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Avian influenza virus in migratory and resident birds during migratory season in Boushehr, Iran

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Abstract: Avian influenza (AI) is an infectious disease of birds caused by type A strains of the influenza virus. Among these AI viruses, only serotypes H5 and H7 are considered highly pathogenic in poultry. However, serotype H9N2 has also been found to produce severe respiratory tract infections in chickens. Migratory birds of the world are a natural reservoir of influenza viruses of all subtypes. This is the first report of isolation and molecular survey of AI virus from migratory and wild resident birds in Boushehr, Iran. These areas are at risk for the spread and transmission of influenza A viruses. A total of 443 fecal specimens (fresh droppings and cloacal swabs) were collected from migratory and wild resident birds in the Boushehr wetlands from October 2009 to June 2010. The AI virus was identified by reverse transcriptase polymerase chain reaction (RT-PCR) using a set of primers specific to the nucleoprotein of AI virus, and for positive samples, it was tested again by RT-PCR with specific primers for the H9, H7, and H5 subtypes. Low pathogenic AI viruses (H9 subtype) were detected by RT-PCR and virus isolation, but no highly pathogenic viruses were found during the period of study. The 1st and 2nd positive cases were in slender-billed gulls (*Larus genei*) from resident birds in the Helleh wetlands, and the 3rd positive case was in a mallard (*Anas platyrhynchos*) hunted on the Helleh wetland.

Key words: Avian influenza, Iran, migratory birds, wild birds

Migratory birds are important to public health because they can be infected by a number of pathogenic microorganisms that are transmissible to humans (1). Avian influenza (AI) is a contagious viral disease and is worldwide in distribution. It is thought that wild living water birds, particularly wild waterfowls, are natural reservoirs for all of the AI viruses (2,3). All of the known subtypes of influenza A virus have been isolated from wild birds living in aquatic environments, mainly dabbling ducks. This indicates that surveillance for influenza A virus in migratory birds can be of major value as a sentinel system to prevent outbreaks in domestic poultry (3). The first documented outbreak of highly pathogenic avian influenza (HPAI) in the wild bird population was in 1961, when an outbreak in common terns (*Sterna hirundo*) killed about 1600 birds in South Africa (4). When screening wild birds in search of

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the Newcastle disease virus (known to be spread by wild birds), during an outbreak of Newcastle disease in poultry, in California in 1974 (5), revealed that low pathogenic avian influenza (LPAI) A virus could be isolated from wild birds. Further screening soon revealed that species living in aquatic environments such as ducks, gulls, geese, and shorebirds harbored the LPAI A virus strains of many different subtypes and probably acted as a reservoir for these strains (6). The influenza A virus, including all of its subtypes and most of their subtype combinations, is commonly found in aquatic birds such as ducks, geese, gulls, and shorebirds, while only a limited number of subtypes have been found in non-avian hosts. Therefore, waterfowl, in particular wild dabbling ducks (genus *Anas*), are thought to constitute the main natural viral reservoir for the LPAI virus, from which strains occasionally arise that are transmitted to other species, including humans and poultry. This paper reports the isolation of AI from migratory birds in the wetlands of Boushehr province of Iran, which is close to some major broiler and layer breeder areas during the avian migratory season and resident birds, from October 2009 to June 2010.

The present study was conducted in the wetlands of Boushehr in southern Iran (Table). A total of 443 fecal specimens (212 fresh droppings from migratory birds and 110 fresh droppings from wild resident birds, and 121 cloacal swabs from migratory birds) were collected. Some of the birds were trapped and for the bird species that could not be trapped, fresh dropping samples were collected from the ground at locations where large numbers of the birds congregated. Fecal samples of both migratory and resident birds were collected from several sites in Bushehr Province, which is known for the arrival of migratory birds during the avian migratory season (Table). Only fresh and wet samples were collected. Cloacal swabs and fresh dropping samples were collected using cotton swabs and immediately put into vials containing virus transport media (Hanks balanced salt solution containing 0.5% lactalbumin, 10% glycerol, 200 U/mL penicillin, 200 µg/mL streptomycin, 200 µg/mL polymyxin B sulfate, 250 µg/mL gentamicin, and 50 U/mL nystatin) on wet ice and then frozen to −70 °C. All of the samples were processed for virus isolation in embryonated chicken eggs and tested by reverse transcriptase polymerase chain reaction (RT-PCR).

Virus isolation and characterization of the samples collected were performed at the Razi Vaccine and Serum Research Institute, Shiraz, Iran. According to the method described by Swyane (7), 200 µL of the original specimens were inoculated into the allantoic cavity of 9- to 11-day-old embryonated chicken eggs. The amino-allantoic fluids were harvested and analyzed for hemagglutination activity (HA) (7). When the HA titers were negative, the allantoic fluids were passaged once again in embryonated chicken eggs. A hemagglutination inhibition (HI) assay was used for virus isolate subtyping, and was performed using H5, H7, and H9 subtype-specific reference anti-sera obtained from Istituto Zooprofilattico Sperimentale delle Venezie, Italy (7). Samples were collected from different species, with the majority of the samples originating from ducks, geese, gulls, Ardeidae, and common fowls (Table).

RNA was extracted from all of the amnio-allantoic fluid samples using viral Gene-spin™ viral DNA/RNA extraction kit (Vetek, Korea), following the manufacturer's instructions.

To identify the AI virus by RT-PCR, 2 primers based on a conserved sequence of the nucleoprotein (NP) gene of influenza viruses were used (8). The NP specific primers used were: NP1200 (forward): 5'-CAG (A/G) TACTGGGC (A/T/C) ATAAG (A/G) AC-3' and NP1529 (reverse): 3'-GCATTGTCTCCGAAGAAATAAG-5' (8). Each positive reaction was tested for H9, H7, and H5. To detect specific subtypes of the AI virus by RT-PCR, 3 sets of primers, each based on conserved sequences of a single HA subtype, were used (8). Samples were amplified by 1-step RT-PCR. RT-PCR was carried out as previously described (8) in a reaction mixture (25 µL) containing 2.5 µL of 10X reaction buffer (Promega, Madison, WI, USA), 2.5 µL of dNTP blend (2.5 mM each of 4 dNTPs, Promega), 0.2 µL of AMV reverse transcriptase (9 units/µL, Promega), 0.3 µL of RNase inhibitor (40 units/µL, Promega), 0.5 µL of Taq DNA polymerase (9 units/µL, Promega), 1 µL of each primer (10 pmol each), 1 µL of RNA template (about 1 ng), and 17 µL of water. The PCR condition for the amplification of H5, H7, and H9 was 42 °C for 45 min (reverse transcription), 95 °C for 3 min, 35 cycles of 95 °C for 30 s (denaturation), 50 °C for 40 s (annealing), and 72 °C for 40 s (extension), followed by 72 °C for 10 min (final extension) (8).

Table. List of bird families, species, location of sampling, and assay results.

Family	Species	Location	Assay results						
			Number of samples		Virus isolation (HA positive)		Subtyping by RT_PCR		
			Fecal	Cloacal swab	No.	Type of sample	H5	H7	H9
Anatidae (ducks)	Shelduck (*Tadorna tadorna*) Slender-billed gull (Larus genei) Wigeon (*Anas penelope*) Mallard (*Anas platyrhynchos*) Common teal (*Anas crecca*) Common pochard (*Aythya ferina*)	Mond & Helleh	65	42	1 2	Cloacal swab Fecal	- -	- -	+ +
Ardeidae	Great white egret (Casmerodius albus)	Mond & Helleh		10					
Scolopacidae	Bar-tailed godwit (*Limosa lapponica*) Eurasian curlew (*Numenius arquata*)	Mond & Bushehr		14					
Rallidae	Eurasian coot (*Fulica atra*)	Kabgan area	24						
Otididae	Houbara (Chlamydotis undulata)	Mond & Helleh	23	25					
Burhinidae	Stone curlew (Burhinus oedicnemus)	Mond & Bordekhon	87						
Laridae	Slender-billed gull (Larus genei)	Bandargah	43	10					
Sternidae	Caspian tern (Sterna caspia) Great crested tern (*Sterna bergii*) Lesser crested tern (*Sterna bengalensis*) Bridled tern (*Sterna anaethetus*)	Nakhilo, Omolkarm, Kharkoo Islands	80	17					

From October 2009 to June 2010, fresh dropping samples and cloacal swabs were collected from migratory and resident birds in the wetlands of Bushehr in Iran, and were tested for the presence of the influenza A virus by virus isolation and RT-PCR. Efforts were made to ensure the majority of these samples were collected at different locations in Bushehr Province. RT-PCR was performed with H5, H7, and H9, an expected amplification of 488 bp with H9 subtype specific. The AI virus was isolated from 3 of 443 samples processed for virus isolation and confirmed by RT-PCR. Positive HI results were shown only by the specific serum against H9N2. Specific serums against H5N1 and H7N1 were unable to inhibit HA activity of the virus. Viruses of the H9 subtype were obtained from 2 resident birds and the other was from a migratory bird. The 1st and 2nd LPAI positive cases were in slender-billed gulls (*Larus genei*) from resident birds in the Helleh wetlands, and the 3rd H9 positive case was in a mallard (*Anas platyrhynchos*) hunted on the Helleh wetland (9). No samples from the fresh dropping samples and cloacal swabs in any of the wetlands in Bushehr province were positive for H5 and H7 by RT-PCR and virus isolation during the study period.

Wetlands birds are thought to constitute the major natural reservoir for the AI A virus. Oslen (10) stated that LPAI viruses can be found in numerous bird species, but it is unclear in which of these species influenza viruses are endemic, and in which the virus is a temporary pathogen. Species in which influenza viruses are endemic share the same habitat, at least part of the year, with other species in which influenza viruses are frequently detected, including geese, swans, rails, petrels, and cormorants. In these birds, influenza virus prevalence seems to be lower than in dabbling ducks, but it should be noted that studies on these species are limited, and it is possible that peak prevalence has been missed because of its seasonal nature or location (10,11). All influenza virus subtypes and most hemagglutinin/neuraminidase combinations have been detected in the bird reservoir and poultry, whereas relatively few have been detected in other species. LPAI viruses can be found in numerous bird species (10). Although many wild bird species may harbor influenza viruses, the birds of wetlands and aquatic environments constitute the major natural LPAI virus reservoir. LPAI viruses have been isolated from at least 105 wild bird species of 26 different families (10). Influenza A virus has been detected in 1 shorebird sample obtained from Delaware Bay (United States) and 1 from South Korea (11). In the present study, we found 2 positive samples in slender-billed gulls, which were resident in Boushehr Province. Influenza A H9N2 viruses have been detected worldwide in poultry, and currently are endemic among poultry species in Asia (12,13). Between 1998 and 2000, H9N2 viruses were reported in Middle Eastern countries and were responsible for widespread and serious disease in commercial chickens in Pakistan (14), Iran (15,16), the United Arab Emirates (17), and Saudi Arabia (18). Numerous infections of poultry and other birds with the subtype H9 during 1995 originated from separate introductions from feral and migratory birds (18). In northern Iran, 5 subtypes of the AI virus, H3N8, H7N3, H8N4, H9N2, and H10N7, were isolated from migratory birds during a surveillance campaign in 2003 and 2004 (19). During this campaign in the autumn and winter of 2003 and 2004, 472 fecal samples were collected from migratory birds in northern Iran, and AI viruses were detected from the samples (19). Feridouni (19) reported that AI viruses can easily reach the wetlands of Iran from Siberia, and that these viruses could perpetuate in water fowl in this region during autumn and winter. Thus, Iran is one route of migratory wild, local wild, and feral birds. Holdings where wild birds and domestic birds share the same habitat due to agricultural practices are at the highest risk for outbreaks (20), suggesting that wild bird transmission is the most common route. The H9 AI virus has been isolated in wild ducks throughout the world (21). An important result is that wild bird surveillance maybe a tool for obtaining strains of the influenza virus that can be used for vaccine development as well as diagnostic tests and reagents, as they are indeed similar to outbreak strains. However, a LPAI outbreak at a poultry farm could cause a large economic loss for the poultry industry; therefore, it could be expected that LPAI would be transmitted to poultry farms in Iran. The current increased interest in influenza virus surveillance in wild and domestic birds provides a unique opportunity to increase our understanding not only of HPAI epidemiology, but also of the ecology of LPAI viruses in their natural hosts, at

the same time and for the same cost. This is the first study on migratory and resident birds in southern Iran. Although our findings support a circulation of H9N2 subtype in migratory birds, it seems further samples of migratory birds are necessary for better understanding of the ecology of the influenza virus in migratory birds.

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References

1. Reed, K.D., Meece, J.K., Henkel, J.S., Shukla, S.K.: Birds, migration and emerging zoonosis: West Nile virus, Lyme disease, influenza A and enteropathogens. Clin. Med. Res., 2003; 1: 5-12.
2. Račnik, J., Brigita, S., Tomi, T., Marko, Z., Alenka, D., Uroš, K.: Evidence of avian influenza virus and paramyxovirus subtype 2 in wild-living passerine birds in Slovenia. Eur. J. Wildl. Res., 2008; 54: 529-532.
3. Wallensten, A.: Influenza A virus in wild birds. PhD Dissertation. Department of Molecular and Clinical Medicine (IMK), Division of Molecular Virology, Faculty of Health Sciences, Linköping, Sweden. 2006.
4. Becker, W.B.: The isolation and classification of Tern virus: influenza A-Tern South Africa 1961. J. Hyg., 1966; 64: 309-20.
5. Slemons, R.D., Johnson, D.C., Osborn, J.S., Hayes, F.: Type A influenza viruses isolated from wild free flying ducks in California. Avian. Dis., 1974; 18: 119-22.
6. Webster, R.J., Bean, W.J., Gorman, O.T., Chambers, T.M., Kawaoka, Y.: Evolution and ecology of influenza A viruses. Microbiol. Rev., 1992; 56:152-179.
7. Swanye, D.E., Asenne, D., Beard, C.W.: Avian influenza. In: Swanye DE, Glisson JR, Jackwood MW, Pearson JE, Reed WM, Eds. Isolation and identification of avian pathogens. 4th edn. American Association of Avian Pathologists, Kennet Square. 1998; 150-155.
8. Lee, M.S., Chang, P.C., Shien, J.H., Cheng, M.C., Shieh, H.K.: Identification and subtyping of avian influenza viruses by reverse transcription-PCR. J. Virol. Methods, 2001; 97: 13-22.
9. Mullarney, K., Svensson, L., Zetterstrom. D., Grant, P.J.: Collins Bird Guide. 2nd edn. Harper Collins Pub. Ltd., London. 2009.
10. Olsen, B., Munster, V.J., Wallensten, A., Waldenström, J., Osterhaus, A.D.M.E., Fouchier, R.A.M.: Global patterns of influenza A virus in wild birds. Science, 2006; 312(5772): 384-388.
11. Munster, V.J., Baas, C., Lexmond, P., Waldenström, J., Wallensten, A., Fransson, T., Rimmelzwaan, G.F., Beyer, W.E., Schutten, M., Olsen, B., Osterhaus, A.D., Fouchier, R.A. Spatial, temporal, and species variation in prevalence of influenza A viruses in wild migratory birds. PLoS. Pathog., 2007; 3: 61.
12. Li, C., Yu, K., Tian, G., Yu, D., Liu, L., Jing, B., Ping, J., Chen, H.: Evolution of H9N2 influenza viruses from domestic poultry in mainland China. Virol., 2005; 340: 70-83.
13. Xu, K.M., Li, K.S., Smith, G.J.D., Li, J.W., Tai, H., Zhang, J.X., Webster, R.G., Peiris, J.S.M., Chen, H., Guan, Y.: Evolution and molecular epidemiology of H9N2 influenza A viruses from quail in southern China, 2000 to 2005. J. Virol., 2007; 81: 2635-2645.
14. Naeem, K., Ullah, A., Manvell, R.J., Alexander, D.J.: Avian influenza A subtype H9N2 in poultry in Pakistan. Vet. Rec., 1999; 145: 560.
15. Nilli, H., Asasi, K.: Natural cases and experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. Avian Pathol., 2002; 31: 247-252.
16. Nilli, H., Asasi, K.: Avian influenza H9N2 outbreak in Iran. Avian Dis., 2003; 47: 828-831.
17. Manvell, R.J., McKinney, P., Wernery, U., Frost, K.: Isolation of highly pathogenic influenza A virus of subtype H7N3 from a peregrine falcon (Falco peregrinus). Avian Pathol., 2000; 29: 635-637.
18. Banks, J., Speidel, E.C., Harris, P.A., Alexander, D.J.: Phylogenetic analysis of influenza A viruses of H9 haemagglutinin subtype. Avian Pathol., 2000; 29: 353-360.
19. Fereidouni, S.R., Aghakhan, M., Werner, O., Starick, E., Bozorghmehrifard, M.H.: Isolation and identification of avian influenza viruses from migratory birds in Iran. Vet. Rec., 2005; 157: 526.
20. Gilbert, M., Chaitaweesub, P., Parakamawongsa, T., Premashthira, S., Tiensin, T., Kalpravidh, W., Wagner, H., Slingenbergh, J.: Free-grazing ducks and highly pathogenic avian influenza, Thailand. Emerg. Infect. Dis., 2006; 12: 227-34.
21. Lebarbenchon, C., Chang, C., van der Werf, S., Aubin, J.T., Kayser, Y., Ballesteros, M., Renaud, F., Thomas, F., Gauthier-Clerc, M.: Influenza A virus in birds during spring migration in the Camargue, France. J. Wildl. Dis., 2007; 43: 789-793.