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Article

Determination of efficacy of different Newcastle disease vaccine used in broiler chickens

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Abstract: The present study was designed to determine the protection afforded by different vaccination programs against the Newcastle disease virus. A serological survey on the prevalence of antibodies to Newcastle disease virus (NDV) was carried out in broilers chicken in Dinajpur districts. For this reason, a total of 75 serum samples were collected form broiler chickens and samples were divided into five groups, each group contain 15 chickens. Group E did not receive any vaccine and served as a negative control group. Groups A-D were vaccinated with different vaccination programs against NDV. From this experimental work the principal objectives of the present investigation it may be stated that production of Hl-antibody was higher in birds of group D vaccinated with CevacVitapest-LR compared to those of group A vaccinated with Medivac NDLaSotaR, group B vaccinated with BCRDV(R) and group C vaccinated with Izovac. B, HitchnerR. Thus, the CevacVitapest-LR was found to be superior to some extent than Medivac ND-LaSota, BCRDVR, Izovac B, Hitchnert. 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 considerately greater problems in young susceptable birds than Hitchner B1 strain and even through LaSota induces a stronger immune response.

Keywords: Newcastle disease; broiler chicken; vaccine; haemagglutination inhibition

1. Introduction

Newcastle disease (ND) is an acute, contagious infection of pet, free living and domestic birds (Alexander, 1997). The virus is distributed all over the world either as naturally circulating virus or as a vaccine virus. It has been established in at least 241 species of birds representing 27 of 50 orders of the class Aves (Alexander, 1995a). 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 chickens (Alexander, 1991). As regards epidemic incidences, NDV causes disease in intensive poultry and is responsible for high economic losses up to 100% mortality (Alexander, 1991; Awan *et al*., 1994).The causative agent, Newcastle disease virus (NDV), is an enveloped RNA virus, belonging to the genus Avulavirus in the family Paramyxoviridae (Mayo, 2002). Moreover, ND is recognised as an enzootic disease in most countries of Africa, Asia and some countries of Europe (Awan *et al*., 1994; Alexander, 1995b and Ballagi-Pordany *et al*., 1996). In case of Bangladesh, ND has been described as endemic with prevalence of viscerotropic velogenic strains (Islam *et al*., 1995). Strains of NDV have been classified into five pathotypes including viscerotropic velogenic, neurotropic velogenic, mesogenic, lentogenic and asymptomatic enteric (Office International des Epizooties, 2008). The asymptomatic enteric form of infection is caused by lentogenic strains (Lancaster, 1981) that results in no clinical signs or pathology and is detectable only by virus isolation from the gut or feces and

by presence of specific antibodies (French *et al*., 1967; Mc Ferran *et al*., 1968). Complete histories of the B, and LaSota strains provided accounts of their interesting discoveries (Hitchner, 1975; Goldhalf, 1980). Hitchner's form (Hitchner and Johnson, 1948) is a mild or inapparent respiratory infection of chickens caused by lentogenic strains. Mortality by this strain is rare in birds of any age. Islam *et al*., 1995 observed one valogenic Okinawa and one mesogenic Meyazaki isolate as rapid eluter from chicken erythrocytes.The disease caused by virulent NDV is a major disease problem in the poultry of Africa and Asia (Awan *et al*. 1994). It affects the nervous, respiratory and gastrointestinal systems (Brown *et al*., 1999; Alexander, 2003). Many of the vaccination programs have been used in commercial chicken flocks to achieve reasonable protection against NDV (Khalifeh *et al*., 2009). Conducted an investigation to determine the correlation between HAI antibody titre by Amin *et al*. (1987) and also Control of ND by vaccination is a routine in commercial chicken flocks in many countries. Inactivated vaccines have been used for inducing mainly systemic immunity (Rauwa *et al*., 2009). 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 adjuvant (Biggs *et al*., 1988; Alexander, 1991). 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 may induce cell-mediated immunity, humoral immunity, local immunity and passive immunity (Alexander, 1991 and 1997). Serological testing for antibody to NDV has primarily utilised either the hemagglutination inhibition (HI) test or virus neutralisation test. The HI has been used as the standard test (Adair *et al*., 1989; Allan *et al*., 1974; Brown *et al*., 1990; Alexander, 199l). 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 are suspicious of prophylactive nature of the agent. A number of relevant questions are faced by scientists and field veterinarians as to the immunogenicity, retention of virus titre, stability and such other qualities of vaccine. In order to address one such query, the present study was undertaken with to determine the maternally derived antibody and the antibody titre in broiler chicks following different vaccination. Also evaluate the comparative antibody production of different vaccines used in this experiment.

2. Meterials and Methods

2.1. The study area and samples collection

The study was carried out in Dinajpur district from January 2011 to June, 2011. A total of 75 blood samples from different (A-E) group vaccinated with different vaccine and non-vaccinated were collected from commercial broiler farms, f or Haemagglutination Inhibition (HI) test.

2.2. Propagation of virus in chicken embryo inoculation

Lyophilized virus suspension used as antigen in HA/HI test was thawed and treated with antibiotic (Gentamycin 1.0 mg/ml) of which 0.1 ml was inoculated into each of five l0-day-old embryonated chicken eggs through air sac route. The eggs were then incubated at 37° C and observed twice daily for five days. The embryos that died within 24 hours of inoculation were discarded and those dying within and remained alive for 4 days were chilled at 4°-8° C for l-2 hours (Cottral, 1978).

2.3. Harvesting and storage of allantoic fluid as antigen

Eggs were chilled at 4°C for two hours to kill the embryo and to reduce the contamination of allantoic fluid (AF) with blood and other extraneous material during harvesting. Each egg was swabbed with cotton soaked with 70% alcohol to disinfect and remove any dust from the shells. The sterilized forceps and scissors were dipped in absolute alcohol and flamed immediately before use. The egg shell above the air space was removed and AF was collected aseptically with sterile.

2.4. Collection and processing of blood for HI test

Collection of blood was done from the wing vein with the sterile syringe and needle and placed in slanting position for 1 hr at room temperature. Then the clot was detached from the wall of the syringe carefully, allowed it to settle down and afterward serum was collected. Serum collected so was centrifiged at 1500 rpm for 15 minute to obtain clear serum and then stored at -20°C temperature until used.

2.5. Collection and preparation of 0.5 and 2% chicken red blood cell

Chicken blood was collected from by heart puncture directly with sterile syringe and needle containing anticoagulant (Alsever's solution) at the rate of 5 ml for 5 ml blood. Following collection, blood sample was washed with PBS and cenfrifuged at the rate of 500 rpm for 5 minutes. The supernatant was discarded and cRBC was collected and then 2% and 0.5% cRBC were prepared in PBS for slide and micro HA tests and HI test respectively. The unused cRBC suspension was stored at VC until used.

2.6. Haemagglutination test (HA) procedure

Presence of NDV antibody was detected by hemagglutination inhibition test as described by OIE (2000). A cut off titer of 1:4 was considered specific indicating that the birds had been previously exposed to the virus, while titers less that these values were considered nonspecific (Numan *et al*., 2005).

2.6.1. Slide haemagglutination 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 case.

2.6.2. Micro-haemagglutination test in a V bottom micro well plate to determine HA units (4 HA/25 µl)

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. 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. 50 µ1 of antigen 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 50 µ1 solution from the well ll. Well 12 was taken as control. 50 µl of 0.5% cRBC suspension was added in to the each well of the row A. The plate was allowed to stand for 45 minutes 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 titration was determined as the highest dilution giving completes HA (No streaming); this represented 1 HA unit (HAU) and was calculated accurately from the initial range of dilutions (OIE, 2004).

2.7. Haemagglutination inhibition test (HI) procedure

HI test was performed to determine the HI titre of the sera samples collected from the chickens. The HI titre 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 antigens and decreasing serum method procedure following Anon (1971). The Haemagglutination titre of HA antigen was first determined by HA test. The antigen was then 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.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 minutes at room temperature. Then 50 µl of 0.6% chicken R13C suspension was added into all well. Then the mixture was again kept at room temperature for 60 minutes. 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.

3. Results

The study was conducted to determine the persistence of antibody (MDA) in broiler chicks, as well as, to evaluate the level of antibody production in such birds following vaccination with Medivac ND-LaSotaR (LaSota strain), BCRDVO (F strain), Izovac B I HitchnerR (B I strain) and Cevacvitapest-LR (LaSota strain). Prior to vaccination, blood samples were collected to measure MDA on day three of age of broiler chicks which were then divided into five groups as required. Four groups of birds were vaccinated primarily at day five of age of birds and also at 21 days, while one group was maintained as unvaccinated control. Antibody produced in chicks following administration of these vaccines through intraocular route (eye drop), as well as, MDA persisted until day 17 in unvaccinated control birds. Post-vaccination sera along with that from unvaccinated

control obtained from randomly selected 10 birds of each group on 15, 19 and 31 days of age were subjected to HI test.

3.1. Determination of HI-antibody titres following vaccination with Medivac ND-LaSotaR, BCRDVR, Izovac B1 Hitchner and Cevacvitapest-LR

For this purpose, 75 day old broiler chicks were equally divided into five groups namely A, B, C, D snd E. Group A, B, C and D were vaccinated with Medivac NDLaSotaR, BCRDVOP, Izovac B I HitchnerR and Cevacvitapest-LO respectively at five days of age through intraocular route, and then boostered at 2l days age of birds, while Group E was maintained as unvaccinated control. Sera samples were collected on day 3, 15, 17, 19 and 31 from all the groups.

3.2. HI titres of serum of birds of Group A vaccinated with Medivac ND-LaSotagR

Birds of this group were vaccinated with Medivac ND-LaSota at five days of age and then at 21 days of age. Sera samples were procured from l0 randomly selected birds on each occasion of day 15(10 DPV), 19(14 DPV) and 31(26 DPV) days of age of birds. The schedule thus planned provided opportunities to look for antibody engendered in birds on two occasions each after single vaccination (day 15,19) and then having boostered (day 31) afterwards, thus, at l0 days (day 15 of age of birds) post administration of vaccine (DPV), sera samples from randomly selected birds revealed HI titres varying between 64-128 with a Mean±SD of 89.60±33.05. Thereafter, on day 19 of age of chicks (14 days post vaccination) the range of HI titre of sera samples was 64-128 having a Mean±SD of 102.40±33.05. On the other hand, sera obtained on, 31 days of age of chicks (10 DPV), the range of HI titre 128-256 with a Mean±SD of 192.00167 .46.

3.3. HI titres of serum of birds of Group B vaccinated with BCRDV@ (F-strain)

Birds of this group were vaccinated with BCRDV at five days of age and then at 21 days of age. Sera samples were obtained from 10 randomly selected birds on each occasion of day 15, 19 and 31 days of age. Thus, 10 days (day 15 of age of birds) after administration of vaccine, sera samples from randomly selected birds revealed HI titres varying, between 64-128 with a Mean±SD of 83.20±30.91. Thereafter, on day 19 of age of chicks (14 days post vaccination) the range of HI titre was 64-128 having a Mean± SD of 102.40 ± 33 .04 .On 31 days of age of chicks, the range of HI titre 128-256 with a Mean± SD of 204.80±66.09.

3.4. HI titres of prevaccinated serum and unvaccinated/control (Group E) birds

The HI antibody titres withMean± SD of pre-vaccinated birds and those of group E (unvaccinated/control). It was noticed that maternally derived III antibody during pre-vaccination stage of all the birds on day three of age of chicks ranged from 32-64 with a Mean±SD of 48.00 ± 16.87. Sera samples from unvaccinated control group E were obtained on day 15, 17, 19 and 31 of age of birds. It was observed that on day 15, maternal antibody (Mab) titre was within the range of 16-32 with aMean±SD of 24.00±8.43. Thus, it appeared that matenal (Passive) antibody almost declined to minimal on day 15 of age of birds. Afterwards the Mean±SD of such antibody were 11.20±4.13, 5.20±1.93 and 3 .40±0.97 on day 17, 19 and 31 of age of birds respectively.

3.5. Comparative HI titres of serum of birds vaccinated with different vaccines

Birds of the four groups such as A, B, C and D were administered with Medivac ND-LaSota, Baby Chick Ranikhet Disease vaccine (BCRDV), Izovac B1 Hitchner and Cevacvitapest-L respectively and 10 sera samples obtained randomly from each group on 15, 19 and 3l days of age were subjected to HI test. 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 sits 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±3091, in case of Izovac B, Hitchner was 80.00±43.33, and in case of Cevacvitapest-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 B, Hitchner and Cevacvitapest-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 Cevacvitapest-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 Cevacvitapest-L respectively as shown in Table 1.

Table 1. Comparative HI titres of serum of birds vaccinated with different vaccines.

Age of birds	Day post vaccination	Antibody titres (Mean±SD)				
		Group-A (vaccinated with ND-LaSota)	Group-B (vaccinated with BCRDV)	Group-C (vaccinated with fzovac BlHitchner)	Group-D (vaccinated with Cevac Vitapest-L)	Group-E, unvaccinatod/ control)
Day-3	-	-	-	-	-	48±16.87
Day-15	10	89.60±33.05	83 ±43.33	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±6746	204.80±66.90	192±67.46	320±173.31	3.40±0.97

Legends: Not done; SD=standard deviation; ND=Nexvcastle disease; BCRDV=Baby Chick Ranik-het Disease Vaccine.(3.5 under hoba)

3.6. Mean of post vaccination HI titres of serum compared to unvaccinated control

An illustrative elucidation of Mean±SD HI titres, of vaccinates administered with Medivac ND-LaSota (Group A), BCRDV (group B), Izovac B, Hitchner (Group C) and Cevacvitapest-L (Group D). Compared with those of prevaccinated and non-vaccinated control birds (Group E). 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 Medivae ND-LaSota, Baby BCRDV, Izovac B, Hitc liner, Cevacvitapest-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 as shown in Table 2.

Table 2. Mean of post vaccination HI titres of serum compared to unvaccinated control.

Serum sample No	3 days aged birds (Prevaccination)			15 days aged birds (Unvaccinated/Control)			17 days aged birds (Unvaccinated Control)			19 days aged birds (Unvaccinated/ Control)			32 days aged birds (Unvaccinated/ Control)		
	HI titre	Range	Mean ± SD	HI titre	Range	Mean ± SD	HI titre	Range	Mean ± SD	HI titre	Range	Mean ± SD	HI titre	Range	Mean ± SD
1	64	32-64	48.00	32	16-32	24.00	8	8-16	11.20	4	4-8	5.20 ±	4	2-4	3.40 ±
2	32		±	32		± 8.43	8		± 4.13	4		1.93	4		0.97
3	32		16.87	16			16			4			4		
4	32			32			8			4			4		
5	64			16			16			8			2		
6	64			32			16			8			4		
7	64			16			8			4			4		
8	32			16			8			8			2		
9	64			16			8			4			4		
10	32			32			16			4			2		

Legends: SD = Standard deviation, HI=Haemagglutinattion inhibition.

4. Discussion

A comparative illustration of 1-11 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. 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±SD of sera on these occasions clearly indicate a higher value of CevacVitapest-L (LaSota strain) than Medivac ND-LaSota (LaSota strain), BCRDV (F strain), Izovac B1, Hitctrner (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 mentionedm. Lancaster (1966) 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 started that HI and VN antibodies through follow a similar course but VN antibody persist longer and in relatively higher titres (Haplin, 1978). Concerned with the role of HI with the challenge infection with NDV, it was observed (Amin *et al*., 1987). Sera samples of birds possessing HAI titre of 80 or above revealed a level of VNI of 102.48 or above when the birds

demonstrated protection against challenge infection with virulent NDV. On the other hand, sera samples possessing HAI titre of 40 or less revealed VNI of 101.3 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 CevacVitapest-LR compared to those of goup A vaccinated with Medivac NDLaSotaR, group B vaccinated with BCRDVR and group C vaccinated with Izovac. B, HitchnerR. Thus, the CevacVitapest-LR was found to be superior to some extent than Medivac ND-LaSota, BCRDVR, Izovac B, Hitchnert. 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 considerately greater problems in young susceptable birds than Hitchner B1 strain and even through LaSota induces a stronger immune response (OIE Manual, 4h Edition,2000). In view of the above discussion it is necessary that the following aspect of vaccination with BCRDV, B, strain and LaSota strain may be performed in future. Inspite of vigorous vaccination schedules, ND is still a havoc to the poultry industry and a number of outbreaks have been recorded even in vaccinated chicken flocks (Siddique *et al*., 1986). Other factors like poor vaccine quality is a common problem in developing countries and can be the result of poor manufacturing standards, lack of adequate storage facilities, application of expired vaccine batches, faulty application and vaccine handling during transportation (Vui *et al*., 2002). Heat stress and water deprivation also lead to production of steroids and thus resultantly immunosuppression (Sil *et al*., 2002).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 adjuvant. 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.

5. Conclusions

From the study it may be concluded that Persistence of MDA retained until the age of 17 days of broiler chicks. The three commercial vaccines, containing LaSota strain, were found to elucidate slightly higher HI antibody response compared to those containing B 1 and BCRDV (F strain) of NDV respectively .The level of protection of commercial broilers was satisfactory by hyper-immunizing the hens before laying and by adopting good managemental conditions.

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Conflict of interest

None to declare.

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