



Original Article

Comparative Study on Production of Antibody in Broilers Vaccinated with Four Lyophilized Newcastle Disease Vaccine

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A comparative efficacy of four lyophilized Newcastle disease vaccine, namely, Medivac ND-LaSota®, BCRDV®, Izovac B1 Hitchner® and Cevac Vitapest-L® in regard to the production of Hemagglutination Inhibition (HI)-antibody was accomplished. For this, a total of 75 chicks were equally divided into five experimental groups such as, A, B, C, D and E. The HI antibody titers of group A vaccinated on 5 and 21 days of age with Medivac ND-LaSota® fluctuated among the Mean \pm SD of 89.60 ± 33.05 , 102.40 ± 33.05 and 192 ± 67.46 at 15 (10 DPV), 19 (14 DPV) and 31 (26 DPV) days of age respectively. As regards, group B administered with BCRDV® on the same days, the HI titers varied with the Mean \pm SD of 83.20 ± 30.91 , 102.40 ± 33.04 and 204.80 ± 66.09 at 15 (10 DPV), 19 (14 DPV) and 31 (26 DPV) days of age respectively. When considered the HI antibody titers of group C inoculated with Izovac B1 Hitchner® on days 5 and 21 of age, HI titer exhibited the Mean \pm SD of 80.00 ± 43.33 , 96.00 ± 33.73 and 192.00 ± 67.46 at 15 (10 DPV), 19 (14 DPV) and 31 (26 DPV) days of age respectively. Elucidation of HI antibody titers of group D birds receiving Cevac Vitapest-L® on same days were recorded to be of Mean \pm SD of 96.00 ± 33.73 , 115.20 ± 26.98 and 320.00 ± 173.31 at 15 (10 DPV), 19 (14 DPV) and 31 (26 DPV) days of age respectively. In birds of unvaccinated control group E, HI titers were found to be ranged from 32-64, 16-32, 8-16, 4-8 and 2-4 with Mean \pm SD of 48.00 ± 16.87 , 24.00 ± 8.43 , 11.20 ± 4.13 , 5.20 ± 1.93 and 3.40 ± 0.97 on 3, 15, 17, 19 and 31 days of age respectively. Thus, it was found that birds of group A, B, C and D vaccinated with Medivac ND-LaSota®, BCRDV®, Izovac B1 Hitchner® and Cevac Vitapest-L® induced slightly higher level of HI antibody titers than that of BCRDV. Maternally derived antibody (MDA) persisted to a minimal level until the age of chicks of day 17 and later on MDA declined.

Keywords: Vaccine, Antibody Titer, Newcastle Disease Virus

Introduction

Newcastle disease (ND) is an acute, contagious infection of pet, free living and domestic birds. The causative agent is Newcastle disease virus (NDV) belongs to the genus *Rubulavirus* and falls in the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*¹. The virus is distributed all over the world either as naturally circulating virus or as a vaccine virus. ND is widely variable in type and severity of the disease it produces. It is complicated because different isolates and strains of the virus may induce variations in the severity of the disease even in a given host, such as, the chickens². A variety of NDV isolates and strains have been recorded around the world²⁻³.

ND may appear as Pneumotropic (Respiratory), Neurotropic (Nervous), Viscerotropic (visceral organs) and Velogenic viscerotropic (digestive system and other visceral organs). Among the strains that are of comparatively less virulence (Lentogenic) are B₁, F and LaSota which have been widely used as vaccine. The least pathogenic (Lentogenic) strains B₁⁴, LaSota⁵, and F⁶

are employed in birds of all ages by intra-nasal or intra-ocular instillation, admixture with drinking water, or spraying.

The control of ND relies on the use of safe and effective vaccines. Live vaccines prepared with lentogenic strains of NDV are now more commonly used in broilers than vaccines prepared from chemically inactivated strains of NDV, mixed with adjuvant². This is because live freeze-dried vaccines can be produced on a large scale at a relatively low cost. The vaccines are easy to administer on a large scale, and rapidly stimulate humoral, cell-mediated and mucosal surface immunity.

Infections with NDV (either naturally or NDV vaccines) may induce cell-mediated immunity, humoral immunity, local immunity and passive immunity¹. Humoral immunity can be detected and measured by several serological tests². Serological testing for antibody to NDV has primarily utilized either the hemagglutination inhibition (HI) test or virus neutralisation test (VN). Recently, enzyme-linked-immunosorbent assay (ELISA) has replaced the HI test^{2, 7-8}.

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In Bangladesh, various live vaccines containing lentogenic strains of NDV are imported, but efficacy of these vaccines in relation to climatic condition, distribution and transportation are not investigated properly and thoroughly. Sometimes, the farmers/rearers are suspicious of prophylactic nature of the agent. A number of relevant questions are faced by scientists and field veterinarians as to the immunogenicity, retention of virus titer, stability and such other qualities of vaccine.

In order to address one such query, the present study was undertaken to determine the maternally derived antibody of broiler chicks; to determine the antibody titer in chicks following vaccination with Medivac ND LaSota® (LaSota strain), BCRDV® (F-strain), Izovac B1 Hitchner® (B1 strain) and Cevac Vitapest L® (LaSota strain), and to evaluate the comparative antibody production of the vaccines used.

Materials and Methods

Newcastle disease vaccines

Four Newcastle disease lyophilized (freeze-dried) vaccines were used in this study including (i) Medivac ND-LaSota® vaccine of LaSota strain (ii) Baby chick Ranikhet Disease vaccine (BCRDV®) of F (Asplin) strain (iii) Izovac B₁ Hitchner® of B₁ strain and (iv) Cevac vitapest-L® of LaSota strain.

Preparation of antigen

The antigens of strain F, Asplin, BCRDV used for Hemagglutination (HA) and HI test were collected from the repository of the Department of Microbiology and Hygiene, Bangladesh Agriculture University (BAU), Mymensingh. The virus was grown in 10-day-old embryonated chicken egg via allantoic sac (AS) route and allantoic fluid (AF) was collected used as antigen described by Cottral⁹.

Vaccination of chicks and collection of sera

A total of 75 day-old chicks with the history of vaccination of parent stock against Newcastle disease (ND) were obtained for the experiment. After collection of blood at day 3 from 10 randomly selected birds, the birds were divided into 5 groups such as A, B, C, D and E and each group was reared separately from day 3. Group A, B, C and D were vaccinated with Medivac ND-LaSota®, BCRDV®, Izovac B1 Hitchner® and Cevac vitapest-L® respectively at 5 days of age through intraocular route, and then boosted at 21 days age of birds, while group E was maintained as unvaccinated control. After collection of blood, serum samples were collected on day 3, 15, 17, 19 and 31 from all the groups.

Collection and preparation of chicken red blood cell (cRBC) suspension

Blood samples collected from chicken with anticoagulant were washed with PBS and centrifuged at 500 rpm for 5 mins. Discarding the supernatant, cRBC was collected and prepared a 2% and 0.5% cRBC in PBS for slide and micro HA test and HI test respectively¹⁰. The unused cRBC suspension was stored at 4°C until used.

Slide hemagglutination test

Drops of 2% chicken red blood cells were placed onto a clean glass slide. One drop of the control and test samples were added and mixed by rotating the slide and results were observed and recorded by comparing with the control samples. The red blood cells were agglutinated in positive case¹¹.

Micro-haemagglutination test

It was carried out by two-fold serial dilutions of the viral suspension in a micro well plate to determine the haemagglutination titre of the HA antigen used (4 HA/25ul). For this purpose a 96 well 'V' bottomed micro plate was taken. Then 50 µl of PBS was dispatched in each well of the row A. Antigen (50 µl) was added to the first well, after thorough mixing serial dilution was continued up to the 11 well of the row A and finally discarded 50µl solution from the well 11. Well 12 considered as a control. Fifty microliter of 0.5% cRBC suspension was added into each well of the row A. The plate was allowed to stand for 45 min for reaction among the antigen and RBC at room temperature. An uniform layer of the agglutinated cells covering the bottom of well of the plate was considered as positive HA and in HA negative case, a sharp buttoning of RBC at the bottom of well of plate. The end point of the HA activity was considered to be the highest dilution of the antigen in which positive pattern of agglutination of RBC was present. The HA titre was calculated as the reciprocal of the highest dilution of antigen in which positive pattern of HA was present¹¹.

Hemagglutination Inhibition test

The test is defined as the determination of antibody titer that inhibits the agglutination of RBC by the Newcastle disease virus. HI test in this study was performed to determine the HI of the sera samples collected from the chickens. The HI titer of sera samples of control chickens were determined to measure the maternal antibody and its persistence. The test was conducted by using constant 4 HA unit antigen and decreasing serum method (β procedure) following Anon¹¹. The antigen was diluted in PBS to yield 4 HA units per 0.25 µl of suspension. The sera were heated at 56°C for 30 minutes in hot water bath before using for the test. For performing the HI test, two fold serial dilution (starting from 1:5) of the serum was prepared in a 'V' bottomed microtitre plate. Then 25 µl of antigen suspension containing 4 HA units was added into all well except well number 12 of A, B, C, D, E, F, G and H as marked on the plate and mixed thoroughly. Well number 12 were kept as control. The serum antigen mixture was then incubated for 45 to 60 mins at room temperature. Then 50 µl of 0.6% chicken RBC suspension was added into all well. Then the mixture was again kept at room temperature for 60 mins. A compact mass of sediment cells covering the bottom of the plate was considered as positive for HI. The serum end point was determined as the highest dilution of serum, which inhibited the agglutination of the RBC in the test.

Results

The study was conducted to determine the persistence of maternally derived antibody (MDA) in broiler chicks, as well as, to evaluate the level of antibody production in such birds following vaccination with Medivac ND-LaSota® (LaSota strain), BCRDV® (F strain), Izovac B₁ Hitchner® (B₁ strain) and Cevac vitapest-L® (LaSota strain).

Birds of the four groups namely A, B, C and D were administered with Medivac ND-LaSota, Baby Chick Ranikhet Disease vaccine (BCRDV), Izovac B₁ Hitchner and Cevac vitapest-L respectively and 10 sera samples obtained randomly from each group on 15, 19 and 31 days of age were subjected to HI test. A comparative embodiment is illustrated in Table 1.

It was observed that on day 15, the range of HI titre was almost same (32-128) in case of birds provided with the four vaccines although the Mean±SD of the four sets were different from each other. Thus, in case of Medivac ND-LaSota the Mean±SD was 89.60±33.05 while that of BCRDV was 83.20±30.91, in case of Izovac B₁ Hitchner was 80.00±43.33, and in case of Cevac vitapest-L was 96.00±33.73.

Similar was the picture of range of HI titre (64-128) of sera samples obtained on 19 days of age of birds. However the Mean±SD of individual group of sera was 102.40±33.05, 102.40±33.04,

96.00±33.73 and 115.20±26.98 belonging to birds administered with Medivac ND-LaSota, BCRDV, Izovac B₁ Hitchner and Cevac vitapest-L respectively.

When considered the range of HI titre of the sera samples collected on day 31, it may be observed that the range was (128-256) in each of three vaccines except Cevac vitapest-L where the range was 128-512. The Mean±SD of HI titres were recorded to be 192.00±67.46, 204.80±66.09, 192.00±67.46 and 320.00±173.31 in case of Medivac ND-LaSota, BCRDV, Izovac B₁ Hitchner and Cevac vitapest-L respectively.

An illustrative elucidation of Mean±SD HI titres of vaccinates administered with Medivac ND-LaSota (Group A), BCRDV (group B), Izovac B₁ Hitchner (Group C) and Cevac vitapest-L (Group D), compared with those of prevaccinated and non-vaccinated control birds (Group E) is depicted in Table 2.

It was observed that maternally derived HI antibody during prevaccination stage of all birds of on three days of age of chicks ranged from 32-64 with a Mean±SD of 48.00±16.87.

On day 15, the Mean±SD of HI titres vaccinated with Medivac ND-LaSota, Baby BCRDV, Izovac B₁ Hitchner, Cevac vitapest-L and control group were 89.60±33.05, 83.20±30.91, 80.00±43.33, 96.00±33.73, and 24.00±8.43, respectively. Similar feature was observed at 19 and 31 days when the Mean±SD of HI titres were higher in vaccinated group than control group.

Table 1. Comparative HI titres of sera of four different vaccinated groups of chick

Name of vaccine	15 days age of birds (10 DPV)		19 days age of birds(14 DPV)		31 days age of birds(26 DPV)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
MedivacND-LaSota(Group-A)	64-128	89.60 ± 33.05	64-128	102.40 ± 33.05	128-256	192 ± 67.46
BCRDV(Group-B)	64-128	83.20 ± 30.91	64-128	102.40 ± 33.04	128-256	204.80 ± 66.09
Izovac B ₁ Hitchner (Group-C)	32-128	80 ± 43.33	64-128	96 ± 33.73	128-256	192 ± 67.46
Cevac vitapest-L (Group-D)	64-128	96 ± 33.73	64-128	115.2 ± 26.98	128-512	320 ± 173.31
Unvaccinated/ control(Group-E)	16-32	24 ± 8.43	4-8	5.20 ± 1.93	2-4	3.40 ± 0.97

SD=Standard deviation; HI=Haemagglutination inhibition; DPV=Days of Post Vaccination.

Table 2. Mean of post-vaccination HI titers compared to unvaccinated control group of chick

Age of birds	Day post vaccination (DPV)	Antibody titers (Mean±SD)				
		Group-A (vaccinated with Medivac ND-LaSota)	Group-B (vaccinated with BCRDV)	Group-C (vaccinated with Izovac B ₁ Hitchner)	Group-D (vaccinated with Cevac Vitapest-L)	Group-E (unvaccinated/control)
Day-3	-	-	-	-	-	48±16.87
Day-15	10	89.60±33.05	83.20±30.91	80±43.33	96±33.73	24±8.43
Day-17	12	-	-	-	-	11.20±4.13
Day-19	14	102.40±33.05	102.40±33.04	96±33.73	115.20±26.98	5.20±1.93
Day-31	26	192±67.46	204.80±66.09	192±67.46	320±173.31	3.40±0.97

SD=Standard deviation; - =Not done; ND=Newcastle disease; BCRDV=Baby Chick Ranikhet Disease Vaccine.

Discussion

Vaccination as an effective control measure relies on two aspects. Firstly, vaccination of the parent stock to ensure that the progeny chicks are hatched with a high level of MDA and secondly, vaccination of the chickens with a suitable vaccine at the correct age. The resultant high level of MDA found in the progeny chickens is important in protecting the chickens during 10 to 14 days of age from NDV infection¹².

One of the objectives of the present investigation was to detect the persistence of MDA in experimental chicks. Thus, it was observed (Table 1) that such antibody remains in chicks until 19 days of age following which it declined to minimal. Moreover, in control group E high level of HI antibody titers were found during first two weeks of life which is closely related to the findings of Ibrahim¹³ and Westbury¹⁴. The authors reported that MDA reduces the immune response to V4 vaccination.

It has also been reported that MDA in chicks is retained for 12 days to 15 days¹⁵⁻¹⁶. But after booster vaccination, the antibody levels are increased. Maternally derived antibody or passive immunity is the protective device for the prevention of many diseases in newly hatched chicks. Thus if a breeding hen is vaccinated against ND, some amount of resultant antibody produced and retained in the ova (yolk sac) are transmitted to the chick after hatch. These antibodies in turn protect the chick against ND for the first few days of age. In this regard it was observed that the MDA could be considered as an effective means of protection of the chickens till two weeks of age¹⁵. High level of MDA in day old chicks has been reported by Balla¹⁷ and at day 15 to 20, protection levels declined to minimum. Saeed *et al.*¹⁸ reported that MDA declined to zero at day 25. The immune response is improved in elder chicks as levels of maternal antibody fall and immunological maturity develops¹⁹.

The study was also undertaken to compare the production of antibody by vaccines of four lentogenic strains of Newcastle disease vaccines such as Medivac ND-LaSota (LaSota strain), BCRDV (F strain), Izovac B₁ Hitchner (B₁ strain) and Cevac vitapest-L (LaSota strain).

The results of HI antibody titre revealed that such titers are slightly higher in birds of group D vaccinated with Cevac Vitapest-L than those of group B administered with BCRDV, group C administered with Izovac B₁ Hitchner and also group A having inoculated with Medivac ND-LaSota (Table 1).

A comparative illustration of HI antibody titres of sera samples obtained from birds of group A, B, C and D together with that of persistence of MDA in group E is presented in Table 1 and 2. It may be noted that the range of HI titres of the four vaccinated groups of birds are more or less of similar order when measured on days 15, 19 and 31 of age of birds. However, the Mean \pm SD of sera on these occasions clearly indicate a higher value of Cevac Vitapest-L (LaSota strain) than Medivac ND-LaSota (LaSota strain), BCRDV (F strain), Izovac B₁ Hitchner (B₁ strain).

In this context, the utility of measurement of HI antibodies of sera to qualify the protection capacity of birds from an infection with NDV needs to be mentioned. Lancaster²⁰ observed that serological response of chickens to NDV either from natural infection or vaccination is manifested by the appearance of both HI and VN (virus neutralization) antibodies. It was also stated that HI and VN antibodies through follow a similar course but VN antibody persist longer and in relatively higher titers²¹. Concerned with the role of HI with the challenge infection with NDV, it was observed²². Sera samples of birds possessing HAI titer of 80 or above revealed a level of VNI of 10^{2.48} or above when the birds demonstrated protection against challenge infection with virulent NDV. On the other hand, sera samples possessing HAI titer of 40 or less revealed VNI of 10^{1.3} or less when the birds could not resist challenge infection with NDV.

As regards to the principal objectives of the present investigation it may be stated that production of HI-antibody was higher in birds of group D vaccinated with Cevac Vitapest-L[®] compared to those of group A vaccinated with Medivac ND-LaSota[®], group B vaccinated with BCRDV[®] and group C vaccinated with Izovac B₁ Hitchner[®]. Thus, the Cevac Vitapest-L[®] was found to be superior to some extent than Medivac ND-LaSota[®], BCRDV[®], Izovac B₁ Hitchner[®]. However, as regards vaccination of chicks against NDV in earlier days the use of lentogenic strains are recommended although it should be kept in mind that vaccination with LaSota strains would cause considerably greater problems in young susceptible birds than Hitchner B₁ strain and even through LaSota induces a stronger immune response²³.

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