Outbreak Investigation of officially reported of highly pathogenic avian influenza (H5N8 subtype) in Iran- 2016

Fallah Mehrabadi *1, M. H., Tehrani 2, F., Shoushtari 1, A., Bahonar 3, A. R., Rabiee 3, M. H., Ghalyanchilangeroudi 4, A., Ghafouri 2, S. A., Amirhajloo 2, S.

1. Department of Poultry Diseases, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEEO), Tehran, Iran
2. Department of Health and Management of Poultry Diseases, Iranian Veterinary Organization, Tehran, Iran
3. Department of Food Hygiene & Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*Corresponding Author: mhf2480@yahoo.com

Abstract
On 14 November of 2016, an outbreak of HPAI was reported from a commercial layer farm located in Malard, Tehran province. The aim of study was to investigate HPAI H5N8 outbreaks in Iran. The questionnaire was prepared and completed through interviews with farm owners or field observations at the time of disease onset from November 2016 to February 2017. The HPAI H5N8 infection was confirmed in 30 different locations (10 villages (33.3%), nine layer farms (33%), two broiler breeder farms (6.67%), one layer breeder farm (3.3%), one turkey farm (3.3%), one partridge farm (3.3%), five national parks (16.7%), and one wetland (3.3%) in 12 provinces of Iran. The cumulative incidence rates of disease in villages, layer farms, broiler breeder farms, layer
breeder farms, partridge farms, and turkey farms were 0.02%, 0.87%, 0.55%, 6.25%, 7.14%, and 0.69%, respectively. The findings reflect that among the investigated variables at infected locations, new birds entering the home in villages, live bird markets, inappropriate biosecurity conditions, transporting manure during the breeding period, close proximity of a common road to infected farms, and poultry movement inside (pullet) and outside were the most frequently observed possible risk factors for these outbreaks.

In conclusion, attention should be focused on the study of the dynamics and movements of domestic poultry; the investigation and modification of the structure of industrial poultry farms; training for all related people; enhancing passive surveillance; increase biosecurity; increasing attention of the authorities on the importance of the infection; and supplying the required credits and facilities.

**Keywords**: HPAI H5N8, outbreak investigation, Iran.

**Introduction**

Poultry breeding in Iran is highly important in terms of economy, society, and food security. Because of Iran’s geographical condition is suitable for poultry breeding, this industry has distributed in most parts of the country in recent years. Iran produces the most poultry meat among Middle East countries (Ebadzadeh et al., 2017). Moreover, poultry meat and eggs are the most important sources of protein for Iranian families. The per capita consumption rates of poultry meat, poultry eggs, livestock meat, and fish meat are 26.1 kg, 10.7 kg, 11.43 kg, and 15.2 kg, respectively (Ebadzadeh et al., 2015).
During the past three decades, industrial poultry breeding in Iran has increased impressively. Poultry meat production rose from 110 thousand tons in 1974 to 2122.5 thousand tons in 2015 (an annual average growth rate of 7.67%), and poultry egg production rose from 144 thousand tons in 1974 to 931 thousand tons in 2015 (an annual average growth rate of 4.79%) (Ahmadi et al., 2014; Ebadzadeh et al., 2015). This impressive increasing trend has allowed Iran to meet its own national requirements for poultry meat and export poultry meat to its neighboring countries. In 2013 and 2014, Iran exported 4,708 thousand tons and 6,593 thousand tons, respectively, of poultry meat and 3,813 thousand tons and 713 thousand tons, respectively, of poultry eggs (Hosseinpour et al., 2014).

Most poultry farms in Iran are located in the northern provinces (including Gilan, Mazandaran, and Golestan). The poultry breeding and broiler farms in these provinces house 12 million and 92 million chickens, respectively, which comprise 48% and 21% of Iran’s total capacity. In other words, 420 million of the 1 billion chickens produced annually came from these three provinces (Ebadzadeh et al., 2015).

Backyard poultry breeding has also grown and plays an important role in supplying the protein requirements, incomes, and livelihoods of Iran’s rural families. Moreover, there are numerous live bird markets in different parts of Iran. Most backyard poultry farms and live bird markets are located in Iran’s northern provinces. Indeed, according to the poultry diseases database of the Iranian Veterinary Organization (www.gis.ivo.ir), there are an estimated 50 million backyard poultry farms and 107 live bird markets (LBM) in the country, among which, 14 million birds and 55 LBM are found in the north. Duck and goose are the poultry species bred most often in these provinces, particularly in Mazandaran. Iran is also home to 105 Important Bird & Biodiversity Areas (BirdLife International, 2017), and many wild birds migrate to these places annually. Again,
most of them are located in the northern provinces, particularly Mazandaran (Freydonkenar and Miankale), because these provinces are suitable winter habitats for wild birds.

Poor infrastructure, a lack of quarantine and biosecurity, and high poultry density in these provinces create the conditions for the incidence and spread of poultry diseases. One of the most important diseases is the highly pathogenic avian influenza, which can be introduced to the country through the annual migration of wild birds.

Avian influenza disease is caused by type A influenza viruses belonging to the orthomyxoviridae family. There are 16 HA and 9 NA subtypes for avian influenza viruses, and among them, three subtypes (H5, H7, and H9N2) are more important in poultry because of their pathogenesis and effects on public health and the economy (Swayne et al., 2013; Farsad et al., 2016). The H5 and H7 subtypes have two pathotypes: low pathogenicity avian influenza (LPAI) and high pathogenicity avian influenza (HPAI). The latter is responsible for high mortality rates in birds (OIE, 2017a).

In September 2016, an AI H5N8 subtype virus of clade 2.3.4.4 was isolated from migratory wild birds in Ubsu-Nur Lake in Russia, revealing the threat facing all countries in the migratory pathway of wild birds which can transmit the virus (FAO, 2016). Because of its H5N8 subtype, HPAI has been reported in many Asian, European, and African countries, including Iran in 2016 and 2017. Since 2016, this subtype has been reported in 47 countries and more than 130 species of wild birds in addition to chickens (FAO, 2017).

In this study, the HPAI H5N8 subtype outbreaks in Iran in 2016 were investigated and the epidemiological findings and lessons learned are explained herein.
Material and Methods

Industrial poultry farms

Industrial poultry farms are located in all 31 provinces of Iran in varying densities. Broiler farms are located in 31 provinces, layer farms in 29 provinces, pullet farms in 20 provinces, broiler breeder farms in 26 provinces, and layer breeder farms in 9 provinces. The highest density belongs to the northern provinces (Gilan, Mazandaran, and Golestan), Isfahan and Fars provinces (Central Iran), East and West Azerbaijan, Razavi Khorasan, Tehran, Alborz, and Qom. Based on agricultural statistics, 18,237; 1,642; 267; 699; and 21 broilers, layer, pullet, broiler breeder, and layer breeder farms, respectively, have operating licenses. These poultry farms have capacity rates of 369907, 87285, 19691, 26198 and 1393 birds, respectively (Ebadzadeh et al., 2015). According to the IVO database (www.gis.ivo.ir), more than 5,000 additional poultry farms are operating without any official license.

Backyard birds

According to the IVO database (www.gis.ivo.ir), backyard birds exist in more than 60000 villages in Iran (Fallah Mehrabadi et al., 2016), and there are 43 centers for the reproduction and breeding of native species with a capacity of 517,970 birds. The species most bred is traditionally chicken, while in the northern provinces (particularly Mazandaran), domestic duck and goose are bred the most.

History of the disease

Active surveillance for avian influenza (H5 and H7 subtype) was conducted in all LBM and selected villages (168) of the country from 17 September 2016 to 7 October 2016. In each Unit, based on a 5% prevalence and 95% confidence level, with the aim of finding at least one infected
bird (EC, 2010), sixty cloacal swab samples were taken from chickens and domestic waterfowl birds (goose and duck). A total of 10,163 swab samples (6,378 in LBM and 3,785 in villages) were taken from domestic ducks and geese. All swab samples were examined for H5 using Real time RT-PCR (Monne et al., 2008), and all samples were negative.

After that, on November 14\textsuperscript{th}, 2016, an outbreak of highly pathogenic avian influenza was reported from a commercial layer farm located in Malard, Tehran province. The disease was confirmed to be the virus HPAI H5N8 subtype of clade 2.3.4.4 (Ghafoori et al., 2017).

The influenza spread to other provinces and continued until 30 January 2017. This study was conducted from November 2016 to February 2017.

**Sampling**

From each commercial affected farm, 5–10 recently dead birds were sampled. The collected samples consisted of trachea tissue, lungs, brain, spleen and cecal tonsil.

**RNA Extraction**

Separation of RNA virus from prepared samples was performed using High Pure Viral Nucleic Acid Kit made of (Roche, Germany) and according to the factory's instructions.

**Detection of H5**

For detection of influenza H5 subtype, specific primer and probe that designed for conserved regions in the HA2 subunit of the H5 were used (Monne et al., 2008). The PCR Kit- QuantiTect Probe RT (Qiagen, Germany) were used for Real Time RT-PCR. The following thermal protocol was used: 20 min at 50°C and 15 min at 95°C, followed by 40 cycles at 94°C for 45 s and 54°C for 45 s.

All outbreaks were confirmed by the Central Veterinary Laboratory of the Iran Veterinary Organization and the Avian Influenza National Laboratory of Razi Institute. All samples from confirmed outbreak cases in the country were sent to the OIE/FAO Reference Laboratory in Italy.
(IZS Ve. Legnaro–Padova) where they were also confirmed. At the same time, an outbreak of the HPAI H5N1 subtype was detected on 15 January 2017. The outbreaks was observed in Backyard ducks in a house near Gilapey village in Mahmood abad city, Mazandaran province and 10 out of 230 domestic ducks, were died (OIE, 2017b).

**Epidemiological investigation and questionnaire**

To investigate the possible transmission route of the virus and risk factors of the disease, a questionnaire was prepared and completed through interviews with farm owners or field observations at the time of disease onset. The most important variables investigated on industrial farms were the density of poultry farms, the entrance of vehicles and personnel to the farms 21 days before onset of disease, age of flocks, status of age of the flocks (one age group or multiple ages), workers' residency on the farms, type of breeding system, farm fencing status, number of farms belonging to the owner, manure collection conditions during the breeding period, biosecurity and heath management of the farms, and distance to migratory habitats of wild birds. The most important variables investigated on backyard poultry farms were the purchase of new birds (especially aquatic birds) 21 days before the onset of disease, type of birds kept in the families, distance to live bird markets, and distance to migratory habitats of wild birds.

**Control measures**

Once the disease was confirmed in an industrial farm, village, national park, or wetland, control measures were taken according to the European Union guidelines in the affected unit (up to a 1-km radius), the quarantine zone (up to a 3-km radius), and the surveillance zone (up to a 10-km radius) (EC, 2006).
In the infected premises

All infected and exposed poultry on infected industrial farms were destroyed, and all carcasses and eggs were disposed of. On breeder farms, all eggs produced in the hatchery in the 21 days before disease onset were destroyed. All poultry in the infected villages were also destroyed (up to a 1-km radius).

On broiler farms in this zone, all poultry that were of slaughter age were sampled and, upon disapproval of infection, were sent to the nearest slaughterhouse. Moreover, the transportation of manure in this area was prohibited. Restocking of all industrial farms in this range was also prohibited for up to 42 days after cleansing and decontamination of infected premises. In and around ponds and parks, the carcasses of dead birds were gathered and sanitized, and the areas were washed, cleared, and received ongoing clinical care for up to 21 days to determine possible losses. Activities of live bird markets other than the purchase and sale of poultry bred with foci traditionally in the provinces were restricted.

In quarantine (protection) zone

Clinical surveillance and sampling were performed in all industrial and backyard poultry farms in a 3-km radius. Sixty cloacal swab samples were taken from each location within a 3-km radius of affected farms. Based on a 5% prevalence and 95% confidence level (EC, 2010).

Poultry on broiler farms that were of slaughter age were sampled and, after disapproval of infection, transferred to the nearest slaughterhouse in compliance with sanitary conditions. With the goal of reducing the density of farms, the poultry on layer farms within a 3-km radius of any affected layer farm were sampled; if the results were negative, the poultry were sent to the nearest slaughterhouse. Pullet farms within this radius were subjected to clinical investigation and sampling. If the results of two serological tests (HI) within 2 weeks and molecular tests disproved
infection, authorization was given to transport the poultry to layer farms (to continue the production cycle) where the poultry was monitored for up to 21 days. The transferal of eggs from farms within the zone (3-km radius) was not restricted if surveillance and monitoring were conducted, infection was disproved, and eggs were disinfected and transferred only to authorized centers. Restocking on all industrial farms in this range was prohibited for up to 42 days after cleansing and decontamination of infected locations. All layer farm poultry older than 80 weeks were sent to the slaughterhouse. Transportation of manure in this zone was prohibited.

**Surveillance zone**

Clinical investigations were carried out to detect disease and notification was made of any losses on industrial farms or backyard poultry farms. Moreover, biosecurity measures were strengthened by presenting farmers with educational material. Transportation of manure in this zone was prohibited. The poultry on all layer farms aged greater than 80 weeks was sent to the slaughterhouse.

**Notification**

Farmers breeding poultry industrially were notified to strengthen biosecurity measures. Families breeding backyard poultry were notified not to purchase or sell poultry, especially aquatic birds, and to report any mortality to veterinary services. These notifications were carried out using both nonofficial (SMS, brochure) and official (veterinary organization site, national media) methods. In counties with infected farms, all farmers were advised not to restock until the veterinary organization approved it.

**Results**

HPAI H5N8 infection was confirmed in 30 different locations in 12 provinces of Iran (Tehran, Qom, East Azerbaijan, Gilan, Mazandaran, Golestan, Razavi Khorasan, Markazi, Qazvin,
Kermanshah, and Isfahan) (Table 1). An additional outbreak of HPAI H5N1 was detected and confirmed in domestic ducks in Mazandaran at the beginning of 2017. Monthly reported outbreaks were shown in graph 1.

In the 30 recorded outbreaks, 10 villages (33.3%), nine layer farms (33%), two broiler breeder farms (6.67%), one layer breeder farm (3.3%), one turkey farm (3.3%), one partridge farm (3.3%), five national parks (16.7%), and one wetland (3.3%) were infected (Figure 1).

The cumulative incidence rates of disease in villages, layer farms, broiler breeder farms, layer breeder farms, partridge farms, and turkey farms were 0.02%, 0.87%, 0.55%, 6.25%, 7.14%, and 0.69%, respectively (Table 2).

Infected bird species included domestic chicken, turkey, ostrich, duck, and goose in villages and whooper swan, corvus cornix, anser fabalis, phoenicopterus roseus, laridae, ardeidae, gruidae, tachybaptus ruficollis, aythya fuligula and phoenicopreta in national parks and wetlands.

The average age of the infected poultry flocks was 32.71 ± 22.04 weeks in the layer farms. The maximum and minimum ages of flocks on infected farms were 76 and 14 weeks, respectively. Six farms had a flock with one age group, one farm had five flocks with different age groups, and two farms had two flocks with different age groups. The average age of the poultry flocks on broiler breeder farms was 38.81 ± 10.86 weeks. Maximum and minimum flock ages on infected farms were 79 and 27 weeks, respectively. One farm had a flock with one age group and one farm had two flocks with different age groups. The only affected layer breeder farm had three flocks with different age groups and the average poultry age was 21 weeks. Flocks on the infected turkey and partridge farms were 104 and 90 days old, respectively.

In total, 1,098,983 birds (Table 2), 1,283,710 edible eggs, 839,174 broiler breeder eggs, and 248,580 layer breeder eggs were destroyed during these outbreaks.
Clinical sign

In these outbreaks, the data showed that sudden and rapid death (increased mortality rate) and cyanosis of the wattle were the most observed clinical signs in chickens (commercial and backyard). Decline in production was not observed on most farms in the first three days. In layer farms, mortality was initiated in one or more cages together. Generally, not more than one dead bird was observed in any one cage, and because of this, farmers suspected an issue such as insufficient water for the deaths. Tracheal and internal visceral organ hemorrhagic, hyperemia, and necrosis of the spleen were also observed. In turkey, sudden and rapid death (increased mortality rate), lethargy, and cyanosis of the carcass crown and comb were observed.

In domestic duck and goose, the most frequently observed clinical signs were neurological (shaking the head and paralysis) and necrosis of the pancreas. In ostrich, lethargy and neurological signs (paralysis of neck and wings), and in crow, lethargy and lacking the ability to fly were the most frequently observed signs.

Epidemiological findings

The findings reflect that among the investigated variables at infected locations, new birds entering the home in villages (21 days before onset of disease), live bird markets, inappropriate biosecurity conditions, the density of poultry farms, transporting manure during the breeding period, close proximity of a common road to infected farms, and poultry movement inside (pullet) and outside (at the end of the breeding period) the farms 21 days before onset of disease were the most frequently observed possible risk factors for these outbreaks.

Discussion
The detection of the avian influenza on industrial farms in Iran represented weaknesses and passivity in surveillance and disease reporting. Detection, diagnosis, and rapid response are the most effective measures for preventing and controlling the highly pathogenic avian influenza, and if carried out well, these measures will bring the rapid spread of infection under control. The outbreaks of HPAI H5N8 in Iran occurred in areas with the highest density of poultry breeding and caused direct and indirect losses as well as social effects. The detection of the first outbreak on an industrial farm and the detection of several outbreaks in a short period of time indicate a delay in the identification of the disease and failure in the early detection of diseases in backyard poultry. The source of the infection on the first identified farm was not recognized. However, domestic poultry, particularly ducks and geese, were being sold in villages near these farms and near Mazandaran province, from where some domestic ducks are supplied to other places. Therefore, the most likely source of the virus was in Mazandaran province.

Delayed reporting of the first outbreak caused the infection to spread to other nearby farms. Other likely causes of the spread are the existence of common roads for farms, vehicles for the transportation of eggs, association between workers, and the movement of workers to nearby villages. Further spread of infection was prevented by depopulating units within a 3-km radius of the infected farms, quarantining, and conducting other control measures.

Findings such as onset of disease coinciding with the seasonal migration of wild birds and mortality in migratory birds in the Meighan wetland and Miankale National Park where no domestic birds exist indicate that the virus might have been introduced to the country by a migratory bird. This conclusion was also expressed in the FAO report after the H5N8 virus was detected in migratory birds at Lake Ubsu-Nur (FAO, 2016). Because of the existence of suitable winter habitats for migratory birds and their movement within the country due to local weather
conditions, food resources, and access to open water, the virus could have been spread by a migratory bird in the country and infected backyard poultry, especially where the birds had close contact.

The most probably factors for the occurrence of infection in domestic poultry were 1) the entrance of new birds (Gazvin and Mazandaran), especially ducks and geese (with LBM sources). Other studies have also identified LBMs as a risk factor for AI (Capua and Marangon, 2000; Kung et al., 2007; Fallah Mehrabadi et al., 2016); 2) location near the wetland or wintering places for migratory birds (Provinces include: Markazi, Mazandaran, Gilan, and Kermanshah) that can allow direct or indirect contact between wild and domestic birds; and 3) poultry density. The greatest number of outbreaks among domestic poultry were observed in places where the poultry density was high (Gilan and Mazandaran). The high density of poultry could lead to the rapid transmission of the virus among birds, and the virus could persist in the environment for a long time (Pavade et al., 2011). In a study conducted by Pavade et al., high poultry density was associated with an increase in incidence of outbreaks (Pavade et al., 2011).

In these outbreaks, layer farms were the worst infected. Layer farms in Iran, similar to some other countries, do not have high-level biosecurity. Some possible risk factors for the occurrence of infection on these farms are 1) high worker traffic inside and outside the farms; 2) daily movement of vehicles for the transportation of eggs and manure; 3) movement of poultry at the end of the breeding period or pullets 21 days before disease onset using untrained workers on the affected farms (Qom, Khorasan Razavi, and Golestan); 4) Location near the wetland or wintering sites of migratory birds (East Azerbaijan, Qom); and 5) high density of layer farms in the infected area (Located more than 10 farms in 3 km radius).
In a study conducted in the Netherlands in 2003, most of the infected farms were layer farms, and low level of biosecurity and frequent contact between farms were the main risk factors (Thomas et al., 2005). In other studies, the main risk factors of HPAI were non-native employees, birds aged more than 20 to 30 weeks (McQuiston et al., 2005), poultry movement, density of farms (Capua and Marangon, 2000; Kung et al., 2007), and location of farm within a 1-km radius of an infected farm (Mannelli et al., 2006).

Likewise, several breeder farms were affected by the outbreaks. Some of the possible risk factors for infection on breeder farms are 1) Movement between breeding and layer farms by farmer who owned both farms (Gilan); 2) close proximity to the infected layer farm (Khorasan Razavi), and 3) close proximity to the wintering habitats of migratory birds (Mazandaran).

Because turkeys raised in Iran are transmitted to other provinces for slaughter, a turkey farm in Isfahan was probably infected by infected transport vehicle (unpublished data in IVO). The occurrence of infection on this farm played a key role in the spread of the infection in Isfahan and other provinces. After observing dead turkeys on the farm, the farmer sent the live turkeys to the slaughterhouse, because of lack of assurance for compensation. Because of mortality among the birds, they were sampled and tested for HPAI in the slaughterhouse, and infection was confirmed. Turkeys are highly sensitive to the HPAI virus. A study carried out in Italy indicated that existence of turkey farms can lead to increased risk of HPAI (Mannelli et al., 2006).

One covey of raised partridges was illegally transported from an infected area in Isfahan to Razavi Khorasan province. This covey was tested in Khorasan, and infection was confirmed. The birds were destroyed before the initiation of any clinical sign. In another study, the legal and illegal transportation and trade of live birds were risk factors for HPAI (Capua and Marangon, 2000; Kung et al., 2007).
Based on published study, the age is not a determinant factor for HPAI (Bertran et al., 2016). In this study, the diversity of the age of the infected flocks was observed.

In spite of the rapid development of the poultry industry in Iran, the infrastructure of this industry is not well developed; therefore, there is many problems. Some factors provide the conditions for the easy spread of most diseases, including HPAI. Old buildings, lack of renovation for equipment, low biosecurity levels in many farms, the high density of poultry farms, existence of illegal farms, lack of existence of an integrative system are structural factors. Also transportation of slaughtered poultry into other provinces, and weakness of education at different levels posed problems and challenges for the industry.

The outbreaks discussed herein had direct and indirect socio-economic effects. All expenses for culling and compensating farmers were paid by the government. The outbreaks were not predicted, and therefore imposed additional expenditures on the government. In Iran, authorized poultry farms are guaranteed that the government will compensate farmers for their losses when the farms are destroyed due to HPAI. Farmers were compensated (in US dollars) $2.03 for each layer chicken, $3.30 for each broiler chicken, $6.00 for each breeder chicken, $25.35 for each turkey, $4.60 for each backyard chicken or duck, and $0.40 for each embryonic egg.

The compensation paid to the farmers equaled 75% of the value of the birds, litters, and animal products that were destroyed. Incomplete compensation and delayed payment led to dissatisfaction among farmers. In a 2004 outbreak in Thailand, farmers were compensated for 100% of their losses in the first phase, but only 75% in the second phase, because the epidemic was widespread and devastating for Thai farmers (Thanawat et al., 2005).

In this outbreak, the price of eggs decreased in the initial stage of the outbreak; then it increased because of the culling of eggs and the evacuation of some farms, especially those with poultry
aged above 80 weeks. Exportation of live chickens and eggs to neighboring countries was also cut as a consequence of the outbreak.

Restricting poultry movement, especially for broilers, led to reductions in the price of the poultry and dissatisfaction among farmers in affected provinces. Some pullet farms in the infected provinces could not sell their pullets, and their poultry were kept with older poultry on the farms. A lack of collaboration between villagers, especially pigeon raisers, in culling their birds (because of the high value of their birds) was another consequence of the outbreak. Such issues in addition to a lack of sufficient knowledge concerning the importance of HPAI, especially its public health importance, led to the lack of collaboration with the veterinary organization. If veterinary services could perform well to implement effective control measures, HPAI could be successfully controlled and prevented. If the veterinary authority is reduced, the potential for prolonged disease activity and difficulty in eradicating it arises (Pavade et al., 2011).

Conclusion

Currently, the highly pathogenic avian influenza is considered to be the most important infectious threat to the poultry industry of Iran. In the African-Western Eurasian flyways, the wetland systems in the country (especially in the northern provinces) provide suitable winter habitats for migratory wild birds. The West Siberian/Caspian/Nile populations of ducks, geese, and swans regularly migrate and stop off in Iran (Nourani et al., 2014). The proximity or even mixing of wild bird-inhabited areas and densely-populated domestic bird areas could be a big potential risk for the introduction of influenza viruses into the country. Introducing hunted migratory birds into local LBMs also raises this potential risk. In turn, the LBMs of northern provinces, especially in Mazandaran (Freydonkenar and Jouybar cities) are in continuous trade throughout the countryside.
Attention should be focused on the study of the dynamics and movements of domestic poultry; the investigation and modification of the structure of industrial poultry farms; training for villagers, farmers, clinicians, and related guilds and their employees; enhancing passive surveillance of migratory and domestic birds; heightening active clinical surveillance on industrial farms (especially layer farms); recommendations to poultry owners to increase biosecurity; increasing attention of the authorities on the importance of the infection; and supplying the required credits and facilities. If these conditions are ignored, the transmission and spread of HPAI in the country will be almost impossible to halt.

Conflict of interest
We declare that there is no conflict of interest.

Acknowledgement
The authors of this research thank the Directorate of Health and Management of Poultry Disease of the Iran Veterinary Organization for their support.

References


OIE, 2017b. Update on highly pathogenic avian influenza in animals (type H5 and H7) [Internet]. Available at: http://www.oie.int/wahis_2/temp/reports/en_fup_0000230559_20170505_133144.pdf.


<table>
<thead>
<tr>
<th>Province name</th>
<th>Num of infected premises</th>
<th>Num of Death birds</th>
<th>Num of Destroyed birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tehran</td>
<td>4</td>
<td>7382</td>
<td>521939</td>
</tr>
<tr>
<td>Alborz</td>
<td>1</td>
<td>750</td>
<td>30245</td>
</tr>
<tr>
<td>Qom</td>
<td>1</td>
<td>75</td>
<td>119504</td>
</tr>
<tr>
<td>East Azarbaijan</td>
<td>1</td>
<td>463</td>
<td>52537</td>
</tr>
<tr>
<td>Markazi</td>
<td>2</td>
<td>88</td>
<td>849</td>
</tr>
<tr>
<td>Mazandaran</td>
<td>6</td>
<td>571</td>
<td>30351</td>
</tr>
<tr>
<td>Qazvin</td>
<td>1</td>
<td>10</td>
<td>282</td>
</tr>
<tr>
<td>Kermanshah</td>
<td>1</td>
<td>31</td>
<td>49</td>
</tr>
<tr>
<td>Golestan</td>
<td>1</td>
<td>2927</td>
<td>158368</td>
</tr>
<tr>
<td>Gilan</td>
<td>5</td>
<td>13992</td>
<td>69444</td>
</tr>
<tr>
<td>Esfahan</td>
<td>4</td>
<td>726</td>
<td>5084</td>
</tr>
<tr>
<td>Khorasan-Razavi</td>
<td>3</td>
<td>6000</td>
<td>110431</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>32115</strong></td>
<td><strong>1098983</strong></td>
</tr>
</tbody>
</table>
Table 2 cumulative incidence rate and Relative Risk of HPAI H5N8 in different poultry type of Iran 2016

<table>
<thead>
<tr>
<th>Premises type</th>
<th>Number of premises</th>
<th>Number of infected</th>
<th>Incidence %</th>
<th>RR* (CI** 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villages</td>
<td>60000</td>
<td>10</td>
<td>0.016</td>
<td>1</td>
</tr>
<tr>
<td>layers</td>
<td>920</td>
<td>9</td>
<td>0.97</td>
<td>58.69 (23.90-144.11)</td>
</tr>
<tr>
<td>Broiler breeder</td>
<td>364</td>
<td>2</td>
<td>0.55</td>
<td>32.97 (7.25-149.94)</td>
</tr>
<tr>
<td>layer breeder</td>
<td>16</td>
<td>1</td>
<td>6.25</td>
<td>617 (50.93-2760.93)</td>
</tr>
<tr>
<td>partridge</td>
<td>14</td>
<td>1</td>
<td>7.14</td>
<td>400 (54.55-2933.19)</td>
</tr>
<tr>
<td>turkey</td>
<td>145</td>
<td>1</td>
<td>0.69</td>
<td>41.37 (5.33-321.17)</td>
</tr>
<tr>
<td>Wild Birds</td>
<td>320</td>
<td>6</td>
<td>0.02</td>
<td>0.07- 0.04</td>
</tr>
<tr>
<td>total</td>
<td>61779</td>
<td>30</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

*RR=Relative risk

**CI= Confidence Interval
**Chart 1:** Number of reported HPAI H5N8 outbreaks in Iran, 2016, according to the date of report (Month)
Figure 1: Distribution of Infected unites with HPAI H5N8 by unit types in Iran-2016