Iranian Journal of Virology 2010;4(2): 7-16
©2010, Iranian Society for Virology

Original Article

Evidence of H1 and H3 Influenza Virus Infection in Captive Birds in Tehran from November 2008 to February 2009

Heydarchi B[1, 2], Hosseini SM[1]*, Tavassoti-Kheiri M[2], Sheykhi N[3], Mousavi SF[1], Khaltabadi-Farahani R[1], Bashar R[2], Fotouhi F[2], Bolurieh T[2]

1. Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, GC, Iran.
2. Influenza Research Lab, Pasteur Institute of Iran, Tehran, Iran.
3. Department of Clinical Sciences, Faculty of Specialized Veterinary Science, Science and Research Campus, Islamic Azad University, Tehran, Iran.

Abstract

Background and Aims: Influenza A virus infects birds and some mammalian including human. H1 and H3 subtypes are circulated in both human and birds' population. To determine the prevalence of the mentioned subtypes in birds, different captive bird species in Tehran zoo, and affiliated centers in Tehran were investigated for virus infection.

Methods: In this study, Between November 2008 and February 2009, 76 cloacal swabs and serum samples were collected from 5 orders of *Anseriformes*, *Galliformes*, *Columbiformes*, *Pelicaniformes* and *Phoenicopteriformes*. Antibody surveillance was undertaken by haemagglutination inhibition assay and for detection of influenza virus genome RT-PCR technique was used.

Results: In total, 57.7% and 88.75% of bird sera were seropositive against H1 and H3 viruses respectively. The highest GMT value and the greatest antibody titers were observed in *Galliformes* order particularly in chicken species. Influenza A genome was not detected in any of the samples by RT-PCR using M gene.

Conclusion: Results of this study indicated that seropositive birds were infected during the last or possibly previous years with H1 and H3 virus strain.

Keywords: Hemagglutination Inhibition Test (HI); Real time polymerase chain reaction (RT-PCR); Captive birds; Tehran

Introduction

Influenza A viruses which belong to *Orthomyxoviridae* family could infect different avian and mammalian species. The natural reservoir of influenza A viruses are aquatic birds such as *Anseriformes* and *Charadriformes* in which different combinations of all 16 HA and 9 NA has been found (1).

Although there has been no report of avian influenza isolation from chickens, turkeys and other *Galliformes* species in their wild states and they are not natural reservoir of influenza viruses (2), humankind activities such as captivity, domestication and agriculture have changed the natural ecosystem of birds. Zoological collections are man-made systems where captive birds have close contact to free-flying birds, park keeping staff and visitors to parks and zoos. Variety of host bird species in these new niches may alter the ecology and epidemiology of influenza viruses therefore viruses could transmit interspeciesly and be adapted in new hosts (3).

***Corresponding author:** Seyed Masoud Hosseini, Ph.D, Department of Microbiology, Faculty of Biological Sciences, Shahid Beheshti University, GC, Tehran, Iran.
Tel: (+98) 21 29 90 27 21, Fax: (+98) 21 22 73 60 44
Email: Ma_Hosseini@sbu.ac.ir

[Downloaded from journal.isv.org.ir on 2026-11-08]
[DOI: 10.21859/isv.4.2.7]

Although H1N1 and H3N2 viruses are currently circulating among human population (4), but it is believed that all influenza A viruses in mammalians are originated from the avian genes (2). In the 20th century, Asian influenza pandemic of 1957 caused by a reassortant H2N2 virus that some genes of it came from avian viruses and in H3N2 virus of 1968 pandemic (Hong Kong influenza pandemic), the genes of virus originated from reassortment of human and avian genes (5, 6).
In recent years, direct transmission of H5N1 (4, 7), H7N7 (8) and H9N2 (9) viruses from birds to human have been reported. Therefore it is necessary to monitor places such as parks and zoos to track down the influenza A viruses. The present study was undertaken to provide insight about the possibility of influenza H1 and H3 viruses' infection in captive birds of man-made systems such as zoo and parks where the birds have close contact to human and other influenza viruses' hosts.

Methods

Sample collection

From November 2008 to February 2009 (during influenza season in Tehran), 76 cloacal swabs and serum samples were collected from birds in Tehran Zoo, Saiee Park and Pardisan Park. Studied birds included, 3 species of Anseriformes order (plus 5 hybrid species of ducks), 7 species of Galliformes order, 1 species of Columbiformes order, 1 species of Pelicaniformes order and 1 species of Phoenicopteriformes order. Cloacal samples using Dacron swabs were transferred into the viral transportation medium (VTM). The VTM was prepared with sterile glycerol-PBS (1:1) solution (pH 7.2) containing Benzyl-Penicillin, Streptomycin and Amphotericin. The samples containing VTM was centrifuged at 3000 × g for 10 min and the supernatants were stored at -20°C for further process. To harvest sera, blood samples were centrifuged at 2500 ×g for 15 min and stored at -20°C for further studies.

Viruses

NIBSC standard human influenza A/ New Caledonia/ 20/ 99 (H1N1) and A/ Panama/ 2007/ 99 (H3N2) viruses used in this study were passaged in Madine Derby canine kidney cells (MDCK) in Influenza Unit of the Pasteur Institute of Iran (10).

Vaccination of chickens

Positive control serum for HI assay was not accessible. Therefore, it was decided to immunize seronegative chickens (confirmed with ELISA) with inactivated human influenza vaccine influvac 2008/2009 (Solvay Pharmaceuticals B.V. Weesp, The Netherlands) containing A/Brisbane/59/2007 (H1N1) and A/Brisbane/10/2007 (H3N2) antigens. Group 1, including 4 chickens was vaccinated by injecting 0.25 ml of vaccine (half-dose) containing 7.5 μg of each antigen. Group 2, including 4 chickens, were injected by full-dose vaccine (0.5 ml and 15 μg of each antigen) whereas 2 chickens of group 3 (control group) were not vaccinated against influenza viruses. Vaccination was performed intramuscularly in the breast muscle of birds at day 0 and 21. Chicken sera were collected at days 0 (before first injection), 21 (before second injection) and 35 to obtain antibodies against H1 and H3 viruses.

Serological assay

The Haemagglutination Inhibition (HI) assays were carried out to determine antibody titers against A/New Caledonia/20/99 (H1N1) and A/Panama/2007/99 (H3N2) viruses in serum samples (11). To measure antibody titers, beta procedure of HI test was used (12). The sera of all the species except chickens were pre-treated with 10% chicken red blood cells to remove non-specific hemagglutinating reagents. Also sera of hunting birds (pheasant and partridge), quail and guinea fowl were treated in a water bath at 56°C for 30 minutes. Afterward a 2-fold serial dilution of the treated sera was made in 96-well U shaped plate using PBS (pH 7.2). A 25μl of 4 HA unit antigen was added to each well. The plates were incubated for 30 minutes at room temperature, and finally 1% chicken RBCs suspension (in PBS containing 0.5% v/v bovine serum albumin) added and the plates again incubated for 40 minutes at room temperature. HI end point titers were determined as the reciprocal of the highest dilution that produced complete inhibition of haemagglutinin activity. A titer of 16 and

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

higher was considered as a positive. Results were validated by negative control serum with titer less than 8, and positive control serum with a definite titer more than 16. In HI assay for each serum sample, RBC control well, serum control well (to control the removal of non-specific hemagglutinating reagents in serum) and virus control well was conducted.

RT-PCR

RNA extraction from cloacal specimens was performed using RNXTM-Plus solution (CinnaGen, Tehran, Iran). The procedure was carried out according to the manufacture's recommendation and RNA used for cDNA synthesis. Briefly, the mixture of 10µl RNA and 2µl Uni12 primer (5'-AGC-AAA-AGC-AG-<G>-3') was incubated at 70°C for 5 minutes and immediately chilled on ice. Thereafter, the components of RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Canada) including 5 x reaction buffer (4µl), RibolockTM RNase Inhibitor 20U/ml (1µl), dNTP mix 10mM (2µl) and ReverAidTM M-MuLV Reverse Transcriptase 200u/µl (1µl) was added to the tube containing RNA and primer. The tube was incubated for 5 minutes at 25°C followed by 42°C for 60 minutes and finally at 70°C for 5 minutes. M primers targeting matrix gene of influenza type A viruses were used in PCR (MF: 5' –GACTCAAATGTCAAGAACCTTTA-3, MR: '5'- CCACTTATTTCCTCTCTGTTTAG-3') to amplify a 132bp fragment. The PCR reaction mixture consisted of 6µl cDNA, 12.5µl Taq DNA Polymerase Master Mix RED kit (Ampliqon, Denmark) which contained Tris-HCl 150mM pH 8.5, (NH4)2SO4 40mM, MgCl2 1.5mM, 0.2% Tween 20, dNTPs 0.4mM, Ampliqon Taq DNA Polymerase (0.05Unit/ul), 1µl of each forward and reverse of M-primers (Table 1) and distilled water to the final volume of 25µl. The thermocycling conditions were 95°C for 5 minutes (primary denaturation), 35 cycles of 94°C for 40 seconds (denaturation), 59°C for 40 seconds (annealing) and 72°C for 40 seconds (extension), and the final extension at 72°C for 5 minutes which terminated the PCR reaction. The PCR products were analyzed by running 2% agarose gel electrophoresis.

Sensitivity of RT-PCR

The sensitivity of RT-PCR was assessed by ten-fold serial dilution of extracted H1N1 RNA (A/ New Caledonia/ 20/ 99). Subsequently, RT-PCR was performed according to the procedure described above. The highest dilution of RNA with positive RT-PCR was considered as the sensitivity cut-off.

Results

Vaccination of chickens

The serologic results of vaccinated chickens are summarized in Table 1. Mean titer of HI antibodies against human vaccine antigens in full-dose vaccinated chickens were higher than half-dose vaccinated (4 fold for H1 and 2 fold for H3 antibodies). Control group showed no antibody titers in HI assays.

Prevaccination chicken sera (day 0) were seronegative for human antibodies. Antibody mean titers against vaccine antigens at day 35 were significantly higher than those at day 21. First vaccine injection resulted in H3 antibody titer production in 75% and 100% of half-dose and full-dose vaccinated chicken respectively, whereas H1 antibody was not detected in any of the sera. At day 35 (2 weeks after second injection), 34% of half-dose vaccinated chickens had H1 antibody titer and 100% showed antibody against H3 antigen. Group 2 (full-dose vaccinated) had 100% seropositivity for each vaccine antigens. Positive serum resulted from vaccination was used as control serum in HI assay.

Serological assay

In total, 57.7% (41 of 71) of bird sera were positive for antibodies against H1 subtype (HI titer 1:16≤ was considered as positive) and also 62 of the 70 birds' sera were positive for H3 subtype antibody with overall percentage of 88.75%.

HI results for H1 subtype

Results of HI assay against H1 subtype among different bird species is shown in Table 2.

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

Table 1. The results of chicken vaccination.

Day Post-Vaccination	**0**		**21**		**35**	
Antigen Subtypes	H1	H3	H1	H3	H1	H3
Half-dose vaccinated Chickens	0%*	0%	0%	75% (MT: 16)	34% (MT: 16)	100% (MT: 16)
Full-dose vaccinated Chickens	0%	0%	0%	50% (MT: 16)	100% (MT: 64)	100% (MT: 32)
Unvaccinated Chickens	0%	0%	0%	0%	0%	0%

- MT: Mean Titer is the average titer of HI in each group.
*: % represents the percentage of positive serum samples in each group.
- Vaccination was carried out at day 0 and 21. Sera collection was at day 0, 21 and 35.

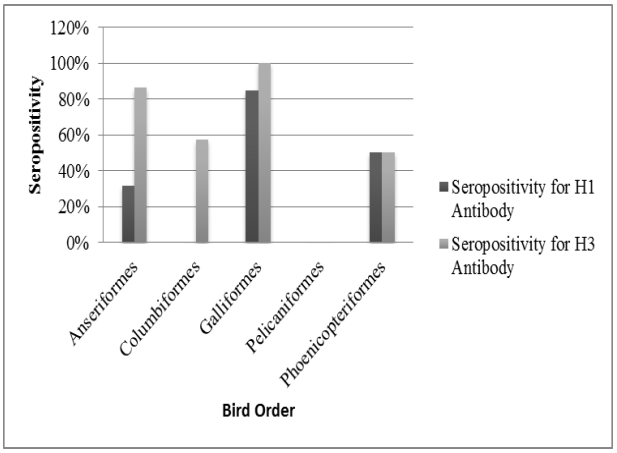


Fig. 1. Percentage of seropositivity against H1 and H3 subtypes in different bird orders.

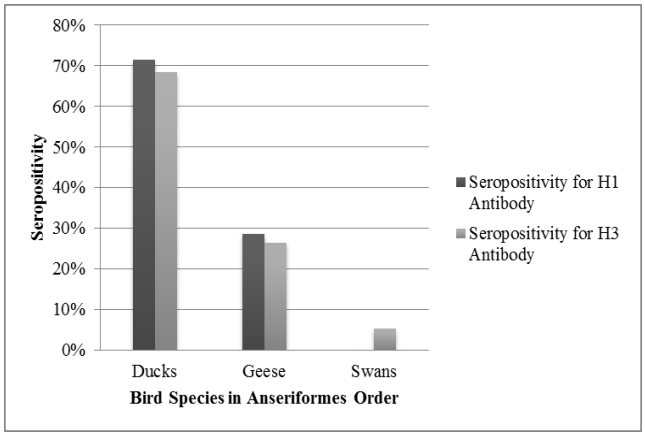


Fig. 2. Contribution of different bird species in seropositivity of *Anseriformes* order.

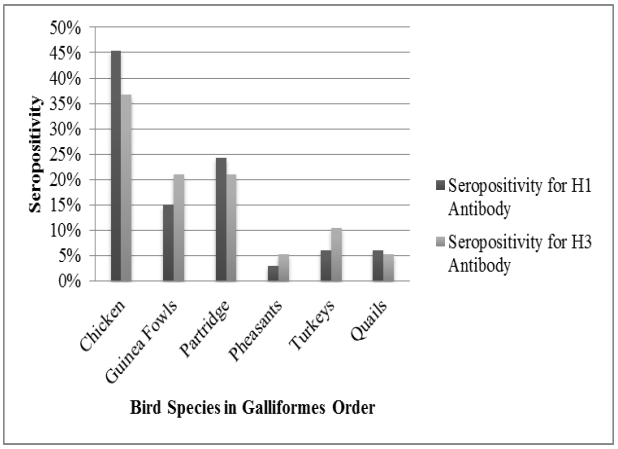


Fig. 3. Contribution of different bird species in seropositivity of *Galliformes* order.

Total Geometric Mean Titer (GMT) value of H1 antibody titer was 16 with the antibody range of 2 to 256. As shown *Galliformes* had the greatest GMT value of 30.33 while that in *Anseriformes* was 9.07. *Phoenicopteriformes* came afterward with the GMT value of 8 followed by *Columbiformes* (3.62) whereas the only sample of *Pelicaniformes* had antibody titer of 8.

Of *Anseriformes* order, Geese species had GMT of 10.55 which was higher than Ducks species with GMT of 8.72. In serum of Swan sample no antibody titer against H1 subtype was detected. In *Galliformes* order, the highest

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

Table 2: Distribution of Antibody Titers against Avian Influenza H1 Subtype Using Haemagglutinaton Inhibition Assay

Bird **Order**/Species	**No. of Samples**	**No. of Positive Samples**	**% of Positive Samples**	**Antibody titer against H1 subtype using HI assay**									
				1:2	**1:4**	**1:8**	**1:1**	**1:32**	**1:64**	**1:12**	**1:256**	**1:51**	**GMT**
Anseriformes													
Ducks	16	5	31.25%	1	2	8	4	1	-	-	-	-	8.72
Geese	5	2	40%	-	-	3	2	-	-	-	-	-	10.55
Swans	1	0	0%	-	-	1	-	-	-	-	-	-	-
	22	7	31.81%	1	2	12	6	1	-	-	-	-	9.07
Columbiformes													
Pigeons	7	0	0%	2	4	1	-	-	-	-	-	-	3.62
Galliformes													
Chickens	15	15	100%	-	-	-	-	6	2	5	2	-	73.51
Guinea fowls	8	5	62.5%	-	-	3	4	1	-	-	-	-	13.45
Partridges	8	8	100%	-	-	-	5	1	2	-	-	-	24.67
Pheasants	2	1	50%	-	-	1	-	-	1	-	-	-	22.62
Turkeys	4	2	50%	-	-	2	2	-	-	-	-	-	11.31
Quails	2	2	100%	-	-	-	1	1	-	-	-	-	22.62
	39	33	84.61%	-	-	6	12	9	5	5	2	-	30.33
Pelicaniformes													
Pelicans	1	0	0%	-	-	1	-	-	-	-	-	-	-
Phoenicopteriformes													
Flamingos	2	1	50%	-	1	-	1	-	-	-	-	-	8
Total	**71**	**41**	**57.7%**	**3**	**7**	**20**	**19**	**10**	**5**	**5**	**2**	**-**	**16**

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

Table 3: Distribution of Antibody Titers against Avian Influenza H3 Subtype Using Haemagglutinaton Inhibition Assay

Bird **Order**/Species	**No. of Samples**	**No. of Positive Samples**	**% of Positive Samples**	**Antibody titer against H3 subtype using HI assay**									
				1:2	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**	**1:256**	**1:512**	**GMT**
Anseriformes	16	13	81.25%	-	-	3	9	2	2	-	-	-	18.22
Ducks	5	5	100%	-	-	-	3	2	-	-	-	-	21.11
Geese	1	1	100%	-	-	-	-	-	1	-	-	-	-
Swans													
	22	19	86.36%	-	-	3	12	4	3	-	-	-	19.94
Columbiformes													
Pigeons	7	4	57.14%	-	-	3	2	2	-	-	-	-	14.49
Galliformes	14				-								
Chickens	8	14	100%	-		-	-	4	2	5	3	-	90.5
Guinea fowls	8	8	100%	-	-	-	-	4	4	-	-	-	45.25
Partridges	2	8	100%	-	-	-	-	3	3	2	-	-	58.68
Pheasants	4	2	100%	-	-	-	1	-	-	1	-	-	45.25
Turkeys	2	4	100%	-	-	-	3	1	-	-	-	-	19.02
Quails		2	100%	-	-	-	-	1	-	1	-	-	64
	38	38	100%	-	-	-	4	13	9	9	3	-	57.36
Pelicaniformes													
Pelicans	1	0	0%	-	-	1	-	-	-	-	-	-	-
Phoenicopteriformes				-									
Flamingos	2	1	50%		1	-	-	1	-	-	-	-	16
Total	**70**	**62**	**88.57%**	**-**	**1**	**7**	**18**	**20**	**12**	**9**	**3**	**-**	**33.29**

[DOI: 10.21859/isv.4.2.7] [Downloaded from journal.isv.org.ir on 2026-11-08]

GMT value of H1 antibody was found in chickens (73.51) followed by Partridges (24.67), Pheasants and Quails (22.62), Guinea fowls (13.45) and Turkeys (11.31).

Figure 1 represents data on the distribution of H1 antibody among the birds' sera. As shown 57.7% (41/71) of serum samples had antibody. *Galliformes* had the highest seropositivity prevalence (33/39, 84.61%) against H1 subtype whilst 31.81% (7/22) of *Anseriformes* and 50% (1/2) of *Phoenicopteriformes* were found seropositive. In *Columbiformes* and *Pelicaniformes* no antibody titer of H1 subtype was detected. Figure 2 demonstrates the contribution of each bird species in seropositivity of *Anseriformes* order against H1 subtype. Ducks had the seropositivity of 71.42% (5/7) whereas the seropositivity of Geese was 28.57% (2/7). As illustrated in figure 3, the highest contribution of seropositivity in *Galliformes* belonged to chicken species (45.45%, 15/33) followed by 24% (8/33) in Partridge species, 15.15% (5/33) in Guinea fowl species, 6% (2/33) in Turkey and Quail species and 3% (1/33) in Pheasant species.

HI results for H3 subtype

Out of 70 serum samples collected from different bird species, 62 samples showed antibody titer against H3 subtype with total GMT value of 33.29 in antibody range of 4 to 256. *Galliformes* had the highest GMT value of 57.36 (Table 3) following by *Anseriformes* (19.94), *Phoenicopteriformes* (16) and *Columbiformes* (14.49). The sample of *Pelicaniformes* had no antibody titer against H3 subtype (titer of 8).

Analysis of data implied that in *Anseriformes* order, GMT value in Geese was 21.11 in comparison with the GMT value of 18.22 in Ducks. Swan serum had no antibody titer against H3 subtype. In *Galliformes* order, the greatest GMT value was in chicken species (90.50) whereas GMT value was 64 in Quail species, 58.68 in Partridge species, 45.25 in Guinea fowl and Pheasant species and 19.02 in Turkey species. Prevalence of antibody titer against H3 subtype in 70 birds was 88.57% (Figure 1). The highest seroprevalence rate of 100% was in *Galliformes* (38/38). *Anseriformes* showed H3 antibody seropositivity of 86.36% (19/22). It was seen that 57.14% (4/7) of *Columbiformes* and 50% (1/2) of *Phoenicopteriformes* were seropositive for H3 antibody in serum samples. Pelican serum was not seropositive for H3 subtype antibody.

Figure 2 represents the distribution of bird species against H3 subtype in *Anseriformes*: Seropositivity for H3 antibody was 68.42% (13/19) in Ducks, 26.31% (5/19) in Geese and 5.2% (1/19) in Swans. The highest seropositivity in *Galliformes* order was related to chickens (36.84%, 14/38) followed by 21.05% (8/38) in Guinea fowls and Partridges, 10.52% (4/38) in Turkeys and 5.26% (2/38) in Pheasants and Quails (Figure 3).

In tested birds of Pardisan Park, 44.4% (4/9) of serum samples had H1 antibody whereas H3 antibody was observed in all 9 sera. Thirteen of the 30 (43.3%) birds' sera of Saiee Park had antibody titers against H1 subtype as compared to 25 of the 29 tested sera (86.2%) that showed titers for H3 antibody. Of 32 birds sera belonged to Tehran Zoo, 24 (75%) were positive for H1 antibody and 28 (87.5%) had antibody titers against H3 subtype.

Molecular assay

The sensitivity of RT-PCR was determined to be 0.1ng template RNA genome. No avian influenza viruses' genome was detected in any of 76 cloacal samples collected from Tehran Zoo, Saiee Park and Pardissan Park in Tehran between November 2008 and February 2009.

Discussion

To assess the prevalence of H1 and H3 viruses in captive birds of Tehran, cloacal swabs and serum samples were collected from Tehran Zoo, Saiee Park and Pardisan Park. Our study is one of the rare surveillance of H1 and H3 influenza viruses among a variety of captive bird species in Iran. Studied sites had the importance in the ecology of influenza viruses due to the variety of captive bird species and their close contact with the free flying birds and human. Furthermore, in Tehran Zoo, some influenza hosts such as horse and dog were kept. Diversity of bird species in the studied

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

zoo and parks could lead to a unique condition that facilitates mutations and altering of viruses' ecology to adapt to new hosts including other bird species and human.

Haemagglutination inhibition assay is routinely used to detect serum antibodies of influenza A viruses. The ability of influenza viruses to agglutinate erythrocytes is related to receptors specificity. It has been shown that avian influenza viruses bind preferentially to the N-acetylneuraminic acid 2, 3-galactose (NeuAc2, 3-Gal) linkage, whereas human viruses prefer the NeuAc2, 6-Gal linkage (13). Chicken red blood cells contain both NeuAc2, 3-Gal and NeuAc2, 6-Gal receptors so they can be used in HI assay.

RT-PCR is a valid and universal molecular technique to detect and confirm the presence of influenza viruses' genome even if they are present at a very low level in the laboratory specimens. The primers used to detect influenza type A viruses, were designed from highly conserved regions of M gene and were type specific which could detect M segment of influenza type A viruses with origin of various hosts (14). This assay is more sensitive, more specific and less time consuming in comparison with other diagnostic assays (15). However, the absence of the expected RT-PCR products, i.e. a negative result, does not necessarily mean the absence of influenza A viruses. Results should be interpreted along with information about clinical signs and epidemiological data.

Interestingly, during our study period, there was no report of death or clinical signs of influenza disease among the captive birds, whereas the birds were not vaccinated already against influenza A viruses. Although the results could be attributed at least partially to the presence of an undetectable amount of genomic RNA, based upon the sensitivity of the test, our findings demonstrated that no RNA genome of influenza A viruses was present in the samples under study.

Despite H1 and H3 are most currently subtypes in human population and there is limited information on the prevalence of these two subtypes among birds, but some previous studies have been reported presence of antibodies to H1N1 and H3N2 viruses in wild and domestic birds in Brazil (16). Our study showed a high seropositivity of 57.7% against H1 subtype and also 88.75% against H3 subtype. *Galliformes* order had the highest seropositivity and antibody titer among tested birds. The present study is supported by other studies reporting that H1 is one of the predominant subtypes among domestic poultry (3). Meanwhile, ability of H3 virus to infect of chickens and turkeys is proved by previous studies (17).

Among all studied birds species, chicken showed the highest seropositivity and GMT value against influenza viruses. In Hong Kong, HI antibodies to A/HKU/10/77 (H1N1) human virus was detected in 19.4% of examined hen sera (18). Ayoub *et al* (19) reported a positive HI antibody titer against human H3N2 viruses in hens and turkeys. In hens, 43- 64.7% were seropositive whereas 70.7- 78% of turkeys had antibodies against human H3N2 viruses. Seropositivity of chickens is noteworthy because this species is considered as a popular domestic poultry especially in rural areas that have close relationship to human.

The results of our study represented seropositivity for H1 and H3 viruses in pheasant species but low GMT value occurrences. Previous studies implied that in infection of pheasant by influenza viruses, low levels of serum hemagglutination inhibition antibody titers would be detected when chicken red blood cells were used in HI assay. More importantly, pheasants can serve as a "carrier" of influenza A viruses because of their continuous asymptomatic infection (20).

On the other hand, previous studies indicate that quails play a significant role in the interspecies transmission of influenza virus from ducks to chickens (21). Domestic poultry such as quail and chicken possess α2, 3 and α2, 6 sialic acid receptors that may help passage of avian influenza viruses from bird species (such as ducks with only α2, 3 sialic acid receptor) to human with predominant α2, 6 sialic acid receptors (22, 23). As sample collection of this study was undertaken in an environment that no pigs were kept, chicken and quail may have

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

a key role in the transmission of influenza virus from birds to human and vice versa.

Anseriformes order especially ducks and geese showed antibody titers against H1 and H3 viruses although H3 seropositivity was more than H1 antibody. This result was similar to other studies representing H3 as one of the predominant subtypes in *Anseriformes* order (24, 25). In this study, seropositivity of ducks against H1N1 and H3N2 were 31.25% and 81.25%, respectively. These results are in accordance with the report of Barbic *et al* (26) in domestic ducks of Croatia. They recorded a positive HI antibody for A/New Caledonia/20/99 (H1N1) and A/Panama /2007/99 (H3N2) viruses in 40.6% and 90.2% of examined sera.

Seropositivity of pigeons (*Columbiformes* order) against H3 subtype could be important because they are not routine captive birds of zoos and parks but their population is dominant bird species in some cities such as Tehran.

Some tested sera had seropositivity against influenza H1 and H3 viruses while influenza A genome was not detected in any of the cloacal samples. As HI assay is able to detect haemagglutination antibodies as soon as 2 weeks to 1 year post-infection, the results of this study indicate that seropositive captive birds were infected during recent year with H1 and H3 virus strain. Although, the antigens used in HI assay were human viruses, the possibility of cross reaction should be considered. Since the sampling locations had unique niches due to the variety of influenza virus hosts, exposure to H1 and H3 viruses from another origin, including free-flying birds, could have given positive results in the HI test.

Acknowledgements

We would like to appreciate Mr. Mobarakeh of Pasteur veterinary diagnostic laboratory for his technical supports. Also we are grateful to veterinarians Dr. Hojjati from Saiee Park, Dr. Masoudi from Pardisan Park and Mr. Elhami from Tehran Zoo for sample collection and clinical examination of birds.

References

1. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev. 1992;56(1):152-79.
2. Alexander DJ, Allan WH, Parsons DG, Parsons G. The pathogenicity of four avian influenza viruses for fowls, turkeys and ducks. Res Vet Sci. 1978;24(2):242-7.
3. Swayne DE. Avian influenza vaccines and therapies for poultry. Comp Immunol Microbiol Infect Dis. 2009 Jul;32(4):351-63.
4. Cox NJ, Subbarao K. Global epidemiology of influenza: past and present. Ann Rev Med 2000;51:407-21.
5. Kawaoka Y, Krauss S, Webster RG. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J Virol. 1989;63(11):4603-8.
6. Taubenberger JK, Reid AH, Krafft AE, Bijwaard KE, Fanning TG. Initial genetic characterization of the 1918 "Spanish" influenza virus. Science. 1997;275(5307):1793-6.
7. Chotpitayasunondh T, Ungchusak K, Hanshaoworakul W, Chunsuthiwat S, Sawanpanyalert P, Kijphati R, et al. Human disease from influenza A (H5N1), Thailand, 2004. Emerg Infect Dis. 2005;11(2):201-9.
8. Lin YP, Lim W, Gregory V, Cameron K, Bennett M, Hay A. Recent examples of human infection by animal and avian influenza a viruses in Hong Kong. nternational Congress Series 2001;219:179-85.
9. Guo Y, Dong J, Wang M, Zhang Y, Guo J, Wu K. Characterization of hemagglutinin gene of influenza A virus subtype H9N2. Chin Med J (Engl). 2001;114(1):76-9.
10. Soltani Z, Hosseini M, Tabatabaian M, Bashar R, Shahidi M, Tavassoti Kheiri M. Detection of human influenza viruses in nasopharyngeal samples by RT-PCR vs Tissue culture. Iranian J Virol. 2008;1(3):1-5.
11. Legnaro P. OIE, FAO and National Reference Laboratory for Newcastle Disease and Avian Influenza. Italy; 2007 July 23rd - August Contract No.: Document Number|.
12. Cross G. Hemagglutination Inhibition Assays. Seminars in Avian and Exotic Pet Medicine. 2002;1(11):15-8.
13. Suzuki Y, Ito T, Suzuki T, Holland RE, Jr., Chambers TM, Kiso M, et al. Sialic acid species as a determinant of the host range of influenza A viruses. J Virol. 2000;74(24):11825-31.

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]

14. Bicharz KD, Smietanka K, Minta Z. Molecular methods for the detection of avian influenza type A viruses. Bull Vet Inst Pulawy. 2006;5: 287-91.
15. Pregliasco F, Mensi C, Camorali L, Anselmi G. Comparison of RT-PCR with other diagnostic assays for rapid detection of influenza viruses. J Med Virol. 1998 Oct;56(2):168-73.
16. Ortiz EJ, Kochel TJ, Capuano AW, Setterquist SF, Gray GC. Avian influenza and poultry workers, Peru, 2006. Influenza Other Respi Viruses. 2007;1(2):65-9.
17. Campitelli L, Fabiani C, Puzelli S, Fioretti A, Foni E, De Marco A, et al. H3N2 influenza viruses from domestic chickens in Italy: an increasing role for chickens in the ecology of influenza? J Gen Virol. 2002;83(Pt 2):413-20.
18. Shortridge K, Webster RG, Kam SL, Gardner JM. Reappearance of H1N1 influenza virus in man: evidence for the persistence of the virus in domestic chickens. Bull World Health Organ. 1979;57(3):475-7.
19. Ayoub NNK, Heider G, Glathe H, Ziedler K, Ebner D, Prusas E. Influenza-A-Antikorper (human) beim Gefl ügel. Mh Vet-Med. 1974;29:139-43.
20. Humberd J, Guan Y, Webster RG. Comparison of the replication of influenza A viruses in Chinese ring-necked pheasants and chukar partridges. J Virol. 2006;80(5):2151-61.
21. Perez DR, Lim W, Seiler JP, Yi G, Peiris M, Shortridge KF, et al. Role of quail in the interspecies transmission of H9 influenza A viruses: molecular changes on HA that correspond to adaptation from ducks to chickens. J Virol. 2003;77(5):3148-56.
22. Kuchipudi SV, Nelli R, White GA, Bain M, Chang KC, Dunham S. Differences in influenza virus receptors in chickens and ducks: Implications for interspecies transmission. J Mol Genet Med. 2009;3(1):143-51.
23. Henning J, Wibawa H, Morton J, Usman TB, Junaidi A, Meers J. Scavengin Ducks and Transmission of Highly Pthogenic Avian Influenza, Java, Indonesia. Emerg Infect Dis. 2010;16 (8):1244-50.
24. Fouchier F, Penel C, Pierre Montero M, Bremond P, Champion S. Integrin alphavbeta6 mediates HT29-D4 cell adhesion to MMP-processed fibrinogen in the presence of Mn2+. Eur J Cell Biol. 2007;86(3):143-60.
25. Ferro PJ, Budke CM, Peterson MJ, Cox D, Roltsch E, Merendino T, et al. Multiyear surveillance for avian influenza virus in waterfowl from wintering grounds, Texas coast, USA. Emerg Infect Dis. 2000;16(8):1224-30.
26. Barbić L, Turk N, Župančić Z, Kovač S, Stojević Z, Draženović V, et al. Antibodies against human influenza viruses in sentinel duck flocks in the ornithological reserve Kopački rit in Croatia. Veterinarski Arhiv. 2009;79 (6):573-82.

[Downloaded from journal.isv.org.ir on 2026-11-08]

[DOI: 10.21859/isv.4.2.7]