Study on Vaccination Efficacy against Avian Influenza in Rajshahi, Bangladesh

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Abstract: Vaccines are used in integrated control strategies to guard poultry against H5N1 high-pathogenicity avian influenza (AI). This study was to evaluate the efficacy of AI vaccine against AI in poultry. About 400 serum samples were collected from the selected 130 farms located at Rajshahi region, Bangladesh. The study was carried out from January, 2013 to January, 2015. The AI vaccine titres were compared in different breed of layer, environmental temperature, feed and egg production. Result showed that the value of titres (mean $\pm$ SD) in Hyline brown, Hyline white, Bovans white and Novogen white were 6.71 ± 0.05 , 6.67 ± 0.06 , 6.79 ± 0.03 and 6.43 ± 0.04 , respectively. The highest serum antibody titres were 6.61 ± 0.24 against AI antibody in $> 26^\circ\text{C}$ environment temperature. The highest and lowest serum antibody value of AI against feed brand (nutrient) were 7.75 ± 0.08 and 5.58 ± 0.22 for Aftab and Quality brand feed, respectively.

Key words: Avian influenza, titres, layer, environmental temperature and egg production.

1. Introduction

Presently, poultry meat and eggs provide the cheapest quality animal protein to millions of people. There are around 15 millions small, medium and large poultry farms in Bangladesh, and approximately 246 million poultry population provide yearly 5,400 million pieces of eggs and nearly 37% of total animal protein [1]. In Bangladesh, a country has a population density of $964/\text{km}^2$ and 257 million poultry [2, 3]. Virus H5N1 infection was first detected among poultry in 2007. By the end of 2013, the country had reported 549 outbreaks among poultry according to the World Organization for Animal Health [4]. The first human case of H5N1 virus infection in Bangladesh was identified during 2008 [5]. The current Asian H5N1 highly pathogenic avian influenza (AI) virus has spread over Asia, Europe

and Africa, as well as affects village and commercial chicken operations in many Southeast Asian countries. The major control strategy for highly pathogenic avian influenza (HPAI) outbreaks in poultry has traditionally been one of eradication, via movement restrictions, slaughter of affected and at-risk birds, and improving biosecurity at poultry facilities to prevent initial infection. However, within the poultry, including ducks and turkeys, and in wildlife species, particularly migrating birds, alternate control strategies must be considered, with vaccines likely to be a key component, which is widespread presence of the current Asian H5N1 virus in village. Effective vaccines that can prevent infection as well as disease and be used in a variety of avian species, are needed for field use.

Currently, available commercial vaccines for AI are killed oil-emulsion virus vaccines. They have mostly been used to control endemic low pathogenic avian influenza (LPAI) in chickens and turkeys, or HPAI

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outbreaks in Pakistan and Mexico [6-8]. The European Union has approved use of inactivated oil-emulsion vaccines for use in Italy, provided that they allow for differentiation of infected versus vaccinated birds [9]. Control of mildly pathogenic avian influenza (MPAI) in Mexico has been undertaken with both killed vaccines and recombinant fowl pox vaccines [8]. The aim of this study was to evaluate the level of antibodies titres against AI in layer chicken vaccinated with inactivated H5N1 and H5N2 vaccine under different breed, egg production, feed of layer and environmental temperature of farms.

2. Materials and Methods

The study was conducted at Rajshahi, Bangladesh from January, 2013 to January, 2015. The whole experimental design was to detect the haemagglutination inhibition (HI) titres of AI that was protect flock from AI infection, and AI vaccine titres were compared under different layer breed, egg production, feed of layer and environmental temperature of examined farms. Total 130 farms were selected from the 400 farms which had having similar management, then each of 50 layer farms was randomly chosen for both observational group and 30 for control group. The AI H5N1 virus vaccine (strain Re-6) manufactured by Merial in France and AI H5N2 virus vaccine (strain A/duck/Postdam/1402/86) manufactured by Intervet in Netherland were vaccinated at the 1st, 5th and 15th weeks (inject subcutaneously with 0.5 mL). The titres were done at 21 d after each vaccination. The experimental layer breeds were Hyline brown, Hyline white, Bovans white and Novogen white. Feeds fed in farms were different in crude protein and energy level according to manufacturer (Quality Feeds Ltd., Dhaka; Nurish Bangladesh, Dhaka and Aftab Bohumukhi Farms Ltd., Dhaka). Environmental temperature were $\leq 20^{\circ}\text{C}$, $20\text{-}26^{\circ}\text{C}$ and $\geq 26^{\circ}\text{C}$ and egg production were 0% (pullet), 70%-80%, 80%-90% and above 90%.

2.1 Preparation of Chicken Red Blood Cell (RBC) Suspension

A total of 5 mL of chicken blood was collected aseptically in a disposable syringe containing 1 mL of sodium citrate (4% solution) as anticoagulant. The blood was centrifuged at 1,500 rpm for 15 min, and the plasma and buffy coat were removed with a pipette. After washing three times with phosphate buffered saline (PBS), 1% suspension in PBS was prepared for HI test.

2.2 Laboratory Test

This study was conducted jointly by Paragon Laboratory, Dhaka, Central Disease Investigation Laboratory (CDIL), Dhaka and Institute of Biological Sciences, University of Rajshahi among 130 layer farms at Rajshahi region in Bangladesh. The selected farms were more resemble in housing, feed, hygiene and management. From each flock, three chickens (a total of 400 chickens where three chickens from each farm, and 10 from large flock) were randomly selected for blood collection. 1 mL of blood was collected aseptically from wing vein of each bird and allowed to clot. Then sera were separated and stored at -20°C until the HI test was carried out. The antigen (AI inactivated vaccine, H5N1) used in this study manufactured by Marial, Nanjing Animal Health Co. Ltd., China. HI test was performed to detect the antibodies against AI.

2.3 HI Test

HI test was done according to the procedure of OIE [10]. Briefly, two fold serial dilution of 25 μL serum was made with PBS in V-bottomed micro titer plates (Nunc) up to the 10th well. 25 μL of four haemagglutinating (HA) units of AI virus or antigen was added up to the 11th well. The plates were kept at room temperature for more than 30 min to fascinate antigen antibody reaction. Then, 50 μL of 1% (v/v) chicken RBC suspension was added to each well. The 11th well contains antigen and RBCs as the positive

control and the 12th well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40 min and agglutination was assessed by tilting the plates. If the samples showed peculiar central button shaped, settle of RBCs were recorded as positive. The maximum dilution of each sample causing HI was considered as the end point, which was used to estimate the HI titres. The HI titres of each serum sample were expressed as reciprocal of the serum dilution.

2.4 Statistical Analysis

The seroprevalence of AI infection in relation to breed of layer, environmental temperature, feed and egg production was compared by means of *F* test. A significant level of $P < 0.05$ and $P < 0.01$ was used.

3. Results and Discussion

3.1 Results of Prevalence of Antibodies against AI in Layer Chickens

HI test was evaluated with different factors, such as breed, egg production, feed of layer and environmental temperature of farms, to find specific immunity against AI. The average antibody level was shown in Table 1, with mean HI titres ($\log_2$) of 7.67 ± 0.07 , 7.33 ± 0.07 and 3.34 ± 0.17 , respectively, in H5N1, H5N2 and control group of layer chicken.

3.1.1 HI Titres in Different Breed

Different breed of commercial layer chickens was also examined in detail. In Hyline brown, Hyline white, Bovans white and Novogen white groups, HI antibody titres varied from 5 to 7 and their respective percentages were 26.9%, 16.9%, 44% and 12.2%, respectively (Table 2). The mean level of antibody

titres was the highest (7.69 ± 0.86) in Hyline white, followed by 7.60 ± 0.13 in Novogen white and 7.25 ± 0.07 in Hyline brown, while the lowest (5.36 ± 0.29) in Bovans white breeds of layer. The variation was due to genetic composition, management and methods of data collection.

3.1.2 HI Titres in Different Egg Production

When compared with egg production of layer farms, the antibody titres had no significance effects in egg production (Table 3). The mean titres value of antibody titres in relation with egg production were observed 6.47 ± 0.20 , 6.57 ± 0.61 , 7.19 ± 0.28 and 6.08 ± 0.48 and their seroprevalence were 63%, 10%, 14.6% and 12.4% in pullet with egg production 0%, layers with egg production 70%-80%, 80%-90% and > 90%, respectively.

3.1.3 HI Titres in Different Feed Brand

AI titres level of nutrient (feeds) evaluated by HI test was shown in Table 4. The prevalence of HI titres was 62%, 18% and 10% in Quality, Nurish and Afteb brand feed fed, respectively. The mean highest to the lowest level of antibody titres were 7.75 ± 0.08 , 7.60 ± 0.11 and 5.85 ± 0.22 in Afteb, Nurish and Quality brand feed in layer flock.

3.1.4 HI Titres in Different Environmental Temperature

The vaccine immunity had no significance effect with environmental temperature, but level of titres was different in various group of environmental temperature (Table 5). The seroprevalence was 43%, 46% and 11%, and the mean HI titres was 6.61 ± 0.24 , 6.49 ± 0.24 and 6.47 ± 0.50 in environmental temperature < 20 °C, 20-26 °C and above 26 °C of the region, respectively.

Table 1 Distribution of HI titres s against AI in serum samples of layer chickens.

Type of vaccines application	No. of observation	HI titres ($\log_2$) (mean $\pm$ SD)	<i>F</i> value	Significance
H5N1 strain vaccine	50	7.67 ± 0.07^a	437.663	***
H5N2 strain vaccine	50	7.33 ± 0.07^b		
Control	30	3.34 ± 0.17^c		
Total	130	6.54 ± 0.16		

^{a-c} Means bearing uncommon superscript differ significantly; *** significantly different at $P < 0.01$.

Table 2 Distribution of HI titres against AI in serum samples of layer chickens in various breed.

Breed	No. of observation	Seroprevalence	HI titres ($\log_2$) (mean $\pm$ SD)	F value	Significance
Hyline brown	35	26.9%	7.25 $\pm$ 0.07 ^b	19.952	**
Hyline white	22	16.9%	7.69 $\pm$ 0.86 ^b		
Bovans white	57	44.0%	5.36 $\pm$ 0.29 ^a		
Novogen white	16	12.2%	7.60 $\pm$ 0.13 ^b		
Total	130	100%	6.54 $\pm$ 0.16		

^{a,b} Means bearing uncommon superscript differ significantly; ** significantly different at $P < 0.01$.

Table 3 Effect of egg production on the distribution of HI titres against AI in serum samples of layer chickens.

Egg production	No. of observation	Seroprevalence	HI titres ($\log_2$) (mean $\pm$ SD)	F value	Significance
0% (pullet)	82	63.0%	6.47 $\pm$ 0.20	1.129	NS
70%-80% (layer)	13	10.0%	6.57 $\pm$ 0.61		
80%-90% (layer)	19	14.6%	7.19 $\pm$ 0.28		
Above 90% (layer)	6	12.4%	6.08 $\pm$ 0.48		
Total	130	100%	6.54 $\pm$ 0.16		

NS: not significant ($P > 0.05$).

Table 4 Effect of nutrition (feed brand) on the distribution of HI titres against AI in serum samples of layer chickens.

Feed	No. of observation	Seroprevalence	HI titres ($\log_2$) (mean $\pm$ SD)	F value	Significance
Quality	81	62%	5.85 $\pm$ 0.22 ^c	18.789	**
Nourish	23	18%	7.60 $\pm$ 0.11 ^b		
Aftab	26	20%	7.75 $\pm$ 0.08 ^a		
Total	130	100%	6.54 $\pm$ 0.16		

^{a-c} Means bearing uncommon superscript differ significantly; ** significantly different at $P < 0.01$.

Table 5 Effect of environmental temperature on the distribution of HI titres against AI in serum samples of layer chickens.

Environmental temperature	No. of observation	Seroprevalence	HI titres ($\log_2$) (mean $\pm$ SD)	F value	Significance
< 20 °C	56	43.0%	6.61 $\pm$ 0.24	0.078	NS
20-26 °C	60	46.0%	6.49 $\pm$ 0.24		
Above 26 °C	14	11.0%	6.47 $\pm$ 0.50		
Total	130	100%	6.45 $\pm$ 0.16		

NS: not significant ($P > 0.05$).

3.2 Discussions of Prevalence of Antibodies against AI in Layer Chickens

Although depopulation programmes have been enforced in many parts, HPAI H5N1 outbreaks have been frequently reported since 2003 in some Asian countries, including China, Indonesia, Vietnam, Thailand and South Korea [11, 12]. It appears that the HPAI H5N1 infection becomes an endemic disease in Asia. Human infection and deaths caused by HPAI

A(H5N1) viruses in several countries [13], such as A(H9N2) virus in Bangladesh [14], A(H7N2), A(H7N9), A(H9N2) and A(H10N8) viruses in China [15], reflect the persistent public health threat posed by different AI A virus subtypes. Subtype H5N1 virus remains endemic among poultry in Bangladesh, China, Egypt, Indonesia and Vietnam [16]. Due to the coexistence of both large-scale poultry farms and backyard-raised chickens, ducks and geese, it is very

difficult to successfully conduct the protection of AI in these areas.

That is why vaccination against AI is encouraged in some Asian countries in conjunction with depopulation in the HPAI-infected farms in limited areas in campaigns against HPAI. In Bangladesh, a pilot project was undertaken for vaccination against HPAI with H5N1 and H5N2 in Gajipur region. Therefore, some selected layer chicken farms were vaccinated in Rajshahi and evaluated it by titres level.

The authors also observed the variation in HI titres and their relation with breed, egg production, nutrition (feed) and environmental temperature. Earlier, Sun et al. [17] evaluated many farms by inactivated oil emulsion vaccines of subgroup H5, and gave satisfactory results, but HPAI outbreaks still occurred in some vaccinated flocks. Smith et al. [18] reported that the appearance of antigenic variants might be a major reason responsible for the failures of vaccination. The authors observed that immunity against AI vaccine varied due to strain, breed, egg production and environmental temperature.

4. Conclusions

The immunity level (HI titres against AI) was better in H5N1 strain. The best titres level against AI serum were observed in Hyline white breed, layer with 70%-80% egg production, fed Aftab brand feed and at environmental temperature 20-26 °C.

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