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Original Article

Avian Influenza (H9N2 Subtype) in Iranian Broiler Farms: A Cross-sectional Study

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ABSTRACT

The present study aimed to determine the seroprevalence of H9N2 influenza in broiler farms at the time of slaughter in Iran. A total of 747 birds were sampled from 74 Farms in 13 provinces within 2013-2016. The obtained sera were investigated using the hemagglutination inhibition (HI) test. Out of 74 sampled farms and 747 birds, 57 farms (77%) and 445 (59.57%) birds were reported to be seropositive. In 2013, 10 farms and 110 birds were sampled out of which three farms (29.6%) and 29 birds (30%) were seropositive. In 2014, 24 farms and 220 birds were sampled out of which 22 farms (91.6%) and 220 birds (86.6%) were positive in six provinces. In 2015, 30 farms and 278 birds were sampled out of which 5 farms (16%) and134 birds (48.2%) were positive in four provinces. Finally, in 2016, 7 farms (70%) out of 10 sampled farms and 62 birds (59%) out of 105 sampled birds were positive for H9N2 in eight provinces. The mean titer of units in 2013 was statistically lower, as compared to that in 2014 ($P<0.01$). In addition, the proportion of positive serum units in 2013 was statistically lower, as compared to that in 2014 ($P<0.001$). In general, the prevalence of H9N2 was high indicating the continuous circulation of the virus in Iran. Given the importance and impact of this virus on the poultry industry, people's livelihood, and public health, more epidemiological studies are needed to evaluate the effectiveness of the adopted measures and methods in controlling the H9N2 virus.

Keywords: Avian Influenza H9N2, Broiler farms, Abattoirs, Iran, Seroprevalence

Grippe Aviaire (sous-type H9N2) dans les Fermes Iraniennes de Poulets de Chair: une Étude Transversale

Résumé: Cette étude visait à déterminer la séroprévalence de la grippe H9N2 dans les fermes de poulets de chair au moment de l'abattage en Iran. Au total, 747 oiseaux provenant de 74 fermes réparties dans 13 provinces ont été échantillonnés entre 2013 et 2016. Les sérums obtenus ont été étudiés en utilisant le test de l'inhibition de l'hémagglutination (HI). Sur 74 fermes et 747 oiseaux échantillonnés, 57 fermes (77%) et 445 (59,57%) oiseaux étaient séropositifs. En 2013, 10 fermes et 110 oiseaux ont été analysés, parmi lesquels 3 fermes (29,6%) et 29 oiseaux (30%) étaient séropositifs. En 2014, 24 fermes et 220 oiseaux ont été échantillonnés, dont 22 fermes (91,6%) et 220 oiseaux (86,6%) provenant de six provinces se sont avérés être positifs. En 2015, parmi les 30 fermes et 278 oiseaux testés, 5 fermes (16%) et 134 oiseaux (48,2%) répartis dans quatre provinces étaient positifs. Enfin, en 2016, 7 (70%) des 10 fermes échantillonnées et 62 (59%) des 105 oiseaux testés étaient positifs pour le H9N2 dans huit provinces. Le titre sérologique moyen en 2013 était statistiquement inférieur

à celui de 2014 (P <0,01). De plus, la proportion d'unités sériques positives en 2013 était statistiquement inférieure à celle de 2014 (P<0,001). Dans l'ensemble, la prévalence du H9N2 était élevée, ce qui indique la circulation continue du virus en Iran. Compte tenu de son importance et l'impact de ce virus sur l'industrie avicole, les moyens de subsistance des populations et la santé publique, des études épidémiologiques supplémentaires sont nécessaires pour évaluer l'efficacité des mesures et méthodes adoptées pour lutter contre le virus H9N2.
Mots clés: Grippe aviaire H9N2, Fermes de poulets de chair, Abattoirs, Iran, Séroprévalence

INTRODUCTION

Iran is the largest contributor to the poultry industry in the Middle East, and broilers have the largest share (Shariatmadari, 2000). The production of chicken meat in Iran in 2010 was 1.765 million tons which ranked seventh among the world's largest producers (FAO, 2018). Due to its affordable price, chicken meat with a total consumption of 26.1 kg is considered the most important source of protein for the households, as compared to red meat and fish with 11.43 and 15.2 kg, respectively. Over the past three decades, the poultry industry has witnessed dramatic growth, and the production of chicken meat has risen from 110,000 tons in 1974 to 2122.5000 tons in 2015 with an average annual growth rate of 7.67%. This growth was so tremendous that produced chicken meat not only supplied the domestic needs but it was also exported to neighboring countries. The export of chicken meat in 2013 and 2014 was 4708,000 and 6593,000 tons, respectively. There are 20,886 broiler breeder farms with a capacity of 393,254 broilers in Iran. According to data recorded in the Veterinary Organization's Diseases Database (GIS), there are more than 5,000 non-licensed farms in the country, apart from the licensed ones. In total, 24,700 broiler units are active in Iran. The poultry industry has been always exposed to disease agents and their problems. The high density of poultry, as well as the unavailability of many infrastructures, including quarantine and appropriate biosecurity principles, has made the conditions favorable for avian disease prevalence. A wide range of pathogens, including influenza, infectious bronchitis, Newcastle disease viruses, avian pneumovirus (APV), and mycoplasma gallisepticum play a significant role in the development of respiratory diseases in poultry (Saif et al., 2013). One of these diseases is influenza A virus subtype H9N2 (H9N2 influenza) which along with the very virulent Newcastle and infectious bronchitis viruses are regarded as the main causes of disease and mortality in broilers. The simultaneous infection in the case of these diseases causes a high mortality rate and extensive damage in broiler flocks (Haghighat-Jahromi et al., 2008; Roussan et al., 2008; Hassan et al., 2016). Avian influenza is caused by type A influenza viruses belonging to the orthomyxoviridae family. In the poultry industry, three subtypes of avian influenza viruses, including H5, H7, and H9N2, are pathogenically and economically important (Saif et al., 2013). H9N2 is a low pathogenic avian influenza (LPAI) and two H5 and H7 subtypes are highly pathogenic viruses or highly pathogenic avian influenza (HPAI) in poultry (OIE, 2015). Despite its low pathogenicity, if H9N2 influenza outbreaks are caused by concurrent bacterial and viral infections, they will be very lethal and lead to economic losses due to reduced egg production and reduced feed intake. Moreover, it is the most important sub-type of influenza in poultry in endemic countries (Thuy et al., 2016). The first report of the isolation of the H9N2 virus from South America was recorded in 1966 when it led to numerous outbreaks in the turkey rearing farms in the United

States (Perez et al., 2003). The H9N2 virus in Asia was first isolated in 1994 in Guangdong, China; thereafter, it spread across Asia, the Middle East, and even Europe (Sun and Liu, 2015). The H9N2 influenza virus is the most common subtype isolated from non-aquatic birds in Asia and Europe. This disease is endemic to the poultry industry in the Middle East and Asia, including Iran, and causes significant damages (Nili and Asasi, 2003). The avian influenza A (H9N2) virus infection was significantly prevalent within 1994-1999, and this virus developed severe problems and diseases in the poultry industry of Iran and Pakistan in 2000 (Alexander, 2007). Although the H9N2 influenza vaccine is used in Iran, it is widely observed in the backyard and industrial poultry despite vaccination (Nili and Asasi, 2003). There are several reports of mortalities in industrial poultry caused by this subtype (Nili and Asasi, 2003), as well as viral circulation and seroprevalence in backyard poultry in the country (Fallah Mehrabadi et al., 2016). Regarding the importance of this disease, the current study was conducted to determine the seroprevalence of H9N2 influenza in broiler farms during slaughter in the selected slaughterhouses in Iran.

MATERIAL AND METHODS

Study population. The study unit included active broiler farms during the research period in the targeted provinces in Iran. This cross-sectional study was carried out from August 2013 to October 2016 during the implementation of the National Avian Influenza Program. Sampling was carried out in the slaughterhouse with the highest capacity and variety of broilers in each of the six provinces of Iran, including Tehran, Qom, East-Azarbayjan, Fars, Khorasan-e-Razavi, and Isfahan (Figure 1). In the present study, the samples were taken from farms for which H9N2 AIV vaccination was not implemented. The sampling was performed on all broiler flocks that were sent for slaughter during sampling time, and 11 serum samples were taken from each broiler flocks.

Sample evaluation method. Before slaughtering, 1 ml blood was taken from each bird and its serum was separated. The Haemagglutination Inhibition (HI) test was performed on each serum sample using 4 HA units of H9N2 antigen which was provided by Razi Vaccine and Serum Research Institute, Karaj, Iran, according to the instructions of Veterinary Organization. The calculation of the antibody titer was accomplished based on serial log2 dilutions of the serums. The serum titers of 4≥ (1/16 dilution) were considered positive (OIE, 2015), and the farms with at least one positive bird were regarded as positive units.

Data analysis. To describe the results, the frequency of samples, serum positive farms, and the arithmetic mean of the farm titer are expressed. The comparison of mean serum titers was carried out with one- way ANOVA and post hoc Tukey test, and serum positive birds and farms proportions in different years were analyzed by the Chi-square test at $P < 0.05$ significance level. The data were analyzed in SPSS software (version 22).

RESULTS

The present study was performed in 13 provinces. A total of 747 birds from 74 epidemiologic units (from slaughterhouses in six provinces) were sampled within 4 years. The highest numbers of sampled farms were located in -e-Razavi, Isfahan, Khorasan and Qom provinces with 25, 19, and 11 farms, respectively (Table 1). Out of 74 farms, 57 farms (77% (86%-65.8%) and confidence intervals of 95%) and 445 birds out of 747 sampled birds (59.57% (63.1%-56 %) and confidence intervals of 95%) were serologically positive (Table 1). In 2013, from 10 broiler farms and 110 birds that were sampled in 4 provinces, 3 units (29.6%) and 29 birds (30%) were seropositive. In 2014, from 24 broiler farms and 254 birds that were sampled in 6 provinces, 22 units (91.6%) and 220 birds (86.6%) were seropositive. In 2015, 30 broiler units and 278 birds were sampled in 4 provinces out of which 5 units (16%) and 134 birds (48.2%) were positive. In 2016, 10

broiler units and 105 birds were sampled in 8 provinces out of which 7 units (70%) and 62 birds (59%) were positive (Table 1). Based on the results illustrated in Table 2, the mean titer of sampled units was 4.54±2.95 within 4 years. The mean titer of units in 2013 was statistically lower, as compared to that in 2014 ($P<0.01$). Nevertheless, there was no significant difference in terms of the mean titer of units in 2013, 2014, 2015, and 2016 ($P>0.05$). The proportion of positive serum units in 2013 was statistically lower, as compared to that in 2014 ($P<0.001$). Nonetheless, there was no significant difference in terms of the proportion of positive serum units between 2013 and other years ($P>0.05$). The proportion of positive serum birds in 2013 was statistically lower, as compared to that in other years ($P <0.05$). The results are presented in Table 3.

DISCUSSION

In the current study, the prevalence of H9N2 infection was very high both in the farms and among the birds indicating that the disease has been hyper-endemic in broiler chickens and the virus has been in high circulation in these farms. The prevalence rate and mean antibody titers were variable in the studied years probably due to the difference in the number of sampled farms and the sampling region. The infection ratio also has a statistically significant difference in the studied years. This difference signifies the difference in infection rate in different years which can be attributed to different reasons, such as the implementation of control measures, difference in poultry density in the studied areas, and the difference in the biosecurity and hygienic level of the studied units. In the current study, the samples were taken from seemingly healthy flocks without any clinical symptoms. Although H9N2 influenza viruses are low pathogenic viruses, high mortality in broilers, as well as decreased egg production in the layer, and broiler breeder flocks will be observed at the presence of favorable conditions, such as co-infections. The current research was a cross-sectionall study for the evaluation of seroprevalence of H9N2 infection in Iran. The results may differ from year to year; however, the important point is that the H9N2 virus is endemic and should not be ignored or underestimated due to its devastating impact on the poultry industry and rural poultry production. In similar studies in Iran, the prevalence of this disease has been reported to be high. For instance, in 2013, the seroprevalence of H9N2 was reported to be 40.6% in broiler flocks in northwestern Iran. In 2014, it was measured at 67.5% at the unit level and 48.9% at bird level in turkey, partridge, and quail breeding farms. Moreover, in 2015, this seroprevalence was reported as 59.4% and 35.9% at unit and bird levels, respectively (Mehrabadi et al., 2018). The seroprevalence was calculated at 86% in rural native backyard poultry in 2013. In 2014, the seroprevalence was 90 and 53.3% in rural areas and zoos, respectively (Ghaniei et al., 2013). Despite widespread vaccination against the disease, there are reports of mortality and reduced production caused by the disease in flocks of broilers even among the vaccinated ones (Nili and Asasi, 2003). In several studies conducted in Iran, H9N2 influenza has always been one of the most important causative agents, especially in respiratory syndromes. In addition to Iran, H9N2 influenza is endemic in many neighboring countries and the Middle East resulting in numerous diseases, losses, and damage every year. In 2017, Hosseini et al. isolated the H9N2 virus from 40% of the broiler flocks sampled in Afghanistan. They reported the high prevalence of H9N2 influenza in poultry flocks in most regions of Afghanistan and the similarity of these viruses to common viruses in Iran and Pakistan (Hosseini et al., 2017). In Iraq, the prevalence of H9N2 was reported in 16% of broiler flocks with no symptom, 100% of broiler and rural flocks with respiratory symptoms, and 57.14% of wild birds (Abdul-Sada, 2015). In another study in Iraq, H9N2 and Newcastle disease viruses were simultaneously isolated in 75% of broiler flocks with respiratory problems, and H9N2 was isolated in 25% of broiler flocks with respiratory problems alone (Al-Mohana et al., 2013). In Jordan, 71% of broiler breeders were

Table 1. Seroprevalence of H9N2 avian influenza in slaughterhouses in different provinces within 2013-2016

Province	sampled Farms	Positive (%)	sampled Birds	Positive (%)
East Azarbaijan	1	1(100%)	5	2 (40%)
Ardabil	1	1 (100%)	12	12 (100%)
Isfahan	25	21 (84%)	225	169 (75.1%)
Alborz	1	1 (100%)	15	15 (100%)
Tehran	1	0 (0%)	9	0 (0%)
KH. Razavi	19	17 (89.5%)	197	86 (43.7%)
Semnan	1	0 (0%)	10	0 (0%)
Fars	6	0 (0%)	65	0 (0%)
Qazvin	1	0 (0%)	11	0 (0%)
Qom	11	11 (100%)	120	110 (91.7%)
Kerman	2	2 (100%)	18	18 (100%)
Kohgiluyeh va Buyer Ahmad	1	0 (0%)	16	0 (0%)
Mazandaran	4	3 (75%)	44	33 (75%)
Total	74	57 (77%)	747	445 (59.6%)

Table 2. Mean titer of H9N2 avian influenza serum antibody in broiler farms of Iran within 2013-2016

Year	Number of Sampled farms	Mean titer H9N2	Standard deviation	P-value
2013	10	2.19	3.532	
2014	24	5.62	2.387	0.009
2015	30	4.14	2.452	0.236
2016	10	4.85	3.843	0.155
Total	74	4.45	2.959	-

Table 3. Seroprevalence of H9N2 avian influenza in broiler farms on farms and Birds level by year

	Farm level					Bird level				
Year	Number of Sampled farms	positive	OR	CI 95%	P-value	Number of Sampled Birds	positive	OR	CI 95%	P-value
2013	10	3	1	-		110	29			
2014	24	22	0.04	.01-.28	<0.001	254	220	0.06	0.03-0.1	<0.001
2015	30	5	2.14	0.41-11.26	0.312	178	134	0.12	0.07-0.20	<0.001
2016	10	7	0.18	0.03-1.25	0.074	105	62	0.25	0.14-0.44	<0.001

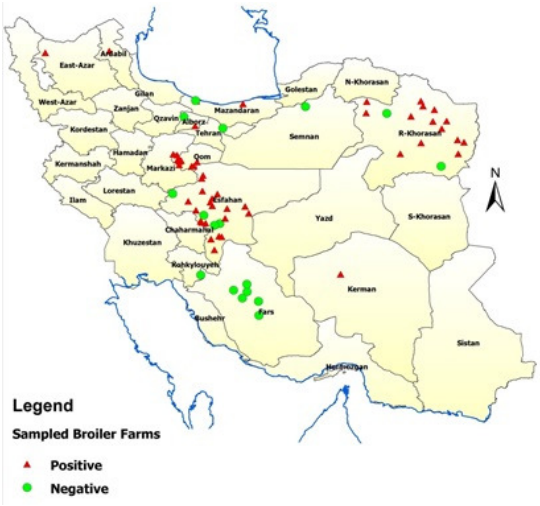

Figure 1. Sampled farms for AI H9N2 in slaughterhouses in Iran within 2013-2016

seropositive for H9N2 (Al-Natour and Abo-Shehada, 2005). In addition, the seroprevalence of H9N2 in this country in 2009 was reported as 54.2% and 78.3%, in broiler flocks and layer farms, respectively (Roussan et al., 2008). In Lebanon, Barbour et al. (2006) reported the isolation of H9N2 in commercial poultry flocks

with symptoms and observed a 32% H9N2 seroprevalence among workers in those farms. In a study conducted in Egypt, the H9N2 influenza virus was detected in 53% of samples; moreover, H9N2 influenza coinfection with infectious bronchitis virus with 41.7% prevalence had the highest simultaneous incidence of infection (Hassan et al., 2016). In the United Arab Emirates, the H9N2 influenza virus was isolated from broiler and quails chicks with mortality within 2000-2003, (Aamir et al., 2007). In Pakistan, the disease has been endemic since 1996 and has caused economic losses to the poultry industry with the annual incidence of the disease in this country (Cameron et al., 2000). Furthermore, the H9N2 influenza virus poses a daunting challenge to public health, apart from economic losses to the poultry industry. In addition, there have been numerous reports of clinical cases of human infection with the H9N2 virus, the breakdown of the interspecies barrier (Lin et al., 2000), and the serological evidence of this infection in humans in Asia, the Middle East, Africa, and North America (Li et al., 2017). In Iran, Heidari et al. reported a 12% H9N2 seroprevalence with HI test in poultry farm workers (Heidari et al., 2016). The H9N2 virus is also of great importance due to the probability of its genetic reassortment with other influenza viruses, including highly pathogenic influenza viruses. So far, the role of H9N2 virus has been identified in the emergence of at least three viruses, including H5N1, H7N9, and H10N8 which can be pandemic and are transmitted directly from birds to humans and cause mortality in the affected people (Pu et al., 2015). In conclusion, based on the results of the present study, H9N2 influenza viruses are at high circulation in broiler farms in Iran. Due to the high density of broiler farms in many areas and the low level of hygiene and biosecurity of some farms, if the conditions are favorable, there is a high probability of the incidence of the disease with clinical manifestations and high mortality and heavy economic losses due to the simultaneous infection of the H9N2 virus with other pathogens. On the other hand, this virus can threaten public health and cause human infection since it can be transmitted to humans in the case of close contact with poultry. Therefore, in order to minimize the potential risks of this virus to the poultry industry and public health, the level of hygiene and biosecurity measures must be increased in farms. Furthermore, more epidemiological studies are needed to evaluate the effectiveness of the implemented measures, including vaccination, and the possible correction of adopted methods.

Ethics

We 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.

Authors' Contribution

Study concept and design: Fallah Mehrabadi, M. H., Motamed, N.,

Acquisition of data: Tehrani, F., Ghalyanchilangeroudi, A., Borhani Kia, A.

Analysis and interpretation of data: Fallah Mehrabadi, M. H.

Drafting of the manuscript: Motamed, N.

Critical revision of the manuscript for important intellectual content: Fallah Mehrabadi, M. H., Tehrani, F., Ghalyanchilangeroudi, A.,

Statistical analysis: Fallah Mehrabadi, M. H.,

Administrative, technical, and material support: Fallah Mehrabadi, M. H., Motamed, N., Tehrani, F., Ghalyanchilangeroudi, A.

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