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## Efficacy of inactivated new castle disease vaccine through in ovo vaccination

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### Abstract

Newcastle disease is a highly prevalent disease of poultry industry all over the world. It causes high economic losses to farmers. Vaccination has been ideal and successful method for prevention of the disease. Many live and inactivated vaccines are available commercially for control of disease. But, major limitation of vaccination method is susceptibility of infection between time of hatch and vaccination. Hence, in ovo vaccination method was developed as alternative method. In present study, 30 specific pathogen free (SPF) embryonated eggs were inoculated with inactivated Newcastle disease vaccine and 30 eggs were kept as control. After 21 days of vaccination, the hatched birds were challenged with virulent strain of Newcastle disease. The antibodies against Newcastle disease were measured by haemagglutination-inhibition (HI) method at various time intervals. The results reported that in ovo vaccination method was efficient to protect the birds from disease.

**Keywords:** new castle disease, in ovo, vaccination, poultry

### 1. Introduction

Newcastle disease (ND) is endemic in many countries since it was found first time in 1926<sup>[1, 2, 3]</sup>. The disease is commonly known as Ranikhet Disease in India<sup>[4, 5]</sup>. ND is caused by Avian Paramyxovirus type 1 (APMV-1) from Paramyxoviridae family. There are different types of ND virus like velogenic, mesogenic and lentogenic depending upon severity and lesions<sup>[6]</sup>. ND can cause morbidity and mortality up to 100% of the chicks affecting any age group. Economically also, ND is a major poultry disease and threat to poultry industry<sup>[4, 7, 8]</sup>. The severity of infection and symptoms are dependent on virulence, host, age, immune status, other diseases and environmental condition<sup>[9]</sup>. ND occurs in a wide range of hosts (241 species) and almost all species of birds are affected<sup>[10]</sup>.

General approaches to control ND are hygiene and vaccination. Hygiene includes cleaning, sanitation, limited access of wild birds and personnel hygiene. Vaccination with proper biosecurity measures is most effective tool to control ND<sup>[11]</sup>. ND can be eradicated by vaccination, but still is not practised in a few countries on a commercial scale<sup>[12, 13, 14]</sup>. There are many approaches for ND vaccination through live vaccines and inactivated vaccines by different routes of administration, but still there are limitation of successful vaccination like early exposure to disease after hatching.

Recently, novel approach of in ovo vaccination has been developed to prevent the occurrence of several poultry diseases<sup>[15, 16]</sup>. In ovo vaccination has several advantages like neonatal resistance, uniform doses, better protection, reduction in labour cost, etc<sup>[17, 18]</sup>. This study was designed to determine the suitability of in ovo vaccination with inactivated ND vaccine and efficacy of the vaccine to protect chicks from ND through serum antibody titre estimation and challenge study.

### 2. Material and methods

#### 2.1 Study design

Sixty healthy specific pathogen free (SPF) embryonated eggs were selected and divided in to two groups as under:

Group 1: Eggs vaccinated with Newcastle Disease Vaccine, Inactivated (30 nos)

Group 2: Eggs kept as control without vaccination (30 nos) SPF eggs were procured from Venkey's India Limited, Pune and no antibodies against Newcastle Disease (ND) were present in them. Eggs were kept in hatcher until 21 days of hatching. At 18<sup>th</sup> day of hatching, eggs from group 1 were inoculated with 0.2 ml Newcastle Disease Vaccine, Inactivated (procured from Hester Biosciences Limited, Ahmedabad) through in-ovo route carefully. Eggs from group 2 were kept as control and not treated with any vaccine throughout the study. After hatching, young chicks from both the groups were kept in in deep litter designated rooms separately so that cross contamination can be prevented. Chicks were kept in controlled environment and fed with healthy diet and pure water.

After 21 days of vaccination, all birds were challenged with 10<sup>5</sup> LD<sub>50</sub> of virulent strain of Newcastle disease virus intramuscularly. Birds were observed for 14 days after challenge for any clinical signs. Dead birds were sent for examination of post-mortem signs of Newcastle disease.

Serum was collected at 03 (at the time of hatch), 07, 14, 21, 28, 35 and 42 days post vaccination. Haemagglutination-Inhibition test was performed to estimate present of antibodies against Newcastle disease.

## 2.2 Data analysis

All data were entered in to Microsoft Excel Worksheet, 2013 and represented as mean  $\pm$  SEM where necessary. Statistical analysis was performed by one way ANOVA method using Microsoft Excel software and p value less than 0.05 was considered as significantly different data with 95% confidence interval.

## 3. Results

There was 100% hatchability observed in all vaccinated and control chicks and chicks were found healthy at time of hatch. After vaccination, serum was collected from chicks at 03 days (at time of hatch), 07, 14, 21, 28, 35 and 42 days. Serum antibody titre against Newcastle disease was measured in log<sub>2</sub> as presented in Table 1.

**Table 1:** Serum antibody titre (log<sub>2</sub> HI units) against Newcastle disease after in ovo vaccination (n=30)

Group name	Days post vaccination (Birds were challenged at 21 days post vaccination)						
	03	07	14	21	28	35	42
Group 1 (Vaccinated)	1.33* $\pm$ 0.08	2.23 $\pm$ 0.07	4.56 $\pm$ 0.18	5.10 $\pm$ 0.13	8.06* $\pm$ 0.17	7.10 $\pm$ 0.1	6.06 $\pm$ 0.09
Group 2 (Control)	0	0	0	0	-	-	-

Data are represented as mean  $\pm$  SEM

\*Data are significantly different (p<0.05) with that of 21 days results

HI of Group 2 are not obtained as birds were dead after challenge at 21 days

Birds from both groups were challenged after 21 days of vaccination and observed for 14 days post challenge. Birds vaccinated with ND vaccine did not show any signs of disease and no mortality was observed. All control birds died within 4 days of challenge with post mortem signs of Newcastle Disease.

## 4. Discussion

In ovo vaccination is the novel approach to protect birds from many respiratory diseases [15, 16, 19]. In current study, inactivated vaccine of Newcastle disease was given in ovo to protect birds against ND, similar research was performed by Ebrahimi *et al.* 2000 [20], where 300 eggs of broiler breeders were selected for in ovo vaccination with Newcastle Disease and Avian Influenza oil emulsion vaccine. In current study, 30 SPF eggs were vaccinated and 30 eggs were kept as control. In ovo vaccination was performed on 18<sup>th</sup> day of hatching. Vaccination procedure was successful in all eggs with 100% hatchability. Oil emulsion vaccine against Newcastle Disease could produce neonatal protection from day of hatching as the results show that antibody HI titre was obtained as 1.33  $\pm$  0.08 as compared to that of control which was found 0 throughout the study. HI titre was found significantly different on 21<sup>st</sup> day of vaccination compared to that of 3<sup>rd</sup> day and 28<sup>th</sup> day of vaccination. It indicates that Inactivated vaccine was sufficient to induce high protective level after 21 days of vaccination and it boosted after challenge at 21 days. Similar observations were found by Okwor *et al.* 2014 [21], who reported that in ovo vaccination could produce effective protection in the terms of antibody titre and sustaining against challenge.

## 5. Conclusion

The study concluded that in ovo vaccination is a good

alternative of other ways of vaccination, particularly against Newcastle disease as the disease is highly important and measures of effective protection are necessary. In ovo vaccination has advantage of neonatal protection with less side effects and decreased labour cost.

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