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Research Article

Seroprevalence of Newcastle disease in layer chickens and pathology in clinically affected chickens at Gazipur, Bangladesh

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ABSTRACT

The goal of this study was to determine the seroprevalence of Newcastle disease (ND) and to evaluate the age based effectiveness of three commonly used ND vaccines (Cevac New-L, Avenew and Newcastle Disease vaccine) in commercial layer chickens in Bangladesh using hemagglutination inhibition (HI) test. Additionally, postmortem and histopathological examinations were also performed in 10 clinically affected chickens to elucidate major pathological changes in different organs. For seroprevalence study, total 200 sera (grower 80 and layer 120) were collected randomly from 20 different vaccinated layer farms (10 grower and 10 layer) and for detection of ND vaccine efficacy, a total of 100 sera were randomly collected from 10 commercial non-vaccinated grower and layer farms (10 birds in each farm) located at Gazipur district from January to June-2020. The overall seroprevalence of ND was 21% followed by, 10% in grower stage (12-16 weeks) and comparatively higher seroprevalence, 26.6%, in layer stage (27-40 weeks). In case of detection of age based vaccine efficacy, blood serum samples were collected at 7th and at 14th day post vaccination (dpv) to determine Ab titre of experimental grower and layer chickens. The mean titer at 7th and 14th dpv in both grower and layer groups were meaningfully greater ($P<0.01$) in comparison to chicken groups before vaccination. Moreover, the GMT of grower chickens were $2^{7.2\ -7.6}$ and $2^{8.00\ -8.1}$ at 7 dpv and 14th dpv, respectively and in case of layer chickens it was slightly higher, $2^{9.1-\ 9.2}$ and $2^{9.6\ -10}$ at 7 dpv and 14 dpv, respectively. In case of pathological findings, affected chickens showed characteristic gross and microscopic lesions particularly in proventriculus, trachea, liver and cecal tonsils. These finding may assist epidemiologist to take appropriate preventive and control strategies against the disease.

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Introduction

Newcastle disease is one of the most serious and infectious, septicemic, fatal along with destructive infectious diseases of poultry caused by a paramyxoviridiae of genus morbilivirus and species Newcastle disease virus (NDV) (Olsen & Orosz 2000). Newcastle disease virus (NDV) are divided into three pathotypes (lentogenic, mesogenic and velogenic) based on their host range. These pathotypes of NDV can emerge at any time with a high economic loss in poultry sector. In Bangladesh, velogenic strain is prevalent and it causes huge economic loss @ US$ 288.49 million per annum (Khatun et al. 2018).

The department of Livestock (DLS), Government of Peoples Republic of Bangladesh, and non-governmental organization (NGOs) are vaccinating birds as a way of preventing ND in Bangladesh. The administration of two types of live vaccines, either lentogenic (F, VG/GA, Lasota strain) or mesogenic (M-strain) and neutralized (dead) vaccines, has recently been used to prevent ND. In some cases, such vaccines and vaccination programmes are found to be ineffective in protecting chickens against ND (Chowdhury et al. 1981). Poor immunization methods and/or immune dampening opportunistic illness (infectious bursal disorders, infectious bronchitis, parasitic infestation) are key causes of vaccine failure, as are the age of birds at the time of vaccination (Dortmans et al. 2014; Dakouo et al. 2020). The amount of maternally produced antibodies (MDA) determines the priming time. Another reason for vaccine failure is significant genetic divergence

between the viruses that causes outbreaks (Dortmans et al. 2012; Dimitrov et al. 2016). Regarding this, several researchers have shown that these vaccinations can protect chickens from sickness even when they do not inhibit viral excretion when infected with heterologous isolates (Roohani et al. 2015; Wajid et al. 2018).

The HI test and the ELISA test are two serological assays that may be used to assess the antibody responses of commercial layer chickens who have been vaccinated against ND (Hossain et al. 2013; Belgrad et al. 2018; Oberländer et al. 2020). The disease's prevention and control are mostly dependent on stringent sanitary practices and well-designed vaccination programmes, taking into account the fact that while ND immunization protects birds from more acute symptoms, viral multiplication and shedding may still occur (Miller et al. 2013). In Bangladesh both live and killed vaccines are used and vaccination schedule is followed as per Directorate of livestock service. Despite the widely use of commercial vaccines, in some cases ND vaccines are less effective (Asmaul et al. 2016). As a result, current study was planned to observe the seroprevalance of ND in vaccinated layer flock and to estimate the age-based effectiveness of the three most widely used vaccines named Cevac New-L (Lasota Strain), Avenew (VG/GA strain) and Newcastle Disease vaccine (Mesogenic strain) in layer chickens using HI test. Finally, pathological examination was carried out to find gross and microscopic changes in organs of clinically affected chickens.

Materials and Methods

Sampling and monitoring seroprevalence of ND

A sum of 200 sera samples (grower 80 and layer 120) used for seroprevalence were randomly obtained from 20 various vaccinated layer farms (10 grower and 10 layer) located at Gazipur district in Bangladesh from January to June-2020. Prior to two weeks after inoculation with commercial ND vaccines, blood samples were taken to separate sera. The HI test was used in the laboratory of Kazi farms in Gazipur for detection of humoral immunity against ND. According to Anebo et al. (2014), layer chickens should have antibody titre 2^{4-5}, 2^{5-7}, and 2^{7-10} at the age of 0-09 weeks, 10-19 weeks and more than 20 weeks, respectively to protect ND. Chickens with low HI titre are recommended for vaccination (Oberländer et al. 2020).

Sampling and evaluating vaccine efficacy

For the evaluation of vaccine efficacy, a total of 10 commercial layer farms with different ages (grower 12-16 weeks and layer 27-40 weeks) were monitored in Gazipur district. A total of 100 blood samples were taken randomly from 10 birds on each farm for preliminary HI test. After that, birds having low Ab titre to protect ND were chosen for application of experimental vaccines. Following the detection of low Ab titre among the 50 birds of different ages, the experimental vaccination programme with 3 vaccines (Cevac New-L, Avenew and ND vaccine) was executed in 2 grower as well as 3 layer farms (Table 1). Finally, blood samples were collected at 7^{th} and 14^{th} dpv from each selected flock for evaluating vaccine efficacy by conducting HI test.

Haemaglutination (HA) and hemaglutination inhibition (HI) tests

HI titre of sera samples taken from chickens was determined using an HI test. HA and HI tests were performed in 96 well v bottomed microtitre plate using 0.5% chicken erythrocyte suspension following the standard procedure (Alexander 1988; Anon 2000). HA test was performed before HI test for determination of 4HA unit. To assess the maternal antibodies and its durability, the HI titre of the sera samples from control group of chicks was measured. Antibody titres of 2^{4-5}, 2^{5-7} and 2^{7-10} were judged protective against ND in layer birds aged 0-9 weeks, 10-19 weeks, more than 20 weeks, respectively; titres below this were classified as non-protective (Anebo et al. 2014).

Pathological study

Necropsy was performed in 10 clinically affected dead and sick birds and tissues were collected from trachea, proventriculus, lung, liver, spleen, and intestine for histopathological examination following standard procedures (Dey 2018).

Data analysis

Statistical Package for Social Science (SPSS) programme was used for analysis of the data. Evaluation of vaccines effectiveness was analyzed by paired t-test and Independent sample test (T-Test).

Results and Discussion

Seroprevalence of Newcastle Disease (ND) after vaccination in different farms in Gazipur District

In 20 different vaccinated flocks, overall seroprevalence was 21% out of 200 sera samples, where the seroprevalence was 10 and 26.6% in grower birds and layer, respectively (Table 2). The results showed that the intensity of ND was comparatively higher in layer birds than grower birds. The findings correlate the findings of Rahman et al. (2002) where the prevalence of ND was 37.5%. Present study results also support the results of Rahman et al. (2012) as the authors found the prevalence of ND in Dhaka, Gazipur, Mymensingh and Bogra districts 50.0, 45.0, 25.0 & 32.5%, respectively.

Table 1: Schedule of vaccination in 5 farms which had low antibody titre

Farms	Name of vaccines(strain)	Amount of vaccine	Route of administration	No. of birds	Age of birds
B	Avinew® (VG/GA)	0.5ml	I/M	10	12wks (Grower)
J	Cevac New L® (LaSota)	0.5ml	I/M	10	27wks (Layer)
E	Cevac New L® (LaSota)	0.5ml	I/M	10	35wks (Layer)
F	Cevac New L® (LaSota)	0.5ml	I/M	10	40wks (Layer)
I	New castle disease vaccine (Mesogenic)	0.5ml	I/M	10	16wks (Grower)

Table 2: Seroprevalene of Newcastle Disease in different farms

Types of bird	Tested sample	Positive case	Seroprevalence	Overall seroprevalence
Grower	80	10	10%	21%
layer	120	32	26.6%	

Table 3: Layer birds of different ages at different farms showing mean of titre (before vaccination).

Farm	Age (Weeks)	Stage	No. of blood serum sample tested	Mean of Titre (Before Vaccination)	Recommendations
Farm-A	8	starter	10	$2^{4.6}$	No Vaccination
Farm-B	12	Grower	10	$2^{3.9}$	Vaccination
Farm-C	39	Layer	10	$2^{8.9}$	No Vaccination
Farm-D	33	Layer	10	$2^{9.1}$	No Vaccination
Farm-E	27	Layer	10	$2^{5.6}$	Vaccination
Farm-F	35	Layer	10	$2^{5.7}$	Vaccination
Farm-G	46	Layer	10	$2^{9.0}$	No Vaccination
Farm- H	41	Layer	10	$2^{8.9}$	No Vaccination
Farm- I	40	Layer	10	$2^{5.8}$	Vaccination
Farm-J	16	Grower	10	$2^{3.6}$	Vaccination

(HI test at experimental "0" day; at Geometric mean log2)

Table 4: Evaluation of vaccine efficacy after 7th and 14th day of following vaccination by different vaccine in different ages layer farms.

Farm		Age (weeks)	Name of Vaccine	Experimental 0 day	7day after vaccination	14th day after vaccination
Grower	B	12	Avinew (VG/GA strain)	$_a2^{3.9}$	$_b2^{7.2}$	$_c2^{8.0}$
	J	16	Newcastle disease vaccine (Mesogenic strain)	$_a2^{3.6}$	$_b2^{7.6}$	$_c2^{8.1}$
Layer	E	27	Cevac new-L (LaSota)	$_a2^{5.6}$	$_d2^{9.1}$	$_d2^{9.8}$
	F	35	Cevac new-L (LaSota)	$_a2^{5.7}$	$_d2^{9.7}$	$_d2^{10.0}$
	I	40	Cevac new-L (LaSota)	$_a2^{5.8}$	$_d2^{9.2}$	$_d2^{9.6}$

a, b, c, d Different letters showing a significant difference between groups within the same time sampling.

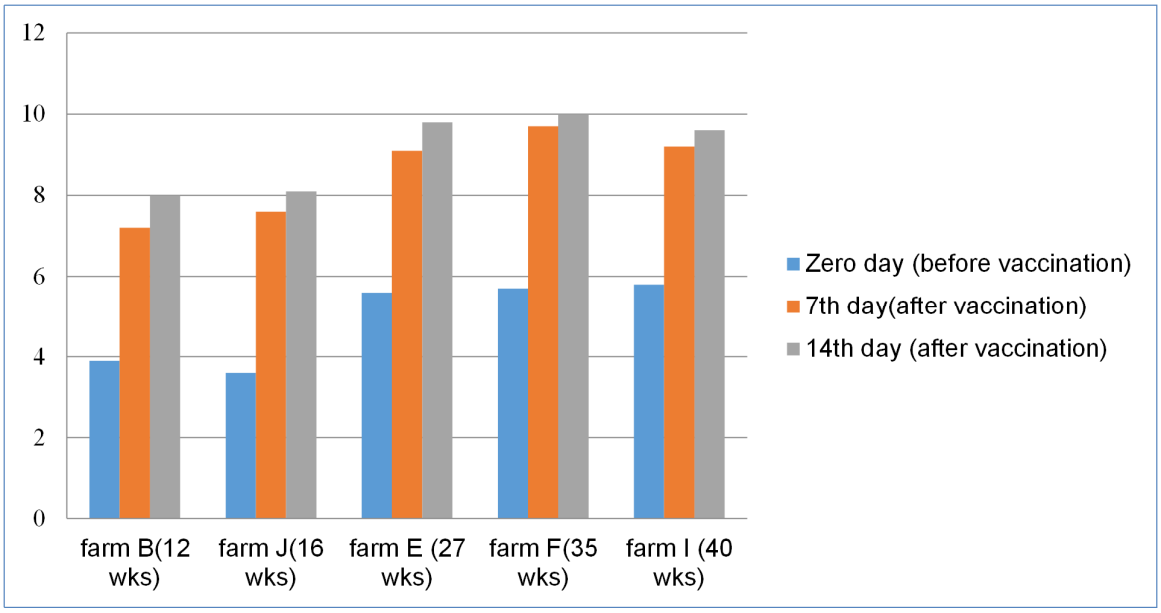

Fig. 1: Graphical presentation of vaccine efficacy after 7th and 14th day of vaccination by different vaccine in different age layer farms.

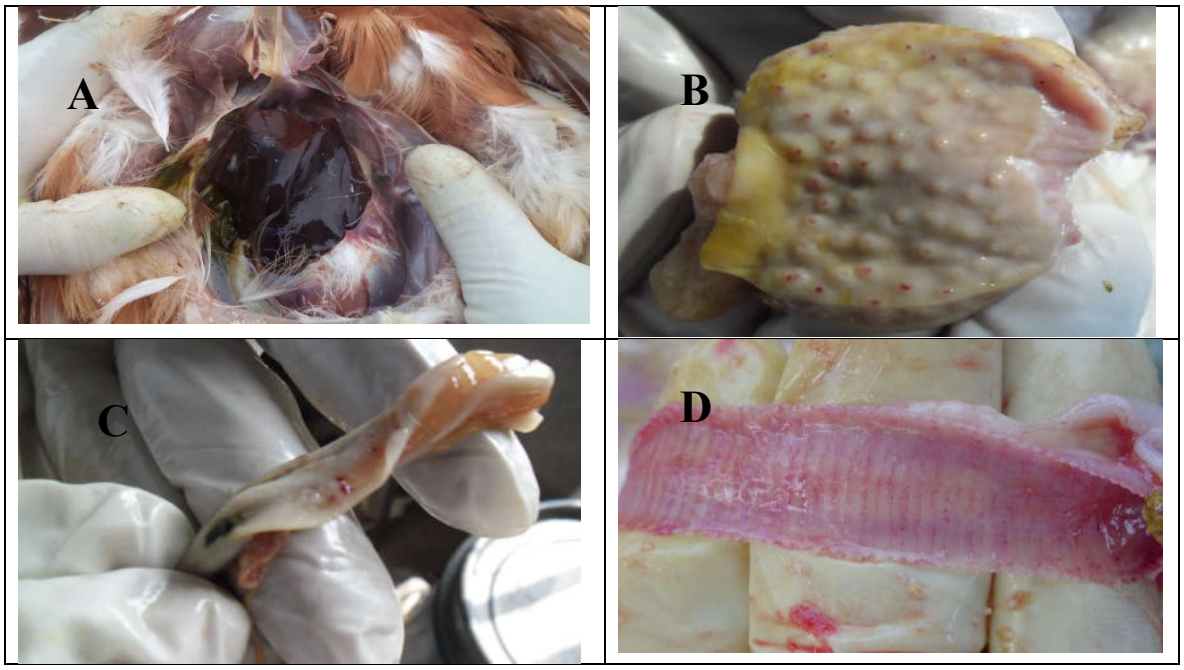

Fig. 2: Gross pathology of affected birds. A; Blackish discoloration of liver due to excessive haemorrhage. B; Haemorrhage on the tip of the proventriculus , C; Hyperemic and necrotic lesion cecal tonsils D; Tracheal mucosa contain many foci of hemorrhage and small clumps of fibrinonecrotic exudate

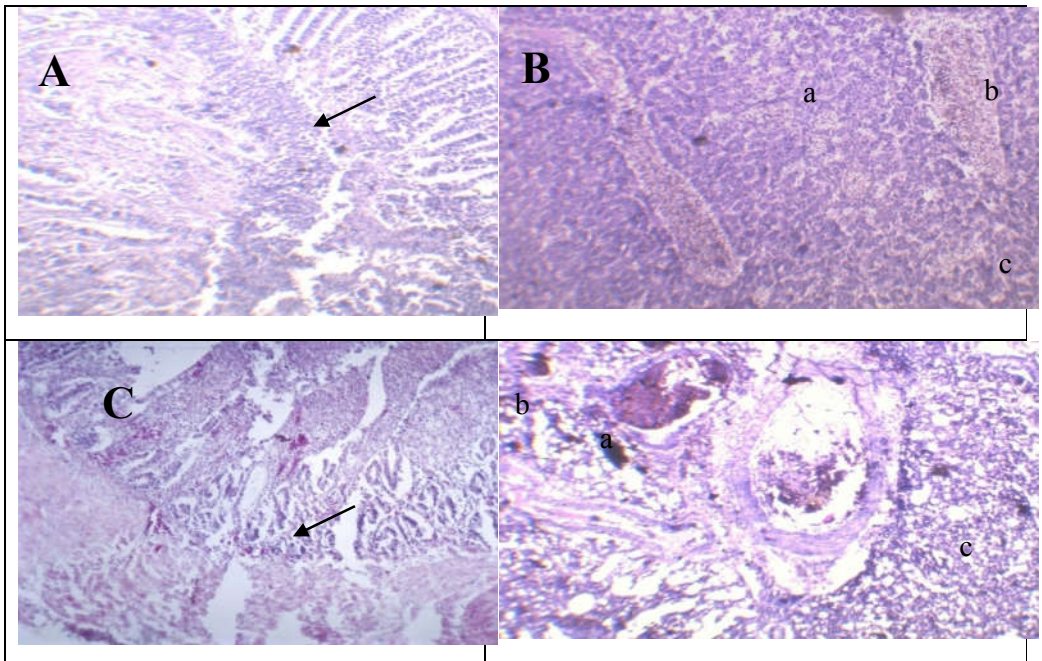

Fig. 3: Histopathology of ND affected birds (H&E X10) A; Proventriculus shows heavy infiltration of inflammatory cells in the mucosa (arrow) B; Liver shows a) Hepatocellular necrosis and infiltrated by mononuclear cells b) Congestion and inflammatory cell in adjacent to portal blood vessel and c) Hemorrhage in liver C; Congestion and hyperemia diffuse lymphoid necrosis in caecal tonsil (Arrow) D; Lung shows a) Haemorrhages b) congestion and c) interstitial pneumonia.

Evaluation of immune response of different vaccines on different age groups of commercial chicken

In order to carry out the evaluation of immune response of vaccination, HI test was performed and found that among the 10 poultry farms, 5 farms had low level of the mean Ab titer which was recommended for further vaccination with our selected vaccines (Table 3). After vaccination with the three selected experimental vaccines (Table 1), there was significant ($p<0.001$) increase of immune response at 7th and 14th dpv (Table 4 and Fig. 1). In case of Grower birds (Group B and Group J), the HI titer for NDV significantly increased at 7 dpv and 14 dpv and the titer was log $_2^{7.2}$ and log $_2^{8.0}$ for group B and log $_2^{7.6}$ and log $_2^{8.1}$ for group J, respectively. This HI test result indicates that immune response was good in both vaccines. On the other hand, the three different age groups (Group I, E and F) of layer birds were vaccinated by Cevac® New-L (LaSota train). In case of 35 wk age group (F), the mean Ab titer was slightly higher, log $_2^{9.7}$ on 7th dpv and log $_2^{10.0}$ on 14th dpv, than other two age groups (I & E) (Table 4 and Fig. 3). This result supports the result of Anebo et al. (2014) as the authors reported that the average Ab titer level by LaSota strain was log $_2^{8.25}$ and by Avinew vaccine was log $_2^{6.5}$ and the Ab titer was log $_2^{7.125}$ and log $_2^{6.5}$ on 7th and 14th days of vaccination, respectively. Present findings also correlate with the findings of some previous studies (Rahman et al. 2004; Banu et al. 2009; Uddin et al. 2014; Asmaul et al. 2016).

Pathological study

Gross lesions

In external examination of dead birds, many birds were found emaciated. The infected birds were found with dark, discolored combs. Eyelid edema and subcutaneous edema were also found in some birds. The post mortem findings of birds include hemorrhage and fibronecrotic exudates in the trachea wall, petechial hemorrhages in proventiculus, congestion in lung, hemorrhages in cecal tonsils and intestine. The liver was congested and black in colour and necrotic foci were found in parietal part of the liver (Fig. 2A-D). In some cases, foamy, purulent, thickened mucoid exudates were found in respiratory tract. Severe mucoid enteritis and ulcers in intestine and on cecal tonsils were also found. Such type of typical lesion in ND affected birds has been reported earlier by Kianizadeh et al. (2002), Kommers et al. (2002), Pazhanivel et al. (2002), Hooper & Selleck (2003), Wakamatsu et al. (2006) and Terregino & Capua (2009).

Microscopic lesions

Histopathological examination showed infiltration of mononuclear cell at submucosa of trachea, congestion, hemorrhage, mononuclear infiltration and necrosis in lung and proventriculus. Such type of lesions were also mentioned by Mishra et al. (2000), Pazhanivel et al. (2002), Srilakshmi et al. (2002). In liver, minute focal area of hepatocellular necrosis infiltrated by mononuclear cells and portal area revealed mild infiltration with mononuclear cells. Such type of changes also narrated by Hooper &

Selleck (2003) and Terregino & Capua (2009). Several descriptive reports of the literature on the histopathological changes following ND infections are related to virulent pathotypes (Beard and Hanson 1984).

Conclusions

Newcastle disease (ND) is still considered as one of the major problems to the poultry industry of Bangladesh. Vaccination is extensively used to protect against ND but field experiences focus its unsatisfactory outcome as far as the control of the diseases is concerned. The present study was undertaken to estimate the seroprevalence of ND and to evaluate the age based effectiveness of three commonly used ND vaccines in Bangladesh as well as find out both gross and microscopic changes in ND infected birds. The overall seroprevalence was higher in layer stage than grower stage and in case of vaccine effectiveness; the results did not show any significant difference among 3 experimental commercial vaccines. Strict quarantine and isolation measures should be put into effect to prevent dissemination of this disease. Depopulation and thorough cleaning and disinfection of premises are necessary and it should be followed by an effective immunization programme.

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Conflict of Interest

The authors declare that no conflict of interest exists.

Authors contribution

All authors contributed equally

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