

## PREPARATION AND EVALUATION OF NEWCASTLE DISEASE OIL EMULSION VACCINES AT HYDROPHILE-LIPOPHILE BALANCE 7.0 USING LASOTA STRAIN-A PRELIMINARY TRIAL

T. Mahboob, M. Arshad, H. Afzal, M. Siddique and G. Muhammad  
*Department of Veterinary Microbiology, <sup>1</sup>Department of Clinical Medicine & Surgery,  
University of Agriculture, Faisalabad-38040, Pakistan*

### ABSTRACT

Two oil emulsified vaccines of Newcastle disease were prepared from Lasota strain. The virus was propagated in 9-day-old chicken embryos through allantoic route. The haemagglutination titre of the allantoic fluid used for vaccine preparation was 2048. The fluid was inactivated by 0.1 per cent formalin concentration of formalin at 37°C for 6 hours. Inactivated allantoic fluid was emulsified with aqueous to oil (mineral oil) ratio of 1:2 (vaccine I) and 1:4 (vaccine II). The hydrophile-lipophile balance (HLB) of the vaccines was fixed at 7.0 using oil phase (Span 80) and aqueous phase (Tween 80) surfactants. Physical characteristics including colour, viscosity, stability and type of emulsion were studied. Sterility and safety were tested. Efficacy of the vaccine was evaluated on the basis of humoral antibody response and post challenge protection in layer chicks. One hundred and twenty, 4-week-old-layer, chicks were divided into 4 groups (A to D) comprising 30 birds each. Group A and B were injected with two experimental vaccines, C with imported oil emulsion vaccine and group D was kept as non-vaccinated control. Statistical analysis showed non-significant difference among four weeks cumulative haemagglutination inhibition mean titres of vaccine-I (256), vaccine-II (243) and imported vaccine (217). All the three vaccinated groups showed significantly higher titres than those of non-vaccinated control group. Both the experimental and imported vaccines gave 80 per cent protection against challenge with field virulent virus at 4 weeks post-vaccination.

### INTRODUCTION

The highly virulent strains of Newcastle disease (ND) virus are responsible for severe economic losses in many concentrated poultry producing areas of the world including Pakistan (Eidson *et al.*, 1982; Azam *et al.*, 1985). Two types of ND vaccines are currently used i.e., live and inactivated. Live vaccines frequently lead to adverse reactions such as mild respiratory disease and drop in egg production. The desirability of eliminating vaccinal reactions suggests that more attention should be focussed on the use of inactivated vaccines in ND control programme. Amongst the inactivated vaccines, the oil-based vaccines seem to offer substantial advantages over the aluminum hydroxide inactivated and the currently available live vaccines (Base and Furminger, 1975).

Presently oil-based vaccines are imported at the expense of substantial foreign exchange. The current research study was therefore conducted as a pilot project for local production of Newcastle disease oil emulsified vaccines.

### MATERIALS AND METHODS

#### Antigen Preparation

Reconstituted lympholized Lasota ND virus was propagated in 9-day-old embryonated eggs via allantoic cavity route. Allantoic fluid (HA titre of 2048) was harvested and inactivated with 0.1 per cent formalin at 37°C for 6 hours. Residual infectivity was checked by inoculating 9-day-old chicken embryos.

#### Vaccine Preparation

Water-in-oil emulsion vaccines were prepared using oil phase (Span 80) and aqueous phase (Tween 80) surfactants which were added to the white light mineral oil at 10 per cent concentration (Stone *et al.*, 1983).

#### Hydrophile-Lipophile-Balance (HLB) Determination

The HLB value of the vaccines was fixed at 7.0 with the help of following formula:

$$z = \frac{ax + by}{a + b}$$

where,

$z$  = Required HLB of emulsion

$a$  = amount of surfactant A (Span 80)

$b$  = amount of surfactant B (Tween 80)

$x$  = HLB value of surfactant A

$y$  = HLB value of surfactant B

Two experimental vaccines were prepared namely vaccine I with 1:2 and vaccine II with 1:4 aqueous to oil ratio.

### Physical properties

Physical properties of the vaccines including appearance, viscosity, type of emulsion and stability were recorded. Viscosity was measured as the time required in seconds for 0.4 ml volume to drop from 'O' 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 emulsion was divided into three aliquotes. One was kept at 37°C, 2nd at 4°C in refrigerator and 3rd at 25-30°C room temperature. Stability was noted as the time until the aqueous phase and oil phase started to separate (Stone *et al.*, 1978; Griffin, 1979).

### Sterility and Safety Tests

Sterility of each vaccine was tested on blood agar and thioglycollate broth while safety test was performed on adult layers.

### Experimental model

One hundred and twenty, 4-weeks-old, layer chickens (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 vaccine-I, B with vaccine-II, C with commercial oil emulsion ND vaccine and group D was kept as non-vaccinated control. Vaccines were applied S/C at the dose rate of 0.5 ml per bird. Serum samples were collected before vaccination and at weekly intervals up to four weeks post-vaccination. Serum samples were assayed for antibodies against ND using haemagglutination inhibition (HI) test (Allan *et al.*, 1978).

At fourth week post-vaccination, 10 birds of each group were exposed to challenge virus, by injecting (I/M) 0.1 ml of virulent field ND virus (HA titre, 512).

### Statistical Analysis

Duncan's New Multiple Range test was applied and significant range of HI log mean titres was calculated among four groups using Mstat-C Computer package.

## RESULTS

### Physical Properties

**Colour:** Both the experimental vaccines had milky white appearance. Imported vaccine had also milky white appearance.

**Viscosity:** Vaccine-I had highest viscosity value (25 seconds) followed by vaccine - II (6 seconds). Viscosity of commercial vaccine was 2 seconds.

**Type of emulsion:** Type of emulsion of both the experimental vaccines and imported one was water in oil.

**Stability:** Both the experimental vaccines and imported vaccine were stable for more than 6 months at 37°C and room temperature. None of the vaccines was stable at 4°C for more than five days.

Table 1: Weekly HI Geomean titres against ND

Weeks	Groups			
	A	B	C	D
0	92	139	70	61
1	121	106	184	53
2	242	211	279	26
3	640	485	367	17
4	640	557	368	23

### Efficacy

#### 1. Post-vaccinal HI Titres

At first and 2nd week post-vaccination, commercial vaccine had the highest GMT, followed by vaccine-I, vaccine-II and control group. At 3rd and 4th week post-vaccination, vaccine-I showed the highest GMT followed by vaccine-II and commercial vaccine. The lowest titres were given by control group (Table 1). Four weeks cumulative mean titres of groups A, B, C and D were 256, 243, 217 and 40 respectively.

#### 2. Protection Against Challenge

Both the experimental vaccines and imported vaccine revealed 80 per cent protection against challenge exposure at 4th week post vaccination (Table 2).

## DISCUSSION

Newcastle disease and other water-in-oil emulsion vaccines consist of an aqueous phase suspended as droplets in mineral oil. The vaccinal

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

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

antigen is contained in the aqueous phase and remains dispersed in the oil or suspending phase through the action of emulsifier. Physical characteristics of the emulsion are affected by factors such as type of emulsifier added, the intrinsic emulsifier activity, the relative volume of the aqueous and oil phases and the emulsification procedures (Herbert, 1978; Rosen, 1978; Stone *et al.*, 1983).

Both the experimental vaccines were sterile and safe. Experimental vaccines prepared in the current studies had suitable viscosity and stability and type of emulsion was water-in-oil. Addition of the hydrophilic emulsifier (Tween 80) and lipophilic emulsifier (Span 80) has been reported to increase emulsion stability, decrease viscosity and increase serological response (Thompson and Batty, 1967; Stone *et al.*, 1981).

Statistical analysis showed that the different aqueous to oil ratios (1:2 and 1:4) had non-significant effect on humoral antibody response and challenge protection. Both the experimental and imported vaccine showed significantly higher geometric titres when compared with control group. However, vaccine with 1:4 aqueous to oil ratio is recommended being less viscous as well as more economical.

The continuous threat of velogenic ND calls for the production of improved ND vaccines. The results of the present and other studies (Cessi and Nardelli 1974; Quaglio *et al.*, 1977; Stone *et al.*, 1980; Robertson, 1981) suggest that oil emulsified vaccines can play a valuable role in minimizing losses resulting from the disease.

Both the trial vaccines proved safe and effective for the control of prevalent Newcastle disease under experimental conditions. However, field trials of these vaccines would be arranged before these can be recommended on mass scale. It is anticipated that this effort would prove as a pilot project for the in-land preparation of not only ND but also other inactivated vaccines and thus saving of substantial foreign exchange.

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