



Research Article

Seroprevalence of Antibodies against Newcastle Disease in Layer Chicken at Cox's Bazar, Bangladesh

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ABSTRACT

This research work was conducted to screen out the seroprevalence and immune status of layer chicken against Newcastle Disease Virus (NDV) in Cox's Bazar, Bangladesh. From 17 different commercial layer farms, a total of 179 serum samples were collected and divided into three age groups such as 20–27 weeks, 36–42 weeks and 48 to above weeks. Haemagglutination Inhibition (HI) test was performed and calculated geometric mean titers for layer groups of 20–27 weeks, 36–42 weeks and 48 to above weeks of age were $\log_2 8.39$, $\log_2 7.67$ and $\log_2 10.66$, respectively. Though all birds showed positive specific immunity of 100%, means good mean titers that was protective to ND, still the birds remained susceptible to ND. So vaccination should be intensified by screening immune status of the flock against ND through analyzing the HI titers to NDV.

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INTRODUCTION

Newcastle Disease (ND) is a highly contagious viral disease that attacks many species of domestic and wild birds which also locally known as Ranikhet Disease (Barman, 2002). The causal agent is Newcastle Disease Virus (NDV) which is a negative-sense single-stranded (ss) RNA virus belonging to the family *Paramyxoviridae* (Hossain et al., 2010). Based on the pathogenicity, NDV is classified into highly virulent (velogenic), intermediate (mesogenic) or avirulent (lentogenic) strain (Lamb et al., 2000). The infections of poultry range from latent to rapidly fatal depending upon the pathotype of virus involvement (Alexander, 2003). Transmission of ND occurs by inhalation or ingestion through direct contact between healthy birds and discharges from infected birds through feces and secretions from nose, eyes and mouth (NABC, 2007).

It is noticed as ND is the most significant viral disease of poultry in the world including developing countries. In Africa and Asia, it is a major constraint against the progress of both industrial and village poultry production (Pazhanivel et al., 2002; Alders et al., 2001). The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability (Hossain et al., 2010).

Vaccination has been reported as the only safeguard against endemic ND (Orajaka et al., 1999; Saha et al., 1998). The current vaccination schedule in Bangladesh directed by the Directorate of Livestock Services (DLS) includes administration of a live vaccine *Baby Chick Ranikhet Disease*

Vaccine (BCRDV) of lentogenic F-strain by intra-ocular instillation to chicks followed by a live vaccine *Ranikhet Disease Vaccine* (RDV) of mesogenic Mukteswar (M)-strain by intramuscular injection at 21 days old chicks and 60 days old chicken which is repeated at every 2 months interval. The infection still occurs in Bangladesh every year in the form of epidemic and appears to cause up to 40–60% of the total mortality in poultry population creating one of the major problems in the development of poultry industry in Bangladesh (Biswas et al., 2005).

It is significant to elucidate the immune status of NDV among chickens in order to formulate appropriate vaccination schedule and control measures. So, the study was conducted to determine the seroprevalence and screen immune level of the birds to Newcastle Disease Virus (NDV) among layer chickens in Cox's Bazar district, Bangladesh.

MATERIALS AND METHODS

The study was carried out at three upazillas of Cox's Bazar from June to August, 2012. A total of 17 commercial layer farms which comprised at least thousand birds were randomly selected with the diagnosis of ND. Data were recorded according to clinical signs exhibited by individual bird during illness, strains of birds, number of birds in farm, age, vaccination schedule and date of last vaccination, number of dead birds, treatment measures if taken and information about management system of farm.

Diagnosis

In Upazilla Veterinary Hospital, poultry diseases were diagnosed on the basis of post mortem findings, clinical signs and as well as various laboratory tests such as Haemagglutination Inhibition (HI) test, Enzyme Linked Immune Sorbent Assay (ELISA) technique, Viral Neutralization (VN) test. Currently HI test is most widely used technique to detect antibodies to NDV in poultry sera.

Post Mortem Findings

These findings were varied but generally include haemorrhagic lesions on the proventriculus and intestinal tract which include pin point haemorrhage on the tip of proventriculus and small ecchymotic haemorrhages on the lymphatic nodule, Payer's patches followed by intestinal mucosa. Edematous, haemorrhagic and degenerative ovaries, button ulcer on the caecum and splenomegaly were observed (Figure 1). Other lesions accordingly hyperemia

and congestion in respiratory tract, serous or catarrhal exudates in larynx and trachea, thickened air sacs containing yellow exudates were also frequent.

Clinical Signs

Signs of ND followed as sudden death without sign, ruffled feathers, depression, prostration, edematous head and wattles, nervous signs as paralysis, torticollis and respiratory signs as gasping and coughing.

Collection of Samples

The sample, 1ml of blood from 179 birds (8 samples per thousand birds) was collected from each bird through wing vein puncture; Sera were separated and stored at -20°C until analyzed. Avinew[®] ND, VG/GA antigen lentogenic strain (Advance Animal Science Co. Ltd. Dhaka, Bangladesh) was used in this study to conduct HI test to detect the immune status against NDV.

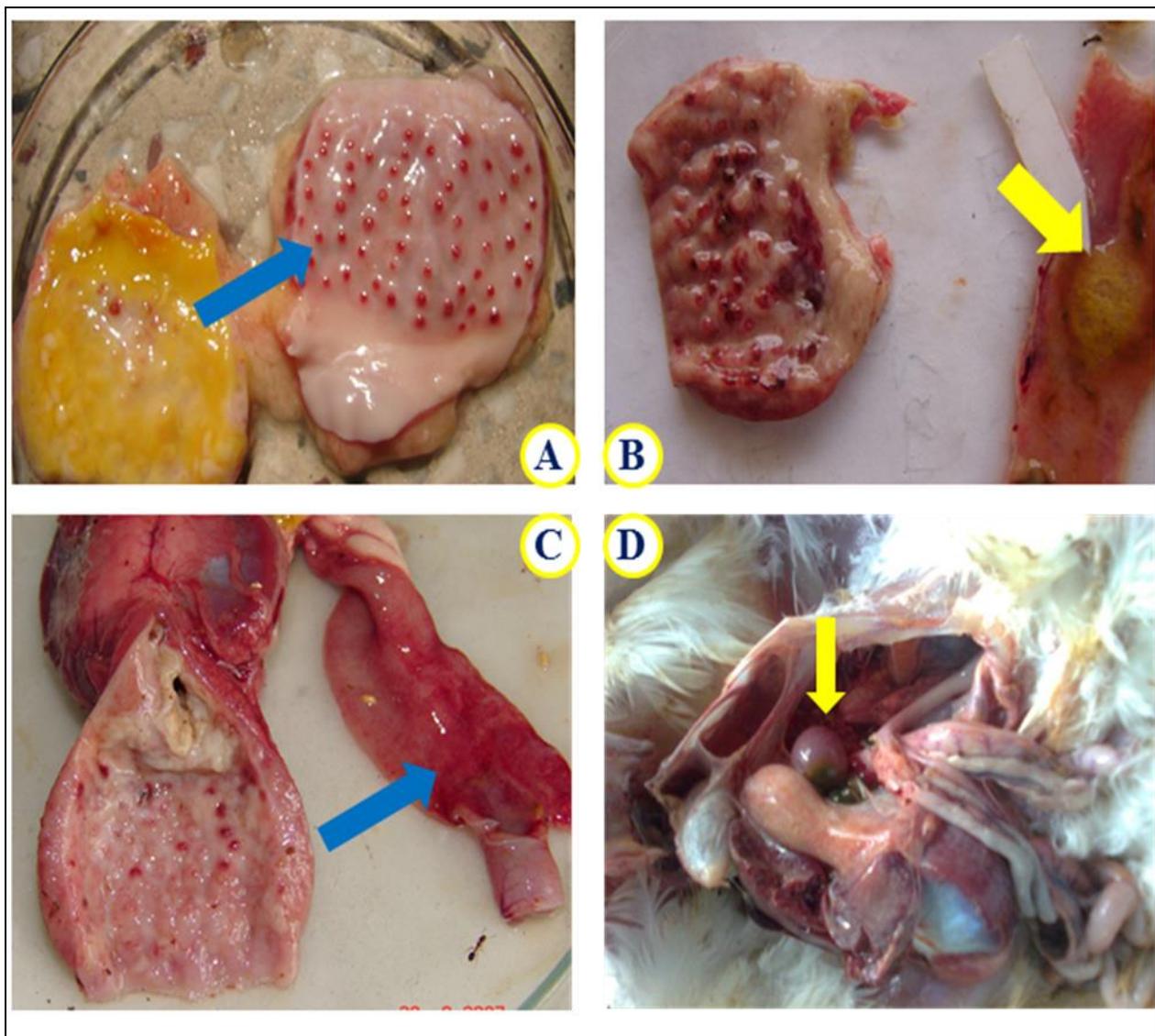


Figure 1: A. Haemorrhage on the tip of the proventriculus; B. Severe haemorrhage on glandular tip and button ulcer in the intestinal mucosa; C. Haemorrhagic intestinal tract; D. Splenomegaly

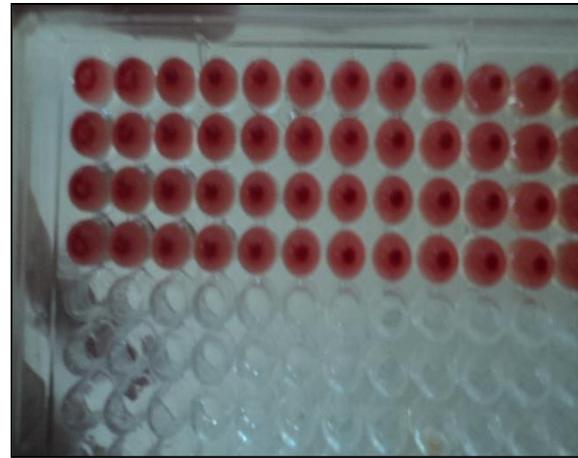
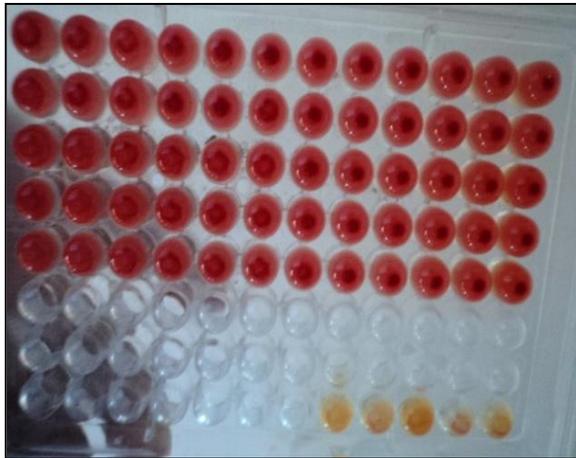


Figure 2: Haemagglutination Inhibition test (HI test) (Buttoning)

HI Test to Screen Level of Antibodies in Blood Sera

HI test was performed in V-bottomed microtitration plates. Test procedure was conducted according to the methodology of OIE Manual (2002). For preparation of 1% chicken Red Blood Cells (RBCs) used in the test, 5ml blood was taken from chicken that had not been primed with Newcastle Disease vaccine. RBCs were collected by centrifuging blood at 1500rpm for 15minutes and these collected RBCs were pooled in an equal volume of Alsever's solution. After that, RBCs were washed thrice in phosphate buffered saline (PBS) before using as 1% (packed cell v/v) suspension. Serum was tested in two fold serial dilutions upto 10th well in microtiter plate. For virus suspension 4 Haemagglutinating Units (HAU) Avinew® ND, VG/GA antigen lentogenic strain was added upto 11th well. After keeping the plates at room temperature for 30minutes 1% chicken RBC suspension was added to each well. The 11th well contains antigen and RBCs as the positive control and

the 12th well contains only RBCs as the negative control. After gentle mixing, the RBCs were allowed to settle at room temperature for 40minutes and agglutination was assessed by tilting the plates. Finally, at the end of 40minutes samples were showing central button shaped settling of RBCs which were recorded as positive and maximum dilution of each sample causing Haemagglutination Inhibition (HI). This was used to estimate the HI titer (Figure 2). The HI titer of each serum sample was expressed as reciprocal of the serum dilution.

Data Analysis

Data that were primarily stored at MS excel (Microsoft office excel-2007, USA). Descriptive analysis was through by STATA version 11 (STATA Corporation, Texas, USA) and results were expressed as proportion ± SEM, 95% Confidence interval (CI) and also followed as logarithm table.

Table 1: Serum samples of layer chicks showing immune response to NDV using Haemagglutination Inhibition (HI) test

Age of Birds(wks)	No. of Sample(s)	Specific immunity	Non-specific immunity	Specific immunity percentage (%)
20-27	99	99	-	100.00
36-42	53	53	-	100.00
48-above	27	27	-	100.00

Table 2: Distribution of layer birds on the basis of Haemagglutination Inhibition (HI) titers obtained against NDV

Age(wks)	No. of Sample(s)	Antibody titers using HI test												GMT
		Log ₂ 1	Log ₂ 2	Log ₂ 3	Log ₂ 4	Log ₂ 5	Log ₂ 6	Log ₂ 7	Log ₂ 8	Log ₂ 9	Log ₂ 10	Log ₂ 11	Log ₂ 12	
20-27	99	0	0	0	7	11	12	6	7	11	22	17	6	8.39
36-42	53	0	0	5	7	6	2	3	0	9	16	0	5	7.67
48-above	27	0	0	0	0	0	0	0	3	6	3	0	15	10.66

RESULT

From the total 179 serum samples of 17 different commercial layer farms, 99 serum samples were ranging from 20-27weeks of age, 53 samples 36-42weeks of age and other 27 samples were 48 to above weeks of age. Birds of all ages were found positive for specific immunity with positive percentage of 100 (Table 1).

But the HI antibody titer varied from log₂4 to log₂12 with a GMT of log₂8.39 at the age of 20-27weeks of age; log₂3 to log₂12 with a GMT of log₂7.67 at the age of 36-42weeks of age and log₂9 to log₂12 with a GMT of log₂10.66 at the age of 48 to above weeks of age (Table 2).

DISCUSSION

All samples irrespective of age represents positive for specific immunity with a positive percentage of 100 where Tariq and Taib (2010) found 26%, 54% and 75% positive for specific immunity at 24-36, 36-48 and more than 48 weeks in chicken which is quite lower than this one. HI test has been widely used for the estimation of titers of specific antibody in the sera of individuals infected with certain viruses including NDV, Influenza virus etc as these viruses can agglutinate erythrocytes (Serrão et al., 2012). Generally ND-HI titer of log₂3 and above is accepted as positive for specific immunity (Sa'idu et al., 2006; OIE 2002; Alexander,

2000). Numan et al. (2005) stated ND–HI titer as $\log_2 7$ to $\log_2 8$ in layer of 17–47 weeks of age that is closer to the present study. This shows that the serum antibody titer is low in some cases and enough to protect the birds from ND infection. There are several possible reasons for the lower level of protection in birds such as poor vaccine quality, not properly maintained vaccination schedule or techniques, impaired immune competence due to immunosuppressive substances in the feed or to immunosuppressive diseases and therefore, unable to protect the chicks from NDV infection.

Hossain et al. (2010) found 98.35%, 92.86%, 97.46% and 97.98% layer chickens had protective HI titers in the summer, rainy, autumn and winter, respectively; Sa'idu et al. (2006) analyzed $\log_2 7.6 \pm 1.62$ in parent stock of layer; this result is similar to the present findings at the age of 36–42 weeks. Birds of this group showed lower antibody levels than the earlier group and showed relatively decreased susceptibility to clinical infection. One of the causes for outbreaks in vaccinated chickens might be the introduction of new ND virus strains against which the local birds have no or very low immunity, leading to vaccine failure.

Group of birds age 48 to above showed higher antibody level than the previous two groups. Similar agreement has been described by Numan et al. (2005) where 100% of layer chickens were positive for specific immunity against NDV in Pakistan. In the present study, the birds in 48 to more weeks of age showed the highest level of antibody titers (GMT 10.66) and showed relatively low susceptibility to clinical infection. The HI titers obtained in the present study were higher than those reported by Biswas et al., 2006 who recorded that 64%, 47.4%, 62.6% and 56.3% of sonali chickens in southern part of Bangladesh had protective HI titers against NDV in autumn, winter, summer and rainy seasons respectively. Other factors like poor vaccine quality is a common problem in developing countries and results poor manufacturing standards, lack of adequate storage facilities, application of expired vaccine batches, faulty administration and handling during transportation (Vui et al., 2002). Heat stress and water deprivation also lead to production of steroids and thus result an immunosuppression. Quality of water which is offered to the birds was also found questionable which might hinder the development of specific immunity. Inappropriate vaccination schedule also leads to the neutralization of maternally derived antibodies and resultantly making the birds more susceptible to the infection.

CONCLUSION

The prevalence of Newcastle Disease is higher level (postmortem findings 45.6%) than the other viral diseases in Bangladesh. Although birds of commercial layer farms had mean titers that was protective to ND, still the birds remain susceptible to ND. To maintain good farm practices it is very important to vaccinate the birds of the flock at proper time with proper dose and schedule regularly. So vaccination should be intensified by monitoring immune status of the flock against ND through analyzing the HI titers to NDV.

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