waterbirds of Neor Lake, Ardabil, northwest of Iran

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Abstract

Avian influenza viruses (AIV) are a highly contagious disease with economic and public health impact that belongs to the Orthomyxoviridae family. Wild migratory waterbirds are known as the main reservoirs of AVI which can distribute viruses to poultry and humans. Considering that Neor Lake, located in the southeast of Ardabil, is the habitat of migratory waterbirds from the countries around the region, there is a risk of transmission and distribution of influenza viruses from wild migratory birds. The present study aimed to monitor the seroprevalence of AI virus H5, H7, and H9 subtypes in migratory waterbirds of Neor Lake, Ardabil, northwest of Iran. A total of 101 blood samples were randomly collected from seven migratory bird species. HI, test was carried out on sera based on OIE protocol to detect H5, H7, and H9 subtypes. In this study, H5N2 and H7N1 antigens were used for the detection H5 and H7 subtypes, respectively. Suspicious samples on the first test were re-examined with H5N1 and H7N7 antigens. H9N2 antigen was used for the detection H9. Results of the tests performed on sera were negative for H5N1, H5N2, H7N1 and H7N7 viruses. Overall, 11.89 % of birds were seropositive for the H9N2 virus. The highest seroprevalence of the H9N2 virus was in Mallard (25 %) and the lowest seroprevalence was in Whooper swan (10.5 %). In this survey, despite seronegative evidence against H5 and H7 of subtypes, because of the prevalence of high pathogenic avian influenza (HPAI) viruses in countries of the region and some areas of Iran, it is recommended to periodically implement surveillance and control programs such as monitoring of circulating AIV in migratory waterbirds of Neor Lake.

Introduction

Influenza viruses are highly contagious, zoonotic, and acute respiratory diseases, which belong to the family of Orthomyxoviridae (1, 2). Influenza viruses are classified as types A, B, and C based on the antigenic differences of nucleoprotein (NP) and matrix protein (M) (2-4). Influenza A viruses infect humans, most avian species such as waterfowl, pets, wild birds domestic poultry, and some mammals (3, 5, 6). Influenza A viruses are classified into two

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High Pathogenic Avian Influenza (HPAI) and low pathogenic Avian influenza (LPAI) based on pathogenicity in birds (2, 7).

HPAI viruses cause a very severe disease with high rates of morbidity and mortality in poultry, in particular in birds of the order Galliformes (mainly chickens, and turkeys) (3, 8, 9). LPAI viruses cause mild and primarily respiratory disease with low economic losses (1, 2). HPAI and LPAI viruses can also be transmitted to humans. (9-11). To date, all HPAI viruses belong to two H5 and H7 subtypes, but all H5 and H7 subtypes are not among HPAI and some of these subtypes have low pathogenicity. The H9 subtype is classified in the group of LPAI (12-14).

Avian influenza viruses (AIV) have been identified from 13 different orders, especially wild waterbirds, among which two orders Anseriforme (ducks, geese, and swans) and Charadriiforme (gulls, terns, shorebirds) play the most important role in virus epidemiology and ecology. (3, 13, 14). The Avian Influenza virus in Wild waterbirds does not have recognizable clinical symptoms (except for H5N1). Wild waterbirds such as ducks are the natural reservoirs of all subtypes of avian influenza viruses that are responsible for the spread of these viruses into poultry and other avian species and also other wild animals, domestic animals, and humans worldwide (10, 15-18).

LPAI virus was first detected in wild waterbirds in the 1970s in the USA and was isolated from poultry in China in the early 1990s (6, 19). In 1991 and 1997, H9N2 virus outbreaks were also reported in other Asian countries, the Middle East, the USA, North America, and European and African countries (3, 19). In Iran, the H9N2 virus (LPAI) occurrence, was first reported from commercial poultry around Tehran and Qazvin in July 1998, and it quickly spread among the country's farms and is now endemic (19).

In 2005, the first occurrence of H5N1 (HPAI) viruses in wild birds was detected at Qinghai Lake in China; after that, subsequently was reported from different avian species such as wild waterbirds and domestic poultry in Asia, Europe, the Middle East, Africa, and North American (8, 20-23). H5N8 virus was first identified in live bird markets in China in 2010 (24). The first outbreaks of the H5N8 virus in domestic and wild birds were reported in 2014 in South Korea, Japan, and Siberia, and were recognized by early 2015 in many Europe, Africa, and America (14, 24). Some subtypes of HPAI viruses are reported in Iran: H5N1, H5N8 and H5N6. The first occurrence of the H5N1 virus was confirmed in wild swan corpses from at Anzali wetland of Iran in February 2006 (25) and the second outbreak was also reported by the Iranian Veterinary Organization (IVO) from dead native chickens in Babolsar Mazandaran province on January 2007 (26). In November 2016, the H5N8 virus was been detected in a layer farm located in Malard, Tehran Province, Iran (11). In November 2016, a die-off of wild birds was reported in the Miankaleh wetland located southeast of the Caspian Sea due to H5N6 subtype of AI virus (11).

Neor Lake is one of the national wetlands of Iran, the habitat of some wild waterbirds during migration from Siberia, Azerbaijan, and northern Russia. Migratory birds usually arrive in October and either remain until February or migrate further south. There is a possibility of new and non-endemic viruses entering to country through migratory birds and the occurrence of new epidemics in native and industrial poultry. Serology is one of the outstanding figures of diagnostic methods for influenza viruses (1, 3, 7). Therefore, a survey of AIV for migratory wild birds is necessary to better understand its epidemiology, to evaluate the infection level and introduction subtypes of AIV. Despite the great importance, there is no information about the outbreak of AIV H5, H7, and H9 subtypes in migratory waterbirds at Neor Lake, it seemed necessary to conduct such research. Thus, the purpose of this study was to survey the seroprevalence of H5, H7, and H9 subtypes of AIV in migratory waterbirds of Neor Lake, southeast of Ardabil City, Iran.

Materials and Methods

Sampling design and data collection

Active surveillance for H5, H7, and H9 subtypes of AI virus was conducted in Neor Lake, located in the southeast of Ardabil city, northwest of Iran from 1 October 2018 to 2 January 2019. A total of 101 blood samples were randomly taken from wild waterbirds belonging to several different bird species including white graylag geese, whooper swan, pelicans, grey herons, mallards, flamingos, and white-winged terns.

1-2 ml of blood was taken from the wing vein from each bird with a 2.5 ml syringe (25). The blood was allowed to clot and preserved at room temperature for one hour to separate the serums. Then each separated serum was placed in a 1.5 ml micro tube (3, 25). The samples were coded, and bird species were recorded, and then the tubes were transferred to the laboratory in a cooler with ice pack, and stored in a freezer at -20 °C (3). After completing the sample collection phase, the stored serum samples were removed from the freezer and prepared at laboratory temperature for the Hemagglutination inhibition (HI) test to differentiate AI virus H5, H7, and H9 subtypes (3, 25).

Serological test

The hemagglutination inhibition (HI) test was used for the detection of H5, H7, and H9 subtypes of the AI virus according to the guidelines of the Office International des Epizooties (OIE) and Iranian Veterinary Organization (IVO) (3, 11, 12). HI, the test was performed in two stages for differential of H5 and H7 subtypes. In the HI test, antigens H5N2 and H7N1 were used for H5 and H7, respectively (27). Suspicious samples on the first test were re-examined with antigens H5N1 and H7N7 in the second HI test (17, 27). In addition, it was investigated H9 subtype using the H9N2 antigen (17). Sera with titers ≥4 (i.e. log2) (above 16) were considered positive in the present study (27).

Results

The number of samples collected from waterbirds in Neor Lake and sera examined for subtypes of AIV are shown in Tables 1 and 2. In this study, 101 blood samples were randomly taken from seven wild waterbird species and all sera were negative for H5N1, H5N2, H7N1, and H7N7 viruses (Table 1). Overall, out of 101 birds sampled, 11.89 % birds were seropositive for LPAI (H9N2) virus. Among seven bird species, four types of bird showed seroprevalence for the H9N2 virus. The highest seroprevalence of the H9N2 virus was in Mallard (25 %) and the lowest seroprevalence was in Whooper swan (10.5 %) (Table 2).

In the HI test, 11.89% of serum samples were found to be positive for H9N2 virus. While 81.11% of serum samples were negative for this virus. In the examined sera, the highest and lowest frequency of positive titers were observed in antibody titer 4 (4.95%) and titer 7 (0.99%), respectively (Table 3).

Discussion

There are reports from different parts of the world about the outbreak of subtypes of AI virus from wild and aquatic birds (1, 11, 17, 27-29). According to the investigations conducted on the genealogy of viruses isolated from these birds, the majority belong to viruses circulating among chickens. Therefore, wild and migratory birds are considered important factors in the epidemiology of influenza viruses (1, 17, 23).

Kocan et al. (1980) with identifying H1N1, H1N2, and H6N1 subtypes of AI virus from migratory ducks in Oklahoma, showed that these viruses can be transmitted to other birds through water contaminated with infected feces. In one investigation on the seroprevalence of AI virus in Italian Waterfowl, 52.2% of ducks were positive (4). Studies by Gilbert et al. (2007) in Thailand showed that free-ranging ducks in winter play an important role in the transmission of HPAI viruses. Kistler et al. (2012) collected 3,205 sera samples from Canadian geese of 9 different states in the United States, of which the seroprevalence of avian influenza A virus was 15%. Zhu et al. (2018)

isolated three different isolates of H9N2 virus from domestic geese with influenza symptoms in China. \Mon et al. (2017) collected 78,804 serum samples from domestic ducks reared outdoors in Myanmar between 2006 and 2019 that 11.9 % of the samples were positive for subtype H5 of HPAI virus.

Table1. Seroprevalence of HPAI viruses in waterbird species of Neor Lake

Waterbirds	No. of tested sample	No. of positive Sample			
		H_5N_1	H_7N_1	H_5N_2	H_7N_7
Mallard	24	0	0	0	0
Whooper swan	19	0	0	0	0
Graylag geese	17	0	0	0	0
Grey Heron	15	0	0	0	0
Pelican	12	0	0	0	0
White-winged tern	9	0	0	0	0
Flamingo	5	0	0	0	0
Total	101	0	0	0	0

No: Number

Table2. Seroprevalence of LPAI virus in waterbird species of Neor Lake

Waterbirds	No. of tested sample	No. of positive sample	
		No	%
Mallard	24	6	25
Whooper swan	19	2	10.5
Graylag geese	17	3	17.6
Grey Heron	15	0	0
Pelican	12	0	0
White-winged tern	9	1	11.1
Flamingo	5	0	0
Total	101	12	11.89

No: Number. %: Percentage

Table 3. Frequency and percentage of detected HI antibody titers against H9N2 virus

HI antibody titer	≤ 3	4	5	6	7
No. of tested samples	89	5	2	3	1
%. of tested samples	88.11	4.95	1.98	2.97	0.99

Titer: i.e. log2. No: Number. %: Percentage

In Iran, there are various reports from different regions about the outbreak of different subtypes of AI virus in different bird species. Fereidouni et al. (2005) examined 217 serum samples prepared from 25 species of migratory waterbirds with ELISA test that influenza virus infection rate was 35.5% in 14

different bird species, and the seroprevalence against avian influenza A virus was significantly higher in Anseriformes (64%) compared to non-Anseriformes (11). Shoushtari et al. (2007) reported the cause of death of 130 swans in the Anzali wetland of Iran using histopathology and TR-RCP methods caused by the H5N1 subtype of HPAI virus. Fereidouni et al. (2017) conducted research on the prevalence of the H9N2 AI virus in 1146 wild waterfowl (from 45 different species) wintering in six different provinces of Iran, the prevalence rate in these migratory waterfowl was 3.05% using the TR-RCP method and in some regions and species up to 8.3% reported. The studies conducted by Hadipour et al. (2011) in 4 different regions of Iran showed that in none of the 400 serum samples belonging to scavenging ducks, antibodies against the H5N1 virus were detected, but seroprevalence H9N2 virus there was 80.92%. The survey conducted on migratory aquatic birds of the six selected habitats of Urmia Lake in 2011-2019 showed that 5% of the samples were positive for AIV (30). Azizpour (2023) reported that all sera tested from different bird species in live bird's markets of Ardabil city were negative for H5N1, H5N2, H7N1, and H7N7 avian influenza viruses, While 18% of the sera were diagnosed as positive for H9N2 virus.

In this study, no antibody against HPAI viruses (H5 and H7 subtypes) was detected in any of the serum samples belonging to migratory waterbirds, but LPAI (H9N2) virus was detected in 11.89% of the samples. This survey is the first report to assess the epidemiology of HPAI virus H5 and H7 subtypes and LPAI virus H9 subtype in migratory waterbirds of Lake Neor, Ardabil, northwest of Iran. Seroprevalence against the H9N2 virus in mallard, graylag geese, white-winged tern, and whooper swan were 25 %, 17.6%, 11.1%, and 10.5%, respectively. In our investigation presented here, the highest rate of seropositive was observed in mallard. This finding is consistent with previous studies (1, 3, 4, 23) waterbirds that wild waterbirds especially mallards may play a major role in the epidemiology of the avian Influenza virus in Iran.

Conclusion

The results of this study show low seroprevalence of the LPAI (H9N2) virus and its circulation in different species of migratory waterbirds of Neor Lake. However according to the findings of this survey, there was no seropositive against H5 and H7 subtypes of the HPAI virus in the migratory waterbirds. Considering that Lake Neor is the habitat of migratory waterbirds passing through the countries of the region and because of the outbreak of HPAI viruses in the countries of the surrounding region, especially the Republic of Azerbaijan, which is adjacent to the northern provinces of Iran. There is a risk of transmission and spread of non-endemic viruses to poultry and humans by migratory birds as the main reservoir of AI viruses. Considering major public health concerns and significant economic losses regarding influenza viruses, special attention to biosecurity issues such as preventing direct and indirect contact of poultry with migratory waterbirds, strict observance of health principles, and implementation of surveillance and control programs such as continuous monitoring of circulating influenza viruses in migratory waterbirds of Neor Lake, especially during the migration season and periodic vaccination of native poultry around Neor Lake are recommended.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of wildlife birds were followed.

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