Bangladesh Veterinary Journal (2018) 52(1-4): 7-14
pISSN 0378-8113 •eISSN 2708-6054
https://doi.org/10.32856/BVJ-2018.02

Research Article

OPEN ACCESS

Different Regimes for Challenge Instigation against Newcastle Disease Virus in Layer Birds

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Abstract

Newcastle disease is a highly contagious disease of poultry and causes huge economic loss in Bangladesh. In the study, the efficacy of different vaccination programs against Newcastle disease virus was performed to examine duration and level of antibody in two layer farms with different diluents. The experiment was carried out in Dinajpur district and serological test was performed in the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during the period of July, 2017 to December, 2017. A total of 200 blood samples were randomly collected from different age groups of layer birds which were vaccinated with commercially available two live and one killed vaccine namely Avi ND LaSota, CEVAC NEW L and ITA-New (ND). Pre-vaccination serum antibody titers (Mean ± SD) of group A birds were 5.97±0.75. Firstly, birds were vaccinated with Avi ND LASOTA® vaccine, and CEVAC® NEW L vaccine. MASTERBLUE powder and ADVANCE NON-FAT were used as diluents in vaccination. After 40 days of post-vaccination, sera samples were procured and showed serum antibody titer (Mean ± SD) 7.0±0.93. In case of farm-B, before vaccination antibody titers levels against NDV were Mean ± SD 5.85±0.75. Birds of farm-B were vaccinated with Avi ND LaSota® vaccine. After 40 days of post-vaccination, titer was Mean ± SD 6.7±0.93. Then birds were vaccinated with ITA-New (ND)® which were killed vaccine. After 40 days of post-vaccination, the titer was Mean ± SD 8.15±0.93. MASTERBLUE powder and ADVANCE NON-FAT are suitable diluents to be used in vaccination against Newcastle disease because tap water may inactivate the vaccine due to its impurity in addition to containing higher level of chlorine and even the type of pipes or vessels used to distribute the drinking water. The results showed that the level of protection of vaccinated birds was satisfactory.

Keywords: Newcastle Disease, Vaccine, Diluents, Haemagglutination Inhibition

INTRODUCTION

Newcastle disease (ND) was first recognized in 1926 and continues to be a problem for poultry producers. At least four defined panzootics have been recognized (Miller *et al.*, 2013), which negatively affect not only economic livelihoods, but also human welfare by decreasing food supplies (Alders, 2014). From 2006 to 2009, the most widespread animal diseases in terms of the number of countries affected were rabies, Newcastle disease and Bovine tuberculosis. ND ranked as the fourth most important disease in terms of the number of livestock units lost for poultry species, behind highly pathogenic avian influenza, infectious bronchitis, and lowly pathogenic avian influenza (World Bank, 2011). The disease is caused by only the virulent strains of avian paramyxovirus serotype-1 (AMPV-1) and APMV-1, and it is synonymous with Newcastle disease virus (NDV) (OIE, 2012). Strains are defined as virulent if they have three or more basic amino acids at position 113–116 of the un-cleaved fusion protein cleavage site (F0) with a phenylalanine at position 117 or obtain an intracerebral pathogenicity index (ICPI) value of P 0.7 in day-old chickens (*Gallus gallus*) (OIE, 2012). NDV is known to infect over 236 species of birds (Kaleta und Baldauf, 1988) and besides poultry species virulent NDV (vNDV) strains are commonly found in pigeons and double crested cormorants (Diel *et al.*, 2012) and occasionally in some other wild bird species. All over the world, poultry industry is facing severe economic losses with every passing year. In several developing countries, ND is endemic and has the greatest impact on villages where people's livelihood depends upon poultry farming (Rezaeianzadeh *et al.*, 2011). ND is fatal and still top ranked poultry disease. Annual losses caused by this dis-

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ease worldwide are in millions of dollars. ND is an economically important disease and a major threat to poultry industry (Narayanan *et al.*, 2010). ND causes huge economic losses to the commercial poultry farmers around the world. Livestock and poultry are an important sector in the economy of Bangladesh.

Commercial poultry farming has been growing during the last two decades and has now become one of the most important agri-business in Bangladesh. The poultry in Bangladesh includes mainly chickens, ducks and pigeons, which are kept in different production systems. Newcastle disease, popularly known as Ranikhet disease, is one of the important diseases affecting the commercial as well as backyard poultry worldwide causing significant economic losses. It affects almost all species of birds causing a highly contagious and rapidly spreading disease with high morbidity and mortality and severe drop in egg production. ND is endemic in Bangladesh claiming significant mortality (Talha *et al.*, 2001; Kafi *et al.*, 2003; Barman *et al.*, 2010). At present there are about 268.43 million chickens and 52.29 million ducks in our country (DLS, 2016) and the investment in this sector is increasing day by day. The development of poultry sector is seriously hampered by some infectious and non-infectious diseases. Although numerous live and inactivated vaccines have been developed to control the disease, the incidences of ND outbreaks in commercial poultry have gradually increased since the 1990s (Alexander, 1999). 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 attenuated vaccines prepared from lentogenic strains such as Hitchner B1, Lasota, Clone 30 and VG/GA are widely used because they provide high efficacy of protection through the induction of both systemic and local immunity (Sil *et al.*, 2002). Many of the vaccination programs have been used in commercial chicken flocks to achieve reasonable protection against NDV.

For confirmation of ND, the OIE prescribes isolation of NDV in embryonated chicken eggs and their identification using haemagglutination (HA) and hemagglutination inhibition (HI) test with an NDV-monospecific antiserum (OIE, 2009). Reverse transcription-polymerase chain reaction (RT-PCR) has been established to identify NDV (Gohm *et al.*, 2000; Hu *et al.*, 2010). The efficacy of vaccinations can be estimated best with challenge experiments but they are expensive and time consuming (Czifra *et al.*, 1998). Therefore, serological tests are frequently used to assess protective response; Haemagglutination inhibition (HI) test is the method of choice. It detects antibodies against the haemagglutinating epitopes of the avian paramyxo-virus 1 (PMV-1) (Czifra *et al.*, 1998). 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 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. The present study was conducted to evaluate the serum antibody titers level of layer birds against different Newcastle disease vaccines and effect of diluents in vaccination against Newcastle disease virus after vaccination of layer with lasota strains of Newcastle disease vaccine and killed vaccine in layer farms.

MATERIALS AND METHODS

Two-layer farms were selected for the experiment. The whole experiment was conducted during the period from July- 2017 to December- 2017 in the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. A total number of 200 blood samples of which 140 samples from Shamim poultry farm and 60 samples from Israfil poultry farm (pre-vaccinated and post vaccinated) were collected from wing veins. The chickens were divided into three groups based on ages of birds. Group A and B were containing 70 birds each with their age 8 months and 14 months respectively. Group C was selected with 60 birds with their age 8 months. At the beginning of the study, blood samples were collected from pre-vaccinated birds of both farms and tested to evaluate the serum antibody titer against Newcastle disease by Haemagglutination Inhibition test. The second step of experiment included vaccinated birds with different live vaccines with diluents. After 40 days, blood samples were collected and HI test performed. Nobilis ND Lasota (Newcastle disease vaccine Lasota strains with EID50) antigen was used for HI test.

Vaccines used in this study

Three Newcastle diseased vaccines were used in this study (2 live vaccines and 1 killed vaccine) and these vaccines were purchased from reputed pharmaceutical company of Bangladesh namely, i) AVI ND LaSota® (Laprovet Ltd), ii) CEVAC NEW L (ACI Ltd) and iii) ITA-New (ND) (Laprovet Ltd). These vaccines were kept at 2 – 8°C until used. Two types of diluents were used in this study. They were, 1) MASTERBLUE-stabilizer (NFA Veterinary Pharma. Co. Ltd) and 2) ADVANCE NON-FAT (Advanced Nutrition Ltd).

Experimental layout

The entire study was divided into two major steps. Firstly, detection of serum antibody titer of birds (pre and post vaccination) and secondly, detection of the effects of diluents. A total number of 200 blood samples (wing vein of bird) were collected from different age layer birds with disposable syringes. In case of Shamim layer farm (farm-A), blood samples were collected randomly from at two episodes. Firstly, blood samples were collected from pre-vaccinated birds of Group-A and Group-B. Then birds were vaccinated with Avi ND LaSota® and CEVAC NEW L. As diluents MASTERBLUE powder and ADVANCE NON-FAT were

used. Secondly, blood was collected 40 days of post vaccination. A total number of 120 serum samples were collected and tested to evaluate serum antibody titer level against ND by HI test.

In case of farm-A, experimental birds were divided into two groups namely Group-A (8 months) and Group-B (14 months) according to age. Birds of Group-A and Group-B were vaccinated with Avi ND LaSota®, and CEVAC® NEW L. MASTERBLUE® (stabilizer) and ADVANCE NON-FAT (skim milk) were used as diluents in vaccination. Birds of both Group-A and Group-B were divided into six subgroups, and Table 1 presents grouping of birds.

Table 1: Groups and Sub-groups of birds of Farm A used in this study

Groups	Subgroups	Vaccines + Diluents used
Group-A (8 months of age; n=70)	A1	Avi ND LaSota®+MASTERBLUE® (stabilizer)
	A2	Avi ND LaSota®+ADVANCE NON-FAT
	A3	Avi ND LaSota®+Tap Water
	A4	CEVAC® NEW L+MASTERBLUE® (stabilizer)
	A5	CEVAC® NEW L+ADVANCE NON-FAT
	A6	CEVAC® NEW L+Tap Water
Group-B (14 months of age; n=70)	B1	Avi ND LaSota®+MASTERBLUE® (stabilizer)
	B2	Avi ND LaSota®+ADVANCE NON-FAT
	B3	Avi ND LaSota®+Tap Water
	B4	CEVAC® NEW L+MASTERBLUE® (stabilizer)
	B5	CEVAC® NEW L+ADVANCE NON-FAT
	B6	CEVAC® NEW L+Tap Water

Experimental birds of farm-B were vaccinated with Avi ND LaSota® and ITA-New (ND)® without any diluents. Blood samples were collected randomly from at three episodes. Firstly, blood was collected from pre-vaccinated birds of group-C. Then vaccinated with Avi ND LaSota® with skim milk. Secondly, blood samples were collected 40 days of post vaccination. Then birds were vaccinated with ITA-NEW (ND)- killed vaccine. Thirdly, blood samples were collected 40 days of post vaccination. Serum samples were collected and tested to evaluate serum antibody titer level against ND by HI test.

Collection of serum from blood samples

Blood samples were collected from the selected farms at Dinajpur district on the basis of age, 3 groups named as A, B, C according to age of the birds (Table 1). The blood samples were collected aseptically from the wing vein using 1 ml disposable sterile syringes respectively. After collection of blood, the syringes with blood were kept at 4 – 8°C for overnight, so that blood can clot in one side of the syringe. Then the clotted blood was removed carefully with sterile needle and sera were poured into sterilized graduated centrifuge test tubes. For each syringe separate needle was used. The sera were subjected to centrifugation at 1000 rpm for 10 minutes for purification. Then the clear sera were collected and kept in clean sterilized Eppendorf tubes and stored at –20°C for further use.

Preparation of chicken red blood cell (1 % v/v) suspension

Chickens blood samples were collected from a live bird market. Blood was collected in a 15ml falcon tube containing 5 ml Alsever's solution and anticoagulant. The collected blood was then centrifuged at 1500 rpm for 5-7 minutes and the supernatant was poured off. PBS (1X) was added into the falcon tube containing blood and centrifuge at 1500 rpm for 5-7 minutes. This step is repeated for 4-5 times for washing chickens blood. 1% v/v suspension of chicken RBC was prepared by adding PBS.

Haemagglutination test (HA)

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).

Micro-haemagglutination test

The V bottom micro well plate was used to determine HA units (4HA/25µl). It was carried out by two-fold serial dilutions of the viral suspension in a micro well plate to determine the haemagglutination titer of the HA antigen used. For this purpose, a 96 well "V" bottomed micro plate was taken. Then 25µl of PBS was dispatched in each well of the row A. 25µl of antigen was added to the first well, after thorough mixing serial dilution had continued up to the 11 wells of the row A and finally the discarded 25µl solution from the well 12 was taken as control. 25µl of 0.5% cRBC suspension was added into each well of the row A. The plate was allowed to stand for 45 minutes for reaction between the antigen and RBC at room temperature. A uniform layer of the agglutinated cells covering the bottom of well of the plate was considered as positive HA and a sharp buttoning of RBC at the bottom of well was considered a negative HA. 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, 2000).

Haemagglutination inhibition test (HI)

PBS 25µl was dispended into 12 wells of one row of the plastic v-bottomed 96 plates. Two-fold serial dilution of field serum 25µl was made up to 11th plate. An equal volume of 4HA unit of ND virus was added into each well up to 12th wells. The mixture was kept for 30 minutes at room temperature, then 25µl of 1 % (v/v) chickens RBC suspension was added to all wells. The RBC is allowed to settle down for 40 minutes. Agglutination was assessed by tilting the plates. The samples showing peculiar central button shaped settling of RBCs were recorded as positive. The last wells, which had a complete inhibition, was considered as the HI antibody titer (OIE, 2000).

Statistical analysis

Various data were collected and summarized in the computer program MS EXCEL (Microsoft Co.). All data were analyzed by SPSS version 21 by performing t-test and F-test. Values were expressed as mean ± SD. Significance was determined when $P<0.05$, which means significant at 5% level of significance.

Table 2: Detection of HI-antibody titers of birds vaccinated with Avi ND LaSota® and CEVAC® NEW L in Farm-A

HI titer	1:2 (2^1)	1:4 (2^2)	1:8 (2^3)	1:16 (2^4)	1:32 (2^5)	1:64 (2^6)	1:128 (2^7)	1:256 (2^8)	1:512 (2^9)	Mean±SD	P-value
Pre-vaccination	-	-	-	-	7	9	4	-	-	5.97 ± 0.75	0.023*
Post-vaccination	-	-	-	-	32	23	36	29	-	7.0 ± 0.93	

*Significant at $P<0.05$

RESULTS

The study was conducted to determine immune response of NDV in layer chickens vaccinated with different vaccines and diluents. Prior to vaccination, blood samples were collected to measure the antibody titer. Pre-vaccinated birds were kept as control group.

Detection of HI-antibody titers of farm-A

Birds of farm-A were vaccinated with Avi ND LaSota® and CEVAC® NEW L vaccine. Pre-vaccinated antibody titer's level against NDV varies from $\log 2^5$ to $\log 2^7$ and Mean ± SD were 5.97±0.75. After 40 days of post-vaccination, 120 sera samples were procured to determine the immune response against NDV vaccine, the titer varies from $\log 2^5$ to $\log 2^8$ and Mean ± SD were 7.0±0.93. Significant ($P<0.05$) differences were observed in titer levels between pre and post vaccination stages, and results are shown in Table 2. In addition, effects of vaccine type were also considered in all birds of Farm-A. In case of Avi ND LaSota® vaccine, pre-vaccination serum HI titers (Mean±SD) were 5.9±0.96 and post-vaccination serum HI titers (Mean ± SD) were 6.98±0.92. When, CEVAC® NEW L vaccine was used, pre-vaccination serum HI titers (Mean±SD) were 5.83±0.98 and post-vaccination serum HI titers (Mean±SD) were 6.83±0.78. However, the variation was highly significant ($p<0.001$) and value was higher when Avi ND LaSota® was used in birds (Table 3).

Detection of HI-antibody titers of birds in Farm-B

Before vaccination, antibody titers levels against NDV varied from $\log 2^5$ to $\log 2^7$ and Mean±SD 5.85±0.75. Af-

ter vaccination, H1-antibody titers of birds were detected to determine the level of titer and results are shown in Table 4. Birds of firm-B were first vaccinated with Avi ND LaSota® vaccine. After 40 days of post-vaccination, the titer varied from $\log 2^5$ to $\log 2^8$ and Mean±SD were 6.7 ± 0.93. Then birds were vaccinated with ITA-New (ND)® (killed vaccine). In sera sample collected at 40th day of post vaccination, the titer varied from $\log 2^6$ to $\log 2^9$ and Mean±SD 8.15 ± 0.93. Significant ($P<0.01$) differences were observed in titer levels among pre and post vaccination stages.

Table 3: Comparison of serum HI-antibody titers among different ND vaccines in Farm-A

Vaccines used	Serum HI titer (Mean±SD)		P value
	Pre-vaccination	Post-vaccination	
Avi ND LaSota®	5.9 ± 0.96	6.98 ± 0.92	0.000
CEVAC® NEW L	5.83 ± 0.98	6.83 ± 0.78	0.000

***Significant at P<0.001

Table 4: Detection of HI-antibody titers of birds vaccinated with Avi ND LaSota® and ITA-New (ND)® in Farm-B

HI titer	1:2 (2^1)	1:4 (2^2)	1:8 (2^3)	1:16 (2^4)	1:32 (2^5)	1:64 (2^6)	1:128 (2^7)	1:256 (2^8)	1:512 (2^9)	Mean±SD	P-value
Before Vaccination	-	-	-	-	7	9	4	-	-	5.85 ± 0.75	
Vaccination with Avi ND LaSota®	-	-	-	-	2	6	8	4	-	6.7 ± 0.93	0.002**
Vaccination with ITA-New (ND)®	-	-	-	-	-	1	3	7	9	8.15 ± 0.93	

**Significant at P<0.01

Comparison of HI-antibody titers in serum among different ND vaccines, diluents used in Group-A and Group-B

Birds of Group-A were divided into six subgroups (A1, A2, A3, A4, A5 and A6) according to vaccines and diluents used. Table 5 shows that serum HI titers (mean±SD) significantly ($P<0.05$) increased after vaccination in all sub-groups of Group-A. Considering the diluent, serum HI Titer was significantly higher in MASTERBLUE, moderately higher in ADVANCE NON-FAT compared to tap water. There is no significant ($P>0.05$) difference in serum HI Titer of Sub-groups where MASTERBLUE® and ADVANCE NON-FAT were used as diluent (Table 5).

Similar trend was observed in serum HI titers (Mean±SD) of birds of different Sub-groups of Groups-B and results are presented in Table 6. Birds of Group-B were divided into six subgroups (B1, B2, B3, B4, B5, and B6) according to vaccine and diluents used. Serum HI titers (mean±SD) significantly ($P<0.05$) increased after vaccination in all sub-groups. Serum HI titer was the highest in sub-groups (Sub-groups B1 and B4) where MASTERBLUE® was used as diluent with both vaccine.

Comparison of egg production performance of immunized layer birds

Before vaccination, production performances of layer birds were low. However, egg production increased after vaccination. In case of farm-A, previous production performance was 75% and after vaccination it became 89%. In case of farm-B, previous production performance was 72% and after vaccination it became 84% (Table 7).

Table 5: Comparison of HI-antibody titers in serum among different ND vaccines, diluents used in Group-A

Sub-groups	Vaccines+Diluents used	Serum HI titer (Mean±SD)		P value
		Pre-vaccination	Post-vaccination	
A1	Avi ND LaSota®+MASTERBLUE®	5.6 ± 0.84	6.8 ± 0.42^{a}	0.013*
A2	Avi ND LaSota®+ADVANCE NON-FAT	5.3 ± 0.95	6.5 ± 0.70^{a}	
A3	Avi ND LaSota®+Tap Water	5.3 ± 0.23	5.9 ± 0.74^{b}	
A4	CEVAC® NEW L+MASTERBLUE®	5.3 ± 0.95	6.6 ± 0.52^{a}	0.021*
A5	CEVAC® NEW L+ADVANCE NON-FAT	5.2 ± 0.79	6.4 ± 0.57^{b}	
A6	CEVAC® NEW L+Tap Water	5.2 ± 0.45	5.8 ± 0.79^{c}	

*Significant at P<0.05

Table 6: Comparison of HI-antibody titers in serum among different ND vaccines, diluents used in Group-B

Sub-groups	Vaccines+Diluents used	Serum HI titer (Mean±SD)		P value
		Pre-vaccination	Post-vaccination	
B1	Avi ND LaSota®+MASTERBLUE®	6.5 ± 0.85	8.1 ± 0.74	0.02*
B2	Avi ND LaSota®+ADVANCE NON-FAT	6.2 ± 0.79	7.7 ± 0.88	
B3	Avi ND LaSota®+Tap Water	6.2 ± 0.79	7.3 ± 0.74	
B4	CEVAC® NEW L+MASTERBLUE®	6.5 ± 0.85	8.0 ± 0.66	0.032*
B5	CEVAC® NEW L+ADVANCE NON-FAT	6.3 ± 0.67	7.8 ± 0.79	
B6	CEVAC® NEW L+Tap Water	6.3 ± 0.57	7.2 ± 0.42	

*Significant at P<0.05

Table 7: Comparison of egg production performance among birds of Firm-A and Farm-B

Study area	Egg production performance	
	Pre-vaccination	Post-vaccination
Farm-A	75%	89%
Farm-B	72%	84%

DISCUSSION

The aims of this study were to determine the antibody titer level against different ND vaccines in layer birds after vaccination. In the research work, sera samples were procured from 20 randomly selected birds of Farm-A (Shamim Layer Farm) to determine the HI-antibody titers. Antibody titers levels against NDV vary from $\log 2^5$ to $\log 2^7$ and Mean±SD 5.97±0.75. Birds of farm-A were vaccinated with Avi ND LaSota® and CEVAC® NEW L vaccine. The titer varies from $\log 2^5$ to $\log 2^8$ and Mean±SD were 7.0±0.93. This finding was supported by previous observations of Motitschke und Jungback (2012). They reported that Ab titer of 85.9% (n=79) was the protective level ($\geq \log 2^4$) while 7.6% (n=7) were found below the protective level ($\log 2^1$ to $\log 2^2$). On the 7th day of post vaccination, antibody titer varies from $\log 2^5$ to $\log 2^9$ with log GMT 2.35 and geometric mean titers 121.8. On the 14th day antibody titers range from $\log 2^3$ to $\log 2^7$ with log GMT 2.72 and geometric mean titers 38.1 and on the 21st day of post vaccination, an-

tibody titer varies from $\log 2^3$ to $\log 2^7$ with log GMT 2.35 and geometric mean titers 43.3. In the case of Farm-B (Ishrafil Layer Farm), antibody titers levels against NDV vary from $\log 2^5$ to $\log 2^7$ and Mean±SD were 5.85±0.75. Birds of farm-B were vaccinated with Avi ND LaSota® vaccine. The titer varies from $\log 2^5$ to $\log 2^8$ and Mean±SD were 6.7±0.93. Then birds were vaccinated with ITA-New (ND)® which were killed vaccine. Post vaccination titer varies from $\log 2^6$ to $\log 2^9$ and Mean±SD 8.15±0.93. This finding was supported by previous observation of Samad *et al.* (2007), who conducted a study with two ND killed vaccine named as "Newcevac Nobilis®" and "Nobivac ND broiler®" in 210 broiler and layer breeder birds of seven different flocks of breeds and ages' with history of regular vaccination. They summarized that vaccination with ND killed vaccine provoked a high level of humeral immunity.

Age of birds, type of vaccine and diluents used were considered in this study. Birds of two age groups were included. Serum titer levels were significantly increased in all birds after immunized with vaccines irrespective of age, vaccines and diluents. Serum HI titer was significantly higher in MASTERBLUE, moderately higher in ADVANCE NON-FAT compared to tap water. There was no significant difference between MASTERBLUE® and ADVANCE NON-FAT. This finding was supported by some previous observations. AL-Mayah *et al.* (2009) reported that chicks vaccinated with ND vaccines when prepared in reverse osmosis water vaccination showed a high HI titer as compared with that chicks vaccinated with ND vaccines prepared in tap water only. When distilled water, tap water + powder skim milk and reverse osmosis water were used in drinking vaccination against ND, HI tests showed a high immune response to ND vaccine among all chicks and only chicks vaccinated with ND vaccine prepared in the tap water showed a low immune response. Narayanan *et al.* (2010) have stated that in the absence of stabilizers, vaccines administered in water are likely to be inactivated by free chlorine or other metals. Powdered stabilizer has ability to preserve the viability of live vaccine reconstituted in water containing free chlorine. Maintenance of viable vaccine during administration is required for effective immune stimulation and response after vaccination. The chlorinated water with vaccine also demonstrated that the virus could not withstand the detrimental effect of 4 ppm of chlorine in the water. Skim milk has been found to be essential in stabilizing vaccines whereas 1×PBS has been shown to protect viable vaccine in water. In spite of vigorous vaccination schedules, ND is still 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 resultant in immunosuppression (Sil *et al.*, 2002). The control of ND relies on the use of safe and effective vaccines.

ND is considered as one of the major threats to the poultry industry in Bangladesh because of its high morbidity, mortality and reduced productivity of eggs. ND alone is responsible for at least 40-60% mortality of the total population of poultry in Bangladesh. Despite extensive use of vaccines, outbreaks of ND are still recorded due to failure of effective cold chain system, which is required for the maintenance of efficacy of vaccines. However, in developed country, extensive use of currently available vaccines, strict quarantine combined with rapid diagnostics, biosecurity, stamping out and other containment measures seem to keep ND under control. The results showed that the level of protection of vaccinated layer birds were satisfactory, which may be due to hyper immunizing the birds and adopting good management conditions. Live vaccine and killed vaccine induced a significant antibody titer. This is indicated that the vaccine was absolutely effective against NDV and Killed vaccine provides higher protection level than live vaccine. MASTERBLUE (stablilzer) and ADVANCE NON-FAT powder is suitable diluents to be used in vaccination against ND vaccine with tap water as tap water may inactivate the vaccine due to its impurity in addition to containing higher level of chlorine and even the type of pipes or vessels used to distribute the drinking water.

ACKNOWLEDGEMENTS

This research work was supported by Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh.

CONFLICT OF INTEREST

None to declare.

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