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Preparation and Evaluation of Newcastle Disease Oil Emulsion Vaccine at Hydrophile Lipophile Balance 7.0 Using Mukteswar Strain

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Abstract

Mukteswar strain of Newcastle disease (ND) virus was propagated in 9-day-old embryonated eggs via allantoic route. The harvested allantoic fluid with haemagglutination (HA) titre of 1024 (EID_{50} $10^{9.3}$) was inactivated using 0.1 percent formalin solution. Two experimental vaccines were prepared in light mineral oil with aqueous to oil ratio of 1:2 (MK-VAC-I) and 1:4(MK-VAC-II).The hydrophile lipophile balance (HLB) of the vaccine was fixed at 7,0 using oil phase (span 80) and aqueous phase (tween 80) surfactants. One hundred and twenty, four week old layer chicken were divided into four equal groups (A to D). Groups A and B were injected with two experimental vaccines and C with commercial oil emulsion vaccine keeping D group as non-vaccinated control. Highest four week cumulative haemagglutination inhibition titres were shown by commercial vaccine (226) followed MK-VAC-I (217) MK-VAC-II (171) and lowest by control group. Both the vaccine showed 80 percent protection against virulent virus challenge comparable to the commercial one.

Introduction

The use of inactivated oil emulsion vaccine becoming more popular day by day in prevention and control of poultry diseases. It has been reported that these vaccine can elicit high levels of circulating antibodies and provide protection against challenge with virulent viruses such as Velogenic viscerotropic ND and highly pathogenic avian influenza (Brugh *et al*., 1979).

Killed and live attenuated ND vaccines with various regimens are being used to control the disease but non of the procedure is entirely successful in preventing the disease (Landgraf and Vielitz, 1970). Live vaccines are prepared from LaSota, Mukteswar, Asplin and Kornorrov strains. Live vaccines are although inexpensive easy to administer and give high antibody titres but these titres are maintained relatively for shorter periods. In contrast oil-based killed vaccines are little expensive but give good titres over longer periods (Brugh and Siegel, 1978). Problem encountered with live attenuated virus vaccines is that it may cause overt disease or introduce a foreign strain which may upset the immunoprophylaxis (Jeffery, 1985). Inactivated vaccines which are prepared by destroying the infectivity with chemicals such as formalin, betapropiolactone and phenol are becoming more popular (Mohanty and Dutta, 1981). These oil-emulsion vaccines are prepared by combining appropriate quantities of mineral oil, emulsifier and the desired antigen in various mechanical devices to incorporate aqueous antigen within the surfactant covered particles.

Mahboob *et al*. (1997) prepared oil-emulsion vaccines of ND using LaSota strain. The haemagglutination inhibition (HI) cumulative mean titres (CMT) and protection against virulent virus challenge was comparable with commercial oil-emulsion vaccine. The present experiment has been designed to use Mukteswar strain of ND.

Materials and Methods

Cultivation of ND virus: Freeze dried vaccinal Mukteswar strain of ND virus was reconstituted with phosphate buffered saline (PBS) and inoculated to 9-day-old chicken embryo via allantoic route. Harvested allantoic fluid was titrated by haemagglutination (HA) test and was confirmed as ND virus by HI test, using known ND antiserum (Allan *et al*., 1978). Allantoic fluid with HA titre of 1024 (EID_{50} $10^{9.3}$/0.1 ml) was inactivated by 0.1% formalin. Residual infectivity was tested by inoculating the inactivated allantoic fluid in embryonated eggs (Stone, 1988; Mahboob *et al*., 1997).

Preparation of oil-emulsion vaccines:

Adjustment of hydrophile lipophile balance (HLB): Tween 80 and span 80 were used as aqueous and oil phase surfactants, respectively at 10 percent concentration of the oil phase. HLB value of the experimental vaccines were fixed at 7.0 using formula as described by Stone (1988) and Mahboob *et al*. (1997).

Preparation of emulsion: Inactivated allantoic fluid was emulsified with white light mineral oil (containing span 80 and tween 80) in two aqueous to oil ratios e.g; MK-VAC-I with 1:2 and MK-VAC-II with 1:4 aqueous to oil ratios. Both the vaccines were homogenized at 20500 rpm for 30 seconds using ultraturrax T_{25} homogenizer.

Physical properties: Physical properties including appearance, stability, type of emulsion and viscosity were recorded as described by Stone *et al*. (1978).

Experimental model: Total of 120 four-week-old layer chicken (Previously immunized with live LaSota vaccine at 12th day of age) were divided into four groups (A to D) of

30 birds each. Group A was injected with MK-VAC-I, group B with MK-VAC-II, group C with commercial oil-emulsion vaccine and group D was kept as non-vaccinated control. Vaccines were applied S/C at dose rate of 0.5 MI.

Collection of serum samples: Serum was collected at day zero of vaccination then at weekly interval up to four weeks post vaccination (PV) from randomly selected five birds of each group.

Measurement of humoral response: Serum samples were assayed for antibodies against ND using haemagglutination inhibition test (Allan *et al*., 1978).

Experimental Challenge: At fourth week PV 10 birds from each group were challenged by injecting 0.1 MI I/M of velogenic ND virus (isolated from field outbreak) with HA titre of 512 and ELD_{50} 10^9/0.1 ml.

Statistical analysis: MI log mean titres were analyzed statistically using Duncan's new multiple range test.

Results

Physical properties: Both the experimental vaccines were stable for more than 8 months at 37°C and room temperature (25-30°C). Non of the vaccines was stable at 4°C for more than 5 days. MK-VAC-I had the highest viscosity (25 Sec.) followed by MK-VAC-II (6 sec.) and commercial vaccine (2 sec.). Type of emulsion of both the experimental vaccines was water in oil and had milky white appearance.

Table 1: Weekly HI geomean titres against ND virus antigen

Weeks	Groups*			
	A	B	C	D
0	92	61	85	70
1	120	92	200	60
2	184	160	290	45
3	557	422	367	55
4	422	390	323	38
GMT**	217	171	226	52

*Each group consists of 30 birds. Group A and B were injected with MK-VAC-I (1:2) and MK-VAC-II (1:4) respectively, C was injected with commercial vaccine while D was kept as non vaccinated control. Vaccines were inoculated S/C at a dose rate of 0.5 cc at four weeks of age; **Cumulative mean titres

Post-vaccinal HI titre: At first and second week post vaccination commercial vaccine had highest HI geomean titres (200 and 290) followed by MK-VAC-I (120 and 184) and MK-VAC-II (92-160), whereas control group showed geomean titres (GMT) of 60 and 45. At 3rd and 4th week post vaccination MK-VAC-I had highest GMT (557 and 422) followed by MK-VAC-II (422 and 390) and commercial vaccine (367 and 323). Lowest GMT were shown by control group (Table 1). When five-week cumulative mean titres (CMT) were compared commercial vaccine had highest CMT (226) followed by MK-VAC-I (217) and MK VAC-II (171).

Table 2: Protection against challenge with virulent field strain of ND virus

Group	No. of dead birds at day post-challenge					Total dead	Protection (%)
	3	4	5	6	7		
A	-	2	-	-	-	2/10	80
B	-	2	-	-	-	2/10	80
C	-	2	2	-	-	2/10	80
D	3	3	2	-	-	8/10	20

At 4th week post vaccination 10 birds from each group were exposed to experimental challenge. The virulent fief virus with HA titre 512 (ELD_{50} 10^9/0.1 ml) was injected I/M to the birds

Protection against challenge: Both the experimental vaccines and commercial vaccine had 80 percent protection against challenge while control group failed to face the challenge (Table 2).

Discussion

Parentely inoculated antigen contained in oil-emulsion adjuvant generally stimulate higher and more persistent antibody titres than equivalent amounts of antigen inoculated without adjuvant (Stone *et al*., 1978). One major constrain in developing oil-emulsion is the difficulty of preparing stable water in oil-emulsion with low viscosity. The adjuvant effect of these vaccines depends on a stable emulsion of the water-in-oil type and low viscosity is essential to assure injectability and ease of handling (Stone *et al*., 1983).

Present research studies were conducted to develop a located oil emulsion vaccine and to study humoral response protection against challenge and the effect of aqueous to ratios on physical characterization. Both of the experimental and the imported vaccines had the milky white appearance. According to Griffin (1979) clarity or transparency may gained either by having both phases of the same refractive index or by dispersing the internal phase in a such same particles (>0.2 µl) that refraction does not occur, because the particle size of the emulsion is much smaller than t wavelength of light.

Experimental MK-Vac I (w/o 1:2) had the highest viscosity followed by MK-Vac II and commercial vaccine. Stone *et al*. (1978) and Stone (1988) observed that as aqueous to ratio increased the viscosity of emulsion decreased and the vaccines containing only, the aqueous phase or oil phase surfactant had much more higher viscosity as compared to those containing both aqueous and oil phase surfactants Both the experimental the vaccines and commercial vaccine were stable for more than 8 months at 37°C and exists room temperature (25-30°C) but none was stable more than 5 days at 4°C. These finding were supported by

previous work done by Stone *et al*. (1980, 1983), Stone (1988, 1991) and Mahboob *et al*. 1997).

Type of emulsion was water-in-oil (w/o) in both the experimental vaccines and the commercial vaccine. According to Griffin (1979) w/o emulsions conduct electricity poorly, may be diluted with oil or solvents, feel more like oil, resist drying or loss of water, difficult to wash away and are less corrosive. Type of emulsion depends upon quantity of two phase and viscosity of phases. Regarding the hurnoral antibody response highest CMT were shown by commercial vaccines followed by MK-Vac I and II.

Both the experimental vaccines and commercial vaccine showed 80 percent protection against challenge. Statistical analysis showed that aqueous to oil ratios (1:2 and 1:4) had non-significantly different effect on HI titres and challenge protection tests. HI geomean titres of both experimental and commercial vaccine significantly differs from non-vaccinated control group. Vaccine II proved to be better being less viscous. The results of present study were comparable with results of previous work done by Stone (1988) and Mahboob *et al*. (1997).

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