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PREPARATION AND EVALUATION OF VITAMIN E ADJUVANTED OIL EMULSIFIED INFECTIOUS BRONCHITIS EXPERIMENTAL VACCINE

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ABSTRACT

The present study was conducted to prepare oil emulsified (OE) infectious bronchitis (IB) experimental vaccines. The vaccines were prepared using the vaccinal strain H-120 Infectious Bonchitis virus (IBV). The virus was cultivated in 9-day old embryonated eggs via allantoic cavity route. Allantoic-amniotic fluid (AAF) was collected and inactivated with formalin @ 0.12%. Water in oil emulsion was prepared by adding one part of AAF to four parts of mineral oil containing water phase (Tween 80) and oil phase (Span 80) surfactants. Hydrophile lypohile balance (HLB) of the emulsion was maintained at 7.0. Two oil emulsified experimental vaccines were prepared. Vaccine-I was prepared without vitamin E and Vaccine-II with vitamin E (300 mg/ml). A total of 120 day-old broiler breeder chickens were divided into 4 groups, A, B, C, and D, each having 30 birds. At the age of 21 days, experimental Vaccine-I, experimental vaccine-II and commercial IB killed (H-120) vaccine were inoculated @ 0.5 ml in the birds of groups A, B and C, respectively. Group D was maintained as nonvaccinated control. Efficacy of the vaccines was evaluated on the basis of humoral immune response (haemagglutination inhibition antibody titres) against IB in the four groups. The seven weeks cumulative mean antibody titres (CMT) of each group were calculated. The highest CMT was observed in group B (130), followed by group C (69), group A (58) and group D (17). Statistical analysis showed that haemagglutination inhibition (HI) antibody titres in group B (vaccine- II) were significantly higher than those of groups A, B and C ($P< 0.05$).

Key words: Vitamin E, adjuvant, oil emulsified IB vaccine, broiler breeder, humoral immune response.

INTRODUCTION

Infectious bronchitis (IB) is an economically important viral disease of chickens caused by Infectious Bronchitis virus (IBV), which has many serotypes. Each serotype can cause IB in chicken i.e. broilers, layers and breeders. The strains, which are more prevalent in Pakistan are H120, M41, H52, D274 and D1466. The vaccines mostly used in Pakistan are prepared from H120 and M41 strains (Muneer *et al*., 1999). The morbidity of IB has been reported upto 100%, while mortality is about 25% but may reach as high as 75% or more in very young chicks (Anjum, 1997).

Both live and inactivated virus vaccines are used for immunization against IB. Live vaccines are used in broilers and for the initial vaccination of breeders and layers. Although live vaccines are generally inexpensive, easy to administer and give high titers but the titers are maintained for shoter period of time. The oil based vaccines are little expensive but give good titers, which are maintained over a long period of time (Burgh and Siegel., 1978). Inactivated vaccines are perpared by treating the virus with various chemicals such as formaline, beta propiolactone and phenol or with physical means. Inactivated oil emulsion vaccines are used primarily at point of lay in breeders and layers. Infectious bronchitis virus strains used for live vaccines are frequently attenuated by serial passages in embryonated chicken eggs. Extensive passage is avoided to prevent a reduction in immunogenicity. The degree and stability of such attenuation probably varies among vaccines. Evidence that some vaccines show increase in virulence after back passage in chickens demonstrates the potential for enhancement of virulence of such vaccines by a cyclic infection in a flock (Saif *et al*., 2003).

The influence of vitamin E on the development and functional activity of the immune system in broilers and their resistance to infectious diseases is well known (Franchini *et al*., 1986). It increases humoral immunity in chickens (Tengerdy and Brown, 1977) and turkeys (Franchini *et al*., 1990). It also increases phagocytosis, probably by regulating the biosynthesis of prostaglandins and their effect on the functional activity and proliferative capacity of immune system cells such as B and T lymphocytes, macrophages, polymorpho-nucleated cells, dendritic cells and plasma cells (Panganamala and Cornwell, 1982). The present paper describes immunopotentiator effect of vitamin E when used as an adjuvant in IB oil based vaccine in broiler breeder chicks.

MATERIALS AND METHODS

Cultivation, harvesting and inactivation of virus

Live attenuated IBV (H-120 vaccinal strain) was procured from the local market and admixed with 2 ml saline solution containing gentamicin (200 µg/ml) with pH 7.0. This virus suspension was processed for vaccine preparation. Embryonating chicken eggs (9-days-old) were inoculated with 0.1 ml of the virus suspension through allantoic route, as described by Senne (1989). The embryonic stunting growth was recorded, allantoic-amniotic fluid (AAF) of each egg was collected on 36 hour post-inoculation, pooled in sterile glass flask and stored at −20°C for further processing. The EID_{50} of AAF was calculated by the method described by Reed and Muench (1938) and recorded as $10^{5.48}$/ml. The EID_{50} of AAF was adjusted to $10^{3.5}$/ml. For inactivation of IBV, formalin was added to AAF at the rate of 0.12%, incubated at 37°C for 48 hours and transferred to a refrigerator (+4°C) for further processing.

Sterility and safety testing of AAF

Ten ml of AAF was centrifuged at 6000 rpm for 15 minutes. The sediment was streaked on Thioglycholate agar, MacConkey's agar, Mycoplasma broth and Sabourad's agar to see any bacterial or fungal contaimination. The 9-day-old embryonating chicken eggs were inoculated with 0.1 ml of inactivated AAF via allantoic route. After 168 hours of inoculation, lack of stunting effect of the fluid was considered as complete inactivation of the virus (Hussain, 2000).

Preparation of oil emulsified IB vaccines

The parrafin oil was used as oil adjuvant in the two experimental vaccines. The amount of aqueous phase (Tween-80) and oil phase (Span-80) surfactants added to parafin oil was 10%. The hydrophile lipophile balance (HLB) of oil emulsion was fixed at 7.0 with the help of following formula (Stone, 1988; Mahboob *et al*., 1997).

$$z = \frac{ax + by}{a + b}, \quad \text{where}$$

z = Required HLB of emulsion; a = Amount of surfactant A; b = Amount of surfactant B; x = HLB value of surfactant A and y = HLB value of surfactant B

Vaccine-I was prepared without vitamin E and vaccine-II with vitamin E (300 mg/ml). Water in oil emulsion was prepared by adding one part of diluted AAF in four parts of parafin oil containing surfactants. Both the vaccines were homogenized with the help of a homogenizer (Ultra Turrax T_{45}) at 4000 rpm for 4 minutes.

Physical properties

Physical properties including colour, type of emulsion, stability and viscosity (flow time) of both the vaccines were recorded. Viscosity was measured as the time required in seconds for 0.4 ml volume to drop from '0' mark of one mL glass pipette. Type of emulsion was confirmed by putting two drops of vaccine on glass slide, then mixing each drop separately with mineral oil and distilled water. A water in oil emulsion readily mixed with oil. For stability testing, the emulsion was divided into three aliquotes, the first was kept at 37ºC, 2nd at 4ºC in refrigerator and 3rd at 25-30ºC (room temperature). Stability was noted as the time until the aquous phase and oil phase started to separate (Stone *et al*., 1978; Mahboob *et al.,* 1997).

Experimental model

A total of 120 day-old broiler breeder chicken were procured from the local market. The birds were primed with attenuated live IB vaccine at the age of day 1. At the age of 21 days, the birds were divided into 4 groups (A to D) each having 30 birds. The birds of group A and B were administered experimental vaccine-I and vaccine-II, respectively at dose rate of 0.5 ml subcutaneously. The birds of group C were given commercial OE vaccine (without vitamin E) at the same dose rate, while group D was manintained as non-vaccinated control. Blood samples were collected on weekly intervals by randomly selecting 5 birds from each group on day 0, 7, 14, 21, 28, 35 and 42 post vaccination. Serum was separated and inactivated at 56°C for 30 minutes to destroy complement and stored at -20°C for subsequent use in haemagglutination inhibition (HI) test to measure antibody titres against IB. Trypsin-induced haemagglutination and haemagglutination inhibition assays were performed in "U-shaped" microtiteration plates (Corbo and Chummingham, 1959; Mahmood *et al*., 2004). Geometric mean titres (GMT) at each week were calculated in all the four groups. Cumulative mean titres (CMT) of day 0, 7, 14, 21, 28, 35 and 42 post vaccination were calculated for each experimental group.

Statistical analysis

The data on weekly antibody titres of four groups were subjected to statistical analysis, using analysis of variance technique. Differences among means were compared by Duncan's Multiple Range test (Steel *et al*., 1996). A computerized programme MSTATS was used for the statistical analysis of the data.

RESULTS AND DISCUSSION

Physical properties

Colour of both the experimental vaccines was milky white, viscosity (flow time) was 6 seconds and type of emulsion was water in oil. Both the vaccines were stable for more than 4 weeks at 4ºC, whereas at 37ºC and room temperature the vaccines were stable for one week.

Post-vaccinal GMT against IB

At day zero, non significant difference (P>0.05) was recorded among GMTs of groups A, B, C and D (Table 1). It indicates correct randomization of experimental birds in the four groups. At day 7 and 14 post-vaccination, all the three vaccinated groups A, B and C showed significantly higher titres (P<0.05) than the un-vaccinated control group D. Group B had significantly higher titres than groups A and C (P<0.05), whereas the difference between the titres of groups A and C was non-significant (P>0.05). At day 21, 28, 35 and 42 post-vaccination, the differences among GMTs of all the groups were significant (P< 0.05); the highest titres were recorded in group B, followed by group C, A and D.

Cummulative mean titers (CMT)

When weekly antibody titres against IB were cumulated for 42 days post-vaccination, the differences among CMTs of all the four groups were significant (P<0.05). The highest CMT was recorded in group B, followed by groups C, A and D (Table 1).

Table 1: Geometric Mean Titres (GMT) and Cumulative Mean Titres (CMT) against IB in four experimental groups

Days post-vaccination	Groups			
	A	B	C	D
0	32^{a}	37^{a}	37^{a}	32^{a}
7	32^{b}	48^{a}	37^{b}	24^{c}
14	37^{b}	64^{a}	42^{b}	21^{c}
21	48^{c}	147^{a}	56^{b}	18^{d}
28	97^{c}	294^{a}	111^{b}	12^{d}
35	128^{c}	338^{a}	147^{b}	8^{d}
42	97^{c}	388^{a}	14^{b}	5^{d}
CMT	**58^{c}**	**130^{a}**	**69^{b}**	**17^{d}**

Mean values with different superscripts within a row differ significantly (P<0.05).

The above results show that the oil emulsified experimental vaccine-II with vitamin E produced significantly higher (P<0.05) antibody titres against IB compared to experimental vaccine-I (without vitamin E) and the commercial vaccine. Franchini *et al.* (1991) showed increased immune response to the viral antigen (Newcastle disease virus) by using vitamin E at concentration of 30% of the oil phase in viral inactivated emulsified vaccines. Vitamin E enhanced the imune reponse by interacting with the immune-competent cells involved in the inflammatory reaction that follwed inoculation of emulsified vaccines.

Droke and Loerch (1989) determined the effects of one or two i/m injections of vitamin E on performance, health status and serum antibody response to *Pasteurella haemolytica* vaccination in steers. In all the trials, the performance and average number of days sick per steer were not affected by single injection of vitamin E. In these trials, serum antibody response to *P. haemolytica* vaccination was enhanced with the use of vitamin E. Vitamin E has been reported to increase humoral immunity in chicken, turkeys and mammals, and increases phagocytosis, probably by regulating the biosynthesis of prostaglandins and their effect on the functional activity and proliferative capacitiy of cells of immune system such as B and T lymphocytes, macrophages, dendritic and plasma cells (Tengerdy *et al.,* 1981).

Presently, in Pakistan the oil emulsified IB vaccines containing an immunomodulator are not produced. On the basis of the present study, it was concluded that vitamin E showed immunostimulatory effect when incorporated into oil emulsified IB vaccine @ 150 mg/dose.

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