Development of in ovo vaccine against Newcastle disease of birds

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In ovo vaccination is an emerging trend in the poultry industry because of its advantages like negligible manpower involvement, induction of neonatal resistance and better protection. This study describes that following in ovo administration of lentogenic F-strain of Newcastle disease virus, the vaccine virus replicated in embryos and newborn chicks, did not hamper hatchability, successfully induced antibody response and conferred protection against the disease in broiler birds. The F-strain vaccine virus has the potential for application as in ovo vaccine against Newcastle disease of commercial chicken.

Keywords: Chicken, in ovo vaccination, Newcastle disease, F-strain.

Newcastle disease (ND), caused by avian paramyxovirus, is a highly contagious and fatal disease of chicken and numerous other avian species worldwide. The disease has substantial economic impact in the poultry industry. Newcastle disease virus (NDV) isolates widely vary in virulence and are classified as highly virulent (velogenic), moderately virulent (mesogenic), or low virulent (lentogenic) on the basis of their pathogenicity for chicken. Vaccination is the principle method of controlling ND. Despite the availability of a good number of conventional vaccines using strains like B1, F, Clone 30, La Sota, Mukteswar or other lentogenic/mesogenic strains of NDV, vaccination failure is common due to non-maintenance of cold chain, poor selection of vaccine strain, insufficient dose, presence of maternal antibody, and faulty vaccination schedule.

In poultry, vaccines are generally administered as aero-sols, oculo-nasal drops, through drinking water or by injection. Recently, an in ovo vaccination technique has been developed that has several advantages over the above-mentioned methods, like neonatal resistance, administration of uniform dose of virus and better protection, administration of vaccine in eggs en masse, reduced labour cost and farmers’ involvement. As the name implies, in ovo vaccine is administered in embryonated eggs with the help of an in-egg vaccine delivery system, and soon after hatching birds are protected against the disease. In ovo vaccination has been proved to be effective against Marek’s disease (MD), and infectious bursal disease (IBD) of poultry. However, several live vaccines cannot be administered in ovo, mainly because vaccine viruses cause considerable embryo mortality, reduced hatchability, or development of clinical disease after birth. Selection of highly attenuated virus strain, virus modification, and inoculum dose that exerts least harmful effects on embryos or neonatal chicks is critical in the development of a live vaccine for in ovo application. Here we have studied the suitability of lentogenic F-strain of NDV and standardized its dose for effective in ovo vaccination in broiler chickens.

The F-strain of NDV, obtained from Indian Veterinary Research Institute, Eastern Regional Station, Kolkata was propagated in 10–12-day-old chicken embryos and virus concentration (EID50 – 50% egg infectious dose) in allantoic fluid determined. The total set of experiments was divided into five experimental groups (E1–E5) and two control groups (C1 and C2). Broiler chicken eggs from ND-vaccinated flocks were incubated for 18 days when they were injected. In experimental groups (E1–E5), the embryonated eggs were manually injected with F-strain virus in different virus concentrations (Table 1) following a standard method. Briefly, vaccine inoculum (0.1 ml/egg) was deposited by inserting nearly the entire length of the 1.25-in long 22-gauge hypodermic needle into the egg vertically through a hole punched in the large end of the eggshell. The holes were then sealed and the eggs incubated for hatching. In control groups (C1 and C2), the embryonated eggs were injected with 0.1 ml of sterile PBS (pH 7.2) following the above method, incubated for hatching and the chicks were vaccinated with commercial F-strain vaccine by oculo-nasal route on the day of hatching (group C1) or 7 days of age (group C2). Hatched birds in all but E5 groups were reared under laboratory condition separately with ad lib feed and water. On the 28th day of age about 50% of birds in each group, except E5, were separated, marked and given booster vaccination orally with commercial La Sota vaccine. There was no unvaccinated control and performance of in ovo vaccination in terms of hatchability, survival, development of humoral immunity and protection against the disease was compared with conventional post-hatch vaccination. Data were subjected to analysis of variance (two-way ANOVA) for comparing the antibody response among different groups and ages of birds.

Following natural infection or vaccination neutralizing antibodies directed against NDV surface glycoproteins, the haemagglutinin-neuraminidase (HN) and fusion (F) proteins develop in the body. Though a number of serological tests can detect the antibody response, haemagglutination inhibition (HI) test is frequently used in the poultry industry. The antibody level (titre) detected by

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To examine the vaccine virus replication following in ovo vaccination, liver, spleen and lungs from dead-in-shell embryos and 1- and 7-day-old chicks were homogenized with sterile PBS to 10% (W/V) suspension, centrifuged at 10,000 rpm for 20 min at 4°C and the supernatant was membrane-filtered (0.45 μm). Chicken embryos (10–11-day-old) were inoculated with 0.1 ml of the filtrate into the allantoic cavity and incubated. Replication of NDV in the allantoic fluid was diagnosed by haemagglutination and HI test following standard methods12,13.

Hatchability is an economically important parameter for the poultry industry. Previous studies have also shown that suitable in ovo vaccines do not adversely affect hatchability or survival of hatchet chicks.12,13. In our experiment neither sham injection nor F-strain vaccine virus caused embryo mortality or seriously hampered hatchability when administered to 18-day-old embryos, except when applied in high doses of virus inoculum (E1 and E2 groups; Table 1). This indicated safety of the in ovo administration method per se, as well as, of the F-strain NDV for embryo vaccination. However, hatching time increased by about 6 h (maximum) in experimental groups. Survival of hatched chicks was also not affected, except for the E1 group, where few chicks died within two days of hatching with respiratory distress. Thus, in ovo administration of F-strain vaccine virus in suitable doses did not compromise hatchability or survival of hatchet chicks.

HI serology results showed that following primary vaccination antibody titre sharply increased till the second or third week of age, followed by a steady decline over weeks. The antibody response was poor in the control group (C1, C2): only 42.1% and 88.8% birds were sero-positive in these control groups respectively, in the third week of age (Figure 1). In contrast, in ovo vaccinated birds developed high antibody levels: all or nearly all the birds were sero-positive from the first to the third week of age (Figure 1). The HI antibody titres in experimental groups (E1–E3, E5) were significantly higher (P ≤ 0.01) than in routine vaccination groups (C1, C2) (Figure 2). In the sixth week, about 11.11–66.66% of birds remained sero-positive in experimental groups without
booster vaccination compared to 0% in routine groups (Figure 3). This clearly showed the superiority, in respect of sero-conversion and antibody titre, of in ovo vaccination over routine post-hatch vaccination. Experiences with in ovo vaccination against MD and IBD also showed similar results\textsuperscript{5,15,16}.

Broiler birds are generally reared up to 6–7 weeks when they are marketed. Following in ovo as well as post-hatch vaccination, it was observed that the HI titre sharply declined in the fourth week of age, indicating that the specific antibody levels might not protect birds up to their market age (Figures 2 and 3). However, booster vaccination mounted an anamnestic response in all birds, including C1, C2 and field-reared E5 group of birds, evidenced as a rise in HI titre \textsuperscript{22} within a week; the HI titres continued to increase till the last day of antibody measurement (Figure 4).

Protection against infection is the ultimate aim of vaccination. Following challenge virus infection, 89–100% of birds in post-hatch vaccination (C1, C2) group and 37.5–88.9% of birds in in ovo vaccinated (E1–E5) group which received no booster vaccination died (Table 2). High mortality rate was correlated with low or even undetectable levels of HI antibody titre during the challenge experiment. However, protection was markedly improved following booster vaccination. Compared to about 11–20% mortality in E4 and routine vaccination (C1, C2) groups, all birds in other in ovo vaccinated groups survived. Dead birds had characteristic haemorrhagic spots on proventriculus and gizzard. Overall, in ovo vaccination conferred higher level of protection compared to conventional vaccination post-hatch.

In the present study, the eggs originated from ND-vaccinated parent stock and were expected to carry maternally derived antibodies. Maternal antibodies inhibit replication of vaccine virus and thus prevent successful vaccination in early days of life\textsuperscript{17}. This was evident in control groups, especially C1, in which the primary vaccination was not successful. In contrast to the primary post-hatch vaccination, an increasing level of protective GMT of antibody was recorded in in ovo vaccinated groups of birds from the first week of age, indicating no hindrance of maternal antibody on successful in ovo vaccination. Similar observations have been reported by other workers\textsuperscript{5,10}; however, more vaccine virus may be required to successfully vaccinate embryos carrying high levels of maternal antibody\textsuperscript{18,19}.

![Figure 2. HI antibody response in chickens following in ovo or post-hatch vaccination with F-strain NDV. GMT, Geometric mean (of HI) titre.](image)

![Figure 3. HI antibody response in chickens receiving no booster vaccination. *Few chickens had no detectable HI titre and, as such, geometric mean values were not available.](image)

![Figure 4. HI antibody response in chickens following administration of booster vaccine.](image)

Table 2. Comparative protective efficacy of in ovo and post-hatch vaccination against virulent NDV challenge in chickens

<table>
<thead>
<tr>
<th>Group</th>
<th>Percentage protection*</th>
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<tbody>
<tr>
<td></td>
<td>No booster vaccination</td>
</tr>
<tr>
<td>C1</td>
<td>0</td>
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<tr>
<td>C2</td>
<td>11.11</td>
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<tr>
<td>E1</td>
<td>50</td>
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<td>E2</td>
<td>62.5</td>
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<tr>
<td>E3</td>
<td>37.5</td>
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<tr>
<td>E4</td>
<td>11.11</td>
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*% Protection = (Number of birds survived/Number of birds challenged) \times 100%.
Following *in ovo* administration, vaccine virus replicates in embryonic tissues. This early virus replication leads to the development of protective immunity at the time of hatching. Generally, higher the recoverable virus titre and longer the presence of virus in hatched birds, longer is the immunity. In our study the virus was detected in visceral organs of dead-in-shell and one-day-old chicks, but not from seven-day-old chicks, indicating replication of vaccine virus in embryonic tissues. However, development of vaccine-induced immunity possibly restricted the growth of the virus in older chicks.

Selection of virus strain is critical in the development of a suitable *in ovo* vaccine. The lentogenic B1 strain NDV, which is non-pathogenic for newly hatched chickens, kill embryos when inoculated into 18-day-old chicken embryos. Chemical modification using ethylmethylsulphonate or inactivation of vaccine virus might be required to reduce the pathogenicity of the virus for embryos. This in turn may increase the cost and/or reduce the immunogenicity of the vaccine. Recombinant fowlpox vector expressing NDV F and HN proteins, and RNA editing-defective NDV mutant with low-level V protein expression have been reported to be safe and effective for *in ovo* administration. However, efficacy of these vaccines has either not been tested in commercial chickens or not tested beyond the second week of age in chickens. Our study showed that lentogenic F-strain of NDV without modification could be safely used for *in ovo* vaccination in chicken. However, this primary immunization alone may not provide life-long immunity against the disease in broiler birds and hence a booster vaccination is also recommended.

Proper dose of virus to be administered *in ovo* is also critical for achieving satisfactory hatchability and immune response. Our results showed that embryonic and post-hatch mortality increased when eggs were inoculated with higher dose of virus (Table 1). Similar results were noted by Giambrone et al. However, administration of very low dose of vaccine virus may lead to insufficient antibody response. Following *in ovo* vaccine administration, we have also recorded a dose-dependent antibody response in the first two weeks of age according to the amount of virus injected. However, beyond this the HI titres did not follow the vaccine virus concentration (Figure 2). The level of protection against lethal challenge was also vaccine virus dose-dependent (Table 2). For successful embryo vaccination, the dose selected should induce sufficient protective response even in birds having maternal antibodies. At the same time it should remain safe for embryos having low level of passive antibodies and not hamper hatchability and survival of chicks. In our study F-strain virus in a dose of 2.24 log EID₅₀/egg was most appropriate for *in ovo* vaccination of commercial broiler birds. In summary, *in ovo* vaccination using F-strain of NDV was safe and effective in protecting broiler chickens against ND. Large-scale field trials may be necessary before positioning the vaccine for mass immunization in poultry industry.


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