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Oral delivery and efficacy of collagen encapsulated Newcastle disease virus vaccine in Aseel chicken under field conditions

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Abstract
Background: The objective of the study was to evaluate immunogenicity of orally administrated live ND vaccine encapsulated in collagen beads to Aseel chicken backyard poultry under field conditions.
Methods: The chicks were randomly divided into three groups of each 50 birds. The chicks were vaccinated on 21st day with live ND LaSota vaccine mixed with collagen beads (NDV-CG-BDs) were administrated though oral routein group I. In group II, the birds were vaccinated on 21 days of age with the same batch of live lentogenic ND LaSota vaccine through oral route. In group 3, no vaccines were given throughout the study. Haemagglutination inhibition (HI) test was used to assess the humoral antibody response for Newcastle disease. There were no untoward reactions in vaccinated birds throughout the study period of 45 days.
Result: Collagen beads for Newcastle disease vaccine at the dose rate of one to two beads orally was dispensed to one month old Aseel chickens and 0, 7, 14, 28, 45 and 62 days post vaccination blood sample in filter paper was collected for titre estimation using Haemagglutination inhibition test (HI). Oral delivery of commercial lasota vaccine mixed with collagen beads in the present study elicited protective antibody titre for 45 days without interfering production and weight gain. The present study concluded that, the poultry farmers can easily adopt the technology at the farm site and control the Newcastle disease in backyard poultry.

Keywords: Collagen beads, lentogenic live-virus vaccine, oral delivery, Backyard poultry

Introduction
Rural backyard poultry farming has emerged as strong agro based industry for last one decade; though contribute upto 30% to the national egg production. Major problems in rural backyard poultry rearing are losses due to Newcastle disease. The control of ND is by vaccination and with available vaccines involves maintaining cold chain, catching and handling of individual birds (Nasser *et al*., 2000) [4]. Thus control of NDV by vaccination is considered to be a safe prospect, and also a key to new science of village chicken production and it should be continuous, sustainable and effective in containing the disease. Commercially available Lasota strain vaccine is not giving permanent protection with single dose and the bird's needs to be handled frequently to restrain for administering the vaccine. Since, a lentogenic live-virus vaccine strain Lasota was encapsulated in collagen beads to enable oral delivery of the vaccine to chicken can give permanent protection without restraining the birds for administration of vaccine to the rural backyard poultry.

Materials and Methods
Collagen beads and vaccine
Collagen beads making kit was purchased from Translational Research Platform for Veterinary Biologicals (TRPVB), Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai. Commercial NDV lentogenic vaccine live (LaSota strain) was purchased from locally available in medicals. Each dose of vaccine contains LaSota Strain ≥ 10^6 EID50.

Experimental Design
A total of 150 day old Aseel chicken was purchased from Postgraduate research Institute, Kattupakkam. Those chicks were, distributed to a progressive farmer, in Chengalpet District and reared in backyard system of poultry rearing. The chicks were primed with commercial vaccine RDV-F1 followed by booster a lentogenic live-virus vaccine strain lasota was

encapsulated in collagen beads to enable oral delivery of the vaccine to chicken at the age of one month old and followed by every 2 months once. Blood sample were collected from 8 birds in each groups on 0 day for prevaccination titre and 7, 14,28, 45 and 62 day for post vaccination titre. The blood collection strips were undergone for the micro titre plate haemagglutination inhibition (HI) test. The production performance of Aseel chicken was assessed at 15 days interval from 1st weeks onwards.

Preparation of 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 given 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 CaCl2 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.

For haemagglutination inhibition test, the HI antibody titer ≥ $\log2^4$ i.e. ≥ 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 $\log2^4$ (OIE, 2021; Van Boven *et al*., 2008) [2, 7]

Body weight

Body weight measurement was taken randomly from ten birds (n=10) from each group on 0, 7, 14, 28, 45 and 62 days post vaccination (dpv).

Result and Discussion

Vaccination for Newcastle disease (ND) is routinely practiced in countries where virulent strains of the Newcastle disease virus (NDV) are endemic and in countries where virulent strains do not exist slow infection by a field strain with low virulent may have significant economic effect for the poultry producer. Vaccination as mean of protecting birds against ND is mostly used injectable vaccines, not giving permanent protection with single dose and the birds needs to be handled frequently to restrain for administering the vaccine and priming and booster dose vaccine with F1 and Lasota and followed by RDVK is recommended in field condition to control ND. To get remedy for this problem vaccine were prepared by scientist of Translational Research Platform in Veterinary Biologicals (TRPVB), TANUVAS and distributed to farmers and efficacy of vaccines also recorded by several workers (Reynolds and Maraqa, 2000 and Olawale, *et al*., 2021) [3, 1]. The result of immunization of chicken shown in table-1. The IHA antibody titre was found to have significantly increased ($p<0.01$) at post vaccination. There was no mortality after vaccination for 90 days. HI titres were decreased after 45 days of vaccination and similar report was recorded by Rahman *et al.* (2004) [5]. Similarly post vaccinal titres for commercial ND vaccine was recorded. Collagen beads for NDV vaccine oral delivery proved to be highly significant ($p<0.01$) compared to the commercial lasota vaccinated group and unvaccinated controls throughout the study**.** The protective HI titer of ≥ $\log2^4$ was observed from 7th days post vaccination and maintained up to 45 days post vaccination in both the vaccinated groups. The high titres of antibody were noticed between 28-45 days of post vaccination and there was no significance ($p>0.05$) among the treatment and control group. However, there was significant ($p>0.01$) antibody titre among treatment and control group on 7, 14, 28 days of post vaccination. The production and weight gain of the birds were not affected. The immediate intake and processing of vaccine in host immune system enhance antibody titre in 7th day itself. The maintaining of antibody titre was recorded upto 45 days of post vaccination in both treatment and control group. The sudden drop of antibody may be indicating the requirement of booster vaccine. 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. Hence, the collagen beads encapsulated with lasoat strain vaccine can be used in backyard system of poultry rearing. TANUVAS Collagen beads for NDV Vaccine oral delivery in the present study elicited protective antibody titre irrespective of age and bird variety but sustainable immunity was noticed until 45 DPV which was comparable to the existing commercial RDVK vaccine (Durairajan *et al.*, 2017) [6]. Duration of immunity is less in both the vaccine delivery system.

Table 1: Indirect Haemagglutination (IHA) titre

	Treatment group				Contol (commercial vaccine group)		
S. No	**Age at wks**	**DPV**	**Titre range**	**Average titre±SD**	**Titre range**	**Average titre±SD**	**P Value**
1.	2	7 days	32-64	41.14±5.52	16-32	22.86±3.02	0.02^{*}
2.	4	14 days	64-128	100.57±5.52	32-64	45.71±6.05	0.004**
3.	6	28 days	128-256	182.86±12.09	128-256	201.14±24.19	0.06*
4.	8	45 days	128-512	292.57±24.19	128-512	329.14±63.23	$0.72^{N.S}$
5.	10	62 days	128-256	182.86±72.57	32-64	45.7±6.05	0.001**

N.S. – Not Significant; ** Significant at P<0.01; * Significant at $p<0.05$

Table 2: Average body weight of the bird

Trait (Body Weight (g)	**Control**	**Treatment**	**P value**
	Mean ± S.E.	**Mean ± S.E.**	
Birth	39.26 ± 0.26	39.24 ± 0.34	*P>0.05*
2 weeks	95.12 ± 0.48	95.06 ± 0.44	
4 weeks	210.04 ± 0.71	210.16 ± 0.70	
6 weeks	350.46 ± 0.90	348.92 ± 0.93	
8 weeks	519.94 ± 1.26	520.36 ± 1.27	
10 weeks	702.16 ± 1.39	700.74 ± 1.95	

Conclusion

From the results obtained, it is evident that, Collagen beads for NDV Vaccine oral delivery are proving to be effective, economic, user friendly, helping to maintain continuous in field conditions without depending skilled personals and also labor saving for immunizing rural backyard chickens. The protective antibody titre elicited from 7th day of post vaccination and maintained for upto 45 of post vacciantion.

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