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EFFICACY OF AVIAN INFLUENZA VIRUS LOCALLY MANUFACTURED AND IMPORTED VACCINES

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ABSTRACT

Monovalent H7 and H9 inactivated Avian Influenza Virus (AIV) vaccines were developed and its efficacy was compared with the locally available and imported vaccines including monovalent (Flu-Vac H7, Gallimune H9) and bivalent (Avi-Flu H7+H9, Otto-flu H7+H9) using inoculation in five broiler breeder flocks under field conditions. The sero conversion in the inoculates was studied using haemagglutination inhibition (HI) test. On day 21 post-vaccination; in case of H7N3 virus vaccines, the antibody titer; the Geometric Mean (GM) HI titer recorded were as 55.7 in group A (the group inoculated with Gallimmune; Merial vaccine) chickens, 52.0 in group B (the group inoculated with locallly manufactured Avi-Flu vaccine) 59.7 in group C (the group inoculated with locally manufactured Otto-Flu vaccine), 29.9 in group D (the group inoculated with newly developed local vaccine) and 2.5 in group E (un-inoculated control group). In case of H9N2 virus vaccines, on day 21 post vaccination, the GM HI titers of chicks in group A, B, C, D and E were recorded as 27.9, 68.6, 64.0, 24.3 and 2.5, respectively. This study indicated that the locally prepared AI vaccines also provoked quite good HI antibody titers in poultry flocks in Pakistan.

Key words: Avian Influenza, vaccine preparation, vaccine efficacy comparison.

INTRODUCTION

The Avian Influenza (AI) outbreaks continue to cause very high economic losses to poultry farmers ever since its reported existence in poultry in 1994 in Pakistan. These outbreaks inflict economical losses to farmers as the AIV infected birds suffer from poor growth and production performances, and infectious agent may cause very high morbidity and mortality in the infected flocks (Jaffery, 1988). The etiological AI virus (AIV) types associated various clinical signs in poultry in Pakistan have been identified as H7N3, H9N2 and H5N1 (Muhammad *et al.*, 1997; Naeem *et al.*, 1999; Muneer *et al.*, 2001).

The phylogenetic studies on these H9N2 AIV isolates obtained from Saudi Arabia, Pakistan and Iran indicate a close relationship amongst them and suggest their common origin. The outbreaks of 1990 with H9N2 in poultry and other wild birds originated from the introduction of feral birds (Banks *et al.*, 2000).

Clinical signs of AI in poultry are quite variable and depend upon various factors such as host species, age, sex, concurrent infections, immune status and microbial contamination level in the environment. It has been reported that in poultry, the AI virus infection may be either asymptomatic or symptomatic in nature. The AI infection can affect bird's respiratory system and its egg production potential in addition to high morbidity and high mortality (occasionally 100 percent) in the infected flocks (Easterday *et al.*, 1997). Based on virulence, the AIVs can be grouped in two categories as highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) types (Capua *et al.*, 2000).

In view of the above explained importance of AIVs, the present research work was designed with the objective to develop inactivated; monovalent or bivalent; AIV vaccines and compare its efficacy with the already available locally manufactured or imported vaccines.

MATERIALS AND METHODS

Avian Influenza H7N3 and H9N2 virus isolates from poultry flocks in Pakistan were used for preparation of inactivated vaccines. The isolates were obtained from the Microbiology Department, University of Veterinary and Animal Sciences Lahore and separately propagated in 9-11day old developing chicken embryos, incubated in the laboratory.

The AI virus in collected Allantoic Fluid (AF) was diluted in sterile isotonic buffer (PBS at pH 7.2), so as to obtain its 10^3–10^4 dilution. A 0.1 ml quantity of dilution was inoculated in to the allantoic-cavity of 9-11 day-old embryonated eggs which were incubated at 37°C with 85% relative humidity. On candling, the inoculated eggs indicating dead embryos within 12 hrs post incubation were discarded and those surviving there-after up to 72 hrs Post Inoculation (PI) were chilled at 4°C for overnight before harvesting of AF. For harvesting, the

blunt-wide end of the egg was disinfected using 70% alcohol, and the egg-shell was cut using sterile scissor. The AF was collected using sterile glass pipettes avoiding inclusion of any yolk or albumin in the harvest. The AF from inoculated embryos indicating HA activity was pooled, clarified, tested for absence of bacterial contamination, and stored at 4ºC.

Calculation of Embryo Infective Dose$_{50}$ (EID$_{50}$): The EID$_{50}$ of the isolate was calculated using the formula described by Reed and Munch, 1938

Testing for the efficacy of inactivation Process: The efficacy of inactivation process of AI virus (es) was examined using the chicken embryos. A total of 10 aliquots of 0.2 ml volume from the each virus batch were collected and each aliquot was pass aged (in-duplicate) through the 10-day-old developing chicken embryos. This newly developed vaccine was also inoculated on blood agar and nutrient broth for detection of any bacterial contamination.

Method of vaccine preparation: The oil based vaccine against H9N2 and H7N3 AIVs was prepared by mixing equal amounts of Montanide and viral suspension ($10^{8.6}$ EID$_{50}$/ml) using electric churner in a sterilized closed container so as to obtain a homogeneous virus and Montanide mixture suspension. This preparation was tested for presence of bacterial and viral contamination in 21-day old chicks and also by inoculating on culture media.

Testing of AIV Vaccine for Safety: Safety of newly prepared AIV-vaccine was evaluated by administering its recommended and three times higher doses to batches of ten 3-week old chickens. The vaccine-inoculated chicks were observed for two weeks for the presence of any clinical illness.

Evaluation of potency of AIV vaccine: The potency of newly prepared AIV vaccine was evaluated by testing its ability of inducing sero-conversion in the experimentally inoculated chicks.

Comparison of efficacy of existing and newly developed vaccines: Three types of AIV vaccines (locally manufactured n=2; imported n=1sample) and one sample of vaccine developed in the present study were used for observing their comparative efficacy in terms of antibody production in chicks which were divided in various experimental groups.

Experimental Chicks: A total of 100 day-old broiler chicks were divided in five groups (A, B, C, D and E) each consisting of 20 chick and groups were identified by applying different color marks.

Avian Influenza Virus Vaccination: At the age of one week each experimental chick was administered AIV vaccine as per following experimental design:

GROUP A. Flu-Vac; H7 and Gallimune H9 monovalent vaccines manufactured by Merial France.
GROUP B. Avi-flu; H7 and H9 bivalent vaccine manufactured by Avicenna labs, Pakistan.
GROUP C. Otto-flu; H7 and H9 bivalent vaccines manufactured by Ottaman labs, Pakistan.
GROUP D. Inoculated with the newly developed H7 and H9 monovalent vaccines.
GROUP E-The chicks in group E were reared as unvaccinated controls.

Collection of blood samples: Blood samples from each experimental chick in every group were collected at one week interval for determining HI antibody production against the vaccinated AI viruses. One ml blood sample was collected from each chick and serum was separated. These serum samples were used in HI test for conducted to confirm the presence of antibodies against AIV types H_7 and H_9 as per procedure described by Beard and Thayer (1998).

Briefly, the HI antibody titrations were conducted using H7N3 and H9N2 AIVs. Four HA units of virus suspension were used for conducting HI test with sera samples collected at days 7, 14 and 21, using 0.5% chicken RBC suspension. Negative and positive H7N3 anti-sera were used as HI controls. All titration were carried at room temperature (22-25º C).

Each group comprised 20 birds. Group A, B, C and D were inoculated with Flu-Vac (H7) and Gallimune (H9), Avi-Flu (H7+H9), Otto-Flu (H7+H9) and self made H7N3 and H9N2 vaccines respectively. Group E was kept as un-vaccinated control.

RESULTS

Immune Response of Chicks in Group A inoculated with H7N3 virus (Flu-Vac) vaccine (Table-1)

At day 07 post vaccination (PV), all the chicks were tested for development of HI antibody against the H7N3 virus. The HI serum antibody titer in the vaccine inoculates ranged from 1:4 to 1:8. A titer of 1:4 was recorded in eight chicks and titer of 1:8 was detected in twelve birds. The flock GMT value was calculated as 6.1.

On day 14 PV, the HI titers registered an increase and the titers were recorded from 1:8 to 1:32. Serum samples from three chicks showed HI titer of 1:32, ten chicks indicated HI titer of 1:16 and seven chicks had HI titer of 1:8. The GMT HI was 13.9. On day 21 PV, one chick showed a HI titer of 1:128; 13 chicks HI titer of 1:64 and 06 chicks titer of 1:32. The GM HI titer was calculated as 55.7.

HI Antibody response of Chicks in Group B inoculated with H7N3 (Avi-flu) vaccine: On day 07 PV, the minimum HI titer of 1:2 and maximum titer of 1:16 was detected in serum samples of two chicks. A titer of 1:4 and 1: 8 was detected in sera each of 08chicks. The group GMT was recorded as 5.7.

Table 1. Immune response of various groups vaccinated with H7N3 vaccines.

PV period	Groups**	Distribution of HI Titers*										GMT
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	
7th day	A	-	8	12	-	-	-	-	-	-	-	6.1
	B	2	8	8	2	-	-	-	-	-	-	5.7
	C	1	7	11	1	-	-	-	-	-	-	6.1
	D	3	7	8	2	-	-	-	-	-	-	5.7
	E	8	10	2	-	-	-	-	-	-	-	3.2
14th day	A	-	-	7	10	3	-	-	-	-	-	13.9
	B	-	-	8	11	1	-	-	-	-	-	13.0
	C	-	-	1	11	8	-	-	-	-	-	21.1
	D	-	5	6	7	2	-	-	-	-	-	9.8
	E	3	17	-	-	-	-	-	-	-	-	3.7
21st day	A	-	-	-	-	6	13	1	-	-	-	55.7
	B	-	-	-	-	3	11	6	-	-	-	52.0
	C	-	-	-	-	7	8	5	-	-	-	59.7
	D	-	-	1	7	5	7	-	-	-	-	29.9
	E	14	6	-	-	-	-	-	-	-	-	2.5

On day 14 PV, the HI titers in serum samples of vaccinated chicken ranged from 1:8 to 1:32. At that day, an HI titer of 1:32 was observed in serum sample of one chick, titer of 1:16 in serum samples of 11 birds and titer of 1:8 in 08 birds. The GMT HI titer was calculated as13.0. On 21st day PV, serum samples of 03 birds had titer of 1:32, sera of 06 birds had titer of 1:128 each and serum samples of 11 birds a titer of 1:64 each. The group GM HI titer was recorded as 52.0.

Post vaccination H7N3 virus antibody response of chicks in Group C vaccinated with H7N3 (Otto-flu): On day 07 post-vaccination, an HI titer of 1:2 was recorded in serum sample of one chick and a titer of 1:4 was observed in each serum sample of 07 chicks; a titer of 1:8 in each serum sample of 11 birds and a titer of 1:16 was recorded in serum sample of one bird.

On 14th day post vaccination, the serum HI antibody titers ranged from 1:8 to 1:32. Serum samples from eight birds indicated HI titer of 1:32 each, 11 sera a titer of 1.16 and serum of one bird had HI titer 1:8. The group GM HI titer was calculated as 21.1.

On day 21, the chickens registered a good rise in their HI antibody titers against H7 virus. At that day PV HI titer of 1:32, 1:64 and 1:128 were recorded in serum samples each of 07, 08 and 05 chickens, respectively. The GMT HI titer of chicken at day 21 was calculated as 59.7.

HI antibody response of chicks in Group D inoculated with the H7N3 virus vaccine prepared in the present study: On day 07 PV, HI titers of 1:2, 1:4, 1:8, and 1:16 were recorded in sera of 03, 07, 08 and 02 chicks, respectively. The HI GMT on day 07 was calculated as 5.7. On day 14 PV, the serum HI antibody titers of chicks in group D ranged from 1:4 to 1:32. The HI titers of 1:4, 1:8, 1:16 and 1:32 were recorded in serum samples of 05, 06, 07 and 02 chicks, respectively. The group GMT HI titer was recorded as 9.8.

On day 21 PV, the HI antibody levels of this group chickens registered increase. The HI titers of 1:8, 1:16, 1:32, 1:64 and 1:128 were recorded in serum samples of 01, 07, 05, 06 and 01 chick, respectively. The group GMT titer on day 21 was calculated as 29.9.

Serum HI antibody titers of chicks in unvaccinated control Group E: On day 07, the chicks in group E also indicated negligible levels of serum HI antibody titers against H7N3 AI virus. A titer of 1:2, 1:4 and 1:8 was recorded in serum samples from 08, 10 and 02 chicks, respectively. The group GM HI titer was calculated as 3.2. On day 14, the HI titers of chicks ranged from 1:2 to 1:4. A total 17 chicks indicated a serum titer of 1:2 and 03 chicks indicated serum titer of 1:4. The GM HI titer of group was recorded as 3.7. On day 21, HI titers ranged between 1:2 and 1:4. A total of 14 birds indicated HI titer of 1:2 and 06 chicks indicated a HI titer of 1:4. The group GM HI titer was calculated as 2.5. On day 07, the serum HI titer against H9N2 virus vaccination was quite negligible as a titer of 1:2 was shown by 07 birds and a titer of 1:4 was recorded in 13 chicks. The group GMT was recorded as 3.2.

On day 14, the serum HI titers ranged from 1:4 to 1:16 with six birds showing a titer of 1:4; 08 birds 1:8 and 06 chicks a HI titer of 1:16. The GMT HI was found to be 8. On day 21 PV, an HI titer of 1:16 was recorded in 05 birds, a titer of 1:32 in 14 birds and a titer of 1:64 in one bird. The group GM HI titer against H9N2 virus was calculated as 27.9.

HI antibody response of chicks in Group A against H9N2 (Gallimune) vaccine (Table-2)

Table 2. Immune response of various groups vaccinated with H9N2 vaccines.

PV period	Groups**	Distribution of HI Titers*										GMT
		1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	
7th day	A	7	13	-	-	-	-	-	-	-	-	3.2
	B	2	7	8	3	-	-	-	-	-	-	6.1
	C	1	5	12	2	-	-	-	-	-	-	7.0
	D	-	7	7	6	-	-	-	-	-	-	8.0
	E	10	7	3	-	-	-	-	-	-	-	3.2
14th day	A	-	6	8	6	-	-	-	-	-	-	8.0
	B	-	1	6	12	1	-	-	-	-	-	13.0
	C	-	-	3	9	8	-	-	-	-	-	19.7
	D	-	5	6	7	2	-	-	-	-	-	9.8
	E	4	16	-	-	-	-	-	-	-	-	3.5
21st day	A	-	-	-	5	14	1	-	-	-	-	27.9
	B	-	-	-	-	4	10	6	-	-	-	68.6
	C	-	-	-	-	6	8	6	-	-	-	64.0
	D	-	-	3	8	4	4	1	-	-	-	24.3
	E	14	6	-	-	-	-	-	-	-	-	2.5

HI antibody response of chicks in Group B inoculated with Avi-flu (H9N2 virus) vaccine: On 14th day PV, a rise in antibody HI titers of chicks in group B was noted. At that day, HI titers of 1:4, 1:8, 1:16 and 1:32 were recorded in the serum samples obtained from 01, 06, 12 and 01 experimental chick, respectively. The HI GMT was recorded as 13.

On day 21 PV, a good increase in the HI titers. HI titers of 1:32, 1:64 and 1:128 were recorded in sera obtained from 04, 10 and 06 birds. The GM HI was observed at a value of 68.6.

HI antibody response of chicks in Group C inoculated with Otto-flu H9N2 vaccine: On day 07, PV, HI titers of 1:2, 1:4, 1:8 and 1:16 were noted in sera of 03, 07, 08 and 02 chicks, respectively. The H9N2 virus GMT HI titer in vaccinates at day 07 was recorded as 7.

On day 14 PV a rise in serum HI titers in H9N2 virus vaccinates was noted. Serum samples from 03 chicks indicated a titer of 1:8, from 09 chicks an individual titer 1:16 each and from 08 birds an individual titer of 1:32 each. The group GMT HI titer was calculated at 19.7.

On day 21, PV, a further rise in HI titers in vaccinates against H9N2 virus was recorded through HI test. A titer of 1:32 was recorded in each of the 06 chicks, 1:64 in each of the 08 birds and 1:28 was recorded in 06 birds. The GM HI antibody titer was at a value of 64.

Antibody response of chicks in Group D vaccinated with own manufactured H9N2 vaccine: On day 07 PV, the serum HI titers of 1:4, 1:8 and 1:16 were recorded in each of the sera from 07, 07 and 06 chicks, respectively. The group GM HI titer was calculated as 8.00.

On day 14 PV, the recorded serum HI antibody titers were better than those at day 07. Of the total birds, each of the 05 chicks indicated HI titer of 1:4; each of the six chicks had HI titer of 1:8; each of the 07 chicks indicated a titer of 1:16 and of the 02 birds each indicated HI titer of 32 each. The GMT HI titer was determined to be 9.8.

On day 21, PV, the HI titer further improved. A total of 03 birds had a titer of 1:8 each, four a titer of 1:16 each, four 1:32 each; one 1:64 and one bird had a titer of 1:128. The GM HI titer was calculated at a value of 24.3.

Antibody titers of chicks in unvaccinated controls Group E: On day 07, a total of 10 chicks had maternal antibody titer of 1:2 each; 07 chicks a titer of 1:4 and of 03 chicks each indicated a titer of 1:8 H9N2 virus. The GMT HI titer value at 07th day was calculated as 3.2.

On day 14 the HI titers in these non-vaccinates were still at low levels; each of the 16 chicks exhibiting a titer of 1:4 and 03 chicks indicating a titer of 1:8 each. The GMT HI titer was calculated as 3.5.

On day 21, the HI titers in unvaccinated chicks further waned than those on day 07 (GMT 2.5 Vs GMT 3.2) indicating that the maternal antibody titer was decreasing.

DISCUSSION

In many countries implementing strict bio-security measures at the poultry farms along with the use of inactivated monovalent/polyvalent vaccines against avian influenza, has helped in controlling deadly AI. Inactivated mono-valent and polyvalent virus adjuvant containing vaccines induce antibody production and offer

protection against egg laying declines, bird morbidity and mortality. Presently, in many countries experiencing AI problem in poultry, vaccination is routinely used especially during epizootics of HPAI (Swayne *et al.,* 2000).

In the present study, the efficacy of three different types of local and imported vaccines was compared with one new vaccine type experimentally prepared in the present investigation. The post vaccination HI antibody response of chicks in different groups was determined using standard procedure of Allan and Gough (1974). The highest immune response was observed in vaccinates at the third week post-vaccination. The GMTs of the HI titers of chicks in groups A, B, C and D on 21st day post vaccination were recorded as 55.7, 52, 59.7 and 29.9 against H7N3 virus. The chicks in control group had extremely negligible titer that could be those of maternal immunity. The presence of negligible antibody titer in unvaccinated control birds has also been reported by Swayne *et al.* (2000). The findings of present work are congruent with those reported by above workers. In our study no significant difference in the HI response between H7N3 and H9N2 vaccines inoculation in chicks was observed. This finding is also congruent with the findings of above workers who also observed that if the conditions of poultry rearing and vaccination remain good and the procedures adopted are reliable then optimum sero conversion can be obtained. The information obtained through the present investigation on use of inactivated vaccines against avian influenza is worth evaluation in the context of minimizing losses due to morbidity and mortality inflicted by the deadly AI virus. However, there is need for further investigations to be conducted under field conditions.

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