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Short Communication

Serological Survey of Avian Influenza (H9N2) in Commercial Ostrich Farms in Iran, 2015

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ABSTRACT
The aim of this study was to determine the seroprevalence of avian influenza H9N2 subtype in the industrial ostrich farms and its geographical distribution. This cross-sectional study was conducted from January to June 2015. A total of 40 farms were selected from different provinces of Iran, from each of which 11 ostriches (n=440) were sampled. The sera samples were examined using 4 hemagglutination units of H9N2 antigens. A frequency distribution was used to describe the responses to the survey questions. The mean titers between provinces were compared using one-way analysis of variance. According to the results, 21 (47.5%) out of 40 farms and 108 (24.5%) out of 440 ostriches tested positive in the HI-H9N2 test. There were statistically significant differences between the mean titers of samples in different provinces (P<0.001). The current study was conducted on unvaccinated ostriches. The results showed that H9N2 had a high seroprevalence at both farm and bird levels. The findings of this study can be for the further investigation of infection in ostrich farms in order to consider this species in the surveillance programs of the Iranian Veterinary Organization. The detection and isolation of viruses and epidemiological investigation are necessary for the persistent use of H9N2 vaccines in some ostrich farms.
Keywords: Ostrich, Seroprevalence, H9N2, Avian Influenza, Iran

Enquête sérologique sur l'influenza aviaire dans les fermes commerciales d'autruches en Iran, 2015

Résumé: Le but de cette étude était de déterminer la séroprévalence du sous-type H9N2 de la grippe aviaire dans les fermes industrielles d'autruches et sa répartition géographique. Cette étude coupe transversale a été menée de janvier à juin 2015. Un total de 40 fermes ont été sélectionnées dans différentes provinces d'Iran, parmi lesquelles 11 fermes d'autruches (n = 440) ont été échantillonnées. Les échantillons de sérum ont été examinés à l'aide de 4 unités d'hémagglutination d'antigènes H9N2. Une distribution de fréquence a été utilisée pour décrire les réponses aux questions du sondage. Les titres moyens entre provinces ont été comparés à l'aide d'une analyse de variance à un facteur. Selon les résultats, 21 (47,5%) des 40 fermes et 108 (24,5%) des 440 autruches étaient positifs au test HI-H9N2. Il y avait des différences statistiquement significatives entre les titres moyens des échantillons dans différentes provinces (p <0,001). La présente étude a été menée sur des autruches

non vaccinées. Les résultats ont montré que le virus H9N2 présentait une séroprévalence élevée, tant au niveau de la ferme que des oiseaux. Les conclusions de cette étude peuvent servir à la poursuite des recherches sur l'infection dans les fermes d'autruches afin de prendre en compte cette espèce dans les programmes de surveillance de l'Organisation vétérinaire iranienne. La détection et l'isolement des virus et des enquêtes épidémiologiques sont nécessaires pour l'utilisation persistante des vaccins anti-H9N2 dans certaines fermes d'autruche.
Mots-clés: Autruche, Séroprévalence, H9N2, Grippe Aviaire, Iran

INTRODUCTION

In the recent decades, ostrich farming has been developed in different countries across the world, including Iran, where the commercial ostrich farming began in 1999. Nowadays, this industry is spreading all over the country, especially in the central areas (Khodaei Motlagh, 2015). The growing trend of this industry indicates its compatibility with the weather and climate in different parts of Iran. Different studies on ostrich farming revealed that it is economically beneficial (Kohansal et al., 2014). Industrial ostrich farming is extending globally due to its high-quality leather, low-cholesterol meat, egg, eggshell, and oil. Avian influenza (AI) is a highly infectious viral disease, to which a wide range of birds, including ostriches, are susceptible. The AI viruses (H7N1subtype) were isolated from the South African ostriches in 1991 for the first time, and then in 1994 (H5N9 subtype) (Cooper et al., 2007). Various strains of AI viruses have been isolated from this species, including H5N2, H5N9, H6N8, H7N1, H9N2, and H10N1 (Cooper et al., 2007). Studies have shown that ostriches have an uncertain response to AI infection. It has been reported that highly pathogenic AI (HPAI) and low pathogenic AI (LPAI) viruses, either under natural conditions or in vivo, caused similar non-pathognomonic and non-specific clinical signs. In the mentioned study, despite a high viral replication, mortality occurred just in young birds (Toffan et al., 2010). Respiratory disorders, greenish colored urine, depression, ruffled feathers, and ocular discharges, are reported as AI clinical signs. Even HPAI viruses do not cause severe mortality in ostriches. High rates of mortality in this species are often due to concurrent bacterial infections, high density, poor management, and unsuitable ventilation amongst other factors (Sinclair et al., 2006) (Olivier, 2005; Abolnik et al., 2009). Ostriches can act as a host for assortment when infected with various AI viruses while there are no clinical signs of the disease. The H6N2 outbreak in South Africa in 2002 confirms this finding, and phylogenetic studies revealed that the primary generations of this virus have been aroused from assortment between H9N2 and H6N8 AI viruses (Abolnik et al., 2007). The AI H9N2 infection spread significantly in different countries from 1994-1999. This virus strain was isolated for the first time from South African ostriches in 1995. However, in 2002, the virus strain caused severe epidemics in the poultry industry of Iran and Pakistan (Alexander, 2000). A phylogenetic analysis of samples taken from ostrich farming regions in Southern Africa revealed that H9N2 virus in ostriches was originated from the wild bird population all over the Southern region of Africa (Abolnik et al., 2010b). This virus was isolated in Iran in 1998 for the first time (Nili and Asasi, 2002). It spread throughout the country rapidly and became endemic with a high prevalence in the domestic and industrial poultry (Nili and Asasi, 2002; Fallah Mehrabadi et al., 2016). Various studies have indicated that migratory waterfowls play an important role in AI outbreaks. As the outdoor breeding system is used for ostriches, they are more prone to AI virus transmission from waterfowl. A virus can survive more during the cold months, compared with other months of the year; therefore, this is a seasonal disease. Therefore, it is necessary to undertake surveillance and monitoring programs (as a part of control programs) for better conceptualization of the epidemiology and ecology of the disease in ostrich farms (Olivier, 2005). With this background in mind, the purpose of the present study was to determine the seroprevalence of H9N2 subtype in industrial ostrich farms and its geographical distribution. The pattern of serological titer was assessed in order to prepare some essential information for planning an effective control program.

MATERIAL AND METHODS

Study design and sampling. There are approximately 400 commercial ostrich farms in Iran registered in the Iranian Veterinary Organization (IVO), as a part of the Geographical Information System (GIS). In this system, every farm has a unique 11-digit code. During the study period, there were 105 active farms. This cross-sectional study was conducted on 440 ostriches selected from 40 farms across different provinces from Iran from January to June 2015. The number of sample farms was determined assuming 5% prevalence at the farm level with 95% confidence level and 5% precision regarding the finite population (EC, 2010). For the farm selection, a sample frame list of unvaccinated farms was transferred to a MS Excel® (version 2010) sheet. The final list of farms was generated according to the 11-digit codes of the farms, using the random selection function of MS Excel®. For sampling purposes, 11 ostriches were selected from each of the 40 farms considering 95% probability of identifying at least one positive bird, if the expected seroprevalence of the birds is ≥ 30% (EC, 2010).

Laboratory tests. In this study, 1 ml blood was taken from each bird neck vein (jugular vein), and then the serum was separated. The sera were treated using chicken red blood cells (RBC) with 10% dilution according to the World Organization for Animal Health (OIE) protocol before performing the haemagglutination inhibition (HI) test in order to reduce nonspecific reactions. Thereafter, each serum sample was examined using 4 haemagglutination (HA) units of H9N2 antigens and chicken RBC. Sera with titers of 1/16 or more (titer≥4), based on $\log_2$, and farms with at least one positive bird were considered positive (OIE, 2015).

Data collection and analysis. The study responses and the results of the laboratory tests of each farm were recorded in the IVO-GIS database. Subsequently, other relevant available records in the database were linked to the responses and laboratory results. The data (sera titers) were presented as central tendency, mean, median, and standard deviation. A frequency distribution was used to describe the responses to the survey questions. The mean titers among provinces were compared using one-way analysis of variance (Salman, 2003). $P<0.05$ was considered statistically significant. The SPSS software (version 22) and ArcGIS (version 9.2) were used for statistical analysis and mapping, respectively.

RESULTS AND DISCUSSION

A total of 40 ostrich farms (Figure 1) and 440 birds were sampled. Based on the results, 21(47.5%) out of 40 farms and 108 (24.5%) out of 440 ostriches tested positive in the HI-H9N2 test. The highest relative frequencies of seropositive samples were obtained as 100%, 57.14%, and 54.54% in Lorestan, Alborz, and North-Khorasan/Qazvin, Iran, respectively. All samples were negative in Khuzestan, Kerman, Zanjan, Hormozgan, and Sistan-o-Bluchestan, Iran. Table 1 shows the number of farm and bird samples, in addition to the frequency, mean, and standard deviation of HI (H9) positive birds. Data Analysis revealed a statistically significant difference between the HI (H9) mean titers of samples in different provinces ($P<0.001$). The mean titers were significantly higher in Lorestan, Hamadan, Alborz, North-Korasan, Qazvin, and Khorasan-e-Razavi provinces than others. In this regard,

Table 1. Sero-prevalence of avian influenza H9N2 subtype in commercial ostrich farms in Iran (2015)

Province	Number of ampled farms	Number of positive farms	Number of sampled ostriches	HI-H9N2			
				Number of HI (H9N2) positive ostriches	**Mean titer**	**Standard deviation**	**Coefficient of variation (cv %)**
Lorestan	2	2 (100%)	22	22 (100%)	5.41	0.67	12.31
Hamadan	2	2 (100%)	22	12 (54.5%)	3.64	1.60	43.89
Alborz	3	3 (100%)	28	16 (57.1%)	3.36	1.42	42.29
North-khorasan	1	1 (100%)	11	6 (54.6%)	3.27	2.65	80.95
Qazvin	2	1 (50%)	22	8 (36.4%)	2.64	3.63	137.78
Razavi-khorasan	2	1 (50%)	20	3 (15%)	2.45	0.89	36.21
Esfahan	9	5 (55.6%)	90	26 (28.9%)	2.30	2.23	96.77
Semnan	1	1 (100%)	11	3 (27.3%)	1.55	2.66	172.08
Yazd	2	1 (50%)	22	4 (18.2%)	1.50	1.74	115.93
Kordestan	2	1 (50%)	22	3 (13.6%)	1.05	1.46	139.95
Tehran	1	1 (100%)	15	1 (6.7%)	0.80	1.01	126.77
East azarbayjan	1	0 (0%)	20	0 (0%)	0.75	0.97	128.87
Mazandaran	4	1 (25%)	44	1(2.3%)	0.73	1.17	160.69
Fars	3	1 (33.4%)	33	3 (9.1%)	0.48	0.38	79.27
Khuzestan	1	0 (0%)	11	0 (0%)	0	0	
Zanjan	1	0 (0%)	12	0 (0%)	0	0	
Sistanvabaluchestan	1	0 (%0)	13	0 (0%)	0	0	
Kerman	1	0 (%0)	11	0 (0%)	0	0	
Hormozgan	1	0 (%0)	11	0 (0%)	0	0	
Total	40	21 (%52.5)	440	108 (24.5%)	HI Test: hemagglutination inhibition test		

the highest mean titer was in Lorestan ($P<0.05$). However, there were no significant statistical differences between the mean titers in Hamadan, Alborz, North-Korasan, and Qazvin provinces ($P>0.05$). In Iran, there is no report about the clinical disease and virus isolation of H9N2 in ostriches. However, AI H9N2 subtype vaccine, monovalent or bivalent (AI+ND), are used in some ostrich farms, according to the data available in the IVO GIS database. The current study was conducted on unvaccinated ostriches and indicated a high seroprevalence of H9N2 at both farm and bird levels. Considering that the samples were collected from apparently healthy ostriches (no clinical signs), the high seroprevalence rate may be indicative of circulating H9N2 subtypes among these birds. Our results are in agreement with those of the previous studies regarding the seroprevalence of H9N2 in other avian species in Iran. Ghanei et al. showed that 40.6% of the samples were seropositive in a serological survey of H9N2 among broiler chickens in a slaughterhouse (Ghaniei et al., 2013). In another study conducted by Fathiet al. in broiler farms with clinical disease, 53.33% of the farms were seropositive (Nili and Asasi, 2002). Fallah et al. conducted a study in rural domestic poultry, which revealed that 86% of villages were seropositive for H9N2 (Fallah Mehrabadi et al., 2015). Moreover, in another study carried out by Fallah et al. in a backyard poultry with wild bird species, the premise and bird level seroprevalence of H9N2 in bird gardens were reported as 53.3% and 16%, respectively, while these value were obtained as 90% and 31% in the villages, respectively (Fallah Mehrabadi et al., 2016). In line with the findings of the other similar studies, our esults demonstrated that H9N2 is circulating among commercial and backyard poultry and other bird species in Iran. Several possible explanations exist for the high seroprevalence of H9N2 in ostrich farms due to the fact that there are no documented evidence (published or unpublished) about the clinical disease and virus isolation of H9N2 in ostriches in Iran. One of these explanations is the circulation of this virus strain among various avian species. Ostriches are more probable to be among the affected species because their breeding period is long and that they are reared outdoor; therefore, they have a higher possibility of being in contact with other avian species (e.g., backyard poultry and wild birds). Furthermore, since AI H9N2 subtype viruses are considered as low pathogenic agents, suitable conditions are not provided for the occurrence of clinical disease. Finally, these findings can be used for the further investigation of infection in ostrich farms in order to consider this species in the surveillance programs and control measures implemented by the IVO. The detection and isolation of viruses and epidemiological investigations are necessary for the persistent use of H9N2 vaccine in some ostrich farms.

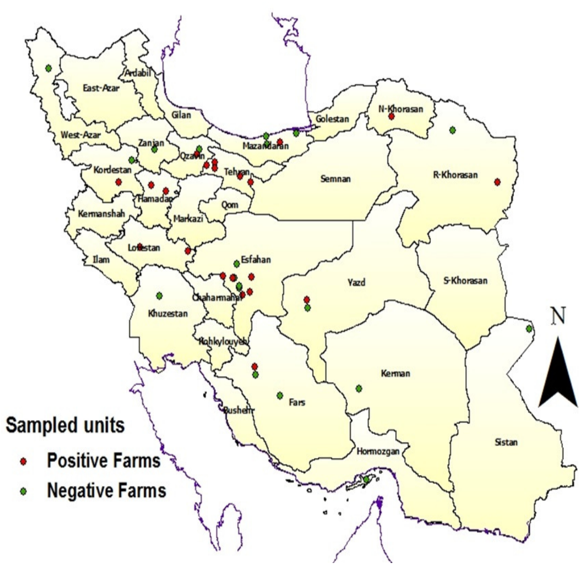

Figure 1. Sampled ostrich farms for the investigation of the seroprevalence of avian influenza (H9N2 subtype) in Iran (2015) (Red: seropositive farms, green: seronegative farms)

Ethics

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

Conflict of Interest

The authors declare that they have no conflict of interest.

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