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

Fallah Mehrabadi 1, M. H., Motamed 2*, N., Ghalyanchilangeroudi 3, A., Tehrani 4, F., Borhani Kia, A. 5

1. Department of Poultry Diseases, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
2. Department of Poultry Vaccine Research and Production, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
3. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Iranian Veterinary Organization, Tehran, Iran
5. Avian Private Veterinary Clinic, Tehran, Iran

Corresponding author email: motamed62@yahoo.com

Abstract

The aim of this study was to determine the sero-prevalence of H9N2 influenza in broiler farms at the time of slaughter in Iran. A total of 747 birds from 74 Farms in 13 provinces were sampled from 2013 to 2016. Sera were investigated using haemagglutination inhibition test (HI) test. Fifty seven (77%) and 445 birds sampled birds (59.57%) were sero-positive. In 2013, 10 farms and 110 birds were sampled in which three farms (29.6%) and 29 birds (30%) were seropositive. In 2014, 24 farms and 220 birds were sampled. 22 farms (91.6%) and 220 birds (86.6%) in 6 provinces were positive. In 2015, 30 farms and 278 birds were sampled that 5 farms (16%) and 134 birds (48.2%) in 4 provinces and finally in 2016, 7 farms (70%) out of 10 sampled farms and 62 birds (59%) out of 105 sampled birds in 8 provinces were positive for H9N2. The mean titer of units in 2013 was statistically lower than that in 2014 (P <0.01). The proportion of positive serum units in 2013 was statistically lower than that in 2014 (P<0.001). Overall the prevalence of H9N2 was high that indicate the continuous circulation of the virus in Iran. Considering the importance and impact of the virus on poultry industry and people Livelihood as
well as public health there is a need for more epidemiological studies to evaluate the effectiveness of the measures and methods taken to H9N2 control.

**Key words:** Avian Influenza H9N2, Broiler farms, abattoirs, Iran, Seroprevalence.

**Introduction**

Iran has the largest 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 was ranked seventh among the world's largest producers (FAO, 2018). Because of the affordable price, chicken meat with a total consumption of 26.1 kg compared to red meat and fish, 11.43 and 15.2 kg, respectively, is considered as the most important source of protein for the households. Over the past three decades, poultry industry has grown dramatically; chicken meat production of 110 thousand tons in 1974 with an average annual growth rate of 7.67% has grown to 2122.5 thousand tons in 2015. The growth of the industry was such that in addition to supplying the domestic needs, a part of the produced chicken meat was also exported to neighboring countries. The export of chicken meat in 2013 and 2014 was 4708 thousand tons and 6593 thousand tons, respectively. In the country, there are 20886 broiler breeding farms with a capacity of 393254 thousand broilers. According to data recorded in the Veterinary Organization's Diseases Database (GIS), except for licensed farms, there are more than 5,000 non-licensed farms in the country. A total of 24700 broiler units are active in our country.

The poultry industry has been always exposed to diseases agents and their problem. The high density of poultry, as well as the lack of availability of many infrastructures, including quarantine and appropriate biosecurity principles, has made the conditions favorable for avian diseases
prevalence. A wide range of pathogens, including influenza, infectious bronchitis, Newcastle disease viruses and avian pneumovirus (APV) and mycoplasma gallisepticum play a role in the development of respiratory diseases in poultry (Swayne et al., 2013). One of the most important of these diseases is the H9N2 influenza which together with very virulent Newcastle and infectious bronchitis viruses are considered as the main causes of disease and mortality in broilers; and the simultaneous infection in the case of these diseases cause 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 including H5, H7 and H9N2 of avian influenza viruses are pathogenically and economically important (Swayne 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). H9N2 influenza outbreaks, despite its low pathogenicity, if caused by concurrent bacterial and viral infections, is very lethal and causes economic damage due to reduced egg production and reduced feed intake, and is the most important sub-type of influenza in poultry in countries to which the disease is endemic (Thuy et al., 2016). The first report of the isolation of the H9N2 virus from South America was recorded in 1966, when it was causing numerous outbreaks in the United States in the turkey rearing farms (Perez et al., 2003). The H9N2 virus in Asia was first isolated in 1994 in Guangdong, China, and then it spreaded 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 poultry industry in the Middle East and Asia, including our country, Iran, and causes significant damages (Nili and Asasi, 2003). Between 1994 and
1999, the virus infection of the H9N2 avian influenza was significantly prevalent, and in 2000, the virus developed severe problems and diseases in poultry industry of Iran and Pakistan (Alexander, 2007). Although the H9N2 influenza vaccine is used in Iran, it is widely observed, despite vaccination, in backyard and industrial poultry (Nili and Asasi, 2003). Several reports of mortalities caused by this subtype in industrial poultry (Nili and Assasi, 2003) As well as viral circulation and sero-prevalence in backyard poultry in the country (Fallah Mehrabadi et al., 2016). Regarding the importance of this disease, this study was conducted to determine the sero-prevalence of H9N2 influenza in broiler farms during slaughter and in selected slaughterhouses in the country.

Materials and Methods

Statistical Population of the Study

The study unit included active broiler farms during the implementation of the project in the targeted provinces in Iran. This cross-sectional study was carried out between August and October of the years 2013 till 2016 at the same time as the implementation of the avian influenza national surveillance program. Sampling was carried out in the slaughterhouse with the highest capacity and variety of broilers in each of the 6 provinces of Iran including: Tehran, Qom, East-Azarbayjan, Fars, Khorasan-e-Razavi and Isfahan. In this study, samples were taken from farms in which vaccination against H9N2 influenza was not performed. Sampling was carried out for all broiler flocks that were sent for slaughter at sampling time; 11 serum samples were taken from each broiler flocks.

Sample Evaluation Method
1 ml blood was taken from each bird before slaughter and its serum was separated. The Haemagglutination Inhibition (HI) test was performed on each serum sample using 4 HA units of H9N2 antigen, provided by the Razi Vaccine and Serum Research Institute, Karaj, Iran and according to the instructions of the veterinary organization. Calculation of the antibody titer was performed based on log2 serial dilutions of the serums. Serums titres of 4 and above (1/16 dilution) were considered positive (OIE, 2015). The farms with at least one positive bird were considered 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. Comparison of mean serum titers was carried out with one way ANOVA and Tukey post hoc test; and serum positive birds and farms proportions in different years were analyzed by Chi-square test at P <0.05 significance level. Statistical analyzes were performed using SPSS software, Version 22.

Results
The study has been performed in 13 provinces. A total of 747 birds from 74 epidemiologic units (from slaughterhouses in 6 provinces) were sampled during 4 years. The highest numbers of sampled farms were located in Isfahan, Khorasan-e-Razavi and Qom provinces with 25, 19 and 11 farms, respectively (Table 1).

57 farms out of the 74 farms (77% (86%-65.8%) and confidence intervals of 95%) and 445 birds out of the 747 birds sampled (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 shown in Table 2, the mean titre of sampled units was 4.54 ± 2.95 for a total of 4 years. The mean titer of units in 2013 was statistically lower than that in 2014 (P <0.01). However, there was no significant differences in terms of the mean titer of units in 2013, 2014, 2015 and 2016 years (P > 0.05).

The proportion of positive serum units in 2013 was statistically lower than that in 2014 (P <0.001), but there was no significant differences 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 than that in other years (P <0.05). Results are shown in Table 3.

**Discussion**

In this 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 were in high circulation in these farms. The prevalence percent and mean antibody titers were variable in the studied years, probably due to the difference in the number of farms sampled and the difference in the sampling region. The infection ratio in the studied years also has a statistically significant difference. This difference indicates the difference in infection percent in different years and can be due to different reasons such as the implementation of control measures, the
difference in poultry density in the studied areas, and the difference in the biosecurity and hygienic level of the studied units. In this study, samples were taken from seemingly healthy flocks without clinical symptoms. Given that the H9N2 influenza viruses are low pathogenic viruses, however, if conditions are favorable, including co-infections, high mortality in broilers, decreased egg production in layer and broiler breeder flocks will be observed. This was a cross sectional study for evaluating of seroprevalence of H9N2 infection in Iran, and results may differ from year to year but the important point is that the H9N2 virus is endemic and should not be ignored or underestimated because of its devastating impact on poultry industry even on rural poultry production.

In similar studies in Iran, the prevalence of the disease has been reported to be high. For example: In 2013, the sero-prevalence of H9N2 was reported to be 40.6% in broiler flocks in northwestern region in Iran, 67.5% at unit level and 48.9% at bird level in turkey, partridge, and quail breeding farms in 2014, and 59.4% and 35.9% respectively at unit and bird levels in 2015 (Fallah Mehrabadi et al., 2017). Sero-prevalence in rural native backyard poultry in 2013 was 86% in rural areas of the country and in 2014, in rural native poultry and bird garden (zoo), it was 90% and 53.3%, respectively, at unit level and 31% and 16%, respectively, at bird level (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, the H9N2 influenza has always been one of the most important causative agents, especially in respiratory syndromes.

In addition to Iran, the H9N2 influenza is endemic in many neighboring countries and the Middle East, causing many diseases and damage every year. In 2017, Hosseini et al., isolated 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 with no symptom in 16% of broiler flocks and with respiratory symptoms in 100% of broiler and rural flocks and in 57.14% of wild birds (Abdul-Sada, 2015). In another study in Iraq, H9N2 and Newcastle disease viruses were isolated simultaneously in 75 percent of broiler flocks with respiratory problems, and H9N2 was isolated in 25 percent of broiler flocks with respiratory problems alone (Mal-Mohana et al., 2013). In Jordan, 71% of broiler breeders were seropositive for H9N2 (Al-Natour and Abo-Shehada, 2005). In Jordan, the sero-prevalence of H9N2 in 2009 was reported 54.2% in broiler flocks and 78.3% in layer farms (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 sero-prevalence among workers in those farms (Barbour et al., 2006).

In a study conducted in Egypt, H9N2 influenza virus was detected in 53% of samples, and in addition, H9N2 influenza co-infection with infectious bronchitis virus, with 41.7% prevalence, had the highest simultaneous incidence of infection (Hassan et al., 2016).

In the United Arab Emirates, during the period 2000 to 2003, H9N2 influenza virus was isolated from broiler and quails chicks with mortality (Aamir et al., 2007). In Pakistan, the disease has been endemic in the country since 1996 and has caused economic damages to the country's poultry industry, and there are incidences of the disease every year in this country (Cameron et al., 2000).
In addition to its economic importance in the poultry industry H9N2 influenza virus is also important in terms of public health. Clinical cases of human infection with the H9N2 virus and the breakdown of the interspecies barrier (Lin et al., 2000) and the serological evidence of this infection in human in Asia, the Middle East, Africa, and North America have also been numerously reported (Li et al., 2017). In Iran, Heidari et al. reported a 12% H9N2 sero-prevalence with HI test in poultry farm workers (Heidari et al., 2016). The H9N2 virus is also important because of the probability of its genetic reassortment with other influenza viruses, including highly pathogenic influenza viruses. Up to now, the role of the H9N2 virus has been identified in the emergence of at least three viruses, including H5N1, H7N9 and H10N8, which have the risk of pandemi and are transmitted directly from bird to human and cause mortality in affected people (Pu et al., 2015). In conclusion, according to the results of this study, H9N2 influenza viruses are at high circulation in broiler farms in our country. 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 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, due to the possibility of transmission of these viruses to humans in the case of close contact with poultry, there is a public health risk and the risk of human infection. Therefore, in order to prevent the potential risks of the virus for the poultry industry and public health, the level of hygiene and biosecurity measures in the farms must be increased. Furthermore, there is a need for more epidemiological studies to evaluate the effectiveness of the measures taken, including vaccination and possible correction of the methods used.

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Conflict of interest
The authors declare that they have no conflict of interest.
References


Table 1: Sero-prevalence of H9N2 avian influenza in slaughterhouse, different provinces, 2013-2016

<table>
<thead>
<tr>
<th>Province sampled</th>
<th>Farms sampled</th>
<th>Positive (%)</th>
<th>Birds sampled</th>
<th>Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Azarbaijan</td>
<td>1</td>
<td>1 (100%)</td>
<td>5</td>
<td>2 (40%)</td>
</tr>
<tr>
<td>Ardabil</td>
<td>1</td>
<td>1 (100%)</td>
<td>12</td>
<td>12 (100%)</td>
</tr>
<tr>
<td>Isfahan</td>
<td>25</td>
<td>21 (84%)</td>
<td>225</td>
<td>169 (75.1%)</td>
</tr>
<tr>
<td>Alborz</td>
<td>1</td>
<td>1 (100%)</td>
<td>15</td>
<td>15 (100%)</td>
</tr>
<tr>
<td>Tehran</td>
<td>1</td>
<td>0 (0%)</td>
<td>9</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>KH. Razavi</td>
<td>19</td>
<td>17 (89.5%)</td>
<td>197</td>
<td>86 (43.7%)</td>
</tr>
<tr>
<td>Semnan</td>
<td>1</td>
<td>0 (0%)</td>
<td>10</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fars</td>
<td>6</td>
<td>0 (0%)</td>
<td>65</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Qazvin</td>
<td>1</td>
<td>0 (0%)</td>
<td>11</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Qom</td>
<td>11</td>
<td>11 (100%)</td>
<td>120</td>
<td>110 (91.7%)</td>
</tr>
<tr>
<td>Kerman</td>
<td>2</td>
<td>2 (100%)</td>
<td>18</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>Kohgeloyeh va buyerahmad</td>
<td>1</td>
<td>0 (0%)</td>
<td>16</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mazandaran</td>
<td>4</td>
<td>3 (75%)</td>
<td>44</td>
<td>33 (75%)</td>
</tr>
</tbody>
</table>
Table 2: Mean titer of H9N2 avian influenza serum antibody in broiler farms of Iran, by year, 2013-2016

<table>
<thead>
<tr>
<th>Year</th>
<th>Num of Sampled farms</th>
<th>Mean titer H9N2</th>
<th>Std. Deviation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>10</td>
<td>2.19</td>
<td>3.532</td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>24</td>
<td>5.62</td>
<td>2.387</td>
<td>0.009</td>
</tr>
<tr>
<td>2015</td>
<td>30</td>
<td>4.14</td>
<td>2.452</td>
<td>0.236</td>
</tr>
<tr>
<td>2016</td>
<td>10</td>
<td>4.85</td>
<td>3.843</td>
<td>0.155</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>4.45</td>
<td>2.959</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Sero-prevalence of H9N2 avian influenza in broiler farms on farms and Birds level, by years.

<table>
<thead>
<tr>
<th>Year</th>
<th>Num of Sampled farms</th>
<th>positive</th>
<th>OR</th>
<th>CI 95%</th>
<th>P value</th>
<th>Num of Sampled Birds</th>
<th>positive</th>
<th>OR</th>
<th>CI 95%</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>110</td>
<td>29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014</td>
<td>24</td>
<td>22</td>
<td>0.04</td>
<td>.01-.28</td>
<td>&lt;0.001</td>
<td>254</td>
<td>220</td>
<td>0.06</td>
<td>0.03-0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2015</td>
<td>30</td>
<td>5</td>
<td>2.14</td>
<td>0.41-11.26</td>
<td>0.312</td>
<td>178</td>
<td>134</td>
<td>0.12</td>
<td>0.07-0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2016</td>
<td>10</td>
<td>7</td>
<td>0.18</td>
<td>0.03-1.25</td>
<td>0.074</td>
<td>105</td>
<td>62</td>
<td>0.25</td>
<td>0.14-0.44</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Figure 1- Sampled farms For AI H9N2 In Slaughterhouses in Iran, 2013-2016