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# Collagen Encapsulated Oral Newcastle Disease Vaccine Induces Protective Antibody Response in Backyard Poultry

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**ABSTRACT**

Newcastle disease (ND) is endemic in India and outbreaks are recorded throughout the year in commercial and backyard poultry farms. The aim of the study was to evaluate the acceptability, safety and immunogenicity of orally administrated live ND vaccine encapsulated in collagen beads to backyard poultry under field conditions. The birds were randomly divided into two groups of each 100 birds. In group 1, live lentogenic ND LaSota vaccine encapsulated in collagen beads (NDV-CG-BDs) were administrated orally at 21 days of age. In group 2, all the birds were vaccinated on 21 days of age with the same batch of live lentogenic ND LaSota vaccine using drinking water. The Newcastle disease humoral antibody response was assessed using haemagglutination inhibition (HI) test**.** The chicks readily taken the live lentogenic Newcastle disease LaSota vaccine encapsulated in collagen beads (NDV-CG-BDs). There were no untoward reactions or mortality throughout the study period of 90 days. There was no significant (*P>0.05*) difference in body weight observed between the groups during the study period. There was no significant difference (*p>0.05*) of mean HI titers in NDV-CG-BDs group in comparison with NDV vaccine administered through water on 7,14,28,45,90 days post vaccination. The protective HI titer of $\geq Log_2 4$ was observed from $7^{th}$ days post vaccination and maintained up to 90 days post vaccination in both the vaccinated groups. In conclusion, the poultry farmers can easily adopt the procedure at the farm site and control the Newcastle disease in backyard poultry.

**HIGHLIGHTS**

- Collagen encapsulated oral Newcastle disease vaccine induced protective antibody response in backyard poultry.
- The protective antibody titer was maintained up to 90 days post vaccination.

**Keywords:** Newcastle disease, LaSota strain, collagen beads, backyard poultry, humoral immune response

Newcastle Disease (ND) is a highly infectious disease of poultry causing economic loss to the poultry farmers (Cattoli *et al.,* 2011; Dimitrov *et al.,* 2017). Newcastle disease is caused by virulent strains of Avian avulavirus type 1 (AAvV-1), belongs to genus *Avian orthoavulavirus 1* (AOAV-1) within the subfamily *Avulavirinae* of the family *Paramyxoviridae* (Rima *et al.,* 2019; Alexander and

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Senne, 2008). Newcastle disease is endemic in India and outbreaks are recorded throughout the year in commercial and backyard poultry farms (Kornel, 2008; Kumar and Koul 2016; Gowthaman *et al.,* 2019).

Free ranging and backyard poultry farming contributes about 11.5% of total egg production of India in the year 2019 (BAHS 2019). Backyard poultry farming in India helps in providing food security to the rural /tribal community, social upliftment and employment to the women (Haunshi and Rajkumar, 2020). Prevention and control of ND in backyard poultry is crucial and is achieved by vaccinating the same using inactivated or live ND vaccines (Senne *et al.,* 2004; Dimitrov *et al.,* 2017; OIE, 2018). The various commercial live ND vaccines include Hitchner-B1, LaSota, V4, NDW, I2 of lentogenic strain; and Roakin, Mukteswar and Komarov of mesogenic strain (Dimitrov *et al.,* 2017; OIE, 2021). However, most of the backyard poultry are not regularly vaccinated against ND due to (i) higher cost of the vaccine, (ii) non-availability of vaccines in remote areas with proper cold chain maintenance, (iii) non-availability of smaller vaccine dose suitable for small poultry farmers, and (iv) lack of awareness among backyard poultry farmers (Madhanmohan *et al.,* 2021). ND vaccines are commonly administrated through parenteral, oral and ocular routes at different stages of a bird's life. Developing an affordable as well as easy delivery method of vaccine to the free ranging and backyard poultry will benefit the rural/tribal community. ND vaccine delivery using novel approach *i.e.* oiled rice (Wambura, 2009) and plant mucilage (Olawaleola *et al.,* 2021) was reported elsewhere.

Natural biodegradable polymers as drug delivery systems have been extensively studied because of its advantages *i.e.* natural product, biocompatible, non-toxic, established structural chemical and immunological properties, readily available and inexpensive etc. (Timbi and Reddy, 1976; Singh *et al.,* 2008). Collagen as a natural polymer has been fabricated into a wide variety of forms including: beads, cross-linked films, meshes, fibers, and sponges (Singh *et al.,* 2008).

The objective of the study was to evaluate the acceptability, safety and immunogenicity of orally administrated live ND vaccine encapsulated in collagen beads to backyard poultry under field conditions.

## MATERIALS AND METHODS

### Collagen beads and vaccine

Collagen beads making kit was obtained from Translational Research Platform for Veterinary Biologicals (TRPVB), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai. Commercial NDV lentogenic vaccine live (LaSota strain; B.No. LA19-013) was purchased from Ventri Biologicals, Pune, India. Each dose of vaccine contains LaSota Strain $\geq 10^6$ $EID_{50}$ per dose.

### Preparation of live NDV vaccine encapsulated in collagen beads (NDV-CG-BDs)

Collagen beads containing live ND LaSota vaccine was prepared as per the manufacturer's instructions. Briefly, live ND LaSota vaccine (200 doses) was reconstituted with six ml of diluents. The reconstituted live ND LaSota vaccine was mixed with 100 ml of collagen solution (Solution A). Solution A was then added drop wise into a beaker containing 200 ml of $CaCl_2$ solution (solution B). Solution B was drained and the collagen beads containing live ND LaSota vaccine were separated using strainer (Fig. 1). Finally, collagen beads containing the live ND LaSota vaccine (NDV-CG-BDs) was used to feed the chicken.

**Fig. 1:** Live lentogenic Newcastle disease LaSota vaccine encapsulated in collagen beads

### Experimental birds

Vanaraja chicks (n=200) of day old age were sourced from College of Poultry Production and Management (CPPM), TANUVAS, Hosur, Tamil Nadu. The experiment was carried out at backyard poultry farm located at Madurai, Tamil Nadu. The backyard poultry farmer consent was obtained before initiating the study. The chicks were provided artificial brooding up to 21 days post hatching. The chicks were provided with starter ration and ad libitum water. All the chicks were vaccinated on 14$^{th}$ day using live ND vaccine B1 type by eye drop method. The chicks were provided with grower ration from second month onwards until the end of this experiment. The birds were reared as semi-intensive system of management.

### Experimental design

The birds were randomly divided into two groups of each 100 birds. In group 1, NDV-CG-BDs (One collagen bead/bird) were given orally at 21 days of age. The NDV-CG-BDs (n=100) were provided in the feeder tray before feeding the starter ration. The NDV-CG-BDs was provided before feeding the starter ration. Bead intake was monitored every 15 minutes up to one hour and it was found that there were no remaining beads in the tray after 15 minutes. In group 2, all the birds were vaccinated on 21 days of age with the same batch of live ND LaSota vaccine using drinking water. Blood samples (0.5ml) from wing vein were collected randomly from twenty birds (n=20) from each group on 0, 7, 14, 21, 28, 45, 60 and 90 days post vaccination (dpv) (Fig. 2). Serum samples were separated and inactivated at 56° for 30 minutes and stored at -20° until further use. The ND humoral antibody response was assessed using Haemagglutination inhibition (HI) test. The safety of the NDV-CG-BDs was assessed. Further, the acceptability of NDV-CG-BDs by the birds was also monitored.

### Haemagglutination inhibition test

Haemagglutination inhibition test was carried out to determine the anti-NDV antibody response as per the O.I.E. Terrestrial Manual 2018. The reconstituted commercial live ND LaSota vaccine (Ventri Biologicals, Pune, India) was used as antigen. Two-fold serial dilutions of serum samples were used to estimate the anti-NDV antibody titers as logarithms to the base two. The HI antibody titer $\geq \log_2 4$ i.e. GM $\geq 4$ was considered as protective titer as per the previous studies. Protection against clinical infection and transmission amongst chickens with NDV is given if at least 85% of a flock has a protective titer of at least $\log_2 4$ (OIE, 2021; Van Boven *et al.,* 2008).

### Body weight

Body weight measurement was taken randomly from twenty birds (n=20) from each group on 0, 21, 45, 60 and 90 days post vaccination (dpv).

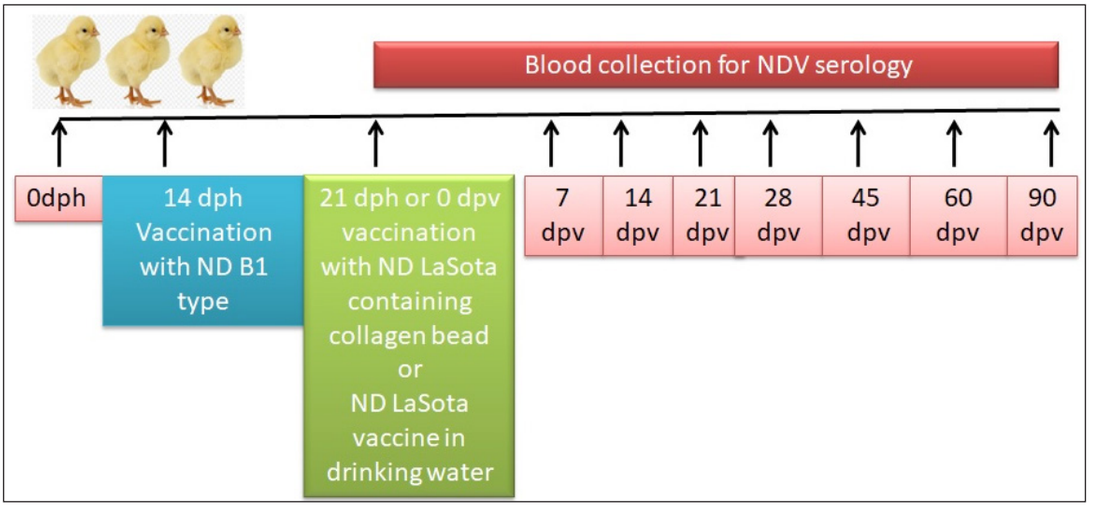


**Fig. 2:** Schematic diagram of vaccination schedule and sample collection. dph – days post hatch; dpv- days post vaccination

## STATISTICAL ANALYSIS

Student t-test was performed to compare the immune response and body weight between the groups. One way ANOVA was performed to compare the immune response at different time point within the group.

## RESULTS AND DISCUSSION

Newcastle disease is a major viral disease that causes death in all age-groups of free ranging and backyard poultry. Control of ND in free ranging and backyard poultry would benefit the poultry farmers in the rural areas. Vaccination practices used in commercial poultry are inappropriate for backyard poultry in rural areas, where cold chain maintenance is difficult, dosage formulations are not available for smaller flocks and vaccine services are provided by the Animal husbandry department at weekends, where birds were brought to the hospital for vaccination (Madhanmohan *et al.,* 2021).

In the present study, there was no mortality observed in the orally administrated NDV-CG-BDs group birds throughout the study period of 90 days. Similarly, there were no untoward reactions in the NDV-CG-BDs group birds during the study period. These results suggested that the orally administrated NDV-CG-BDs are safe to use in backyard poultry. Moreover, in the current study, the NDV-CG-BDs was readily taken by the birds suggested its acceptability.

Average body weight (gram) of the birds from both the groups is presented in table 1. There was no significant (*P>0.05*) difference in body weight observed between the groups during the study period. These results suggested that the orally administrated NDV-CG-BDs do not have any adverse impact on weight gain of the birds.

**Table 1:** Average body weight (gram) of the birds

| | | Body weight (grams) | | |
|---|---|---|---|---|
| **Sl. No** | **Days** | **NDV-CG-BDs group** | **Control group** | **P value** |
| 1 | 0 day | 38.56±0.21* | 38.43±0.21 | 0.67 |
| 2 | 21 day | 148.25±0.30 | 149.23±0.49 | 0.09 |
| 3 | 45 day | 343.8±1.04 | 346.34±1.03 | 0.09 |
| 4 | 60 day | 468.61±0.57 | 467.89±0.62 | 0.09 |
| 5 | 90 day | 890.91±1.68 | 892.67±1.86 | 0.49 |

*average body weight with standard error.

The comparative mean ($Log_2$+SE) HI titer is presented in Fig. 3. The highest mean HI titer was observed on 28 days post vaccination in both the vaccinated groups. There was no significant difference (*p>0.05*) of mean HI titers in NDV-CG-BDs group in comparison with NDV vaccine administered through water on 7,14,28,45,90 days post vaccination. However, NDV-CG-BDs group showed significantly (*p<0.05*) higher mean HI titer on 60$^{th}$ day post vaccination than NDV vaccine administered through water. There was significantly (*p<0.01*) higher mean HI titer on 14,21,28 and 45 days post vaccination in both the vaccinated group when compared with 0 day post vaccination and 7$^{th}$ day post vaccination, respectively. Similarly, in both the vaccinated group significantly (*p<0.01*) higher mean HI titer was observed on 60$^{th}$ and 90$^{th}$ days post vaccination in comparison with 14$^{th}$, 21$^{st}$ and 45$^{th}$ days post vaccination, respectively. Both the vaccinated group showed significantly (*p<0.01*) higher mean HI titer on 45$^{th}$, 60$^{th}$ and 90$^{th}$ days post vaccination when compared with 28 days post vaccination. The protective HI titer of ≥ $Log_2$4 was observed from 7$^{th}$ days post vaccination and maintained up to 90 days post vaccination in both the vaccinated groups.

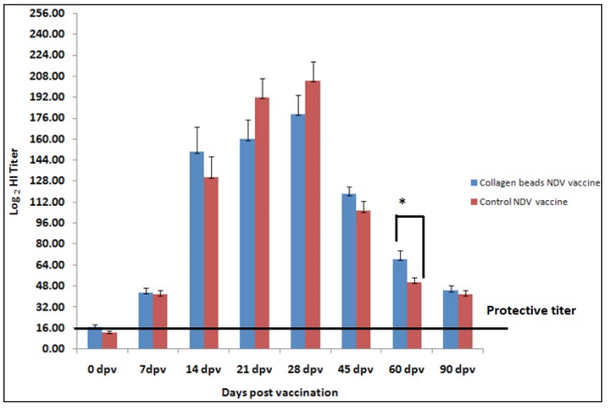


**Fig. 3:** Comparative mean ($Log_2$+SE) HI titer of vaccinated groups. Error bar represents standard error. Solid black line represents the protective HI titer ($log2^4$). * Significant difference (*p≤0.05*)

In this present study, the results suggested that the orally delivered NDV-CG-BDs elicited protective HI titers ($Log_2$4) from 7$^{th}$ days post vaccination and maintained up to 90 days post vaccination. Earlier studies (Allan *et al.,* 1978, Alexander and Senne, 2008) suggested that humoral

antibodies against NDV usually appear 4–6 days post vaccination with live attenuated vaccines. Though the birds were provided with one collagen-NDV bead per bird, there may be a possibility of one or more collagen-NDV beads may be taken by the birds. Nevertheless, blood samples were collected randomly (n=20) from the collagen-NDV beads vaccinated group at different time point to assess the humoral immune response and the results suggested that 100 percent of birds elicited protective HI titer from 7 days post vaccination and maintained up to throughout the study period of 90 days post vaccination which is similar to the vaccine delivered through water. To best of author's knowledge, this may be the first report on evaluation of collagen-NDV beads in backyard poultry under field condition.

In our study, the NDV-CG-BDs were taken by the birds within 15 minutes. It was earlier reported that the virus was viable in the collagen beads for more than 2 hours at room temperature without any reduction in virus titer (https://www.trpvb.org.in/commercialized.html#). This may be the reason for the better humoral immune response elicited by the NDV-CG-BDs group. Protecting chickens against NDV infection is based on both humoral and cell-mediated immune responses (Mariano and Hanson, 1987; Reynolds and Maraqa, 2000). However, in the current study, assessment of cell mediated immune response and challenge study was not carried out. This may be the limitations of the present study. Future study required to assess cell mediated immune response and protection upon virulent NDV challenge in NDV-CG-BDs vaccinated birds.

Further, the NDV- collagen bead preparation is very simple procedure and requires minimum equipment i.e. 10 ml disposable syringe and strainer. The poultry farmers can easily adopt the procedure at the farm site and preparing the formulation using freeze dried commercial vaccine and collagen bead kit and control the ND at backyard poultry.

## CONCLUSION

In conclusion, the orally administrated NDV-CG-BDs are safe to use in backyard poultry and elicited protective HI titers ($Log_2 4$) from 7$^{th}$ days post vaccination and maintained up to 90 days post vaccination.

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