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Influence of Maternal Antibody on the Efficacy of Newcastle Disease Vaccination in Broilers

Pubaleem Deka[1], Sangeeta Das[2] and Pankaj Deka[2*]

[1]Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India.
[2]Department of Veterinary Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-781022, Assam, India.

Authors' contributions

This work was carried out in collaboration among all authors. The author PD designed the study and performed the analysis of the results. Authors PD and SD performed the experiment and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The half-life of maternal antibody (mAb) titres against Newcastle Disease Virus (NDV) were estimated in order to trace its likely declining pattern and the influence on the efficacy of vaccination in broiler chicks. Our study was also conducted to compare two vaccination regimens with live and inactivated NDV vaccine. For this purpose, ninety day-old broiler chicks with known NDV vaccination history of parent stock were randomly allocated into three groups (I) unvaccinated control, (II) Live NDV vaccine, and (III) Live + Inactivated NDV vaccine. Haemagglutination-inhibition (HI) antibodies were determined in the pre- and post-vaccination sera of the experimental chicks. The results indicated that the unvaccinated group showed higher level of mAb against NDV at day 1 with HI antibody titre (Log_2) 9.32 which was maintained up to 7 days of age (8.11) and gradually declined with no measurable antibodies of maternal origin after day 14, signifying the initiation of Newcastle Disease (ND) vaccination at 7 days of age. The vaccinated Group II and III revealed significantly higher HI titre at day 14 (11.52, 12.42), day 21 (12.95, 13.22), day 28 (11.63, 12.18) and day 35 (13.31, 13.39) than the unvaccinated control group I. Moreover, there is no significant difference in humoral immune response among both the vaccinated groups. Thus, our

**Corresponding author: E-mail: drpankajaau@gmail.com;*

study suggests the use of live NDV vaccines for ND prevention in commercial broiler farming over the inactivated NDV vaccines which demands a withdrawal period before slaughter and its economic implication due to additional cost. This study also indicated that the half-life values of mAb and its effect should be considered while customizing an effective vaccine regimen.

Keywords: Broilers; half-life; maternal antibody; newcastle disease virus; vaccination.

1. INTRODUCTION

Poultry keeping is the dominant form of husbandry practice in developing countries like India where both commercial as well as backyard poultry farming system is practiced. The satisfactory poultry production depends on the health status of the birds. Despite all the advantages, the profit of the poultry industry does not meet to the optimum level due to the hindrance caused by some infectious diseases. Among them, Newcastle Disease (ND) also known as Ranikhet Disease (RD) is a highly contagious viral disease of avian species causing serious economic losses in domestic poultry whose listed status with OIE marks its importance to both commercial poultry producers and poultry trading countries [1,2,3].

Vaccination continues to have a great impact in prevention and control of infectious diseases in poultry industry. The ultimate goal of vaccination programme depends on induction of protective immunity in the vaccinated host. More commonly, live vaccines prepared with lentogenic strains of NDV are preferred in broilers over killed vaccines. This is due to the fact that, live vaccines are potentially more efficient in stimulating both wings of immunity and offer feasible mean of mass vaccination at a lower cost. On the contrary, the chemically inactivated vaccines do not induce local immunity and is established slowly. Also, these vaccines add to the production cost and are associated with handling stress of the birds during vaccination [4,5]. However, effective immunization is hardly achievable due to several factors *viz*. type of vaccine, immune and disease status, lack of cold chain mantainence, level of maternally derived antibodies etc [6].

Many researchers established that maternal antibodies play a crucial role in curbing early life immunization in the short lived broiler chickens [7]. But, in due time, the decay of maternally derived antibodies makes the bird vulnerable to infectious disease. Taken together the decline in protection from maternal antibodies and immature immune mechanism are valuable points to be considered in designing effective immunization programs. The purpose of this paper is to consider the half-life of mAb against NDV in broiler chicks and its effect, on customizing effective vaccine regimen.

2. MATERIALS AND METHODS

2.1 Experimental Birds

A total of ninety day-old broiler chicks were procured from a commercial hatchery with known NDV vaccination history of the broiler breeder parent flocks. The chicks were housed in well ventilated and environment controlled animal house of the institution. Commercial broiler feed was provided with *ad libitum* drinking water.

The chicks were divided into three groups (I, II, III). Birds of group I (n=30) served as unvaccinated control as well as used for estimation of half-life of mAb against NDV. The half-life time of mAb was calculated using the following formula [8].

$$\text{mAb half life} = \frac{\text{No. of days to first negative titre}}{\text{Log2 beginning titre} - \text{Log2 end titre(first negative titre)}}$$

The birds of Group II and III were used to investigate the status of humoral immune response following vaccination with inactivated and live vaccine. Two different vaccination strategy was used for group II (n=30) and group III (n=30). The two vaccination programs in the study groups are indicated in Table 1.

2.2 Newcastle Disease Vaccine

Freeze dried live vaccine, LaSota strain (Venkateshwara Hatcheries Pvt Ltd, Maharashtra, India) and ND clone 30 inactivated oil adjuvant emulsion vaccine (Nobilis®, Intervet India Pvt. Ltd., Pune, India) were used for this study.

Table 1. Vaccination programmes in the study groups

Age at ND vaccination	Group I	Group II	Group III
1st day	-	-	NDV Killed (S/C)
7th day	-	NDV Live (intraocular)	NDV live (intraocular)
21st day	-	NDV Live (drinking water)	NDV live (drinking water)

2.3 Serological Analysis

For estimating the half life of mAb, blood samples (n=10) were collected from group I on daily basis up to 30 days of age. Similarly, to evaluate the effect of mAB on immunization, blood samples (n=10) were collected from vaccinated groups at 0, 7, 14, 21, 28 and 35 days post vaccination. Sera were separated and analysed using Haemagglutination Inhibition (HI) test for detecting antibodies against NDV as per standard protocol [9].

2.4 Haemagglutination Inhibition (HI) Test

The HI test was carried out as per the method described in the World Organization for Animal Health (OIE) *Terrestrial Manual*, 2016. Briefly, sera samples were allowed for a serial 2 fold dilution into each of the 12 well of V-bottomed microtitre plate (Cat no: #941396, Tarsons, India) in PBS. For determining the antibody titre, 4 HA unit (50µl) of LaSota (Ventri Biological Private Limited, Maharashtra, India) vaccine strain was added into each well containing (50µl) of serially diluted 2 fold serum. The plate was incubated at room temperature for 30 minutes. 50µl of 1% chicken RBCs were added into each well and the plate was incubated again at room temperature for 30 minutes, where control RBCs had to be settled to a distinct button. The HI titre was determined by recording the highest dilution of serum causing complete inhibition of 4 HAU of the virus.

2.5 Statistical Analysis

The data was analysed by using standard statistical methods (SAS Enterprise Guide 4.3 software).

3. RESULTS AND DISCUSSION

3.1 Estimation of Half-Life of mAb Against NDV in Unvaccinated Broiler Chicks

The geometric mean titre (GMT) and Log_2 HI titre at different age of chicks are depicted in Table 2.

The present study showed that the level of passively transferred anti-NDV antibody in unvaccinated broiler chicks at day 1 of age was high with a GMT of 640. The level of mAb was maintained up to 7 days of age which were declined subsequently with increasing age of the bird. However, there was no measurable antibody of maternal origin at 16 days of age. The conversion of the HI titers into GMT and log2 titer value showed that the mAb against NDV was persistent at low levels up to 14 days with complete decay after 16 days of age.

The half-life of the mAb HI titers were calculated from day 1 of age and observed up to 30th day. However, the first negative mean HI titers were observed at 16th day of life. It was perceived that the calculated half-life values varied according to the age of the birds. Also, as the mAb against NDV was analysed by HI test, the GMT titers didn't decline in a linear pattern and flattened thereafter. But, on conversion of titers into log_2 titers, the decay of mAb (1 day-16 days) showed equitably more linear pattern (Fig. 1). The half-life of mAb varied from 3-2.5 days on average during the first 10 days of life. The highest half-life time was retained for up to 5th day of age. Thereafter, the half life time fluctuated following gradual depletion of maternal antibody.

Comparing our results with previous reports in the literature suggested a divergence in the rate of decay for NDV mAb titers. In the present study, the half-life time of mAb was calculated using the formula mAb half life = No. of days to first negative titre/ log_2 beginning titre – log_2 end titre (first negative titre). The age of the chicks up to first negative HI antibody titre (Log_2) was 15 days and the beginning titre was 9.32 (at day 1 of age) and end titre being 4.32 (at days 16 of age).Using the above formula, the half-life time of NDV mAb were estimated to be 3 days and was found to be persistent at low levels up to two weeks of age. To the contrary, a half-life of 6.3 days and 4.5 days were reported by many scientists [10,11]. The divergence in the results of our study and the above mentioned two studies could be due to the difference in the initial titers of NDV in chicks which is a direct

reflection of the immune status against NDV in the breeder parent. There is no any stringent vaccination program for broilers rather it should be tailored according to the endemicity of the disease, biosecurity level of the farm premises, and level of passively transferred immunity of the birds. Thus, it is important to consider the status of maternally derived antibody level of chicks for strategic vaccination programme.

Table 2. Geometric mean titres (GMT), (Log_2) HI antibody titre and half life estimation of maternal antibodies against NDV

Age (Days)	*GMT*	HI antibody titre (Log_2)	Half life in days	Age interval
1	640	9.32	3.00	1d-16d
2	557	9.11	2.90	2d-16d
3	485	8.92	2.80	3d-16d
4	320	8.32	3.00	4d-16d
5	278	8.11	2.90	5d-16d
6	278	8.11	2.60	6d-16d
7	278	8.11	2.30	7d-16d
8	160	7.32	2.60	8d-16d
9	139	7.11	2.50	9d-16d
10	121	6.91	1.30	10d-16d
11	139	7.11	1.70	11d-16d
12	80	6.32	2.00	12d-16d
13	69	6.10	1.60	13d-16d
14	34	5.08	2.60	14d-16d
15	40	5.32	1.00	15d-16d
16	20	4.32	-	
17	-	-	-	
18	-	-	-	
19	-	-	-	
20	-	-	-	
25	-	-	-	
30	-	-	-	

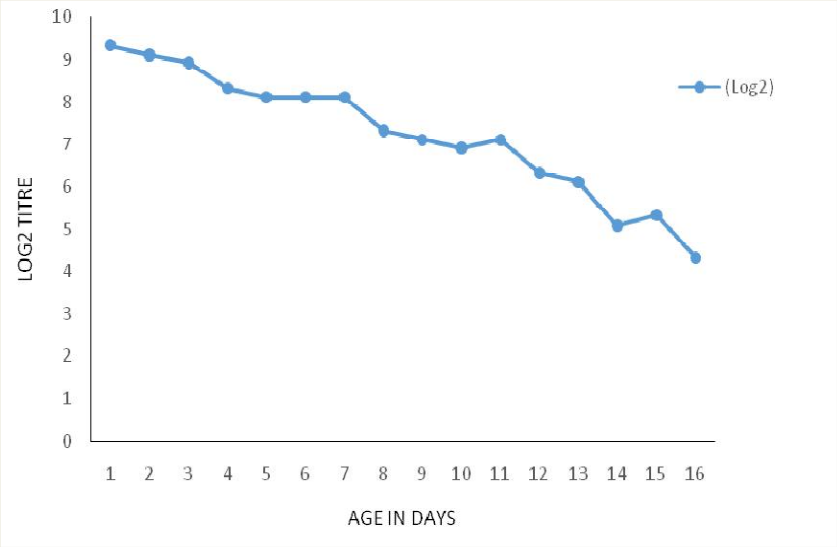

Fig. 1. Graphical representation of mAb decline curve plotted in Log_2 titre

3.2 Effect of Maternal Antibody on Humoral Immune Response of NDV Vaccinated Chicks

The GMT and Log_2 HI titre of the bird's response to vaccination is depicted in Tables 3, 4 and Fig. 2. The HI titre of birds of all the three Groups (I, II and III) showed presence of maternal antibodies on the first day prior to vaccination. In the present study, the effect of mAb on vaccination was determined. It was observed that the HI antibody titres were fairly constant in Group II till day 7 (Log_2 9.52) and on primary vaccination with LaSota strain, live NDV showed significantly increased titres after 14 days. This might be due to the multiplication of the live vaccine virus evoking an immune response [10]. However, the birds in Group III which received the first dosing of inactivated NDV vaccine at day 1 showed a declining trend of HI titre 7 dpv. On booster dosing with live NDV at day 7th revealed significantly higher antibody titre after 14dpv than group II. In this state, the inactivated NDV vaccine may not be effective at day 1 due to neutralization of the vaccine virus by the circulating mAb but it paves way for effective immune response with live NDV [12]. Another finding suggests that inactivated NDV vaccine in 1-day-old chicks with mAb had no immune stimulating effect [13]. A similar study reveals chicks with high mAb were not effectively protected when vaccinated with inactivated NDV vaccine at 1 day of age but was effective when immunized with a live or inactivated vaccine at day 1 followed by revaccination at 21 days of age with live NDV [14].

Our results reflected that both the groups showed slightly decreased antibody titer initially at 28th day of age following booster vaccination [15]. Moreover, the maximum antibody titre was recorded at 35 days of age when the birds were boosted with live NDV at 21st day of age in both the groups. However, in the present study, there is no significant difference between the two vaccination regimens.

Table 3. Geometric mean titre (GMT) of the birds grouped according to treatment (vaccinated/unvaccinated)

Bird groups	Vaccine	HI antibody titre (GMT)					
		1d	7d	14d	21d	28d	35d
I	Unvaccinated	640	278	34	0	0	0
II	Live	787	735	2940	7935	3188	1017
III	Live+Killed	735	253	5487	9554	4653	1077

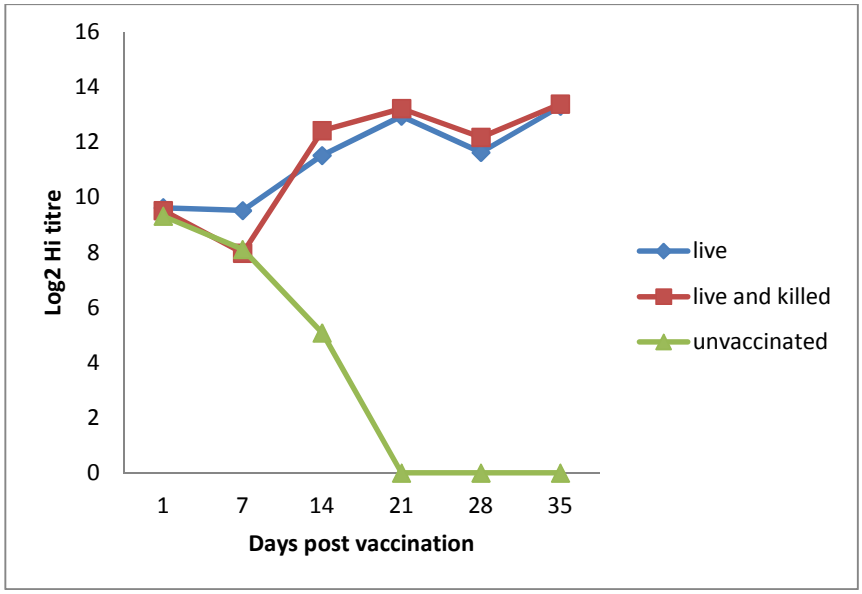

Fig. 2. Serological profile of broiler birds vaccinated with live and killed NDV vaccines

Table 4. Log_2 titre of the birds grouped according to treatment (vaccinated/unvacinated)

Groups	Vaccine	HI antibody titre (Log_2 value)					
		1day	7day	14day	21day	28day	35day
I	Unvaccinated	9.32^a	8.11^a	5.08^b	0.00	0.00	0.00
II	Live	9.62^a	9.52^a	11.52^c	12.95^c	11.63^c	13.31^c
III	Live+Killed	9.52^a	7.98^a	12.42^c	13.22^c	12.18^c	13.39^c

**Values represented with same superscript letters for a HI antibody titre (Log_2) did not differ significantly ($P<0.05$)*

It is usually assumed that high level of maternally derived ND antibody provide protection to the chickens up to 2 weeks of age [15]. In the present study, the unvaccinated group showed higher level of anti-NDV antibodies at day 1 which was maintained up to 7 days of age and gradually declined with increase in age [16]. Even though there is no significant difference in humoral immune response among both the vaccinated groups, revaccination of Group III chicks at day 7 with live NDV vaccine resulted in a rapid response with higher levels of antibody. However, Group II induced higher and more homogenous titres.

4. CONCLUSION

We determined the half-life of mAb against ND in broiler chicks which varied from 3-2.5 days and the highest half-life time was retained for up to 5 days of age. Also, the concurrent administration of inactivated and live NDV vaccines in broiler chicks induced slightly better immune response but there appears to have been an interference phenomenon due to the presence of mAb. But this still does not prove economically viable for the commercial farms due to additional cost of the inactivated NDV vaccine as well as the cost of injecting each individual chick. Also, inactivated vaccines require a withdrawal period before slaughter of the birds. Our study thus, indicated that the half-life values of mAb and its effect should be considered while customizing an effective vaccine regimen and recommended the use of live NDV vaccine in commercial broiler farming.

ETHICAL APPROVAL

All the procedures have been conducted in accordance with the approval from Institutional Animal Ethics committee, Assam Agricultural University, Khanapara, Guwahati-781022 (approval no. 770/GO/Re/S/03/CPCSEA/ FVSc/AAU/IAEC/18-19/649 dated 28.12. 2018).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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