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Original Article

Serological Monitoring of an H5 Inactivated Vaccine in Layer Farms, Iran, 2018

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Abstract

Background and Aims: Avian influenza (AI) is an acute infectious disease of poultry, waterfowl, wild birds, and animals, zoonotically transmitted to humans. Some incidents of HPAI are reported in Iran: H5N1 and H5N8. Iranian Veterinary Organization decides on vaccination (H5) of layer and breeder flocks in high-risk provinces following the outbreak in Iran. This study aimed to evaluate the serum response of the vaccine in the layer flocks of high-risk provinces.

Materials and Methods: Ten laying farms (Size: 30000-50000) were selected from Qazvin (no: 2) and Isfahan (no: 8) provinces that received the H5 vaccine (Four farms: 1 time; 6 farms: 2 times of vaccine shots). Twenty-five blood samples were taken from each flock. The HI test was carried out in a U-bottomed microtiter plate and 4 HA units of homologous antigen.

Results: The mean titers of antibodies in the poultry farms that received the vaccine once were 1.87, while those that received the immunization twice were 4.90 (significant difference; $p<0.05$). Also, if we consider protection baseline 4, 4 out of 6 flocks (~67%) could make it above it. Injection of the vaccine twice also improved CV.

Conclusion: In combination with other control measures such as good biosecurity and monitoring programs, vaccination is considered a suitable and powerful tool to support AI eradication or control programs in endemically infected countries if the Iranian Veterinary Organization (IVO) did regular post-vaccination surveillance and evaluated the flocks for silent infections.

Keywords: Avian Influenza; H5; Iran; Layer; Vaccination

Introduction

Avian influenza (AI) is an acute infectious disease of poultry, waterfowl, wild birds, and animals and an be zoonotically transmitted to humans.

The avian influenza virus (AIV) is a segmented, negative-sense RNA, enveloped virus belonging to the Orthomyxoviridae family.

Hemagglutinin (HA) and neuramin-idase (N) classify AIV into 16 H and 9 N subtypes, respectively. HA undergoes frequent antigenic variation and plays a critical role in the pathogenicity of AIV. Additionally, HA

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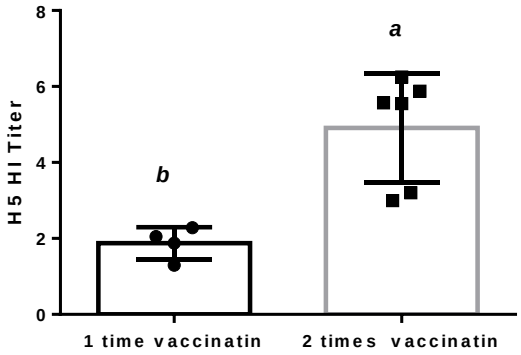
has a vital relation to the host range, antigenicity, and pathogenicity of AIV (1, 2).
H5N1 is the representative subtype of HPAI in Asia and has evolved into over 32 clades distinguished by their haemagglutinin (HA) genes (3-5). In 2010, a novel H5N8 virus (Dkk1203), with genes belonging to the A/Goose/Guangdong/1/1996 lineage H5N1 lineage, was identified in birds at live bird markets in China. Analyzing the topology of the phylogenetic tree, this virus presented longer branches than the previously recognized 2.3.4.1, 2.3.4.2, and 2.3.4.3 subclades, and according to the WHO/OIE/FAO) H5N1 evolution working objective group criteria, these viruses were assigned to 2.3.4.4 clade (6). HPAI A/ duck/Jiangsu/k1203/2010 H5N8 virus of the Asian H5N1 lineage (HA gene belonging to clade 2.3.4) was initially isolated from mallard ducks a live-bird market in eastern China in 2010. In 2013, live poultry markets in the east of China were the first to isolate novel reassortant H5N8 viruses. Then the virus was detected in poultry and wild birds in the Republic of Korea and Japan. Two distinct genetic groups of HPAI H5N8 were identified in phylogenetic analysis in the Republic of Korea, and a characterized virus represented each group: group A (A/broiler duck/Korea/Buan2/2014- like) and group B (A/breeder duck/Korea/Gochang1/2014-like).
In late 2014, HPAI H5N8 viruses were reintroduced into South Korea and Japan, and they were discovered in Europe and North America (7).
Some incidents of HPAI are reported in Iran: H5N1 and H5N8. The H5N1 subtype was first detected and confirmed in wild swan corpse on passive surveillance in Iran on February 13, 2006. Also, In November 2016, HPAI H5N8 was detected in a commercial egg farm in the province of Tehran. Genetic and phylogenetic analysis of the HA gene demonstrated that the Iranian HPAI H5N1 and H5N8 viruses belong to the HPAI H5 virus clades (2.2.1, 2.2.2, and 2.3.2.1c) and 2.3.4.4, respectively (6, 8, 9).
Vaccination of poultry against avian influenza is a response to repeated outbreaks in recent years. Vaccination campaigns have been successful in the short term, but outbreaks have inevitably recurred. Iranian Veterinary Organization decides on vaccination (H5) of layer and breeder flocks in high-risk provinces (Qazvin, Qom, Isfahan) following HPAI H5N8 in Iran as a control tool. This study aimed to evaluate the serum response of the vaccine in the layer flocks of high-risk provinces.

Methods

Sampling: Ten laying farms (Size: 30000-50000) were selected from Qazvin (no:2) and Isfahan (no:8) provinces that received the H5 vaccine (Four farms: 1 time; 6 farms: 2 times of vaccine shots). The minimum age of the sampled herds was 12 weeks, and the maximum was 25 weeks. The first age of vaccination in flocks was two weeks, and the last age was 20 weeks. Blood sampling time was at least three weeks after the first vaccination or two weeks after the second injection. Twenty-five blood samples were taken from each flock. Sampling details are given in Table 1.



	1 time vaccinatin	2 times vaccinatin
Mean	1.87833	4.90774
Std. Deviation	0.420641	1.42451

Fig. 1. Comparison of 1 and 2 times H5 vaccination.

Haemagglutination inhibition (HI) test: The HI test was carried out in a U-bottomed microtitre plate, and 4 HA units of autogenous antigen in 0.025 ml phosphate buffer saline; HI tires were given titer reference number according to Kaleta and Siegmann (10). Geometric mean

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titers (GMT) were calculated for each group of serum samples.

Statically Analysis: Office Excel and Graphpad 6 software analyzed the data. The mean headline of the vaccine group was compared with the two-time t-test vaccine and the mean headline between the herds and ANOVA to compare the data. P <0.05 was considered a statistically significant level.

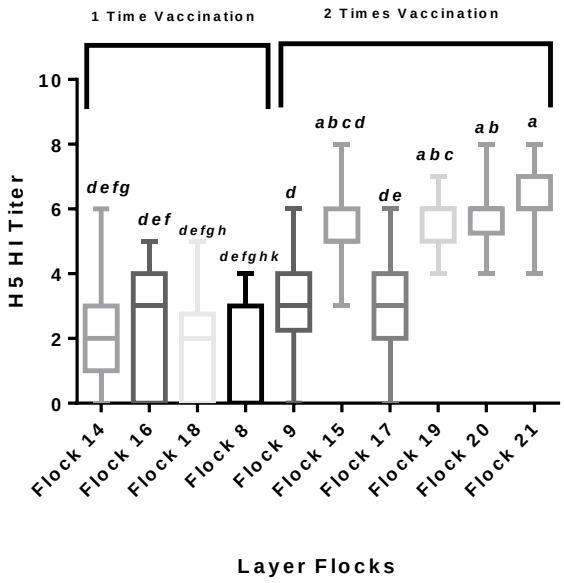

Fig. 2. The HI titers of H5 Avian Influenza of different layer flocks

Results

The mean titers in the farms that received the vaccine once were 1.87, while those that received the vaccine twice were 4.90 (significant difference; p<0.05) (Fig. 1). Also, if we consider protection baseline 4, 4 out of 6 flocks (~67%) could make it above it. The lowest average titer was 1.30, and the highest average titer was 6.25. Injection of the vaccine twice also improved CV. The percentage of individual titers in each group above four was also calculated (Fig. 2 and Table 2).

Discussion

AIV is an emerging threat to public health and continues to cause outbreaks among poultry and humans. In Iran, poultry is ready for sale as meat at 1.5 months of age, but poultry for egg production and breeding stock is kept for up to 1.5 years. There has previously been little information about the influenza vaccination of poultry under field conditions.

Table 1. Layer flocks that involved in this study (Assessment of H5 vaccination).

Flock Code	Province	Sampling Age (Wks)	Time of first vaccination (Wks)	Time of second vaccination (Wks)
F-8	Qazvin	12	5	
F-9	Qazvin	3	5	12
F-14	Isfahan	12	5	
F-15	Isfahan	20	11	15
F-16	Isfahan	7	2	
F-17	Isfahan	25	17	20
F-18	Isfahan	12	6	
F-19	Isfahan	20	12	15
F-20	Isfahan	21	10	16
F-21	Isfahan	16	6	11

In laboratory studies, vaccination against H5 influenza viruses has protected ducks and chickens against lethal challenges (11).

Factors that influence vaccination outcomes include the type and quality of vaccine, vaccination schedule, dose, and method of administration. Importantly there is no single recommended regime for HPAI vaccination of commercial poultry in the endemic situation.

Vaccine-induced immunity is measured by the presence of haemagglutination inhibiting (HI) antibodies in vaccinated birds, and HI titers generally reflect the efficacy of the vaccine and correlate with protection from a virulent H5N1 challenge (12). Based on the OIE's manual minimum HI serological titers in the field, birds should be 1/32 to protect from mortality or greater than 1/128 to provide a reduction in challenge virus replication and shedding. This study showed that flocks should be vaccinated at least twice, and single injection in addition to the low antibody titer, the titer CV is very high.

It should be noted that the vaccine could cause immunity for up to 12 months if vaccinated twice (13). The study showed that injection age did not affect the immunity. H5 hemagglutination inhibition (HI) and virus neutralization (VN) antibodies were observed 40 weeks after vaccination of chickens with two doses and vaccination of ducks with one dose (11).

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Table 2. The HI titers of H5 Avian Influenza of different layer flocks.

Flock cod	Flock 14	Flock 16	Flock 18	Flock 8	Flock 15	Flock 17	Flock 19	Flock 9	Flock 20	Flock 21
Time Of Vaccination	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00
Mean	2.05	2.29	1.88	1.30	5.55	3.00	5.57	3.20	5.88	6.25
Std Deviation	1.65	1.86	1.71	1.69	1.23	1.62	0.85	1.51	0.99	0.90
CV	80.34	81.27	91.08	129.92	22.24	54.07	15.29	47.12	16.88	14.35
Individual titer in each >4 (%)	21.05	28.57	18.75	15.00	40.00	90.00	40.00	100.00	100.00	100.00

Also, 2 of 6 flocks did not have the proper antibody response despite receiving the vaccine twice. It is important to consider the various causes of poor response. If the farmer decides to keep the flock more, It is recommended to do another injection once. The results of this study were able to establish a baseline for an average of around 5. Baseline assists the veterinary organization and veterinarians in evaluating the vaccine and discovering the silent infection. An important characteristic of an effective H5N1 vaccination program is the number of birds protected from virulent challenges, i.e., the "level of flock immunity." The current estimate of this is that ≥60% of birds have HI titers of ≥ 4log2 spread of the H5N1 challenge virus is reduced or prevented (14). The majority of vaccinated flocks in our study (4/10) had HI titers of ≥ 4log2 at 18 Wks and thus were protected. According to the results, not far off that, we have silent infections.

However, after that, protective immunity declined at a variable rate and was associated with, to a degree, the number of vaccinations given. It is speculated that 90% of a flock must be vaccinated to ensure flock immunity. It also requires a high-quality vaccine that elicits a lasting antibody response (15)

In Mexico, for example, vaccination programs against H5N2 epizootics have been underway since 1995(16). In the end, however, extensive vaccination caused antigenic drift from the vaccine strain, contributing to vaccination failure.

In Southeast Asia, H5N1 vaccination programs have been instituted in Indonesia, Hong Kong, China, and Vietnam. The concern in this region is inadequate vaccine coverage. In China, only 20–50% of all flocks were vaccinated, and in Vietnam, only 40–60% (11, 17). Although no outbreaks have been reported in vaccinated flocks, any H5N1 virus introduced into these flocks, e.g., virus shed by asymptomatically infected ducks, maybe further disseminated by vaccinated poultry that is protected only against severe illness (18). Tarigan et al. (2018) show, the HI titers of individual birds in each flock differed significantly from birds in other flocks, indicating that the effectiveness of field vaccination was highly variable and farm-related (12). When used properly, vaccination has been shown to protect poultry against clinical signs and death and markedly reduce virus shedding in vaccinated birds, reducing virus transmission (19, 20). According to the results of this study, at least two vaccinations are required to provide adequate serum protection and titration. However, vaccination must be repeated to achieve higher titers and prevent virus replication. On the other hand, a single vaccination cannot provide the minimum headline needed for protection.

For improvement of vaccination strategy outputs, the following are suggested:1-Sero-Screening of all vaccinated flocks for finding the baseline and silent infection. 2- Periodic sampling and antigen tracking test (Real-time PCR) for flock monitoring 3- Challenge studies to evaluate the vaccine's efficacy on Iranian H5 circulating strains. 4- Increase

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vaccination coverage. 5- Create a program to remove the vaccine policy. 6- Do the HI test with Iranian H5 Antigen**.**

Conclusion

In combination with other control measures such as good biosecurity and monitoring programs, vaccination is considered a suitable and powerful tool to support AI eradication or control programs in endemically infected countries if the Iranian Veterinary Organization (IVO) did regular Postvaccination surveillance and evaluated the flocks for silent infections.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Gauthier-Clerc M, Lebarbenchon C, Thomas F. Recent expansion of highly pathogenic avian influenza H5N1: a critical review. Ibis. 2007;149:202-14.
2. Chatziprodromidou IP, Arvanitidou M, Guitian J, Apostolou T, Vantarakis G, Vantarakis A. Global avian influenza outbreaks 2010–2016: a systematic review of their distribution, avian species, and virus subtype. Syst Rev. 2018;7:17.
3. Kim S-H, Hur M, Suh J-H, Woo C, Wang S-J, Park E-R, et al. Molecular characterization of highly pathogenic avian influenza H5N8 viruses isolated from Baikal teals found dead during a 2014 outbreak in Korea. J Vet Sci. 2016;17:299-306.
4. Chowdhury S, Hossain ME, Ghosh PK, Ghosh S, Hossain MB, Beard C, et al. The Pattern of Highly Pathogenic Avian Influenza H5N1 Outbreaks in South Asia. Trop Med Infect Dis. 2019;4:138.
5. Lee E-K, Kang H-M, Song B-M, Lee Y-NA, Heo G-B, Lee H-S, et al. Surveillance of avian influenza viruses in South Korea between 2012 and 2014. Virol J. 2017; 14:54.
6. Ghafouri SA, GhalyanchiLangeroudi A, Maghsoudloo H, Farahani RK, Abdollahi H, Tehrani F, et al. Clade 2.3. 4.4 avian influenza A (H5N8) outbreak in comercial poultry, Iran, 2016: the first report and update data. Trop Anim Health Prod. 2017;49:1089-93.
7. Kanehira K, Uchida Y, Takemae N, Hikono H, Tsunekuni R, Saito T. Characterization of an H5N8 influenza A virus isolated from chickens during an outbreak of severe avian influenza in Japan in April 2014. Arch Virol. 2015;160:1629-43.
8. Ghafouri SA, Langeroudi AG, Maghsoudloo H, Tehrani F, Khaltabadifarahani R, Abdollahi H, et al. Phylogenetic study-based hemagglutinin (HA) gene of highly pathogenic avian influenza virus (H5N1) detected from backyard chickens in Iran, 2015. Virus Genes. 2017;53:117-20.
9. Ghafouri SA, Mehrabadi MHF, Talakesh SF, Hosseini H, Ziafati Z, Malekan M, et al. Full genome characterization of Iranian H5N8 highly pathogenic avian influenza virus from Hooded Crow (Corvus cornix), 2017: The first report. Comp Immunol Microbiol Infect Dis. 2019;64:73-80.
10. Kaleta E, Siegmann O. Kinetics of NDV specific antibodies in chickens—V. Analysis of frequency distributions of antibody titers against Newcastle disease virus by investigation of random samples in chicken flocks. Comp Immunol Microbiol Infect Dis. 1978;1:83-92.
11. Boltz DA, Douangngeun B, Sinthasak S, Phommachanh P, Midouangchanh P, Walker D, et al. Field assessment of an H5N1 inactivated vaccine in chi-ckens and ducks in Lao PDR. Arch Virol. 2009;154:939-44.
12. Tarigan S, Wibowo MH, Indriani R, Sumarningsih S, Artanto S, Idris S, et al. Field effectiveness of highly pathogenic avian influenza H5N1 vaccination in comercial layers in Indonesia. PloS one. 2018;13:e0190947.
13. Vergara-Alert J, Fernández-Bellon H, Busquets N, Alcántara G, Delclaux M, Pizarro B, et al. Comprehensive serological analysis of two successive heterologous vaccines against H5N1 avian influenza virus in exotic birds in zoos. Clin Vaccine Immunol. 2011;18:697-706.
14. Bouma A, Claassen I, Natih K, Klinkenberg D, Donnelly CA, Koch G, et al. Estimation of transmission parameters of H5N1 avian influenza virus in chickens. PLoS Pathog. 2009;5:e1000281.
15. Savill NJ, St Rose SG, Keeling MJ, Woolhouse ME. Silent spread of H5N1 in vaccinated poultry. Nature. 2006;442:757.
16. Villarreal C. Control and eradication strategies of avian influenza in Mexico. Dev Biol. 2006;124:125-6.
17. Ellis TM, Leung CY, Chow MK, Bissett LA, Wong W, Guan Y, et al. Vaccination of chickens against H5N1 avian influenza in the face of an outbreak interrupts virus transmission. Avian Pathol. 2004;33:405-12.
18. Hulse-Post D, Sturm-Ramirez K, Humberd J, Seiler P, Govorkova E, Krauss S, et al. Role of domestic ducks in the propagation and biological evolution of highly

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pathogenic H5N1 influenza viruses in Asia. Proc Natl Acad Sci. 2005;102:10682-7.
19. Swayne D. Vaccines for List A poultry diseases: emphasis on avian influenza. Dev Biol. 2003;114:201-12.
20. Hamad M, Amen O, Mahmoud M, Hassanin O, Saif-Edin M. Effectiveness of different avian influenza (H5) vaccination regimens in layer chickens on the humoral immune response and interferon-alpha signalling immune marker. Vet Res Commun. 2018;42:145-52.

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