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Quantification of Antibodies Against Avian Influenza Virus Subtype H7N3 in Layer Flocks in Central Punjab (Pakistan)

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ABSTRACT

A serological survey for quantification of antibodies to avian influenza virus (AIV) subtype H7N3 in vaccinated layer flocks was carried out in central Punjab. A total of 288 serum samples were collected (243 from commercial layer farms & 45 from layer breeder farms). Serum samples from commercial layer flocks were divided into four age groups (15-25, 25-35, 35-45, 45-55 weeks) and for layer breeder flocks into three age groups (16, 22, & 26 weeks). Haemagglutination Inhibition (HI) test was performed for the determination of serum antibodies against AIV-subtype H9N2. Calculated geometric mean titers (GMT) for commercial layer groups 15-25, 25-35, 35-45 and 45-55 weeks of age, were found to be 59.7, 104.0, 147.0 and 137.2, respectively. GMT values for layer breeder groups 16, 22, and 26 weeks of age were found to be 111.4, 207.9 and 128.0, respectively. The results showed that the level of protection of vaccinated birds against AIV-subtype H7N3 was found satisfactory in commercial layer flocks as well as in layer breeder flocks.

Key Words: Avian Influenza (AI); Serum Antibodies; Haemagglutination (HA); Haemagglutination Inhibition (HI)

INTRODUCTION

Avian influenza viruses are classified in the family Orthomyxoviridae, genus *Influenza virus* A. The surface is covered by two types of glycoprotein projections; rod-shaped trimers of haemagglutinin (HA) and mushroom-shaped tetramers of neuraminidase (NA). The HA is the major antigen that elicits antibodies which protect against death and clinical signs. These antibodies are HA subtype specific and can last for periods greater than 35 weeks (Brugh & Stone, 1987). Protection by maternal antibodies to homologous HA is probably for the first two weeks after hatching (Swayne *et al*., 2003). Poultry keeping is the dominant form of poultry production in the developing countries. Investment in poultry sector in Pakistan is about one billion US$ and egg availability is increasing 4% annually. Every family in rural areas and every 5th family in urban areas is associated with poultry production activities in one way or the other (Sadiq, 2004). Infectious diseases are one of the main factors constraining the poultry sector. In Pakistan, poultry industry is facing various diseases such as Newcastle disease (ND), Infectious bronchitis (IB), Infectious bursal disease (IBD), Egg drop syndrome (EDS), Hydropericardium syndrome (HPS) and Avian influenza (AI). These diseases are causing high economic losses in terms of high mortality, morbidity, stress, decreased egg production and hatchability all over the world including Pakistan (Alexander, 2000). Avian influenza is a contagious viral disease, world wide in distribution. It affects the chickens of all ages with variable morbidity and mortality. With the highly pathogenic AI viruses, morbidity and mortality rates are very high (50–89%) and can reach 100% in some flocks (Capua *et al.,* 2000). In Pakistan, no one has studied about protection of AI vaccinated birds, either their antibody titers are in protective range or not. In view of this situation, a survey was carried out with the objectives of determining the levels of antibodies against AI vaccinated flocks at different age groups of commercial layers as well as in layer breeders in central Punjab.

MATERIALS AND METHODS

Study area and season. The study was carried out in central Punjab (including Faisalabad, Gojra, Samundri, Kamalia, Sahiwal, Arifwala & T. T. Singh), Pakistan, from October 2004 to March 2005.

Collection of blood samples. A total of 288 blood samples were collected from different commercial layer flocks and layer breeder flocks having a history of vaccination against AIV-subtype H7N3. To perform Haemagglutination Inhibition (HI) test, the blood samples were allowed to clot, sera were separated and frozen at -20^{0}C till further use.

Haemagglutination and haemagglutination inhibition tests. The serum samples were tested to determine the antibodies against AIV-subtype H7N3, using the HA and HI methods described by (Olsen *et al*., 2003). The antigen used was AIV-subtype H7N3 taken from National Agricultural Research Center (NARC), Islamabad, Pakistan.

Washing of RBCs. A total of 5 mL of chicken blood was collected aseptically in a disposable syringe containing 1 mL of sodium citrate (4% solution) as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 min. The plasma and buffy coat was pipetted off. After washing thrice with phosphate buffer saline (PBS), 0.5% RBCs suspension in PBS was made according to instructions in "WHO Animal Influenza Manual (2002), to be used in HA and HI tests.

Test procedure. In order to monitor the antibody titers against AI viruses in commercial layers and layer breeders, HA and HI tests were performed according to the protocols

described earlier by Olsen *et al.* (2003). Briefly, 2-fold serial dilution of the AIV-subtype H7N3 was made in PBS (pH 7.2) in a 96-well micro titration plate. Chicken RBCs were added to each well at 0.5% concentration. The plates were incubated for 30 min at 37°C before recording the haemagglutinating activity. HI titer of each serum sample was also determined. Briefly, 25 µL of the test sera were serially diluted in PBS (pH 7.2) using a 96-well titration plate. To this 25 µL of 4HA unit of AIV-subtype H7N3 was added in each well. The plates were incubated for 30 min at 37°C. Now 50 µL of 0.5% of the chicken RBCs were added to each well and the plates were again incubated for 30 min at 37°C. The results were recorded when complete button formation was observed in the control well and subjected to GMT analysis.

RESULTS

A total of 288 serum samples were collected from different commercial layer farms and layer breeder farms with a history of vaccination against H7N3 subtype, and were subjected to HI test. All samples were found positive for antibodies to AIV-subtype H7N3 with overall positive percentage of 100% (Table I & III).

In commercial layers, 15-25 weeks age old group, the antibody titers ranged from $\log_2$ 16 - $\log_2$ 256 with GMT value of 59.7. Birds of age group 25-35 weeks showed antibody titer range of $\log_2$ 64 - $\log_2$ 256 with GMT value of 104.0. Antibody titers in the age group 35-45 weeks were found in the range of $\log_2$ 32 - $\log_2$ 256 with GMT value of 147.0. Fourth age group of 45-55 weeks showed serum antibodies range of $\log_2$ 64 - $\log_2$ 256 with 137.2 GMT value.

In layer breeders, 16 weeks age old group, the antibody titers ranged from $\log_2$ 64 - $\log_2$ 128 with GMT of 111.4. Birds of age group 22 weeks showed antibody titer range of $\log_2$ 128 - $\log_2$ 256 with GMT value of 207.9. Antibodies in the age group 26 weeks were found in the range of $\log_2$ 64 - $\log_2$ 256 with GMT value of 128.0.

DISCUSSION

Antibody levels with GMT value of 67.29 (67.29/25 µL of serum = $10^{3.43}$/1000 µL of serum) and higher were considered as protective for avian influenza vaccinated birds (Trani *et al.*, 2002). Using this criterion in the present study, the GMT values were calculated (Table II & IV). All vaccinated age groups (except age group 15-25 weeks) were having GMT values higher than described earlier, suggesting that they fall in the protective antibody titer range against AIV-subtype H7N3. Antibody titer range of 15-25 weeks age group was lower than described earlier. Generally people do third shot of AIV-vaccine before production starts i.e. at 16-18 weeks and serum samples taken before or near after vaccination might be having lower antibody titers because in this period their antibody titer is just going to increase. This conclusion is supported by a number of unpublished observations in this country about the benefits of AIV killed vaccines. Naeem *et al.* (2003) also described that avian influenza virus vaccines (H9N2) have been employed during the 1st week of age in broilers and broiler breeders, followed by two more vaccinations at the 8th and 18th weeks in the breeder flocks, with good protection to the flocks against this virus.

Avian influenza of highly pathogenic type was first reported in Pakistan in 1995 (Naeem & Hussain, 1995). The disease caused by subtype H7N3 produced high mortality among the affected flocks especially in the broiler breeder rearing areas of the country. Another influenza outbreak in northern areas of Pakistan was reported in 1999, which resulted in 10-20% mortality with decrease egg production from 10 to 75%. It was found to be H9N2 subtype and was named as A/Chicken/Pakistan/3/99(H9N2) (Naeem *et al*., 1999). Since then the disease has been repeatedly reported from various poultry rearing areas at different locations

Table I. Serum samples of commercial layers showing positive or negative results to AIV-subtype H7N3 using HI test

Age (weeks)	No. of vaccinated samples	Positive samples for AIV antibodies	Negative samples for AIV antibodies	Positive samples % for AIV antibodies
15-25	46	46	-	100
25-35	41	41	-	100
35-45	107	107	-	100
45-55	49	49	-	100
Total	243	243	-	100

Table II. Distribution of vaccinated commercial layers on the basis of $\log_2$ HI titers obtained against AIV-subtype H7N3

Age (weeks)	No. of positive samples for AIV antibodies	Antibody titers using HI test										
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	GMT
15-25	46	-	-	-	2	18	13	8	5	-	-	59.7
25-35	41	-	-	-	-	-	12	26	3	-	-	104.0
35-45	107	-	-	-	-	4	11	47	45	-	-	147.0
45-55	49	-	-	-	-	-	6	32	11	-	-	137.2

Table III. Serum samples of layer breeders showing positive or negative results to AIV-subtype H7N3 using HI test

Age (weeks)	No. of vaccinated samples	Positive samples for AIV antibodies	Negative samples for AIV antibodies	Positive samples % for AIV antibodies
16	15	15	-	100
22	15	15	-	100
26	15	15	-	100
Total	45	45	-	100

Table IV. Distribution of vaccinated layer breeders on the basis of log_2 HI titers obtained against AIV-subtype H7N3

Age (weeks)	No. of positive samples for AIV antibodies	Antibody titers using HI test										
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	GMT
16	15	-	-	-	-	-	2	13	-	-	-	111.4
22	15	-	-	-	-	-	-	4	11	-	-	207.9
26	15	-	-	-	-	-	1	12	2	-	-	128.0

throughout the country. Poorly controlled movement and lack of biosecurity caused AI to become endemic in some poultry populations, especially in Europe and some areas of Asia (Stubbs, 1948). Vaccinated flocks cannot be considered influenza virus-free, but vaccine use typically reduces the amount of virus shed in experimentally vaccinated and challenged birds, thereby reducing shedding and potential transmission of the virus to other birds (Halvorson, 1987). In this scenario, the earlier identified presence of H9N2 and H7N3 in poultry in this country and in other countries in the region, poses a continuous threat for the emergence of more pathogenic strains of both avian and/or human influenza viruses. For this purpose there is a constant need to carry out a coordinated surveillance for influenza viruses both in birds and humans in the country.

CONCLUSION

In conclusion, the level of protection of vaccinated commercial layers as well as layer breeders against AIV-subtype H7N3 was found satisfactory. However, it is highly likely that this virus is spreading among humans, wild birds and poultry and may result in causing new outbreaks after its mutation during interspecies transfer and replication. That's why we should emphasize the farmers who are not vaccinating against AIV, to immunize their birds regularly with inactivated vaccines against AIV-subtype H7N3 as well as against H9N2. In this way chances of emergence of new strains may be reduced to its minimum in the area. If they are not vaccinating their birds with the vaccine virus, there are chances for the field virus to attack and ultimately leading to high mortality or severe decrease in egg production or both.

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