COMPARATIVE EFFICACY OF OCULONASAL AND INTRANASAL ROUTES OF VACCINATION IN INDUCTION OF IMMUNE RESPONSE AGAINST NEWCASTLE DISEASE VIRUS

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ABSTRACT
Vaccination programs to control Newcastle disease include use of low virulent live virus vaccines or inactivated vaccines to induce protective immunity. In order to further characterize the immune response elicited by live virus, we evaluated both cellular and humoral immune response to Newcastle disease virus (NDV) consequent to vaccine application by oculonasal and intranasal routes. Specific antiviral antibodies to NDV were detected as early as 7 days post vaccination (PV). The peak antibody levels were achieved at 2 wk PV and 3 wk PV in chicken vaccinated by oculonasal and intranasal route, respectively. The antibodies persisted upto 7 wk PV albeit low levels. The NDV specific cell mediated immune response (CMI) as detected by lymphocyte proliferation test was induced at 3 wks PV and that persisted upto 7 wk PV in chicken vaccinated by both oculonasal and intranasal route. These results suggest that both oculonasal and intranasal routes of vaccination induce comparable immune responses in chicken vaccinated with lentogenic ND vaccine.

Key words : Chicken; Lentogenic vaccine; Newcastle disease; Systemic immune response.

INTRODUCTION
Newcastle disease (ND) is an OIE List-A disease and is responsible for causing devastating loss to poultry farmers throughout the world. Various vaccines and vaccination strategies have been developed to control the disease, albeit the outbreaks occur. Hence, a strong immune response is intended to protect the birds against the virulent NDV. Both intranasal and intraocular routes of vaccination of chicken with lentogenic strains have been shown to induce high levels of protective immune responses (Russell and Koch, 1993). However, at field conditions lentogenic vaccine strains are given through oculonasal route. Recently, oculonasal route has been demonstrated to induce differential immune responses corresponding to tissue tropism of apathogenic NDV in day old chicks (Rauw, et al., 2009). Moreover, oculonasal route has been attempted to deliver adjuvanted live ND vaccine (Rauw, et al., 2010). Considering the potential of both oculonasal and intranasal routes for delivering adjuvanted ND vaccine, the present study was undertaken to compare the efficacy of lentogenic ‘F’ strain vaccine in the induction of systemic immune responses through intranasal and oculonasal routes.

MATERIAL AND METHODS
The lentogenic vaccine ‘F’ strain of Newcastle disease virus was procured from Division of Avian Diseases, Indian Veterinary Research Institute.
Institute (IVRI), Izatnagar. The virus was propagated in 9-11 days old embryonated chicken eggs. Briefly, 0.2 ml of virus suspension having titer of 10^6 EID_{50}/ml was inoculated in 9-11 days old embryonated chicken eggs and incubated at 37 °C for 72 hrs. The eggs were opened after incubation period and allantoic fluid was tested for presence of virus by spot hemagglutination test using 10% chicken RBCs. The allantoic fluid from positive eggs was pooled and a small aliquot of the pooled virus suspension was tested for sterility. The sterile virus suspension was diluted to 10^7 EID_{50}/ml in Phosphate Buffer Saline (PBS) and was used to vaccinate birds.

Day old broiler chicks were grown in cage system until they were one week of age. The chicks were divided into three groups consisting of 20 birds each. Birds in group-I received 100 µl of 10^{-6.0} EID_{50} of 'F' strain of NDV (NDVF) divided into 50 µl each for ocular and nasal routes. The birds in group-II received 100 µl of 10^{-6.0} EID_{50} of NDVF vaccine through intranasal route (50 µl vaccine in each of the nostrils). The control birds (III) received 100 µl of PBS through intranasal route.

To ascertain humoral immune response, virus specific antibodies were assayed by hemagglutination inhibition (HI) test (Allan et al., 1978) and indirect enzyme linked immunosorbent assay (ELISA) (Shebannavar et al., 2007). Proliferative responses of peripheral blood lymphocyte (PBLs) as an indicator of cell mediated immune response was evaluated by MTT colorimetric assay as described by Bounous et al. (1992).

RESULTS AND DISCUSSION

A virus specific serum antibody was evaluated by HI and ELISA tests (Figure 1). At one week of age before vaccination, birds had maternal antibodies titers ranging form log_{HI} titres of 2.0 to 2.5, which gradually declined to undetectable levels at 3 wks of age (data not shown). Birds vaccinated with NDV vaccine through intranasal route and ocularonasal route induced marginal increase in serum antibodies at 1wk PV with 100.4 and 105.6 corrected value titer by ELISA, respectively, for intranasal and ocularonasal route vaccinated chicks. The antibody levels increased considerably (p<0.05) at 2 wks PV in birds vaccinated through either intranasal or ocularonasal routes. The peak antibody levels in

![Figure 1](image-url): Serum antibody levels in chicks vaccinated with live NDV vaccine through ocularonasal and intranasal route as tested by ELISA.
birds vaccinated through oculonasal route was observed at 2 wks PV (352.9 ELISA value), whereas, in birds vaccinated through intranasal route was observed at 3 wks PV (312.1, ELISA value). Thereafter, the antibody levels declined gradually till 7 wks PV in birds vaccinated through either route. There was no significant differences (p<0.05) between the level of antibodies in birds vaccinated by either routes at any interval. The correlation between HI titers and ELISA corrected values were positive.

The prime aim of vaccination strategies against NDV is induction of strong immune responses such that the host is protected at the face of the infection. Presence of maternal antibodies in chicken provides early protection but also pose hurdle in generation of protective immune responses (Ganapathy et al., 2006). In the present study vaccination of birds at an early age of 1wk in presence of low levels of maternal antibodies generated good immune responses irrespective of their inoculation routes. The immune response to live attenuated vaccine in birds having maternal antibodies is better achieved by respiratory or conjunctival routes than by parenteral routes (Perozo et al., 2008). It is presumed that the virus applied on the nasal or conjunctival surface was able to evade the neutralization effect of serum antibodies and infect the epithelium. Chicks produce IgA, IgG and IgM in their serum, tears, spleen and Harderian gland as a consequence to occulotopical application of live vaccine. The increased number of plasma cells secreting antibodies to viral antigen is seen in Harderian gland. Moreover, increase in the serum and lacrimal fluid antibodies correlated with decrease of virus concentration in Conjunctiva and Harderian gland (Russell and Ezeifeke, 1995) suggesting important role played by Harderian gland in induction of both systemic and local immunity to NDV. Declining maternal derived immunity corresponding to progressive increase in vaccine induced immune response in chicken vaccinated oculonasally with lentogenic strains of NDV has been recently demonstrated (Rauw et al., 2009).

Figure 2: Proliferative responses of PBMCs of chicks vaccinated with live NDV vaccine through oculonasal and intranasal route.
In the present study, peak antibody response was observed at 2 wks PV in group I and 3 wks in group II birds. Our results are in corroboration with the previous studies (Verma et al., 1985; Zoth et al., 2008). The difference in attainment of early peak antibody titres in group I could be due to the differences in antigen handling at the site of application. The replicating antigens which are applied locally reach the circulation and thus stimulate the lymphoid organs. The antibodies in the serum are mainly produced by antibody forming cells in the spleen (Al-Garib et al, 2003). The involvement of ocular route in the group I could have facilitated virus escape to systemic lymphoid tissues or could have stimulated the activated plasma cell localization in the splenic lymphoid organ.

The cell mediated immune response (CMI) was evaluated by MTT colorimetric assay on PBMCs using purified viral antigen as stimulant. The results indicate that the birds vaccinated by intranasal route or oculonasal route developed systemic cell mediated immune response to NDV antigen (Figure 2). The stimulation index of birds vaccinated through intranasal route and oculonasal route at 3wks PV were 0.503 and 0.519, respectively. At 7 wks PV the stimulation index of lymphocytes were 0.293 and 0.313 respectively for intranasal and oculonasal vaccinated birds. Whereas, the mean stimulation index of lymphocytes from control group were 0.087 and 0.062 at 3wk and 7 wk PV, respectively. The proliferative responses observed were significantly higher (p<0.05) in vaccinated birds than non-vaccinated at 3 wks and 7 wks PV. The proliferative response of vaccinated birds decreased by 7 wks PV and the proliferative responses of PBMCs obtained in birds vaccinated through oculonasal route were comparable with those obtained from birds vaccinated through intranasal route. Statistical analysis did not reveal difference between SIs of PBMCs from birds vaccinated intranasally and oculonasally at both 3 wk and 7 wk PV.

Induction of CMIR after immunization against ND has been demonstrated by using MTT assay (Reynolds and Maraqa, 2000). The results are in line with that of Lambrecht et al (2004). They demonstrated that chicken vaccinated with live vaccine produced interferon gamma after antigen recall stimulation from 2 wk to 4 wks post vaccination. Whereas, only some chickens vaccinated with the inactivated vaccine showed a specific response 4 wks after vaccination suggesting long term induction of CMI by live virus application compared to inactivated antigen application. The difference in route of vaccination on development of CMIR has been shown by Hosmani (1997), who observed higher proliferative responses of PBLs to NDV antigen at 4 wks PV in chicks vaccinated through oculonasal route than in chicks vaccinated through oral route. Recently, differences in ability to induce CMI for lentogenic strains varying in tissue tropism have been demonstrated in chicken vaccinated through oculonasal route (Rauw et al., 2009).

In conclusion, the present study showed that both oculonasal and intranasal routes of vaccination are safe, effective and facilitates induction of comparable systemic immune responses to live lentogenic strain of NDV.

REFERENCES