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SEROMONITORING OF AVIAN INFLUENZA H9 SUBTYPE IN BREEDERS AND COMMERCIAL LAYER FLOCKS

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ABSTRACT

A serological survey for detection of antibodies against avian influenza virus (AIV) subtype H9 in vaccinated layer flocks was carried out. Serum samples were divided into age groups A, B, C, D (commercial layers) and E, F, G, H (layer breeders). Haemagglutination inhibition (HI) test was performed to determine serum antibodies against AIV-H9 subtype. Geometric mean titer (GMT) values were calculated. Results showed the level of protection of vaccinated birds was satisfactory.

Key words: Avian influenza virus, subtype H9, serum antibodies, Haemagglutination inhibition.

INTRODUCTION

Avian influenza viruses belong to family Orthomyxoviridae, genus *Influenza virus* A. Their surface is covered by haemagglutinin (HA) and neuraminidase (NA) glycoprotein projections. HA is the major antigen that elicits antibodies which protect against death and clinical signs (Brugh and Stone, 1987). A survey was carried out to determine the levels of antibodies against avian influenza (AI) vaccinated flocks at different age groups.

MATERIALS AND METHODS

The study was carried out in intensive layer farming areas of the Punjab (including Faisalabad, Gojra, Samundri, Kamalia, Sahiwal, Arifwala and Toba Tek Singh), from October 2004 to March 2005. Blood samples were collected from birds having a history of vaccination (type: killed, route: subcutaneous, age of vaccination: mostly at 7^{th}-8^{th} and 18^{th}-19^{th} week) against AIV-subtype H9. Blood samples were allowed to clot, sera were separated and frozen at -20^{o}C till further use. Serum samples were tested to determine the antibodies against AIVsubtype H9, using the HA and Haemagglutination inhibition (HI) methods (Olsen *et al.*, 2003). The antigen used was AIV-subtype H9 donated by National Agricultural Research Center (NARC), Islamabad, Pakistan.

Five ml chicken blood was collected in a disposable syringe coated with sodium citrate as an anticoagulant. The blood was centrifuged at 1500 rpm for 15 minutes (Hussain *et al.,* 2003). After washing thrice with phosphate buffer saline (PBS), 0.5% RBCs suspension was prepared in PBS (Anonymous, 2002). Two fold serial dilutions of the AIV-subtype H9 were made in PBS in 96-well microtitration plates. Washed RBCs were added to each well. Plates were incubated for 30 minutes at 37^{o}C before recording the HA activity. HI titer of each serum sample was also determined. Briefly, 25 µl of the test sera were serially diluted in PBS using a 96-well microtitration plate. To this 25 µl of 4HA unit of AIV-subtype H9 was added in each well. Plates were incubated for 30 minutes at 37^{o}C. After incubation 50µl of 0.5 % of the chicken RBCs were added to each well and plates were again incubated for 30 minutes at 37^{o}C. Results were recorded when complete button formation observed in the RBC control well and spreading RBC pattern in virus control. HI titers were recorded and GMT values were calculated (Brugh, 1978).

RESULTS

A total of 272 serum samples were collected from different commercial layer and breeder flocks, and were subjected to HI test. In commercial layers for birds of group A (15-25 week age), the antibody titers ranged from $\log_2$16-256 with GMT value of 55.7. Birds of group B (25-35 week age) had antibody titer in range of $\log_2$64-256 with GMT value of 104.0. Antibody titers in group C (35-45 week age) were found in the range of $\log_2$64-256 with GMT value of 128.0. Group D (45-55 week age) showed serum antibodies range of $\log_2$32-128 with 73.3 GMT value (Table 1).

In layer breeders, for birds of group E (16 week age), the antibody titers ranged from $\log_2$64-128 with GMT of 111.4. Birds of group F (22 week age) showed antibody titer in the range of $\log_2$128-256 with GMT value of 207.9. Antibodies in group G (26 week age) were found in the range of $\log_2$64-256 with GMT value of 128.0. Group H (35 week age) showed serum antibody titer in the range of $\log_2$128-256 with 168.9 GMT value (Table 2).

Table 1: Distribution of vaccinated commercial layers on the basis of log_2 HI titers obtained against AIV-subtype H9

Group	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
A	15-25	43	-	-	-	2	15	13	8	5	-	-	55.7
B	25-35	98	-	-	-	-	-	31	59	8	-	-	104.0
C	35-45	37	-	-	-	-	-	4	27	6	-	-	128.0
D	45-55	34	-	-	-	-	3	22	9	-	-	-	73.3

Table 2: Distribution of vaccinated layer breeders on the basis of log_2 HI titers obtained against AIV-subtype H9

Group	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
E	16	15	-	-	-	-	-	2	13	-	-	-	111.4
F	22	15	-	-	-	-	-	-	4	11	-	-	207.9
G	26	15	-	-	-	-	-	1	12	2	-	-	128.0
H	35	15	-	-	-	-	-	-	5	8	1	-	168.9

DISCUSSION

Antibody levels with GMT value of 67.29 and higher are considered as protective for avian influenza vaccinated birds (Trani *et al.*, 2002). All vaccinated age groups (except age group 15-25 week) were having GMT values higher than described, suggesting that they fall in the protective antibody titer range against AIV-subtype H9. Antibody titer range of group A (15-25 week age) was lower than protective range. Generally, people do third shot of AIV-vaccine before production starts i.e. at 16-18 weeks of age and serum samples collected before or near after vaccination might be having lower antibody titers because in this period their antibody titer is just going to increase after 8 weeks. Naeem *et al.* (2003) also described that AIV-H9 vaccines have been employed during the 1st week of age in broilers and broiler breeders, followed by two more vaccinations at the 8th and 18th week in the breeder flocks, with good protection to the flocks against this virus. In conclusion, the level of protection of vaccinated commercial layer and breeder flocks against AIV-H9 subtype was found satisfactory.

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