Archives of Razi Institute, Vol. 74, No. 3 (2019) 243-250

Original Article

Prevalence of Avian Influenza in live bird markets, bird gardens, and zoos in Iran in 2015: A cross-sectional study

Fallah Mehrabadi [1] *, M.H., Ghalyanchi Langeroudi [2], A., Bahonar [3], A., Rabiee [3], M.H., Tehrani [4], F., Amirhajloo [4], S., Steneroden [5], K., Salman [5], Mo. D.

1. *Department of Poultry Viral Diseases, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*
2. *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
3. *Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
4. *Department of Health and Management of Poultry Diseases, Iranian Veterinary Organization, Tehran, Iran*
5. *Animal Population Health Institute, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA*

Received 01 February 2019; Accepted 04 May 2019
Corresponding Author: mhf2480@yahoo.com

ABSTRACT

Avian Influenza (AI) H9N2 is endemic in Iran; therefore, it is necessary to estimate the disease prevalence among birds in live bird markets (LBMs) and assess the risk spread across the country. Accordingly, this study aimed to estimate the prevalence of AI subtypes in LBMs, bird gardens, and zoos during October and November 2015 in Iran and investigate the associated risk factors. Data related to independent variables for birds and premises were collected using a prepared questionnaire which included items about previously known potential risk factors associated with avian influenza infection. Serological testing was carried out to detect the antibodies against H5, H7, and H9. Regarding H5 and H7, the antigens H5N2 and H7N1 were used in this study. Positive samples on the first test were examined with the second antigens, namely H5N1 and H7N7. Moreover, sera with titers ≥4 (i.e. $\log^2$) were considered positive and premises with at least one positive bird were considered as positive units. In total, 87 premises were included in this cross-sectional study. Serum samples were examined utilizing hemagglutination inhibition, and RT-PCR was conducted on swab samples. Regarding the molecular test, the RNA was extracted using the High Pure Viral RNA Kit (Roche, Germany). In addition, real-time RT-PCR was conducted based on the described method. The seroprevalence rates of H9N2 were 83.9% and 31.8% at the premises and bird levels, respectively. Totally, 9.2% of pooled swab samples were positive for H9N2. However, all sera and swab samples were negative for H5 and H7. Hot and humid weather (OR=0.13, 95% CI 0.02 – 0.78) as well as bird-keeping condition (i.e., enclosed area) (OR=0.11, 95% CI 0.012 – 1.02) were protective factors for H9N2. High seroprevalence rate of H9 indicates that the disease is endemic in Iranian LBMs. Active surveillance must be carried out in LBMs, especially in the northern provinces of Iran. In addition, cleanliness and improved hygiene would be useful to prevent the spread of disease in LBMs.

Keywords: Avian influenza, Bird garden, Iran, Live bird market, Zoo

Prévalence de l'influenza Aviaire dans les Marchés, les Jardins d'Oiseaux Vivants et les Zoos Iraniens en 2015: une Analyse Transversale

Résumé: L'influenza aviaire (IA) H9N2 est endémique en Iran; il est donc nécessaire d'estimer la prévalence de la maladie parmi les oiseaux sur les marchés d'oiseaux vivants (MOVs) et d'évaluer le risque réparti dans le pays. Dans ce but, cette étude visait à estimer la prévalence des sous-types d'IA dans les MOVs, les jardins d'oiseaux et les zoos iraniens en octobre et novembre 2015 ainsi qu'à étudier les facteurs de risque associés. Les données relatives aux variables indépendantes pour les oiseaux et les différents lieux concernés ont été collectées à l'aide d'un questionnaire comprenant des éléments sur les facteurs de risque potentiellement associés à l'infection par la grippe aviaire. Des tests sérologiques ont été effectués pour détecter les anticorps anti-H5, H7 et H9. En ce qui concerne H5 et H7, les antigènes H5N2 et H7N1 ont été utilisés dans cette étude. Les échantillons positifs du premier test ont été examinés avec les deuxièmes antigènes, à savoir H5N1 et H7N7. De plus, les sérums avec des titres ≥4 (c'est-à-dire log^2) ont été considérés comme positifs et les lieux avec au moins un oiseau positif ont été considérés comme des unités positives. Au total, 87 locaux ont été inclus dans cette étude transversale. Les échantillons de sérum ont été examinés en utilisant l'inhibition de l'hémagglutination, et la RT-PCR a été réalisée par les échantillons sur écouvillon. En ce qui concerne le test moléculaire, l'ARN a été extrait à l'aide du Kit d'ARN Viral Très Pur (Roche, Allemagne). De plus, une RT-PCR en temps réel a été réalisée sur la base de la méthode décrite. Les taux de séroprévalence de H9N2 étaient respectivement de 83,9% et 31,8% aux niveaux des locaux et des oiseaux. Au total, 9,2% des échantillons collectés sur écouvillons étaient positifs pour H9N2. Cependant, tous les sérums et échantillons prélevés étaient négatifs pour H5 et H7. Le temps chaud et humide (OR = 0,13, IC 95%: 0,02 - 0,78) ainsi que les conditions d'élevage des oiseaux (c'est-à-dire, une zone fermée) (OR = 0,11, IC 95%, 0,012 - 1,02) étaient des facteurs de protection pour H9N2. Le taux élevé de séroprévalence de H9 indique que la maladie est endémique dans les LBM iraniens. Une surveillance active doit être effectuée dans les LBMs, en particulier dans les provinces du nord de l'Iran. De plus, la propreté et l'amélioration de l'hygiène seraient utiles pour prévenir la propagation de la maladie chez les LBMs.

Mots-clés: Grippe aviaire, Jardin d'oiseaux, Iran, Marché d'oiseaux vivants, Zoo (Parc zoologique)

INTRODUCTION

Avian influenza (AI) is a serious infection for birds and humans and is caused by several subtypes of influenza virus type A (Schrijver and Koch, 2005; Swayne et al., 2013a). Wild waterfowl are the natural reservoir for these viruses and have been responsible for the introduction of avian influenza into poultry flocks (Sarwar et al., 2013). There are many AI virus strains, which are classified into low pathogenic avian influenza and highly pathogenic avian influenza based on the severity of the disease in birds. To date, four subtypes of avian influenza have been detected and officially reported in Iran. Low pathogenic H9N2 was first reported in 1998 and the disease became endemic in Iran (Nili and Asasi, 2003). The second (H5N1), third (H5N8), and fourth (H5N6) subtypes were reported as highly pathogenic avian influenza. Several studies have found H9N2 in backyard poultry populations in Iran (Majidzadeh-A et al., 2012a; Majidzadeh-A et al., 2012b; Fallah Mehrabadi et al., 2016). Live bird markets (LBMs) are a common source of obtaining poultry and supporting livelihoods in many developing countries, including Iran. The markets are usually in urban areas and provide a variety of poultry products, including live birds for consumption and breeding. Backyard poultry breeding for eggs and meat is a source of income which plays an important economic role in the lives of rural families (Shariatmadari, 2000). According to the data from Iran Veterinary Organization (IVO) (Fallah Mehrabadi et al., 2016), there are an estimated 50 million backyard birds in Iran which provide poultry to LBMs (unpublished data). These markets in Iran are diverse, ranging from the large national Friday Market in the northern province of Mazandaran which supplies domestic ducks and geese to the small local markets that provide domestic, ornamental, commercial, wild aquatic, or mixed-species chickens. These permanent, weekly, daily, or seasonal markets provide slaughter services; however, the customers may get takeaway purchases for home slaughter. The LBMs are most common in the northern provinces of Iran, including Mazandaran, Gilan, and Golestan. In particular, Mazandaran has numerous native aquatic duck and goose species and seasonal migratory birds. Duck meat is a common traditional meal in Mazandaran where integrated rice-duck farming is practiced. Due to the high demand, ducks are also brought from Varamin, Tehran, which is the largest duck-breeding center in the country (Hosseini et al., 2012). The IVO (Fallah Mehrabadi et al., 2016) reported that an estimated 5 million ducks are sold in Mazandaran annually. Migratory birds arrive mostly to the areas of Fereydunkenar and Behshahr in Mazandaran province towards the end of September (Zadegan, 2017) each year. Some species of migratory birds are both legally and illegally hunted and sold in the LBMs alongside live domestic poultry. Highly pathogenic avian influenza has been detected in LBMs in many countries, including Egypt, Nigeria, China, South Korea, Hong Kong, Vietnam, and Bangladesh (Abdelwhab et al., 2010; Negovetich et al., 2011; Aiki-Raji et al., 2015; Bui et al., 2017). It has been shown that the spread and propagation of several diseases, including laryngotracheitis, Newcastle disease, and avian influenza are strongly associated with LBMs (Swayne et al., 2013b). In Iran, the LBMs usually lack adequate housing, cleaning, disinfection, and biosecurity resulting in high bird density and the ideal environment for propagation and transmission of poultry diseases to birds and humans. The likelihood of disease transmission between wild and domestic birds is particularly high due to the high density of the domestic birds (particularly goose and duck) and limited biosecurity measures in LBMs of Iran (Dhama et al., 2008; Majidzadeh-A et al., 2012b). In addition to the LBMs, there are several bird gardens and zoos in Iran where a variety of birds are kept for public display that may be potential sites for avian influenza introduction and spread in Iran. This study was conducted to estimate the prevalence of H9, H7, and H5 avian influenza subtypes and evaluate the risk factors associated with H9N2 in LBMs, bird gardens, and zoos during the annual wild bird migration in Iran.

MATERIAL AND METHODS

Study design. This cross-sectional study was conducted in all active LBMs, zoos, and bird gardens in October and November 2015 during the annual wild bird migration in Iran.

Sampling design and data collection. There are 107, 48, and 28 registered LBMs, bird gardens, and zoos in the database of the Department of Poultry Diseases of the IVO, respectively. Chickens and domestic waterfowl birds (i.e., goose and duck) were sold at most of the LBM premises (Fallah Mehrabadi et al., 2016). Since some of the premises were seasonal and only temporarily active, 87 premises (45 LBMs, 27 bird gardens, and 15 zoos) out of 183 registered ones were included in this study which provided birds during October and November. In total, 100 samples were collected from each premises, 40 birds were selected for blood collection, and 60 different birds were selected for cloacal swab collection. The priority in the collection of biological samples was given to aquatic birds and then chickens. The sellers were asked to select the birds for sampling with the aim of finding at least one infected bird assuming a 5% prevalence using a molecular test with a 95% confidence level. According to the European Commission guideline (2010), one milliliter of blood was collected from the wing vein of the selected birds and placed into a 1.5 ml microtube. The blood samples were then transferred to the central veterinary laboratory in each province, where the blood serum was separated. The cold chain was maintained for all cloacal swab samples, and they were sent to the national reference laboratory of the IVO in Tehran. A questionnaire was prepared to collect data related to independent variables for birds and premises. The items were constructed using previously known potential risk factors associated with avian influenza infection (Bulaga et al., 2003; Fallah Mehrabadi et al., 2016). Descriptive data were collected using the location and size of premises, type and number of present species, weather type (based on the climate classification in Iran), bird-keeping conditions (free-range or enclosed), the way the dead birds were disposed of when the market was closed, the city of origin of birds buyers, the place where unsold birds were kept when the market was closed, disinfection practices when the market was closed, type of market (permanent or weekly), source of birds (city of origin), how birds were offered (live or slaughtered), distance to nearest pond, main road, lake, river, dam, slaughterhouse, distance to the place of residence or temporary seasonal location of the migratory birds, and the kind of market activity (LBM only, public market or live bird and agricultural products market). The questionnaire was completed through a direct interview with bird owners.

Laboratory tests. Serological testing was carried out for the detection of antibodies against H5, H7, and H9 based on the guidelines of the Office International des Epizooties (OIE) and IVO (OIE, 2017). The antigens H5N2 and H7N1 were used for H5 and H7. Positive samples on the first test were examined with the second antigens, namely H5N1 and H7N7. Sera with titers ≥4 (i.e. log2) were considered positive and premises with at least one positive bird were considered a positive unit. Totally, 5 cloacal swab samples were pooled together (resulting in 12 pooled samples for each premise) to perform the molecular test and the RNA was extracted using High Pure Viral RNA Kit (Roche, Germany). Real-time RT-PCR was conducted based on the described method (Monne et al., 2008).

Data Analysis. Data were collected from each premise and were recorded in the IVO database along with the results of the laboratory tests. Subsequently, the data were imported into an excel spreadsheet and analyzed using SPSS software (version 22). Moreover, the Chi-square test was applied to determine the association between each of the independent variables and serological status by each premise as the unit of the study and odds ratios with a 95% confidence intervals (Salman, 2003). P-value less than 0.05 was considered statistically significant. Furthermore, the maps were made using ArcGIS (version.9.2).

RESULTS

According to the results, 87 premises (45 LBMs, 27 bird gardens, and 15 zoos) were included in this study. The premises were distributed throughout Iran, and more than 50% of these premises were located in five provinces, namely east Azerbaijan (14), Gilan (13), Mazandaran (10), Golestan (9), and Tehran (8). The spatial distribution of the sampled premises and H9 status are shown in Figure 1. A total of 3,071 blood samples and 3,869 cloacal swab samples were collected in this study. The cloacal samples were pooled into 770 samples. The premise seroprevalence level of H9 was 83.9% (73/87). Moreover, the seroprevalence rate in LBMs was obtained at 84.4 % (38/45), and it was 83.3% (35/42) at the bird garden and zoo level. Of the 3,071 serum samples, 978 (31.8%) ones were seropositive for H9N2. In addition, the seroprevalence rates of H9N2 were 26.3% (428/1,627), 39.2% (363/926), and 36.1% (187/518) in LBMs, bird gardens, and zoos, respectively (Table 1). All sera samples were negative for H5 and H7. Molecular tests conducted on the 770 pooled samples identified 23 samples on eight premises (9.2%), which were positive for H9. Totally, 23 positive pooled samples consisted of chicken (19), duck (2) and turkey (2) samples. All molecular samples were negative for H5 and H7.

Risk factor analysis. According to the results of the univariate analysis, a statistically significant correlation was observed between the weather type and the bird-keeping conditions (i.e., free-range or enclosed) with H9 infection. Hot and humid weather (OR=0.13, 95% CI 0.02 – 0.78) and bird-keeping condition (i.e., enclosed area) OR=0.11, 95%CI (0.01 – 1.02) were protective factors for H9. Furthermore, there were no statistically significant associations between H9 infection and the other investigated variables (Table 2).

DISCUSSION

This study investigated the presence of H5, H7, and H9N2 infections among birds in LBMs, bird gardens, and zoos in Iran during the annual wild bird migration. All sera and molecular tests were negative for H5 and H7 subtypes. In another study conducted in LBMs in Qom, Iran, no H5 positive birds were detected either on serological or molecular tests (Majidzadeh-A et al., 2012b). However, the H5 subtype has caused several outbreaks among wild and domestic birds in Iran during the past decade. Although the obtained results of this study indicate nonexistent circulation of these virus subtypes in Iran during the time of the study, they do exist in susceptible populations in other countries. The spread of infection to Iran by wild birds during migration is possible and has occurred in other countries (Kim et al., 2012) which necessitates continued monitoring and surveillance of poultry and wild birds for the H5 and H7 subtypes. The seroprevalence of H9N2 in LBMs, bird gardens, and zoos in Iran was high both on the premises level and bird level with the highest observed prevalence in chickens. The H9N2 seropositivity levels were 83.9% and 26.3% in premises and birds, respectively. The H9N2 infection among birds in LBMs has been observed in other countries, and most AI infections in Asia was reported to be due to H9N2 (Choi et al., 2004; Negovetich et al., 2011; Lee et al., 2017). Studies from other countries have found the prevalence of H9N2 in these populations. For instance, the prevalence rates of H9N2 at the bird level in LBMs were estimated at 2.0% in South Korea, 5.4% in Nigeria, 5.2% in China, and 0.2% in Pakistan (Lahore) based on virus isolation, serologic methods, molecular methods, and virus isolation, respectively. Furthermore, the prevalence rate of H9 at the bird level in Iran is higher, compared to other rates in many countries; however, it was similar to that in Bangladesh which was 22.0% using virus isolation (Negovetich et al., 2011; Sarwar et al., 2013; Aiki-Raji et al., 2015; Luan et al., 2016; Lee et al., 2017). Regarding the investigated variables, only weather type and bird-keeping conditions (i.e., free-range or enclosed) had significant impacts on the prevalence of H9 at the premise level. Moreover, the infection rate was lower in premises with hot and dry, as well as hot and humid climates, compared to premises with other weather types. Lower prevalence rates in areas with hot weather may be due to the fact that the transmission of influenza viruses is more efficient under colder conditions (Lowen and Steel, 2014). It is advisable that surveillance systems carefully consider climate parameters in their design. In addition, the prevalence rate was lower in the premises where birds were enclosed, compared to free-range ones. This may be due to the reduced contact between birds when they are enclosed versus the time on which they are allowed to come into contact with other birds. In Thailand, the spread of avian influenza viruses was strongly associated with free-grazing ducks (Thanawat et al., 2005). In this study, H9N2 was detected in the majority of Iranian LBMs which can be a source of infection for domestic and commercial poultry. In the northern provinces of Iran, in particular, migratory wild birds are included in markets during the autumn and winter. Their inclusion in LBMs provides the opportunity for transmission of influenza viruses to different poultry types. Therefore, it is suggested to perform regular and periodic sampling of LBMs for AI. Moreover, the detection of H5 and H7 subtypes can be considered as early warning signs in order to prevent

Table 1. Frequency of H9 seropositive birds in live bird markets, bird gardens, and zoos by species during October-November 2015 in Iran

	Location		
	Bird Market (n=45)	Bird Garden and Zoo (n=42)	Total (n=87)
Bird species	Number of positive samples/samples taken (%)	Number of positive samples/samples taken (%)	Number of positive samples/samples taken (%)
Chicken	368/837 (44)	212/360 (59)	580/1197 (36)
Duck	27/584 (5)	195/569 (30)	222/1243 (14)
Turkey	25/160 (16)	20/63 (32)	45/223 (18)
Pigeon	7/22 (32)	32/97 (33)	39/119 (20)
Quail	1/5 (20)	2/5 (40)	3/10 (10)
Other* species	0/19 (0)	89/260 (34)	89/279 (26)
Total	428/1627 (26)	550/1444 (38)	978/3071 (31)

*Ostrich, partridge, and pheasant

Table 2. Univariate analysis of independent variables for H9 seropositivity in live bird markets, bird gardens, and zoos during October-November 2015 in Iran

Category	Variable	*A. influenza* serology result		OR	OR (95% CI)	P
		Positive	Negative			
Weather Type	Cold and humid	28	3			
	Caspian (moderate and humid)	17	2	0.91	0.13–6.01	0.923
	Mild	16	1	1.71	0.16–17.88	0.652
	Hot and humid	5	4	0.13	0.02–0.78	0.026
	Hot and dry	7	4	0.18	0.03–1.03	0.055
Bird-keeping condition	Free-range	21	1			
	Enclosed	14	6	0.11	0.012–1.02	0.053
The highest number of offered birds	Domestic duck and geese	41	10			
	Other birds	32	4	1.95	0.55–6.79	0.294
Variety of offered birds	Domestic duck and geese	49	9			
	Other birds	24	5	0.88	0.26–2.91	0.837
Actions for dead birds when the market is closed	Not released the carcasses	39	9			
	Release the carcasses	34	5	1.56	0.47–5.13	0.456
Kind of bird buyers	From province	34	7			
	From other provinces	1	0	0.45	0.036–5.68	0.538
	From province and other provinces	3	0	0.91	0.08–9.32	0.940
Places to keep unsold birds when the market is closed	Kept in the city	31	7			
	Moved to other cities	1	0	0.50	0.04–6.22	0.588
	Kept in the city and moved to other cities	6	0	1.75	0.18–16.33	0.623
Disinfection provisions when the market is closed	Manure collected, market disinfected	16	5			
	No action	12	0	4.58	0.49–42.96	0.159
	Market disinfection only during outbreaks	10	2	1.56	0.25–9.64	0.631
Market type	Permanent	12	0	3.85	0.43–34.13	0.204
	Once a week	26	7			
Distance of nearest pond to premises	>3 km	55	9			
	1 to 3 km	1	1	0.16	0.009–2.85	0.215
	<1 km	10	3	0.54	0.12–2.37	0.419
Distance of nearest main road to premises	<1 km	38	8			
	1 to 3 km	13	1	2.73	0.31–24.02	0.364
	>3 km	15	5	0.63	0.17–2.24	0.477
Distance of nearest lake to premises	>3 km	49	6			
	1 to 3 km	4	1	0.48	0.04–5.13	0.552
	<1 km	12	5	0.29	0.07–1.12	0.074
Distance of nearest river to premises	>3 km	31	8			
	1 to 3 km	12	3	1.03	0.23–4.55	0.967
	<1 km	25	3	2.15	0.51–8.96	0.293
Distance of nearest dam to premises	>3 km	54	9			
	1 to 3 km	3	1	0.50	0.04–5.35	0.567
	<1 km	6	3	0.33	0.07–1.57	0.166
Distance of nearest place of residence or temporary season for migratory birds to premises	>3 km	54	9			
	1 to 3 km	2	2	0.16	0.02–1.33	0.092
	<1 km	11	3	0.61	0.14–2.62	0.508
Distance of nearest commercial bird farm to premises	> 3 km	47	9			
	1 to 3 km	11	4	0.52	0.13–2.02	0.351
	<1 km	10	1	1.91	0.21–16.8	0.558
Distance of nearest bird slaughterhouse to premises	>3 km	61	11			
	1 to 3 km	3	2	0.27	0.40–1.81	0.178
	<1 km	2	0	0.58	0.05–6.06	0.648
Source of birds	From other provinces	38	10			
	From the province	35	4	2.30	0.66–8.01	0.190
How birds were offered	Live sale	30	7			
	Live sale and slaughter	5	0	1.54	0.16–14.76	0.705
	Slaughter with separated feathers	3	0	1.03	0.10–10.5	0.978
Kind of market activity	General market	28	5			
	Live birds only	6	1	1.07	0.10–10.9	0.954
	Live poultry and agricultural products	4	1	0.71	0.06–7.78	0.783

commercial poultry of Iran.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Figure 1. Avian influenza subtype H9 in live bird markets, bird gardens, and zoos during October -November 2015 in Iran

Conflict of Interest

The authors declare that they have no conflict of interest.

Acknowledgment

The authors express their gratitude to Dr. Katie Steneroden from Colorado State University, Colorado, USA, for her critical review and editing this manuscript. Moreover, the authors appreciate the support from the Directorate of Health and Management of Poultry Disease, Iran Veterinary Organization, Tehran, Iran.

References

Abdelwhab, E.M., Selim, A.A., Arafa, A., Galal, S., Kilany, W.H., Hassan, M.K., et al., 2010. Circulation of avian influenza H5N1 in live bird markets in Egypt. Avian Dis 54, 911-914.

Aiki-Raji, C.O., Adebiyi, A.I., Agbajelola, V.I., Adetunji, S.A., Lameed, Q., Adesina, M., et al., 2015. Surveillance for low pathogenic avian influenza viruses in live-bird markets in Oyo and Ogun States, Nigeria. Asian Pac J Trop Dis 5, 369-373.

Bui, C.M., Gardner, L., MacIntyre, R., Sarkar, S., 2017. Influenza A H5N1 and H7N9 in China: A spatial risk analysis. PLoS One 12, e0174980.

Bulaga, L.L., Garber, L., Senne, D.A., Myers, T.J., Good, R., Wainwright, S., et al., 2003. Epidemiologic and Surveillance Studies on Avian Influenza in Live-Bird Markets in New York and New Jersey, 2001. Avian Dis 47, 996-1001.

Choi, Y.K., Ozaki, H., Webby, R.J., Webster, R.G., Peiris, J.S., Poon, L., et al., 2004. Continuing evolution of H9N2 influenza viruses in Southeastern China. J Virol 78, 8609-8614.

Dhama, K., Mahendran, M., Tomar, S., 2008. Pathogens Transmitted by Migratory Birds:Threat Perceptions to Poultry Health and Production. Int J Poult Sci 7, 516-525.

EC, 2010. Commission decision 2011/367/EU of 25 June 2010 on the implementation by member states of surveillance programs for avian influenza in poultry and wild birds. Off J Eur Union L 166, 7.

Fallah Mehrabadi, M.H., Bahonar, A.R., Vasfi Marandi, M., Sadrzadeh, A., Tehrani, F., Salmanf, M.D., 2016. Sero-survey of Avian Influenza in backyard poultry and wild birdspecies in Iran—2014. Prev Vet Med 128, 1-5.

Hosseini, S.S., Nikoukar, A., Dourandish, A., 2012. Price Transmission Analysis in Iran Chicken Market. Int J Agr Manag Dev 2, 243-253.

Kim, H. R., Lee, Y. J., Park, C. K., Oem, J. K., Lee, O.S., Kang, H. M., et al., 2012. Highly Pathogenic Avian Influenza (H5N1) Outbreaks in Wild Birds and Poultry, South Korea. Emerg Infect Dis 18, 480-483.

Lee, E.K., Kang, H.M., Song, B.M., Lee, Y.N.A., Heo, G.B., Lee, H.S., et al., 2017. Surveillance of avian influenza viruses in South Korea between 2012 and 2014. Virol J 14, 54.

Lowen, A.C., Steel, J., 2014. Roles of humidity and temperature in shaping influenza seasonality. J Virol 88, 7692-7695.

Luan, L., Sun, Z., Kaltenboeck, B., Huang, K., Li, M., Peng, D., et al., 2016. Detection of influenza A virus from live-bird market poultry swab samples in China by a pan-IAV, one-step reverse-transcription FRET-PCR. Sci Rep 6, 30015.

Majidzadeh-A, K., Ghalyanchi-Langeroudi, A., Soleimani, M., Karimi, V., Morovvati, A., 2012a. Molecular

Surveillance of Avian Influenza in Bird Parks of Tehran, Iran. Iran J Vet Med 6, 165-169.

Majidzadeh-A, K., Soleimanidor, M., Morovvati, A., Karimi, V., Ghalyanchi-Langeroudi, A., 2012b. Molecular Surveillance of Avian Influenza in Live Bird Market of Qom City in Iran. Iran J Virol 6, 33-34.

Monne, I., Ormelli, S., Salviato, A., De Battisti, C., Bettini, F., Salomoni, A., et al., 2008. Development and Validation of a One-Step Real-Time PCR Assay for Simultaneous Detection of Subtype H5, H7, and H9 Avian Influenza Viruses. J Clin Microbiol 46, 1769-1773.

Negovetich, N.J., Feeroz, M.M., Jones-Engel, L., Walker, D., Alam, S.M., Hasan, K., et al., 2011. Live bird markets of Bangladesh: H9N2 viruses and the near absence of highly pathogenic H5N1 influenza. PLoS One 6, e19311.

Nili, H., Asasi, K., 2003. Avian influenza (H9N2) outbreak in Iran. Avian Dis 47, 828-831.

OIE, 2017. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2017. Chapter 2.3.4. Avian influenza (infection with avian influenza viruses) (NB: Version adopted in May 2015).

Salman, M.D., 2003. Animal Disease Surveillance and Survey Systems, Methods and Applications, Blackwell Publishing.

Sarwar, M., Muhammad, K., Rabbani, M., Younus, M., Sarwar, N., Ali, M.A., et al., 2013. Prevalence of Avian Influenza Viruses in Live Bird Markets of Lahore. J Anim Plant Sci 23, 388-392.

Schrijver, R.S., Koch, G., 2005. Avian influenza: prevention and control, Springer, New York.

Shariatmadari, F., 2000. Poultry production and the industry in Iran. World Poult Sci J 56, 55-65.

Swayne, John R., G., Larry R., M., Lisa K., N., David L., S., Venugopal, N., 2013a. Diseases of Poultry, Blackwell Publishing Ltd., Iowa 50010, USA.

Swayne, D.E., Glisson, J.R., McDougald, L.R., Nolan, L.K., Suarez, D.L., Nair, V., 2013b. Diseases of Poultry, Blackwell Publishing Ltd., Iowa 50010, USA.

Thanawat, T., Prasit, C., Thaweesak, S., Arunee, C., Wirongrong, H., Chantanee, B., et al., 2005. Highly Pathogenic Avian Influenza H5N1, Thailand, 2004. Emerg Infect Dis 11, 1664-1672.

Zadegan, S.S., 2017. Fereydoon Kenar, Ezbaran & Sorkh Ruds Ab-Bandans. In: Finlayson, C.M., Milton, G.R., Prentice, R.C., Davidson, N.C. (Eds.), The Wetland Book: II: Distribution, Description and Conservation, Springer Netherlands, Dordrecht, pp. 1-12.