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COMPARISON OF TWO EXPERIMENTAL BINARY ETHYLENIMINE (BEI) INACTIVATED NEWCASTLE DISEASE OIL--EMULSION VACCINES

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Summary. - Mesogenic R2B Mukteswar strain of Newcastle disease virus derived from tissue culture and allantoic fluid was inactivated with binary ethylenimine (BEI). Oil-emulsion vaccines using both substrates were prepared and tested for their potency in chickens. The vaccines elicited a good serological response as assessed by haemagglutination inhibition (HI) test and had an adequate potency as evaluated by challenge test.

Key words: Newcastle disease virus; vaccine strain; vaccine efficacy

Inactivated oil-emulsion Newcastle disease virus (NDV) vaccine is widely used in many countries for immunization of laying chickens. The vaccine is manufactured by inactivating the virus grown in allantoic sac of embryonating eggs with formalin or ß-propiolactone and used as an oil-emulsion vaccine. Preparation of oil-emulsion vaccine requires specific pathogen free (SPF) eggs for cultivation of the virus. The use of cell culture-adapted ND virus as a live vaccine have been reported by many workers (Bankowski *et al.*, 1958; Allan, 1969; July and Hipolito, 1972; Otsuko *et al.*, 1974). In the present investigation an attempt was made to use binary ethylenimine (BEI) inactivated BHK-21 cell culture adapted mesogenic strain of R2B Mukteswar and allantoic fluid infected with R2B Mukteswar strain of ND virus for preparation of the oil-emulsion vaccine. The immunogenic response of the vaccine was evaluated in nine week old broiler chickens by challenge experiments and in haemagglutination inhibition (HI) tests.

Commercial broiler chickens were purchased from Poultry Directorate (Indian Council of Agriculture Research) Rajendranagar, Hyderabad which were immunized against ND in the first week of age. Chicks at the age of nine weeks verified for antibodies to NDV were used. R2B Mukteswar (mesogenic) strain was inoculated into allantoic sac of 9-11 days old embryonated eggs; the allantoic fluid was harvested 48 hr post-inoculation. The virus adapted to BHK-21 cells was also used for vaccine production. A virulent velogenic strain of NDV was obtained from Indian Veterinary Research Institute and used for challenge experiments.

Oil-emulsion allantoic fluid vaccine (OEAFV) was prepared from the allantoic fluid of eggs inoculated with R2B Mukteswar strain; after harvest it was inactivated with a single dose of 0.05%

v/v BEI (at 37 ^{0}C for 24 hr). Inactivated virus was used for preparation of the oil-emulsion vaccine. The oil-emulsion tissue culture vaccine (OETCV) was prepared in BHK-21 cells inoculated with R2B Mukteswar strain; after harvest the virus was inactivated with BEI (37 ^{0}C for 24 hr) and concentrated by ultrafiltration. The concentrated antigen was formulated as oil-emulsion vaccine. Marcol 52, Montanide 888, Tween-80, and antigen (allantoic fluid/tissue culture virus harvest) were used to formulate the vaccine in the proportion of 67.5 : 7.5 : 0.75 : 24.25, respectively.

Virus samples were collected prior to inactivation and at 1, 2, 3, and 4 hr post-inactivation (p.i.). Infectivity titres were estimated in embryonating eggs and used to determine the inactivation kinetics as described by Pay *et al.* (1971). Samples drawn at 24 hr p. i. were inoculated into embryonated eggs to detect the presence of live virus.

The infectivity of NDV as well as of allantoic fluid was determined by using 9–11 days old embryonated eggs. The haemagglutination (HA) titres of preinactivation, postinactivation samples and samples of concentrated tissue culture derived antigen were determined by the method of Hanson (1980). Haemagglutination inhibition (HI) test was performed as described by Hanson (1980) to assess the serum antibody during the 21 day post-vaccination period.

Vaccines were inoculated into 9-week-old chickens by intramuscular route in a dose of 0.1 ml. Four groups (15 birds each) were given neat, 1/5, 1/25, and 1/125 dilutions of OEAFV; similar dilutions of OETCV were inoculated in four groups of 10 birds each. Serum samples prior to vaccine administration and on day 21 post-vaccination were collected for estimation of HI titres.

The chicks were challenged on 21st day post-vaccination with virulent NDV in a dose of $10^{6.0}$ bird infective units (ID_{50}) by intramuscular route. Six controls (primed at 1 week age) were included in the test. Another set of six controls (unprimed) were also challenged along with the vaccinated birds. The chickens were observed for a period of two weeks, the PD_{50} values were calculated by the method of Karber (1931).

Inactivation of allantoic fluid virus and tissue culture virus harvest with BEI was effective, as no live virus could be detected at 24 hr p. i. in embryonated eggs. The mean half life (t 1/2) of inactivation was 27.12 and 20.00 minutes for allantoic fluid and tissue culture harvests, respectively. Infectivity titres of $10^{8.6}$ EID_{50}/0.1 ml and $10^{6.5}$ EID_{50}/0.1 were observed in the allantoic fluid and tissue culture, respectively at the harvests. Variation in inactivation kinetics of these two samples may be attributed to the differences in the virus titres at the time of inactivation.

HA titres of NDV in the allantoic and tissue culture fluids at harvest are given in Table 1. The virus titre in neat tissue culture fluid harvest was low and the titre of 256 was observed in postconcentration samples. Results obtained from protection test and HI test are given in Table 2. Both OEAFV and OETCV

Table 1. HA titres of NDV

	Preinactivation	Postinactivation	Postconcentration
Allantoic fluid	256	256	-
Tissue culture harvest	4	4	256

Table 2. Serological response in groups of chickens vaccinated with OEAF and OETC NDV

Vaccine	Dilution	Log 10 mean HI titre 0 day	21 day	Chickens protected/total challenge	% Protection	PD_{50}
Oil-emulsion allantoic fluid (OEAF) ND vaccine	Neat	0.63	2.16	13/13	100	
	1 in 5	0.69	1.92	13/14	92	
	1 in 25	0.81	1.07	12/15	80	
	1 in 125	0.62	0.69	9/14	64	100.5
Oil-emulsion tissue culture (OETC) ND vaccine	Neat	0.81	2.34	9/9	100	
	1 in 5	0.72	1.74	8/10	80	
	1 in 25	0.56	1.19	9/10	90	
	1 in 125	0.75	0.75	9/10	90	147.9
Unvaccinated control (immunized at first week of age)	-	-	0.63	2/6	-	-
Control (unimmunized)	-	-	nil	0/6	-	-

elicited a satisfactory serological response and had more than the required 50 PD_{50} per dose.

Our results show that BEI inactivated NDV satisfactorily. Inactivation kinetics revealed that the rate of inactivation depended on the virus titre at the time of inactivation. It was possible to achieve a complete inactivation of $10^{8.0}$ EID_{50}/0.1 ml virus within 24 hr. BEI was also used for inactivation of FMD virus (Bahnemann, 1976) as well as rabies virus (Larghi and Nebel, 1980); Buonavoglia *et al.* (1988) reported that this compound can be used for inactivation of NDV. Tissue culture derived R2B Mukteswar strain of NDV offered a suitable substrate for preparation of the oil-emulsion vaccine. Virus yield in tissue culture appeared to be lower than in the allantoic fluid. However, concentration procedures by using effective downstream processing may be utilized for optimization of the antigen content in the vaccine. This will avoid dependence on SPF eggs by manufacturing units. Both OEAFV and OETCV induced satisfactory HI titres and the potency test results indicated that the vaccine performance was adequate.

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