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EVALUATION OF OIL BASED AVIAN INFLUENZA VACCINE (H5NI) PREPARED WITH DIFFERENT CONCENTRATIONS OF ADJUVANT

M. IQBAL, M. NISAR, ANWARUL-HAQ, S. NOOR AND Z. J. GILL

Veterinary Research Institute Ghazi Road, Lahore Cantt, Pakistan

ABSTRACT

Bird flu vaccine from H_5N_1 strain of avian influenza virus was prepared with two concentrations of adjuvant (Montanide ISA 70MVG). Two vaccines (I and II) were prepared containing 50 and 60% Montanide, respectively. Immune response of both the vaccines as single, as well as booster, dose was evaluated in layer birds through haemagglutination inhibition test. Single dose of both vaccines showed poor immune response, while booster dose gave better response with both the vaccines. However, the vaccine prepared with 60% Montanide provided better immune response compared with the vaccine containing 50% montanide.

Key words: Birdflu vaccine, HI titre, H5N1 avian influenza virus, oil based vaccine.

INTRODUCTION

Avian influenza is one of the highly contagious Office of International Epizootics (OIE) list "A" diseases. This diseases is also called as "fowl plague" due to its high mortality in chicken. It has emerged as a disease with significant potential to affect commercial poultry production, resulting in extensive losses. Avian influenza is caused by influenza "A" virus which belongs to family orthomyxoviridae. It is a negative stranded, segmented RNA virus with 16 haemagglutin and 9 neuraminidase types. H_5N_1 is the causative agent of avian flu and is endemic in many bird populations. It is characterized by nasal and lacrimal discharge, reddening of legs and comb, facial swelling, off feed and death. Migratory birds like waterfowl, are known to carry different subtypes of avian flue.

Avian influenza has been prevailing in Pakistan since 1994 (Muhammad, 2006). Early incidence of H_5N_1 was reported in two poultry farms at Charsada and Abbotabad in Northern areas of the country in February, 2006. Human mortality has also been recorded due to H_5N_1 (Subbarao, 1997; Sims *et al*., 2003). Aqueous vaccine against H_5N_1 virus has effectively controlled the disease in the vaccinated flock but due to quick absorption, immunity produced was of shorter duration and frequent booster doses are needed. Keeping in view the duration of immunity and to avoid stress to the birds by repeating aqueous vaccine, an economical and quality oil based avian influenza vaccine from local strain of avian influenza virus subtype H_5N_1 (A/chicken/Pakistan/ NARC-2238106) was prepared by using two concentrations of adjuvant.

MATERIALS AND METHODS

Avian influenza virus (H_5N_1) was grown in 10-day old chicken embryonated eggs. The embryos showing death within 36-48 hours was chilled at 4°C and their chorioallantoic fluid (CAF) was harvested. Sterility test, haemagglutination test and embryo lethal dose 50(ELD_{50}) of the CAF was determined (Allan *et al*., 1978). The CAF was kept at 4°C for 48 hours after addition of formalin (0.12%) and later examined for safety and sterility, as described by Allan *et al.* (1978).

Two batches of oil-based vaccines were prepared using Montanide 50% (vaccine-I) and 60% concentration (vaccine-II). Thirty layer birds were procured from poultry unit of Veterinary Research Institute, Lahore, Pakistan. Ten pullets were bled at random to check the status of birds against avian influenza virus. The birds were divided into three groups A, B and C with 10 birds in each group. Layers of groups A and B were inoculated with vaccine-I and II, respectively @ 0.3 ml/ pullet intramuscularly. Layer birds of group C were kept as control. After 21 days of primary vaccines, the birds of both vaccinated groups were bled to determine the single dose titre along with the control group for comparison.

After first bleeding, each vaccinated group was divided into two subgroups as A, A_1, B and B_1. The birds of A_1 and B_1 subgroups were given booster dose with vaccine I and II, respectively. Blood samples were collected from birds of both groups (single and booster dose) after 30, 60, 90 and 120 days post vaccination. Serum was separated from each sample and stored at -20°C till processing for haemagglutination inhibition (HI) test.

The HI antibody titre was determined following the method described by Allan *et al*. (1978). Geometric mean titre (GMT), cumulative GMT (CGMT) and standard deviation (SD) of HI antibodies of each group were determined and compared following Villages and Purchase (1980).

Table 1: Haemagglutination inhibition titre of various groups of vaccinated birds

Day	Control	Vaccine I, single dose	Vaccine II, single dose	Vaccine I, booster dose	Vaccine II, booster dose
0	0	0	0	0	0
30	0	12.1	13.90	--	--
60	0	10.6	10.60	27.90	34.20
90	0	6.10	8.00	18.40	24.30
120	0	4.20	6.10	16.00	18.40
CGMT*	--	8.25	9.65	20.67	25.60
SD**	--	3.20	2.92	5.13	6.51

*=Cumulative geometric mean titre. ** =Standard deviation.

RESULTS AND DISCUSSION

The HI titres of birds with single shot of vaccines I and II at days 30, 60, 90 and 120 were 12.1: 13.9; 10.6, 10.6; 6.1: 8.0; and 4.2: 6.1 respectively. While, the HI titres of birds with booster shot of vaccine I and II at days 60, 90 and 120 were 27.9, 34.2; 18.4, 24.3 and 16.0, 18.4 respectively. No titre was noted in control birds (Table I).

These results show that when the oil based avian influenza vaccine was given as single shot, the maximum HI titre of birds was observed for both vaccines I and II at day 30. After that, HI titre declined for both vaccines and reached 4.2 and 6.1 for vaccine I and II, respectively, at day 120.

The results of birds boosted with 50 and 60% montanide vaccines showed high HI titre at day 60. After that, it rapidly declined in 50% montanide to 18.4 and 16.0 at day 90 and 120, respectively, compared with 24.3 and 18.4 at day 90 and 120, respectively, for 60% montanide. The GMT of the vaccine having adjuvant concentration 60% was higher compared to the vaccine having 50% concentration of the adjuvant. These results are in line with Tizzard (1996), who found that adjuvat promoted immunogenicity when added to the vaccine.

In the present study, it was noted that oil based avian influenza vaccine produced high protective titre, Ayesha *et al*. (2005) also reported higher HI titre with oil based avian influenza vaccine compared to alum precipitated and alum hydroxide gel containing vaccines. Similarly, adjuvanted vaccine of Newcastle disease enhanced immune response for longer duration (Iqbal *et al*., 2003).

In conclusion, single shot of oil based avian influenza vaccine did not confer protective immune response but boost shot of vaccine confered the protective immune response for longer duration. Furthermore, oil based vaccine prepared with 60% montanide provided higher GMT than that with 50% montanide concentration.

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