Research Article

Retrospective Analysis of Newcastle Disease in Chickens in Barishal District of Bangladesh

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Abstract | Poultry diseases are one of the most important constraints to the sustainable growth of the poultry industry in Bangladesh. Among these diseases, Newcastle disease (ND) is one of the most threats and possess a continuous risk to the sustainable development of this growing sector. The present study was designed to investigate the seroprevalence of the Newcastle disease virus (NDV) in chickens at Barishal district of Bangladesh, from November 2021 to October 2022. During the reporting period, a total of 419 serum samples (227 layers and 192 broilers) were randomly collected from apparently healthy, sick and recovered birds. The haemagglutination inhibition (HI) test was used to analyze the chicken sera samples for determination of the titer of the antibodies against NDV. The test results showed that the overall seroprevalence was 13.84% (n = 58/419). Layer chickens (17.62%) were more prevalent for ND than broilers (9.38%). However, a chi-square ($\chi 2$) statistical analysis revealed that factors such as the age of chickens, seasons, floor condition of the chick's house, flock size, bird health status, and practices for dead bird disposal showed no significant associations ($p > 0.05$) with the prevalence of the disease. In conclusion, this study helps to fill a knowledge gap in the prevalence and distribution of NDV in the Barishal district of Bangladesh, provides a framework for future longitudinal research on NDV risk, and may help to formulate appropriate disease control strategies for commercial chickens.

Keywords | Seroprevalence, Factors, Newcastle disease, HI test, Chickens, Bangladesh

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INTRODUCTION

Newcastle disease (ND) is a highly contagious and acute infectious viral disease of poultry caused by infections with virulent strains of Newcastle disease virus (NDV), also known as avian paramyxovirus 1 (APMV-1) (Butt et al., 2019). The virus itself causes injuries that are clinically identified by respiratory disturbances, gastrointestinal disorders, nervous system impairment and reproductive abnormalities according to their host physiology, pathogenicity, and immune status (Caroline, 2022; Wiseman et al., 2018). Newcastle disease not only leads to low growth performance and high mortality but also adversely affects productivity and economic efficiency in poultry production (Karthikeyan et al., 2020). Various species of birds, both domestic and wild, are susceptible, but chickens are specifically susceptible to Newcastle disease (Getabalew et al., 2019). Newcastle disease causes immeasurable financial

losses and poses a major threat to the poultry industry all over the world (Ross et al., 2022). The poultry sub-sector is an important channel for advancing agricultural growth and diminishing malnutrition for the people of Bangladesh (Da Silva and Rankin, 2014). But different diseases, faulty biosecurity, and inadequate nutrition threaten the growing poultry industry (Wong et al., 2017). Also, the outbreak of this harmful disease is one of the greatest hindrances to the expansion of poultry farms, resulting in significant economic losses (Khatun et al., 2018). Vaccines are used regularly in commercial poultry to minimize the devastating sequel of such outbreaks. Because of unavailability of sufficient data on NDV surveillance and the consolidation of NDV outbreak patterns in poultry, it is very difficult to execute preventive and control measures to restrain frequent outbreaks of this disease in these areas. Hence, the present study was intended to find out the seroprevalence and associated factors of ND potentially affecting the layer and broiler chickens in the Barishal district of Bangladesh, which will help us investigate the epidemiology of the virus in commercial poultry.

MATERIALS AND METHODS

Study Area and Duration

The study was conducted in the Barishal district of Bangladesh. It is located between 22°27' and 22°52' north latitudes and between 90°01' and 90°43' east longitudes. The study was performed in the Department of Poultry Science, Faculty of Veterinary, Animal, and Biomedical Sciences, Khulna Agricultural University, Khulna-9100, Bangladesh, from November 2021 to October 2022.

Design of the Study

All birds (n = 419) were divided into two groups, such as layer (n = 227) and broiler (n = 192) chickens. The randomly selected birds were categorized into three different age groups (i.e., ≤8 weeks, 9-20 weeks, and ≥21 weeks in layers) and (i.e., ≤12 days, 13-30 days, and ≥31 days in broilers). According to the climatic conditions of Bangladesh, the total study period was divided into three conventional seasons, namely summer (March–June), winter (November–February), and rainy (July–October), to observe the seasonal influence. Floor condition of chick's house was classified into three, i.e. concrete, mud poultry house and bamboo/wood. In this study, a flock is defined as all the birds in all the houses on one farm. Flocks were categorized conventionally into three groups such as small, medium and large-sized flocks. Depending on the health status; all the birds were divided into healthy, sick and recovered birds. Dead bird/carcass disposal (proper and improper) practices were studied.

Data Collection

Data were collected using a structured questionnaire. The questionnaire was developed after reviewing several published papers to gather knowledge about ND at the national and international levels. The questionnaire was administered following a 'face-to-face' method to collect data at bird, flock, and farm levels after carefully explaining the purpose of the work to the farm owners. Then, all the data, such as the farmer's name, farm location, flock size, age of chickens, seasons, floor condition, bird health status and dead birds disposal practices were noted and recorded properly.

Collection of Sera Samples

A total of 419 blood samples (3 ml) were randomly collected aseptically using sterilized syringes (5 ml) from the wing veins of the birds. Samples were subsequently allowed to clot at ambient temperature on the bench in the laboratory, kept refrigerated overnight at 4°C, and then centrifuged at 10,000 rpm for 10 min to separate the serum. Serum samples were then transferred to a labeled sterile cryovial tube (1.5 ml) and stored at -20 °C until used.

Serological Testing

The hemagglutination inhibition (HI) test was used to analyze the chicken sera samples for the detection of antibodies against the ND virus. The HI test was conducted according to the procedures reported by Beard and Wilkes (1985) and OIE (2002). The test was performed by running two-fold dilutions of equal volumes (0.025 ml) of phosphate-buffered saline (PBS) and test serum (0.025 ml) in V-bottomed micro-titration plates. Firstly, four haemagglutinating units (HAU) of virus or antigen were added to each well, and the plate was kept at room temperature for 30 minutes at least. Then, 0.025 ml of 1% (v/v) chicken red blood cells (RBCs) was added to each well, and, after gentle mixing, the RBCs were allowed to settle for about 40 minutes at room temperature. Finally, the HAI titer was read from the highest dilution of serum, causing complete inhibition of 4 HAU of antigen. The agglutination was estimated by tipping the plates. Those wells in which RBCs stream at the very same rate as the control wells (holding 0.025 ml RBCs and 0.05 ml PBS only) were considered to show inhibition after greater than or equal to 4 (logarithm to base 2) was counted as positive.

Statistical Analysis

All the data obtained from the questionnaire survey and laboratory findings were entered into a spreadsheet program of Microsoft Excel 2010 for data summary and analyzed to calculate the seroprevalence of ND with the help of Microsoft office excel worksheet 2010. Seroprevalence was calculated as the number of seropositive samples divided by the total number of samples tested. Data were also

analyzed by Pearson's Chi-square ($\chi 2$) test with Statistical Package for Social Sciences (SPSS) version 26 (SPSS Inc., USA) to observe the significant influence of parameters. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

Seroprevalence of ND in chickens at Barishal district of Bangladesh

In this study, a total of 419 birds were examined using hemagglutination inhibition (HI) test, out of which 13.84% (n = 58/419) were recorded as positive for ND. Seroprevalence of ND was recorded at 17.62% (n = 40/227) and 9.38% (n = 18/192) out of 227 and 192 commercial layer and broiler chickens, respectively (Table 1).

Seroprevalence and associated factors of ND in layer chickens

In the present study, the prevalence of ND in layer chickens according to the age, seasons, floor condition, flock size, bird's health status and dead bird disposal practices were studied (Table 2). Insignificantly ($p > 0.05$) the highest prevalence was recorded in the ≥21 weeks age group (21.01%), followed by 15.79% and 9.38%, respectively, in the 9–20 weeks and ≤8 weeks age groups in layers. Though there is an insignificant ($p > 0.05$) association between season and prevalence, highest percentage (22.83%) of Newcastle disease was reported in the winter, followed by 15.71% and 12.31% in the rainy and summer seasons, respectively. From this investigation, it is clear that ND is mostly prevalent in concrete house (21.79%) than bamboo/wood (15.44%) and mud poultry house (0%) in layer chickens. The higher prevalence (21.19%) of Newcastle disease was noted insignificantly ($p > 0.05$) in small-sized flocks (<2000 birds) than 14.29% and 12.50%, respectively, in medium-sized (2000-5000 birds) and large-sized (>5000 birds) flocks in commercial layer chickens. To investigate the seroprevalence of ND in Barishal district depending on the health status of layers, sick birds (24.53%) were found more prevalent for ND than healthy (19.27%) and recovered birds (9.23%). Although the seroprevalence recorded in this study for improper disposal (18.75%) of dead birds was relatively higher than proper disposal (13.73%), the difference was not statistically significant ($p > 0.05$).

Seroprevalence and associated factors of ND in broiler chickens

The prevalence of ND in broilers based on the age, seasons, floor condition, flock size, bird's health status and dead bird disposal practices were analyzed in this study (Table 3). The analysis revealed that an insignificantly ($p > 0.05$) higher prevalence was found in the 13-30 day age group (12.64%), followed by 8.06% and 4.65% in the ≥31 day and ≤12 day age groups, respectively in broilers. Newcastle disease was found almost all year round in broilers but was mostly found in rainy (12.35%) seasons, followed by winter (7.58%) and summer (6.67%) seasons. Insignificantly ($p > 0.05$) higher prevalence of ND was recorded 11.01% in concrete house followed by 8.33% and 6.78% in mud poultry and bamboo/wood house respectively. In broilers, medium-sized flocks having 1000-4000 birds were more prevalent (11.63%) for ND than 9.76% and 6.15% respectively in large-sized (>4000 birds) and small-sized (<1000 birds) flocks. Also, a slightly higher prevalence was reported in sick birds (13.46%) than healthy (8.42%) and recovered birds (6.67%) in broilers, but the difference was not found significant ($p > 0.05$). ND is found more prevalent for those broilers where dead birds are disposed improperly / openly (10.13%) than proper / safe disposal (5.88%) of carcass.

DISCUSSION

In this study, the overall prevalence was found to be 13.84% in chickens. Wodajo et al. (2023), Ahamidou et al. (2023), and Belgrad et al. (2019) reported subsequently 17.06%, 18.6%, and 21.2% overall prevalence of ND in chickens, which are higher than the findings of our study. Conversely, the lower prevalence of Newcastle disease was 11.34% and 13.25% recorded by Sori et al. (2016) and Olorunshola et al. (2022), respectively. Unigwe et al. (2020) found 13.56% positive cases of ND, which is more or less similar to the findings of our study.

From the present investigations, the prevalence of ND was recorded at 17.62% in layers and 9.38% in broiler chickens. Islam et al. (2021) and Geresu et al. (2016) reported 19.4% and 31.61% prevalence of ND in layer chickens, which is higher than the findings of our study. But, in some previous studies, lower prevalence of 14.42%, 9%, and 7.92% were recorded in layer chickens, as reported subsequently by Islam et al. (2023), Islam et al. (2020), and Abbas et al. (2015), which are less than the findings of our study. Al Mamun et al. (2019) recorded 17.54% prevalence in layers, which is in support of our study. Geresu et al. (2016) reported a 12.16% prevalence of ND in broilers, which is higher than the findings of our investigations, whereas Islam et al. (2023), Islam et al. (2020), and Hassan et al. (2016) reported a lower 7.54%, 6.2%, and 8.87% prevalence in broiler chickens, respectively.

In layers, highest prevalence was found in the ≥21 weeks age group (21.01%), followed by 15.79% and 9.38%, respectively, in the 9-20 weeks and ≤8 weeks age groups. These investigations support the findings of Islam et al. (2020), who described 13.7% (>20 weeks), 8.5% (9-20 weeks), and 4.2% (0-8 weeks) positive cases of ND in lay

Table 1: Seroprevalence of ND in chickens at Barishal district of Bangladesh during the period of November 2021 to October 2022

Type of birds	No. of birds examined	No. of birds infected	Prevalence (%)	Total birds examined (n)	Total birds infected (n)	Overall prevalence (%)
Layer	227	40	17.62	419	58	13.84
Broiler	192	18	9.38			

Table 2: Seroprevalence and associated factors of ND in layer chickens at Barishal district of Bangladesh during the period of November 2021 to October 2022

Variables	Category	Birds tested		Test results (n)		Seroprevalence (%)		χ2	*P* value
		n	%	P	N	P	N		
Age of chickens	≤8 weeks	32	14.10	03	29	9.38	90.63	2.615	0.270
	9-20 weeks	76	33.48	12	64	15.79	84.21		
	≥21 weeks	119	52.42	25	94	21.01	78.99		
Seasonal influence	Summer season	65	28.63	08	57	12.31	87.69	3.157	0.206
	Winter season	92	40.53	21	71	22.83	77.17		
	Rainy season	70	30.84	11	59	15.71	84.29		
Floor condition of chicks house	Concrete	78	34.36	17	61	21.79	78.21	1.426	0.232
	Mud poultry house	00	00.00	00	00	00.00	00.00		
	Bamboo/ wood	149	65.64	23	126	15.44	84.56		
Flock size (n)	Small (<2000)	118	51.98	25	93	21.19	78.81	2.202	0.333
	Medium (2000-5000)	77	33.92	11	66	14.29	85.71		
	Large (>5000)	32	14.10	04	28	12.50	87.50		
Bird health status	Healthy	109	48.02	21	88	19.27	80.73	5.097	0.078
	Sick	53	23.35	13	40	24.53	75.47		
	Recovered	65	28.63	06	59	9.23	90.77		
Dead birds disposal	Proper	51	22.47	07	44	13.73	86.27	0.688	0.407
	Improper	176	77.53	33	143	18.75	81.25		

n= number, %= percentage, P = Positive, N = Negative, χ2 = Chi-square value

Table 3: Seroprevalence and associated factors of ND in broiler chickens at Barishal district of Bangladesh during the period of November 2021 to October 2022

Variables	Category	Birds tested		Test results (n)		Seroprevalence (%)		χ2	*P* value
		n	%	P	N	P	N		
Age of chickens	≤12 days	43	22.40	02	41	4.65	95.35	2.349	0.309
	13-30 days	87	45.31	11	76	12.64	87.36		
	≥31 days	62	32.29	05	57	8.06	91.94		
Seasonal influence	Summer season	45	23.44	03	42	6.67	93.33	1.481	0.477
	Winter season	66	34.38	05	61	7.58	92.42		
	Rainy season	81	42.19	10	71	12.35	87.65		
Floor condition of chicks house	Concrete	109	56.77	12	97	11.01	88.99	0.841	0.657
	Mud poultry house	24	12.50	02	22	8.33	91.67		
	Bamboo/ wood	59	30.73	04	55	6.78	93.22		
Flock size (n)	Small (<1000)	65	33.85	04	61	6.15	93.85	1.315	0.518
	Medium (1000-4000)	86	44.79	10	76	11.63	88.37		
	Large (>4000)	41	21.35	04	37	9.76	90.24		

Bird health status	Healthy	95	49.48	08	87	8.42	91.58	1.512	0.469
	Sick	52	27.08	07	45	13.46	86.54		
	Recovered	45	23.44	03	42	6.67	93.33		
Dead birds disposal	Proper	34	17.71	02	32	5.88	94.12	0.593	0.441
	Improper	158	82.29	16	142	10.13	89.87		

n= number, %= percentage, P = Positive, N = Negative, $\chi 2$ = Chi-square value

ers. Similarly, Hassan et al. (2016) recorded the highest prevalence of 61.53% in >20 weeks, followed by 23.07% and 15.38%, respectively, in 8-20 weeks and 0-8 weeks age groups in layer chickens. In our study, broiler chickens were found to be the most prevalent for ND in the 13-30 day age group (12.64%), followed by 8.06% and 4.65% in the ≥31 day and ≤11 day age groups, respectively. This report more or less supports the findings of Sabuj et al. (2019), where they reported the highest prevalence of 8.0% at 11-20 days, followed by 6.9% (21-35 days) and 5.3% (1-10 days). However, a different result was found by Das et al. (2018), who reported the highest prevalence (43.8%) of ND at >30 days, followed by 31.2% at 0-15 days and 25% at 16–30 days age groups in commercial broiler chickens.

Based on seasonal variations, ND was found all the year round in layer chickens but was mostly prevalent in winter (22.83%), followed by rainy (15.71%) and summer (12.31%). This finding was in support of that of Islam et al. (2012), where they reported 15.4% (winter), 8.9% (summer), and 4.4% (rainy) prevalence of ND. Similarly, Al Mamun et al. (2019) described a higher prevalence in winter (25%) than summer (15.33%) and rainy (12.02%) seasons in commercial layer chickens. On the contrary, Newcastle disease was found to be higher during the rainy season (12.35%) compared to the winter (7.58%) and summer (6.67%) seasons in broilers. Our study is in agreement with the findings of Meher et al. (2020), who reported the highest prevalence of ND in rainy (35.48%) seasons, followed by winter (16.67%) and summer (12.24%) seasons. Conversely, the highest prevalence (18.21%) of ND was found in winter by Al Mamun et al. (2019), followed by 12.26% and 5.08% in the summer and rainy seasons, respectively.

From this investigation, ND is found mostly prevalent in concrete house (21.79%) than bamboo/wood (15.44%) and mud poultry house (0%) in layer chickens. On the contrary, higher prevalence of ND is recorded 11.01% in concrete house followed by 8.33% and 6.78% in mud poultry and bamboo/wood house respectively in broilers. Prevalence of ND was recorded higher in mud poultry house (40%) by Meher et al. (2020) followed by bamboo (20.78%) and concrete (14.71%) house in broiler farms. This report is not support of our study. But, Belgrad et al. (2019) reported higher prevalence 67.4% in concrete house than 17.1% and 9.3% in mud poultry and bamboo house respectively. These results are more or less similar to the findings of our study.

In this investigation, it was found that the highest prevalence (21.19%) of ND was reported in small-sized flocks (<2000 birds), followed by 14.29% and 12.50%, respectively, in medium-sized (2000-5000 birds) and large-sized (>5000 birds) flocks in commercial layer chickens. To study the flock-wise variation in layer chickens, Das et al. (2018) reported 45.9%, 29.7%, and 24.3% prevalence of ND in those flocks having 1135-2400, >2400, and 0-1134 birds, respectively. This report is in agreement with the findings of our study. The highest prevalence of ND was found 59.6% in the small-sized flock having ≤10000 birds by Sahoo et al. (2022), followed by 7.1%, 6.4%, and 0%, respectively, in the large, medium, and very large-sized flock having 50001-100000, 10001-50000, and >100000 birds. On the other hand, a higher prevalence (11.63%) was recorded in medium-sized flocks having 1000-4000 birds than 9.76% and 6.15% in large-sized (>4000 birds) and small-sized (<1000 birds) flocks, respectively, in broiler chickens. These results more or less support the findings of Sahoo et al. (2022), where they recorded the highest percentage (21.6%) of ND in the large-sized flock (>5000 birds), followed by 11.7% in the medium (1001-5000 birds), and 10.8% in the small-sized flocks (≤1000 birds) in broilers. However, a different finding was observed by Das et al. (2018); they reported the highest prevalence (62.5%) of ND in those flocks having 1135-2400 birds, followed by 31.2% and 6.2% in the flocks having 0-1134 and >2400 birds, respectively.

To investigate the seroprevalence of ND in Barishal district depending on health status of layers, sick birds (24.53%) were found more prevalent for ND than healthy (19.27%) and recovered birds (9.23%). The seroprevalence of ND was reported higher in sick birds (13.46%) followed by healthy (8.42%) and recovered birds (6.67%) in broiler chickens. Ravishankar et al. (2022) reported the highest prevalence in sick birds (10.6%) followed by healthy (7.8%), dead (5.4%) and recovered birds (1.9%). Similarly, Sahoo et al. (2022) revealed that NDV prevalence was higher among sick birds with respiratory signs (12.7%) as compared to apparently healthy (11.5%) or dead birds (10.0%). Sick birds were found more prevalent (15.21%) for NDV than 9.6% and 9.1% respectively in dead and healthy birds recorded by Joshi et al. (2021). These findings are in support

of our study.

From this investigation, higher percentage of ND is found in layers where improper disposal (18.75%) of dead birds is practiced than proper disposal (13.73%) whereas (10.13%) prevalence recorded in those broilers where dead birds are disposed improperly than proper disposal (5.88%) of carcass. Seroprevalence based on dead bird disposal practices, Wodajo et al. (2023) also reported the higher prevalence of ND in those backyard birds where dead birds are either buried or burnt (24.57%) than thrown nearby (22.29%) and thrown a far (6.70%) from the chick's house.

CONCLUSION

In conclusion, the present study revealed a higher prevalence of ND in chickens at Barishal district with an overall seroprevalence of 13.84% (n = 58/419). ND was prevalent almost all year round and the prevalence of the disease increased with the age of the birds. An effective vaccination program is highly recommended to maintain good farm practices as well as to help reduce, prevent, or eradicate the disease. Therefore, further and detailed studies including molecular investigations are required to identify characteristics of different strains of virus and models of transmission for a better understanding of ND epidemiology to develop and execute an effective control program and minimize the economic losses of ND on commercial poultry farms in the study areas.

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ETHICAL STATEMENT

Ethical approval is not required for this study. No animals were killed for the scientific purpose of this study.

CONFLICT OF INTERESTS

The authors have declared that there is no conflict of interests regarding the publication of this article.

NOVELTY STATEMENT

This is the first report that has focused on individual bird level status of Newcastle disease in commercial chickens in Barishal district of Bangladesh using serological technique i.e. Haemagglutination Inhibition (HI) test. The research has also highlighted the association of various factors with the seroprevalence of ND in chickens of the study areas.

AUTHOR'S CONTRIBUTION

This work was carried out in collaboration among all authors. Authors MSI and DAM designed the study, performed the statistical analysis and wrote the protocol. Authors MMK and SZ contributed to sample preparation and initial draft writing of the manuscript. Authors BM, NSR, ZF, TS and MAJ, managed the literature searches, interpretation of the results and critical review. All authors read and approved the final version of the manuscript.

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