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Article

Comparative efficacy of LaSota, B1 and Mukteswar Strain vaccines for Newcastle Disease Virus (NDV) in layer chickens

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Abstract: The control of Newcastle Disease (ND) relies on the use of safe and effective vaccines. Live vaccines which are prepared with lentogenic strains of Newcastle Disease Virus (NDV) are now more frequently used in broilers and layers 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 relative low cost. The vaccines are easy to administer on large scale, and rapidly stimulate cell-mediated and mucosal surface immunity. The present study was designed to compare the efficacy of LaSota, B1 and Mukteswar Strain vaccines for NDV in layer chickens. Findings of our present study indicated that production of HI-antibody titre was higher in birds of group A (512) vaccinated with ND LaSota, compared to those of group B (256) vaccinated with RDV (Mukteswar strain); group C (128) vaccinated with ND B1 (B1 strain) Hitchner at six weeks after vaccination. Thus, the ND LaSota vaccine was found to be superior to some extent than ND 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 greater problems in young susceptible birds than Hitchner B1 strain and even though LaSota induces stronger immune response.

Keywords: Newcastle Disease Virus; LaSota; B1; Mukteswar strain vaccine

1. Introduction

Newcastle disease (ND) is an acute contagious disease of pet, free living and domestic birds. The causative agent Newcastle disease virus (NDV) is a negative-sense, single-stranded RNA virus which belongs to the genus *Avulavirus* and falls in the family *Paramyxoviridae* (Alexander, 1997). The virus is distributed worldwide either as naturally circulating virus or as a vaccine virus. It has been constant in at least 241 species of birds representing 27 of 50 orders of the class Aves (Alexander, 1995b). ND is widely adaptable in type and severity of the disease it produces. It is complicated because various 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). A variety of NDV isolates and strains have been listed around the world (Ballagi-Pordany *et al.*, 1996). As regards epidemic (epizootic) incidences, NDV causes disease in intensive poultry and is responsible for high economic losses up to 100% mortality (Alexander, 1991; Awan *et al.*, 1994). Moreover, ND is recognized as an enzootic

disease in most countries of Africa, Asia and some countries of Europe (Awan *et al.,* 1994; Ballagi-pordany *et al.,* 1995; 1996). In case of Bangladesh, ND has been delineated as endemic with prevalence of Viscerotropic velogenic strains (Chowdhury *et al.,* 1982; Islam *et al.,* 2003). ND may appear pear 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 B_1, F and LaSota which have been widely used as vaccine. The least pathogenic (Lentogenic) strains B_1 (Hitchner *et al.,* 1948), LaSota (Winterfield *et al.,* 1957), and F (Asplin, 1952) are used in birds of all ages by intra-nasal or intra-ocular routes, admixture with drinking water, or spraying. The asymptomatic enteric form of infection is caused by lentogenic strains (Lancaster, 1981) that produced no clinical signs or pathology and is detected only by virus isolation from the gut or feces and by presence of specific antibodies (French *et al.,* 1964; Simmons, 1967**;** Mc Ferran *et al.,* 1988). Complete histories of the B, and LaSota strains supplied 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. The control of ND depends on the use of safe and effective vaccines. Live vaccines prepared with lentogenic strains of NDV are now more widely used in broilers than vaccines prepared from chemically inactivated strains of NDV, mixed with adjuvant (Biggs *et al.,* 1988; Alexander, 1991). This is due to 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 immunity (Alexander, 1991; 1997). Humoral immunity can be detected and measured by several serological tests **(**Tizzard, 1982**;** Alexander, 1991). Serological testing for antibody to NDV has primarily utilized either the hemagglutination inhibition (HI) test or virus neutralization test (VNT). The HI has been used as the standard test. Recently, enzyme-linked-immunosorbent assay (ELISA) has replaced the HI test (Adair *et al.,* 1989; Brown *et al.,* 1990; Alexander, 1991). In Bangladesh, different live vaccines containing lentogenic strains of NDV are imported, but efficacy of these vaccines in relation to climatic condition, distribution and transportation are not studied properly and thoroughly. Sometimes, the farmers are suspicious of prophylactic nature of the agent. A number of relevant questions are faced by the scientists and field veterinarians as to the immunogenicity, retention of virus titer, stability and such other qualities of vaccine. Therefore, the present work has been undertaken to determine the antibody titer in chicken following vaccination with Medivac ND La Sota® (La Sota strain), RDV® (Mukteswar strain), and Izovac B1 Hitchner® (B_l strain) and to evaluate the comparative antibody production of the vaccines used in this study.

2. Materials and Methods

2.1. Study area

The study was carried out in the experimental animal sheds of experimental farms in Rangpur district of Bangladesh under the supervision of Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh.

2.2. Experimental chickens

A total number of 3000 day-old-chicks of ISA Brown breed with the history of vaccination of parent stock against Newcastle disease (ND) were collected from the Phenix Poultry Ltd., Dhaka and carried to the experimental farm located in Baharkachna, Rangpur, under the supervision of Department of Microbiology, HSTU, Dinajpur. The birds were fed with Quality poultry feed and water and litter maintaining strict biosecurity and other pre-requisites required for rearing such birds.

2.3. Study design

There was total 3000 experimental layer chicken. These birds were divided into three experimental groups (group-A, group-B and group-C). Group-A, group-B and group-C was vaccinated with ND Lasota, RDV and ND B1 respectively. In all groups, first vaccination was performed at day 5, 2^{nd} and 3^{rd} doses were given at day 21, and 56 respectively. First blood collection of all groups of chickens were performed at 10 weeks for HI titre whereas 2^{nd}, 3^{rd} , 4^{th}, 5^{th} and 6^{th} collections were done at 12, 14, 16, 18 and 20 weeks of age respectively.

2.4. Propagation of virus in chicken embryo inoculation

Lyophilized virus suspension used as antigen in HA/HI test was thawed and treated with antibiotic (Gentamicin 1.0 mg/ml) of which 0.1 ml was inoculated into each of five 10-day-old embryonated chicken eggs through air sac (AS) 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 remained alive for 4 days were chilled at 4^{o}-8^{o} C for 1-2 hours (Cottral, 1978).

2.5. 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 into absolute alcohol and flamed immediately before use. The eggshell above the air space was removed and AF was collected aseptically.

2.6. Collection and processing of blood for HI test

Collection of blood was done from the right jugular 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. Collected serum was centrifuged at 1500 at rpm for 15 minute to obtain clear serum and then stored at -20°C temperature until use.

2.7. Collection and preparation of 0.5 and 2% chicken red blood cell (cRBC) suspension

Chicken blood was collected either from the jugular vein or from wing vein 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 centrifuged at the rate of 500 rpm for 5 minutes. The supernatant was discarded and cRBC was collected and then 2% and 0.5% cRBC suspensions were prepared in PBS for slide and micro-HA tests and HI test respectively. The unused cRBC suspension was stored at 4°C until used (Khan, 1992).

2.8. Haemagglutination Inhibition (HI) test procedure

The procedure of HI test was followed as per method described by Anon. (1971) and divided into two parts: microplate HA test to determine HA units (4 Units/25µl) and microplate HI test.

3. Results

The present study was conducted to compare the efficacy of following vaccination with ND-LaSota® (Lasota strain), RDV (Mukteswar strain), and Izovac B1 Hitchner® (B1 strain). After vaccination, sample was collected randomly from 16 birds from 1-unit of 1000 birds. HI titres of serum of birds of Group A vaccinated with ND--LaSota® vaccine are shown in Table 1. Birds of this group were vaccinated with ND-LaSota at 5 days of age and then booster doses were given at 21 and 56 days respectively. First blood collection for HI titer was performed at 10 weeks then at 12, 14, 16, 18 and 20 weeks of age. HI titres of serum of birds of Group B vaccinated with RDV are shown in Table 2. Birds of this group were vaccinated with RDV (Mukteswar) at 5 days of age and then at day 21 and 56 respectively. HI titres of serum of birds of Group C vaccinated with ND B1 (B1 strain) vaccine are shown in Table 3. Birds of this group were vaccinated with ND B1 (B1 strain) at five days of age and then at 21 and 56 days of age.

Comparative HI titres of serum of birds vaccinated with different vaccines are shown in Table 4.

Table 1. HI antibody titres of serum of chickens vaccinated with ND-LaSota® vaccine (Group A).

Sl. No.	Age at wks.	DPV	Titer range	Average titer ± SD
1	10	2 wks.	64-256	128±38.74
2	12	4 wks.	128-256	256±22.61
3	14	6 wks.	256-1024	512±55.34
4	16	8 wks.	256-512	256±42.44
5	18	10 wks.	128-512	256±35.58
6	20	12 wks.	128-256	128±30.21
P value				0.0002**

Legends: ** Means significant at ($p<0.01$) 1% level; P means Probability, DPV=Days post vaccination, SD=Standard deviation

Table 2. HI antibody titres of chickens vaccinated with RDV (Group B).

Sl. No.	Age at wks.	DPV	Titer range	Average titer ± SD
1	10	2 wks.	64-128	64±16.64
2	12	4 wks.	128-256	128±22.18
3	14	6 wks.	128-256	256±22.78
4	16	8 wks.	128-256	128±28.47
5	18	10 wks.	64-128	64±25.47
6	20	12 wks.	32-64	64±8.42
P value				0.0251**

** Means significant at ($p<0.01$) 1% level; P means Probability

Table 3. HI antibody titres of chickens vaccinated with ND B1 (B1 strain) vaccine (Group C).

Sl. No.	Age at wks.	DPV	Titer range	Average titer ± SD
1	10	2 wks.	64-128	128±12.44
2	12	4 wks.	128-256	256±17.87
3	14	6 wks.	128-256	128±12.67
4	16	8 wks.	64-128	128±20.52
5	18	10 wks.	64-128	64±15.42
6	20	12 wks.	32-128	64±18.28
P value				0.0028**

** Means significant at ($p<0.01$) 1% level; P means Probability

Table 4. Comparative HI titres of serum of chickens vaccinated with different ND vaccines.

Sl. No.	Name of unit	Weeks post vaccination (WPV)					
		2 wks.	4 wks.	6 wks.	8 wks.	10 wks.	12 wks.
1	Group A	128	256	512	256	256	128
2	Group B	64	128	256	128	64	64
3	Group C	128	256	128	128	64	64

4. Discussion

It may be noted that the range of HI titres of the three vaccinated groups of birds are more or less of similar order when measured on 10, 12, 14, 16, 18 and 20 weeks age of birds. However, the Mean±SD of sera on these occasions clearly indicate a higher value of ND-LaSota (LaSota strain) than RDV (Mukteswar strain), and Izovac B_1 Hitchner (B_1 strain). In this context, the utility of measurement of HI antibodies of sera to detect the protection capacity of birds from an infection with NDV needs to be mentioned. Lancaster (1966) observed that serological response of chickens to NDV either from natural infection or vaccination is revealed by the appearance of both HI and VN (virus neutralization) antibodies. It was also stated that HI and VN antibodies though follow a similar course, but VN antibody persist longer and in relatively higher titres (Hossain, 1950; Hossain *et al.,* 1972; Haplin, 1978). It should further be mentioned that HI test provides a measurement of the ability of serum from an exposed bird to inhibit agglutination of chicken RBC by NDV, whereas VN or SN indicates the ability of serum to neutralize this infective property of NDV and therefore, provides more precise information about the immune response (Hanson, 1964). 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 study, it may be stated that production of HI-antibody was higher in birds of group A vaccinated with ND LaSota® compared to those of group B vaccinated with RDV (Mukteswar) and group C vaccinated with Izovac B1 Hitchner®. Thus, the ND-LaSota was found to be superior to some extent than Medivac, RDV®, Izovac B1 Hitchner®. However, as regards vaccination of chicks against NDV in earlier days the use of lentogenic strains is recommended although it should be kept in mind that vaccination with LaSota strains would create considerately greater problems in young susceptible birds than Hitchner B_1 strain and even though LaSota produces a stronger immune response (OIE Manual, 4th Edition, 2000). In spite 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. In view of the above discussion, it is essential that the following aspect of vaccination with RDV, B1 strain and LaSota strain may be performed in future.

a) A comparative study on the schedule of vaccination with RDV, B_1 strain and LaSota.
b) Assessment of genetic reversion of F, B, and LaSota strain after using vaccine.
c) Determination of efficacy of RDV, B_1, LaSota and such other strains for mass vaccination on food-based method and
d) Influence of maternal antibody in chicks on the efficacy of Mukteswar, B_1 and LaSota strains of vaccine.

5. Conclusions

In this study, comparative efficacy of different Newcastle disease virus vaccines namely ND-Lasota® (Lasota strain), RDV® (Mukteswar strain) and Izovac B1 Hitchner® (B1 strain) vaccines regarding the production of HI-antibody was accomplished in chickens. The results obtained in this study indicate that the HI antibody titres of chickens of group-A vaccinated with ND-LaSota fluctuated between 64-1024. In Group-B administered with RDV®, the HI titres varied between 64-256. When considered the HI antibody titres of group C inoculated with B1 Hitchner®, HI titre shown 64-256. Thus, it was found that birds of group A, B and C vaccinated with ND-Lasota®, RDV and commercial Izovac B_1 Hitchner®, Group A induced higher level of antibody titers than that of other two groups (Group B and C). From our current study, it may be concluded that among three commercial vaccines used in this work, vaccine containing Lasota strain, was found to elucidate slightly higher HI antibody response compared to those containing B1 and RDV (Mukteswar strain) of NDV respectively.

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Conflict of interest

None to declare.

Authors' contribution

Md. Tazul Islam Sarker, Md. Mostafizer Rahman and Md. Fakhruzzaman designed the study. Md. Tazul Islam Sarker did the actual research work. Md. Fakhruzzaman and Md. Nurnoby Islam drafted the original manuscript, critically reviewed and edited the manuscript. All authors have read and approved the final manuscript.

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