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THE IMMUNITY DURATION AND INTENSITY IN INDUSTRIAL LAYING HENS FOLLOWING VACCINATION WITH INACTIVATED H5N1 AVIAN INFLUENZA VACCINE

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Abstract

After the severe AIV H5 outbreak in Kazakhstan in 2020 the extensive use of AIV H5 vaccines started in the industrial poultry farms to limit the H5N1 influenza spread. Traditional methods, such as stamping out are no longer a viable option in countries where Highly Pathogenic Avian Influenza (HPAI) has become endemic. However, available vaccines and vaccination protocols have been widely researched in laboratory conditions, and limited testing has been conducted on commercial layers for the immunity persistence in field conditions. Immunity persistence after AIV vaccination can be quite different between laboratory and field conditions. Our basic goal was to assess the intensity and duration of immunity in commercial layers following 1, 2 and 3 vaccinations. H5N1 hemagglutination inhibition (HI) antibodies were observed 370 days after vaccination of chickens using three schemes of vaccination. However, at 370 days post vaccination 1 experimental group obtaining one vaccination had comparatively low HI titers (mean titer 6,5±1,2 log2), the other two groups having two and three vaccinations showed 8,8±2,4 mean log2 and 9,4±2,2 mean log2 respectively. The described results showed that inactivated H5N1 vaccine can produce lengthy, intense and homogenous immunity under field conditions following 2 and 3 vaccinations.

Key word: avian influenza immunity; influenza H5N1; vaccination scheme.

Introduction

Avian influenza is an extremely contagious, pantropic infection of poultry causing severe economic loss to the industry. The highly pathogenic subtype H5N1 of Highly Pathogenic Avian Influenza (HAIV) can be quite devastating to a commercial industry. Kazakhstan poultry industry experienced an outbreak of H5N1 AIV beginning in September 2020. The initial infections were detected in a commercial egg-laying flock and a single noncommercial backyard flock [1]. Later on, in October 2020 the outbreak of H5N1 AI spread to other northern regions and till the end of autumn infection appeared in southern commercial egg production flocks. The severe 2020 AIV outbreak brought huge economical losses for the Kazakhstan poultry industry [2]. Some unauthorized resources indicate that the mortality rate amounted to 98 – 100%. Although commonly attested measures for AIV preventing and control are biosafety and biosecurity including adequate and persistent serologic monitoring, vaccination using strong and antigenically homologous vaccine can also be successful. Moreover, vaccination coverage should be sufficient enough and the vaccine application should be easily controlled in the field conditions to prevent recurrence of AIV outbreaks [3]. Despite the fact that no AIV outbreaks have been registered

in vaccinated birds, a new H5N1 virus infecting these birds, e.g. virus introduced by asymptomatically infected birds, may be spread by vaccinated birds that are immunized only against severe desease [4]. After the AIV outbreak in 2020 in Kazakhstan inactivated Russian vaccine called Flu Protect H5 became very popular due to its production of high humoral immunity in poultry. However, for such long –living hens as layers and breeders it is vital to be immune to AIV till the end of their production life. Vaccination of industrial flocks against avian influenza (AI) requires consideration of many different factors, including scheme of vaccination, financial expenses, labor efforts and the availability to implement surveillance and monitoring programs. It is apparent that the effectiveness of a vaccine in laboratory trials conditions may differ from the use of it in industrial flock [5]. Thus, the persistence of AIV immunity in industrial birds have been tested in a very limited research. Many authors have described the prevention of H5N1 disease in vaccinated birds and reduction of virus shedding after vaccination [6, 7]. However, in these studies the immunity was assessed 1-3 weeks post-vaccination and this period is not relevant to field conditions. The immunity response persistence after H5 vaccination under the conditions of big production farm not determined yet. Moreover, dozens of factors may influence the strength and persistence of the immunity after vaccination in field. Prescription and decision for usage of the inactivated vaccine can rely on broader information included in the original registration file, many scientific investigations, controlled trials, and field studies that have been conducted to increase knowledge regarding its characteristics and performances [8, 9]. Field experience from large commercial layers farm use has also enriched the experience and knowledge about the field performance of this vaccine.

The novelty of this work lies in the fact that a large-scale field study of the intensity and duration of AIV immunity in industrial laying hens in the commercial poultry farm was implemented for the first time in Kazakhstan.

The aim of this research was to compare the duration and intensity of H5 immunity in egg producing hens after their vaccination with the FluProtect H5 inactivated vaccine applying three different schemes.

Materials and methods

Flocks. The study was performed on the industrial site in North-Kazakhstan. Layers in three production houses were vaccinated using three different schemes of vaccination. For certain the layers did not have AIV maternally derived antibodies as all of them were received from non-vaccinated parents. For this research we formed three experimental groups from three production houses. During the production period the vaccinated birds were monitored serologically applying HI test. The living standards did not range essentially between the groups. The three experimental groups were kept providing appropriate biosecurity measures.

Vaccination and Sampling. In this study we used a commercially available H5N1 formaline-inactivated vaccine. According to the research plan the three experimental groups were vaccinated using three different schemes of vaccination. The schemes are presented in the table below.

Table 1 – Vaccination schemes of the experimental groups

Experimental group	Vaccination scheme		
#1			at the age of 95-100 days (full dose)
#2	at 1st day of life (half-dose)		at the age of 95 – 100 days (full dose)
#3	at 1st day of life (half-dose)	at the age of 55-60 days (full dose)	at the age of 95-100 days (full dose)

During the whole study period all experimental groups were sampled every 30-day post-vaccination. It is widely attested that 90-95% confidence level is ensured by collecting of 23 to 30 samples per one epizootological unit for accurate monitoring of the flock immunity status.

Serological Analysis. As it is known hemagglutination Inhibition Assay (HI) is an important technique in the research of viruses, especially influenza viruses. This test provides important information on the immune response to viral infections and it is also useful in vaccine development, epizootological studies, and evaluating the efficacy of vaccination programs. Thus, hemagglutination inhibition assay

was used to assess the immunity duration and intensity. The antigen for the HI test was purchased from GD Animal Health, Deventer, Netherlands. The HI test was conducted according to a generally accepted technique. Sera samples were pretreated with chicken erythrocytes to exclude non-specific agglutination.

Geometric Mean Titer (GMT) was calculated for all groups of the experimental birds. GMT was calculated as the antilogarithm of the mean of the logarithms of each value. Negative titres (<16) were regarded as 4 for the calculation of GMT.

Results

Immunity persistence in productive layers after the inactivated H5N1 vaccine was measured using HI test (Fig. 1).

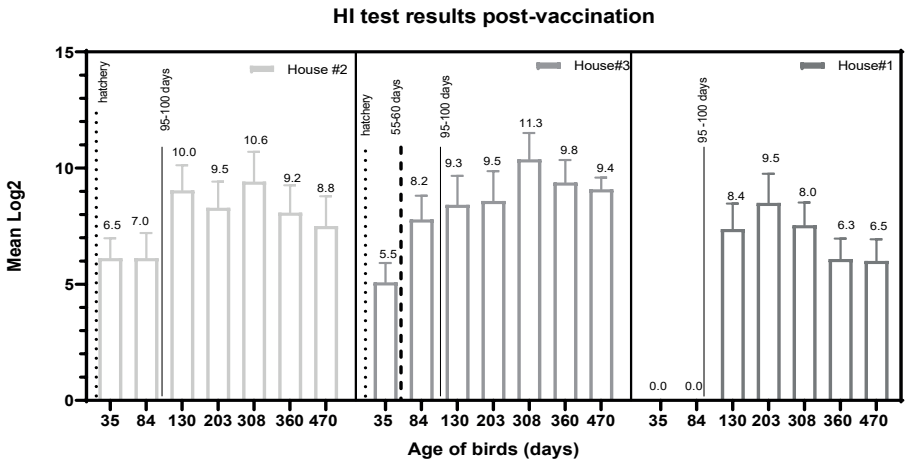

Figure 1 – HI test results showing antibody titters in layers during the whole period of the experiment. Vertical lines mark the period of vaccination

Layers administered a single vaccine dose at the age of 95-100 days showed HI titers (mean titer 8.4 log2) 30 days post-vaccination. Three months later this titer increased non-significantly (mean titer 9.5 log2). Furthermore, HI titers in this experimental group declined throughout the study (mean titer 6,5 log 2) but remained detectable at 470 days of life, till the end of production period. Experimental group number 2 was vaccinated twice: first vaccination was in hatchery using 0,5 of the recommended doses and second vaccination was at 95-100 days at the start of production period with full dose. As it is seen from the Table 2, 35 days post-vaccination chickens had HI titers at the level of 6,5±1,5log2 with 12% of seronegative samples. After 84 days post-vaccination the HI titers increased slightly (mean titer 7,0 ± 1,2 log2) with still some seronegative samples (10%). Furthermore, mean titer in layers rose to 10,0±2,4 log2 post second vaccination and remained nearly unchanged till 360 days of life. Then the titers declined to 8,8±2,4log 2 but remained detectable in 100% of layers till the end of production period. In HI tests conducted in the third experimental group which was vaccinated three times: at hatchery with half a dose of the vaccine, using full dose both at the age of 55-60 days and at the age of 95-100 days, detected extremely minor difference between this group and group number 2 (Table 2).

Table 2 – H5 antibodies in laying hens after inactivated vaccine (1, 2 and 3 vaccinations)

Number of vaccinations	Mean Log2 ± SD/seropositive %				
	dpv	30 dpv	100 dpv	200 dpv	360
Single vaccination	At the age of 95-100 days	8,4± 2,5/100	9,5± 1,8/100	6,3± 2,2/100	6,5± 1,2/100

Continuation of Table 2

Two vaccinations	30 dpv 60 dpv				30 dpb (100dpv)	100 dpb (200 dpv)	200 dpb (300 dpv)	360 dpb (460 dpv)	
	At hatchery (0,5 dose)	6,5 ±1,5/100	7,0 ± 1,2/100	At the age of 95-100 days	10,0± 2,4/100	9,5± 0,8/100	10,6± 2,7/100	8,8± 2,4/100	
Three vaccinations		30 dpv		30 dpb (90 dpv)		30 dpb (100 dpv)	100 dpb (200 dpv)	200 dpb 300 (dpv)	360 dpb (460 dpv)
	At hatchery (0,5 dose)	5,5± 1,1/80	At the age of 55- 60 days	8,2± 2,2/92	At the age of 95-100 days	9,3± 0,7/100	9,5± 1,6/100	11,3± 2,5/100	9,4± 2,2/100

Samples were collected after 30 days post-vaccination as per vaccine manufacturer instruction. dpv – days post primary vaccination, dpb – days post boost vaccination.

The experimental layers had mean titer 5,5±1,1 log2 at the 35 days post-vaccination and some hens were seronegative (20%). After boost vaccination at the age of 55-60 days the HI titer increased to 8,2±2,2, still 8% of samples showed seronegative results. After second boost vaccination at the start of production period the HI titer increased 9,3±0,7 mean log 2 and remained unchanged till the end of production period (mean titer 9,4±2,2 log2) with 100% of seropositive layers.

Fig. 2 shows the changes in HI titers through the whole period of the experiment. HI titers 30 post all boost vaccinations was 8.4, 10.0, 9.3 mean log 2 in 1,2 and 3 experimental groups respectively. All experimental groups had similar HI titters (9,5 log 2) at the age of 130 days (100 days post-vaccination) (Fig. 2).

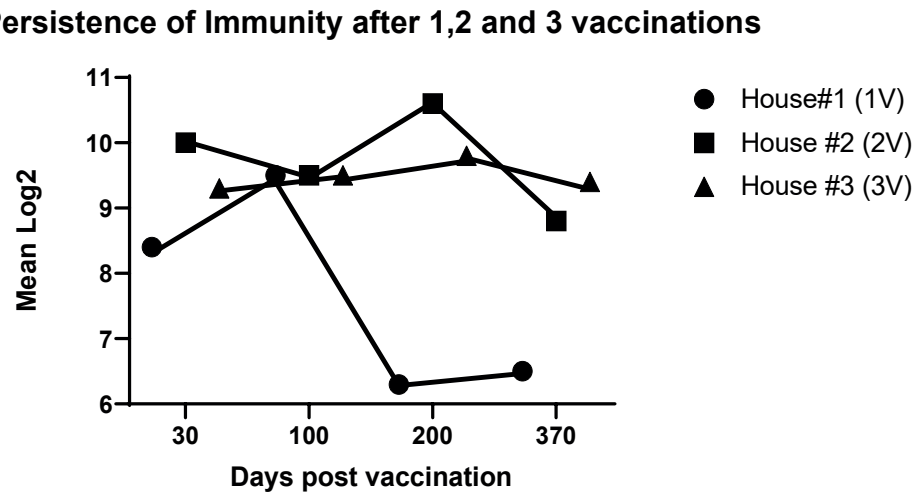

Figure 2 – Immunity persistence in productive layers after 1, 2 and 3 vaccinations. 30 days post vaccination means 30 days post all boost vaccinations in layers having 2 and 3 vaccinations. 370 days post vaccination corresponds to 470 days of life or the end of production period

At 370 days post vaccination 1 experimental group obtaining one vaccination had comparatively low HI titers (mean titer 6,5 log 2), the other two groups having two and three vaccinations showed 8,8 mean log 2 and 9,4 mean log 2 respectively.

Discussion

The antibody responses of the three experimental groups to AI vaccination were different. Experimental group #1 (House #1) achieved 100% seroconversion 30 days post vaccination but by 360 days of age, all of the hens tested had comparatively low titter 6,3 log2. In contrast, experimental groups 2 and 3 were approximately 80% and 92% seropositive, respectively, after first vaccination at 60 days of age by HI. However, 30 days post boost vaccination both groups had remarkably high HI titers. Additionally, both groups had still high HI titers at 360 days of age. The research was implemented on one and the same commercial facility and thus, there were not uncontrolled significant differences between these groups and their environments. There were no substantial vaccine-related differences between the groups. Vaccination of the three groups was carried using the vaccine which was either from the same lot or had very similar viral contents. The adjuvant used was identical. All birds were given the same dose of vaccine at both times and the ages at vaccination were similar among all groups. Based on the clinical signs it can be asserted that there was no challenge with AIV or of any other disease outbreaks in any of the three groups during the experiment. Thus, the differences in HI test results between the groups could be explained only due to the scheme of vaccination. Carol J. Cardona et.al [10] suggest that the immune response measured in experimental group #1 in part resembles a primary antibody response rather than a full anamnestic response. The hemagglutination inhibition titers of all experimental groups began to decline at 360 days of age. However, in the experimental groups 2 and 3 given 1 and 2 boost vaccinations respectively this decrease was slightly different whereas experimental group 1 had significant decline in HI titers to the end of production period. Interestingly, the three groups had the same high HI titers at the age of 130 days (30 days post vaccination or boost vaccination). Perhaps this occurred due to the age, productivity and health condition of layers.

There are scarce studies investigating AIV vaccination of industrial birds under field conditions. Numerous authors describe that H5N1 vaccination has prevented birds [11, 12, 13] from mortality. However, the time limit of the immunity, which is a significant index in the field [14, 15], has not been studied in full volume. Our research results prove that the immunity persistence in layers can differ markedly under field conditions due to the scheme of vaccination. Antibodies were observed in all experimental groups starting the first vaccination. However, the humoral immunity was more persistent, intense and homogenous in layers given two and three vaccinations than that of only one vaccination. Industrial birds grown for egg production, meat, or breeders should be secured from AIV. Kazakhstan poultry industry suggests broilers ready for sale at 39-42 days of age, but layers and breeding stock are maintained for up to 630 days of age. In our study the layers' antibody response was not measured for more than 470 days as birds on this farm are usually kept till 470 days of life. However, AIV immunity having only 6.3 log 2 in HI test lasting to 470 days of life suggests that layers raised for longer period would not be sufficiently secured for life, as intensity of HI antibodies tend to decline with the time elapsed. The antibodies upkeep at the level of 8.8 log2 – 9.4 log2 in the experimental groups #2 and #3 for the whole testing period assumes that application of one or two boost doses in long-living industrial hens such as layers or breeders would be sufficient for longer protection.

Proper vaccination can assist in controlling the highly pathogenic H5N1 viruses' circulation in domestic birds. Certainly, 85% of a flock must be vaccinated having 6.0 log 2 HI titer minimum to ensure flock immunity. Moreover, a lasting antibody response is also required implementing high-quality vaccine. In our study, layers had HI antibodies after inactivated vaccine for 370 days but were not experimentally infected with field virus to confirm protection [16]. Tian et al. [17] described protection from mortality and the virus shedding reduction in birds 52 weeks after primary vaccination with AIV vaccine (9.2 log 2 of HA vs. 6.3 log 2 in our study). Notwithstanding that in our study we did not implement experimental challenge, the results reveal that two and three vaccinations stimulate a 370 days lasting intense immunity in layers. The data from the literature show that under field conditions, most long-living poultry, such as layers and breeders as well as waterfowl and turkeys require a minimum of two or even three vaccinations throughout their life to maintain adequate protection [18, 19]. Single AIV vaccination may be quite sufficient for the protection of short-living birds such as broilers raised for meat.

The immunity duration and intensity in productive laying hens described in this article can assist Kazakhstan authorities in AIV vaccine assessment. In the AIV endemic situation in Kazakhstan the

effective H5N1 vaccine that can induce sufficient immunity in commercial birds will be extremely important for timely AIV outbreaks control, not excluding biosafety and biosecurity measures as well. It is well-known that China and Vietnam realized successful vaccination strategies for excluding the H5N1 transmission from birds to humans. However, after some time AIV outbreaks in poultry repeated. Very likely the two main factors responsible for AIV outbreaks recurring are the practical challenges of implementing the vaccination scheme and the high and rapid AIV antigenic mutability. The ongoing H5N1 circulation in Southeast Asia led to the new strain's formation, demanding that specific vaccine strains be applied in certain countries or regions or renovated every year. In 2003 Hong Kong successfully controlled H5N1 virus transmission by AIV vaccination program [20]. Thus, any industrial flock requires adopted scheme of routine H5N1 vaccination shielding the birds for the whole production period. As it is already known H5N1 has become endemic in Kazakhstan, and as the country is exposed to periodic outbreaks it has become urgent to re-assess the vaccination scheme of AIV vaccination as part of the routine infectious diseases control measures.

Conclusion

The inactivated vaccine application in Kazakhstan was eventually successful in helping to eradicate H5N1 AIV outbreak from the commercial industry, but not as quickly as it might have been. However, there were individual farms that were not successful to eradicate the virus using only one vaccination. The majority of farms that used the inactivated vaccine were multiage egg-production flocks and they used a double vaccination scheme. Our study suggests that using one or two boost AI vaccinations with the inactivated vaccine can provide intense and long – lasting protection to the industrial poultry through its whole production period. However, this vaccination scheme must be applied in association with strict on farm biosecurity to prevent the reintroduction of the virus.

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