

# Preparation and Comparative Evaluation of Selenite Containing Oil-Emulsified Experimental Vaccine Against Newcastle Disease

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## ABSTRACT

Two oil emulsified (OE) experimental vaccines of Newcastle disease (ND) were prepared from Mukhteswar vaccinal strain of ND virus. Sodium selenite was added as source of selenium in experimental vaccine- I (Vac-I) @ of 75 ug/dose (0.5 mL); whereas, experimental vaccine -11(Vac-11) was prepared without selenium. Two hundred, day-old broiler chicks were procured from the local market and divided into four groups (A to D) each having 50 birds. The birds of all the groups were maintained at standard management and housing conditions. All the experimental birds were primed with live ND vaccine (LaSota strain) at the age of day 7. The birds of group A, B and C were revaccinated with Vac-1, Vac-II and commercial OE vaccine of ND, respectively at the age of day 21; whereas, group D was maintained as non vaccinated control. Sera samples were collected from randomly selected 10 birds of each group at weekly interval from day 1 to the age of day 42. Haemagglutination inhibition antibody titres against ND were measured in the sera samples. Seven weeks cumulative geometric mean titres recorded in groups A, B, C and D were 134, 120, 119 and 47, respectively. Statistical analysis indicated that the vaccinated groups (A, B, and C) had significantly higher HI titres than the non-vaccinated control group D. The group A inoculated with Se containing OE experimental vaccine exhibited significantly higher HI titres than the group B and C; whereas, difference in the titre of groups B and C was non significant. The results indicate that selenium (as sodium selenite) added in the oil-emulsified ND vaccines has immunostimulatory effects on humoral immune response against ND in broiler chicks.

**Key Words:** Selenite; Oil-emulsified; Vaccine; Newcastle disease; Chicks

## INTRODUCTION

The influence of the selenium (SE) on the functional activity of the immune system to enhance the antibody synthesis thereby amplifying the immune response to antigenic stimulation is well known (Koller, 1982). Selenium helps in the protection against auto-oxidation of cell membranes by virtue of being a component of an enzyme, glutathione peroxidase, which reduces peroxides before they can attack cell membrane (Laiq, 2000) and is the cellular antioxidant that protects against the cytotoxic capabilities of oxygen metabolites produced by neutrophils in response to bacterial infection (Hogan *et al.*, 1990).

Keeping in view all these aspects, the present research study was conducted to prepare and evaluate Se containing oil emulsified experimental vaccine against ND in broiler chicks.

## MATERIALS AND METHODS

### Preparation of experimental oil-emulsified ND vaccines.

Lyophilized vaccinal Mukhteswar strain of ND virus obtained from a local public research Institute (100 doses) was reconstituted with 2 mL of phosphate buffer saline (PBS) solution. The reconstituted virus was inoculated into 10 chicken embryonated eggs (9 day old) through allantoic route @ 0.1 mL/ embryo. The inoculated eggs were candled

at 24, 48, 72 and 96 h post inoculation (PI). The embryos dying within 24 h PI were discarded because of death due to mechanical injuries. The embryos found dead at 48 and 72 h of PI were chilled at 4°C. All the remaining embryos were chilled at 96 h PI. The allantoic fluid was collected from all the chilled embryos and pooled. The ND virus was identified through haemagglutination and haemagglutination inhibition tests. The EID<sub>50</sub> of propagated ND virus was measured in SPF chicken eggs and adjusted @ 10<sup>8.16</sup> per 0.5 mL (Allan *et al.*, 1978).

The antigen (allantoic fluid containing NDV) was inactivated with 0.1% final concentration of formalin at 37°C for 24 h. After inactivation, the residual infectivity was checked in the 9-day-old embryonated eggs.

Oil phase (span 80) and aqueous phase (Tween 80) surfactants were added to mineral oil (liquid paraffin) @ 10% with final hydrophile Lipophile balance (HLB) of 7.0 and sterilized through autoclaving. One part of inactivated antigen was added into four parts of sterilized liquid paraffin containing surfactants to make water-in-oil (W/O) emulsion at aqueous to oil ratio of 1:4 (Stone, 1988).

Two oil emulsified (OE) experimental vaccines of ND were prepared. Sodium selenite was added as source of selenium in experimental vaccine-1 (Vac-1) @75 ug/dose (0.5 mL); whereas, experimental vaccine -11 (Vac-11) was prepared without sodium selenium.

The sterility of the vaccines was tested in thioglycolate

broth and blood agar (Mahboob, 1996). For safety testing, the two experimental vaccines were inoculated subcutaneously @ 2 mL into five adult birds each. The birds were observed for 15 days for any local or systemic reactions.

**Experimental model.** Two hundred, day-old broiler chicks were procured from the local market. The birds were divided into four groups (A to D) and raised under controlled standard management and housing conditions in the Faculty of Veterinary Science, University of Agriculture, Faisalabad.

The birds of all the groups were primed with live ND (LaSota strain) vaccine at the age of day 7. The birds of A, B and C were re-vaccinated at the age of day 21 as shown in Table I.

**Measurement of humoral immune response.** Blood samples without any anticoagulant were collected by heart puncture of the birds using 5 mL disposable sterile syringes from randomly selected 10 birds of each group at weekly interval from day 1 to day 42 of age. The sera were separated and processed for measurement of haemagglutination inhibition antibody titres against ND virus (Allan *et al.*, 1978).

## RESULTS AND DISCUSSION

Both the experimental OE vaccines were sterile and no growth was observed in thioglycolate broth and blood agar after 72 h at 37°C. Regarding the safety of the vaccines no local or general reaction was observed in adult birds after 15 days. There was non-significant difference in humoral immune response at the age of day one among all the groups. The geometric mean titres (GMT) ranged from 48 to 52 (Table II). At the age of day 7 (before priming), the HI titres decreased in all the groups and difference among the groups was non-significant. At the age of 14 and 21, there was sharp increase in titres. This increase in titres was due to the effect of priming at the age of day 7.

At the age of 28 days (one week post boosting), there was sharp increase in the HI antibody titres in groups A, B and C. The HI titres in group D were significantly lower than the titres of all the groups. At the age of 35 days of age (2<sup>nd</sup> week post boosting), there was gradual increase in the HI titres of groups A, B and C. At the age of 42 days (3<sup>rd</sup> week post boosting), same pattern was recorded.

Cumulative geometric mean titres recorded in groups A, B, C and D were 134, 120, 119 and 47, respectively. Statistical analysis indicated that the vaccinated groups A, B and C had significantly higher HI titres than the non-vaccinated control group D. The group A inoculated with selenium containing OE experimental vaccine exhibited significantly higher HI titres than the group B and C; whereas, difference in the titres of groups B and C were non significant. The results indicate that selenium (as sodium selenite) added in the oil emulsified ND vaccine has immunostimulatory effects on humoral

**Table I. Experimental model**

Group	No. of Birds	Experimental vaccine used at day 21	Dose (ml)	Route
A	50	Vac-I	0.5	S/C
B	50	Vac-II	0.5	S/C
C	50	Commercial OE vaccine	0.5	S/C
D	50	No OE vaccine	-	-

Vac-I: Experimental OE vaccine with 75ug sodium selenite

Vac-II: Experimental OE vaccine without sodium selenite

**Table II. Antibody Titres against ND in experimental birds**

Groups	Geometric Mean titres at the age of (days)							Cumulative Mean Titre
	0	7	14	21	28	35	42	
A	50	22	64	84	194	238	288	134
B	49	24	60	81	180	198	246	120
C	48	21	61	79	189	201	236	119
D	52	21	63	80	32	14	9	39

Priming with Live ND vaccine (Lasota) at the age 7 days

Groups A, B and C were re-vaccinated at the age of 21 days as given in Table I.

immune response against ND in broiler chicks.

The present study confirmed that the selenium enhances the humoral immune response in broiler chickens when it is used at dose rate of 0.05 mg/dose in oil emulsified ND vaccines. This effect probably is connected with a good balance between the inflammatory effect of the mineral oil and the anti-oxidative property of Se on the activity of immune cells (lymphocytes, macrophages and plasma cells) at the point of vaccination.

The effect of selenium on the functional and proliferative activity of immune cells has been exploited by adding into oil- emulsified vaccines in broiler chickens and through parental inoculation in steers and sheep (Droke, 1989; Zhong *et al.*, 1995; Bednarek, 1995). Selenium also potentiates the synthesis of circulating antibodies, when injected into mice prior to or simultaneously with sheep red blood cells antigen and enhances the primary immune response (Spallholz *et al.*, 1973). The immune response of chicks vaccinated with live ND vaccines is significantly improved by selenium supplemented in the diet 14 days before vaccination (Bassiouni *et al.*, 1990).

Selenium is important to maintain host defense mechanisms, including antibody production, prostaglandin production and neutrophils function (Smith, 1985). The neutrophils collected from cows fed Se supplemented diets had increased intracellular killing of bacteria, enhanced viability and reduced extra cellular hydrogen peroxide concentration compared with neutrophils harvested from milk and blood of cows fed Se deficient diets (Gyang *et al.*, 1984). Selenium status of cows also had an effect on the ability of milk neutrophils to kill mastitis pathogens (Erskine *et al.*, 1989).

It has been suggested that like the selenium as an immunostimulant, other immunostimulants used for immunostimulation in poultry viz. vit E and levamisole

(Panigraphy *et al.*, 1979; Bashir, 1994) may be used along with the selenium in combination or alone into the oil emulsified ND vaccines.

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