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Article

Clinicopathological investigation of Newcastle disease in commercial poultry farms at Bogura district of Bangladesh

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Abstract: Newcastle disease (ND) now seems endemic in commercial poultry industry of Bangladesh with continuous significant high prevalence and regular outbreaks. High morbidity, up to 100% mortality rate and high economic loss of ND makes it major constraints of poultry industries. We find out the prevalence of ND in dead, sick and apparently healthy poultry at Bogura district of Bangladesh. From July 2020 to June 2021, we observed a total of 500 birds including 31 sick and dead birds randomly from 10 commercial poultry farms of Bogura district. The prevalence was assessed through clinical observations and the documented history of individual birds. For pathological analysis, 31 samples from sick and deceased birds were collected, and detailed examinations of gross lesions and histopathological changes in the cecal tonsils and proventriculus were conducted. Wide ranges of clinical signs are observed in affected birds and it can be diagnosed based on clinical signs and pathological studies. The prevalence of Newcastle disease was 14.20% in apparently healthy birds and 26.6% in sick and dead birds. ND prevalence increased in summer (56%) and decreased in rainy season (11.5%) and highest at the age group of (5-36) weeks of age. Grossly, numerous infected birds had lesions such as pin-point hemorrhage on proventriculur gland, hemorrhage in the trachea, congested and consolidated lungs, button-like ulcers in the caeca, and hemorrhagic caecal tonsils. Microscopically, there was hemorrhage in the lamina propria associated with lymphatic nodules of the caecal tonsil, sloughing of glandular epithelial cells and infiltration of inflammatory cells in the lumen of the proventriculur gland, congestion around the lobule of the proventriculus, and hyperemia on glandular epithelial cells of the proventriculur gland. This research revealed Newcastle disease remains endemic in Bangladesh's poultry industry, causing high prevalence, seasonal outbreaks, and severe economic losses.

Keywords: Newcastle disease; prevalence; pathological studies; chickens; Bangladesh

1. Introduction

Commercial poultry business is now a significant driver in the nation’s economic growth and job generations (Hamid *et al*., 2017). The poultry industry in Bangladesh has seen remarkable growth over the past two decades, emerging as the fastest-growing livestock subsector. This sector supports the livelihoods of approximately eight million people, including one million business owners, through direct and indirect employment opportunities (Islam *et al*., 2014). Bangladesh's poultry sector plays a critical role in the nation's economic health and welfare.

It provides meat and eggs and cheapest animal protein source for all social groups (Muhammad *et al*., 2015). It provides 37% of nation's entire meat supply and 22 to 27% of protein needs (Hamid *et al*., 2017). On 2020, estimated annual per capita poultry consumption was 7 kg (One Health Poultry, 2021). Poultry farming makes a substantial contribution to income generation and poverty reduction through self-employment, notably for Bangladesh's suffering women and unemployed youth (Akhter *et al*., 2018).

Poultry disease is one of the greatest hindrances to poultry producers' production and economic growth (Islam *et al*., 2016). About 30% of Bangladesh's chickens die as a result of various disease outbreaks (Badruzzaman *et al*., 2015). Climate, geographic location, farm hygiene, management methods, maintenance of bio-security, immune state, chick quality and hatcheries are vital elements that contribute to the spread of poultry disease (Munir *et al*., 2015; Hassan *et al*., 2016). A variety of deadly infectious and non-infectious illness outbreaks frequently that limit the growth of the chicken industry (Akther and Hassan, 2022).

Within infectious diseases, Newcastle disease is most vital viral disease harming Bangladesh poultry industry (Kumar *et al*., 2016; Sarker *et al*., 2021; Bhuiyan *et al*., 2021; Islam *et al*., 2023). The local name for ND on the Indo-Pak subcontinent is Ranikhet disease; it is highly contagious viral disease caused by Newcastle Disease Virus (NDV), belongs to family Paramyxoviridae. This acute and incredibly deadly viral infection affects a wide variety of domestic and wild bird species (Abdisa and Tagesu, 2017). Chicken is most susceptible to Newcastle disease commonly affected poultry flocks. In Bangladesh, ND outbreak in chicken was first reported in 1981. Since, it is regarded as one of the major disease affecting poultry (Kamal *et al*., 2016; Parvez *et al*., 2017). Newcastle disease appears to be endemic in chickens, with consistent prevalence and frequent outbreaks and however, recent studies have reported varying prevalence rates, including moderate levels in Kishoreganj and Gazipur districts, as well as findings from the Central Disease Investigation Laboratories and domestic chicken populations (Belgrad *et al*., 2018; Mamun *et al*., 2019). ND is also endemic Asia, Africa and part of America subcontinent, with highest ND prevalence 82.3% in Pakistan and 63.5 in Nigeria (Ameh *et al*., 2016; Abdisa and Tagesu, 2017; Rahman *et al*., 2017).

The economic impact, production loss, high mortality and morbidity rate of this Newcastle disease have just recently been make it public concern all around the world. ND can have 100% mortality rate in unvaccinated poultry and production losses. ND has posed a serious economic threat to the poultry industry ever since it was first discovered in 1926 (Akhter *et al*., 2018). In Bangladesh, only in family rearing backyard poultry having annual economic loss of 2561 taka due to facing ND outbreak, and estimated annual national financial loss was US $288.49 million (Khatun *et al*., 2018).

Vital clinical symptoms of ND are weakness, depression, and decrease in egg production, loss of appetite, cyanosis of the comb and wattle, thirst, immobility, greenish watery diarrhea, weight loss, nasal and ocular discharges and high mortality (Nooruzzaman *et al*., 2022). ND major gross lesions includes petechial hemorrhages in digestive system and proventiculus, multifocal button like ulcers with raised borders, severe hemorrhage and consolidation of lungs, hemorrhages in the lymphoid tissues of respiratory and digestive system, especially in cecal tonsil, trachea, air sacs, brain, and spleen (Nooruzzaman *et al*., 2022; Shanmuganathan *et al.*, 2023).

Newcastle disease is a fatal infection prevalent in Bangladesh with varying degrees of severity in context to geographical locations. Considering these facts, the expansion of the chicken industry depends on preventing and treating a severe disease like Newcastle disease infection. To effectively prevent and control Newcastle disease, it must be understood in its current form, most susceptible age group, temporal pattern and prevalence of the disease. We hypothesized ND is endemic in the commercial poultry industry of the Bogura district of Bangladesh, exhibiting significant seasonal and age-related prevalence patterns, along with characteristic clinicopathological manifestations. The research question coined that, what are the prevalence, seasonal patterns, age-related susceptibility, and clinicopathological characteristics of Newcastle disease in commercial poultry farms in the Bogura district of Bangladesh? Therefore, the present study was carried out to investigate the existing prevalence, temporal pattern and age group susceptibility of Newcastle disease at different poultry farms at Bogura district of Bangladesh. This research provides critical insights into the prevalence and pathological features of ND, enabling better disease management strategies, targeted vaccination programs, and mitigation of economic losses in Bangladesh's poultry industry.

2. Materials and Methods

2.1. Ethical approval

No ethical approval was required to conduct this study.

2.2. Study areas and period

A total of 500 birds, including sick and deceased ones, were randomly sampled from several commercial poultry farms in Bogura district, Bangladesh, over a period spanning from July to June, 2021 (Figure 1). Laboratory examinations were carried out at the Department of Veterinary and Animal Sciences under the Faculty of Veterinary and Animal Sciences at Rajshahi University. The study focused on identifying sick and deceased chickens in the area. Representative tissue samples, specifically from the caecal tonsils and proventriculus, were collected from naturally occurring cases of illness and infection in chickens from various commercial poultry farms (Grameen Poultry and Hatchery, Babu Broiler House, Ismail Store, Ashikur Layer House, Nur Islam Farm, Jony Farm, Sentu Farm, Milon Poultry Farm, Bablu Farm, and Shamim Farm).

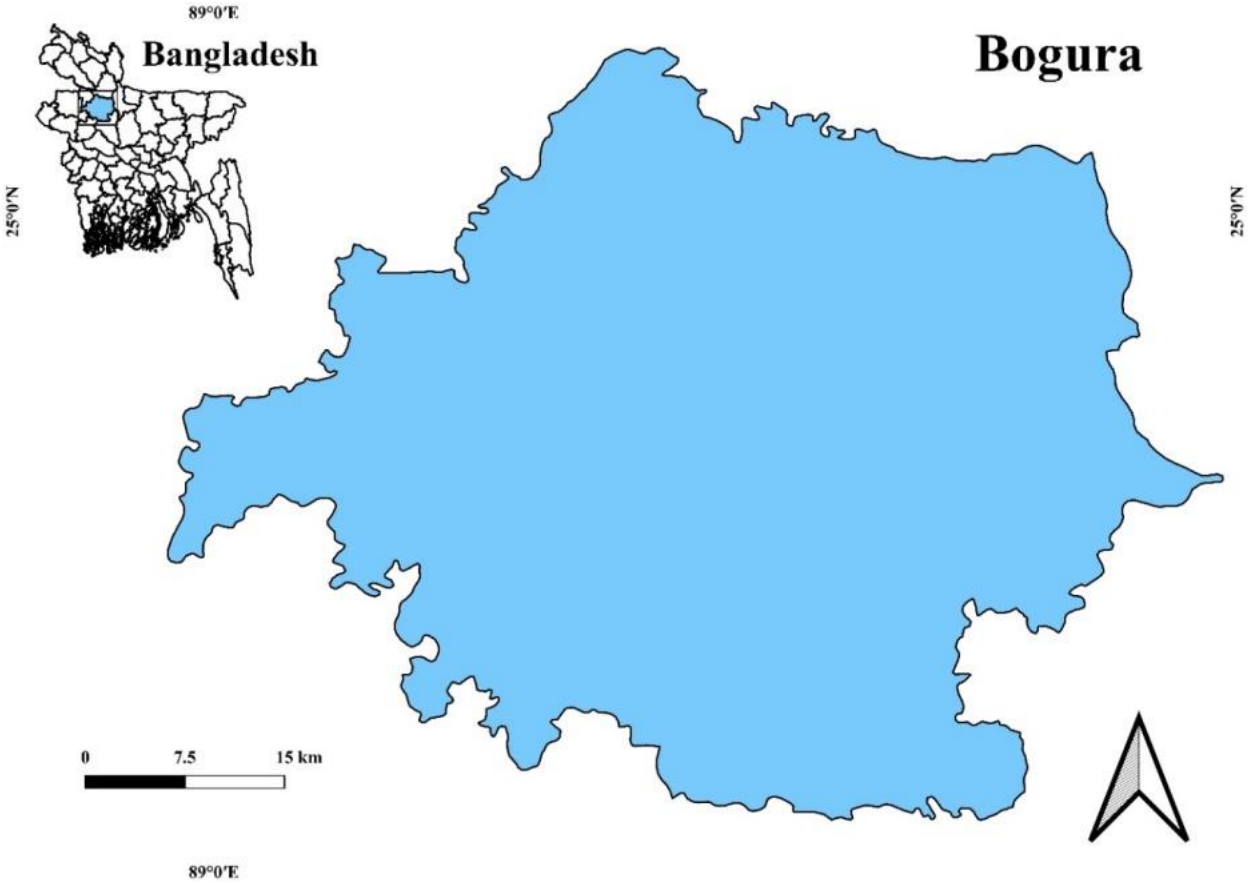

Figure 1. Samples were collected from different poultry farms of Bogura district, Bangladesh.

2.3. Clinical observations

The general health condition of the chickens was recorded. The clinical signs were observed and recorded during the physical visit of the farms and the farmer's complaints in respect of the diseases were also emphasized.

2.4. Pathological findings

The affected birds were brought to the Veterinary Pathology laboratory immediately after clinical observation for necropsy examination. Postmortem examination was performed as per standard procedure. The visible gross morbid lesions were recorded systematically and preserved at 10% neutral buffered formalin solution, following fixation in 10% neural buffered formalin, samples are dehydrated in a series of ascending grades of alcohol, cleared in several changes of xylene and infiltrated with different grades of melted paraffin in the oven at 56 ˚C. The tissues will be then embedded in paraffin and finally the sections will be cut at 5-µm thickness using rotatory microtome (using a photomicrographic kriis, Germany, model-MBL 2100, Camera Labomud, Inc. USA). Then the sections will be stained with Hematoxylin and Eosin (H&E) staining method (Bancroft and Gamble, 2007). The finally prepared slides were examined under microscope using various objectives and typical histopathological lesions were recorded.

2.5. Diagnosis of disease

The disease was diagnosed on the basis of owner's statement, clinical signs observed and laboratory diagnosis with gross tissue changes and characteristic microscopic features of disease.

2.6. Statistical analysis

The results were presented as means ± SD. Statistical differences in infected and non-infected parameters were evaluated via *t*-test (SPSS version 26.0, a statistical package, SPSS Inc). $P<0.05$ was regarded as statistically significant in each case. The map of the study site was prepared using QGIS version 3.22. In this study the prevalence of disease was calculated by the following statistical formula-

$$\text{Prevalence } (\%) = \frac{\text{NDV infected birds during specified time period}}{\text{Total birds during the same time period}} \times 100$$

3. Results

3.1. Prevalence and frequency of different clinical signs in ND

The overall prevalence of Newcastle disease was 14.20% in apparently healthy chickens and 26.65% of sick and dead chickens. Whereas 15.38% in cecal tonsils, 33.33% in proventriculus out of 31 sick and dead chickens were infected by ND (Tables 1 and 2).

Table 1. Prevalence of Newcastle disease in apparently healthy birds at different poultry farms.

Farm numbers	No. of birds randomly examined	No. of (+ve) cases	Prevalence of the cases at different farms (%)	Total no. of (+ve) cases	Overall prevalence among examined cases (%)	*P*-value
1	50	11	22	71	14.20%	0.004**
2	50	7	14			
3	50	8	16			
4	50	6	12			
5	50	8	16			
6	50	6	12			
7	50	5	10			
8	50	7	14			
9	50	7	14			
10	50	6	12			

S = Significant at level ($P<0.05$)

Table 2. Prevalence of Newcastle disease in different organs of sick and dead birds.

Samples	No. of samples examined	No. of (+ve) cases	Prevalence of cases from different organs (%)	Total no. of (+ve) cases	Overall prevalence (%)	*P*-value
Cecal tonsils	13	2	15.38	8	26.6%	0.0005***
Proventriculus	18	6	33.33			

S=Significant at level ($P<0.05$).

The prevalence of NDV was highest at the age of (5-36) weeks of age and lowest at the age of (0-40) days old (Table 3). The prevalence of NDV was highest 56% in summer season and lowest 11.5% in rainy season (Table 4).

Table 3. Prevalence of ND on the basis of age.

Age	Percentage	*P*-value
0-40 days	10.6%	
(5-36) weeks	54.2%	0.06ns
more than (5-36) weeks	35.1%	

NS = Non-significant at level ($P>0.05$).

Table 4. Prevalence of ND on the basis of season.

Season	No. of samples	No. of NDV affected birds	Percentage	*P*-value
Summer	320	180	56%	0.0002***
Winter	140	26	18.5%	
Rainy	26	3	11.5%	
Autumn	14	2	14%	

S = Significant at level (P<0.05).

Among the different clinical signs, depression, off-feed, greenish watery diarrhea and dehydration were detected in all infected birds (100%). Other signs were ruffled feather; sneezing and vent picking were observed in 72%, 16% and 48%, respectively (Figure 2).

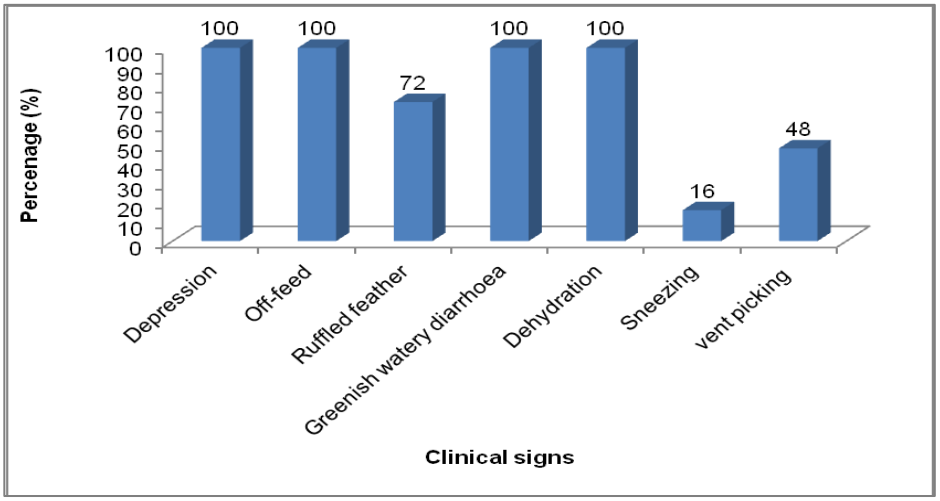

Figure 2. Frequency of different clinical signs and symptoms observed in sick and dead birds.

3.2. Postmortem observations of ND

To determine the gross pathological lesions of ND, necropsy examination was performed. The frequencies of gross pathological lesions in ND infected birds were presented in (Figure 3). Among the different postmortem observations, the highest percentage of birds showed pin point hemorrhage at the tip of the proventriculur gland (Figure 4), hemorrhages in the trachea (Figure 5b), congestion and consolidation of lungs (Figure 6a), button like ulcer in the caeca (intestine) (Figure 6b), and inflammation and hemorrhages in cecal tonsils (Figure 6c).

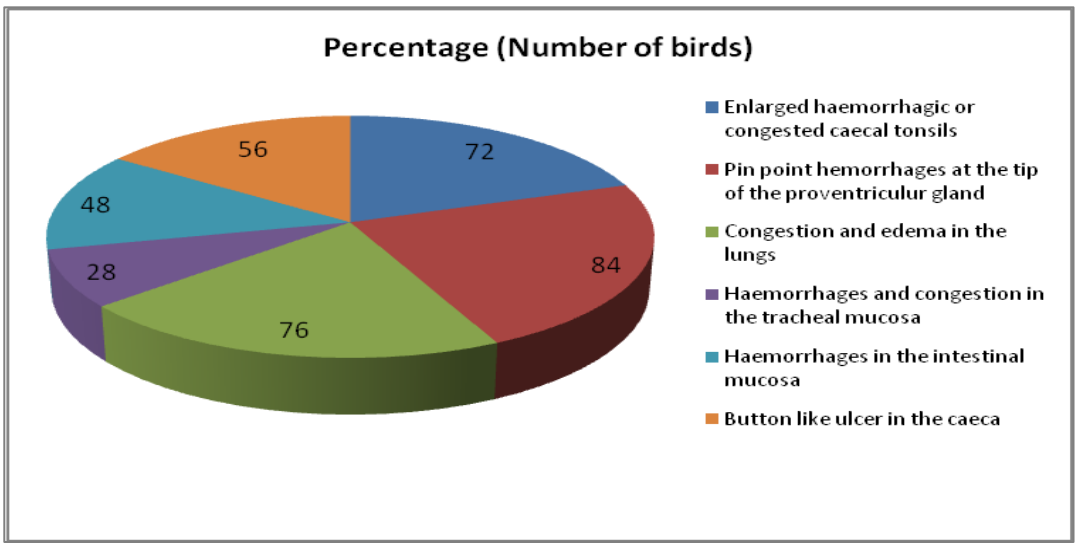

Figure 3. Percentage of gross lesions observed in Newcastle disease affected chickens.

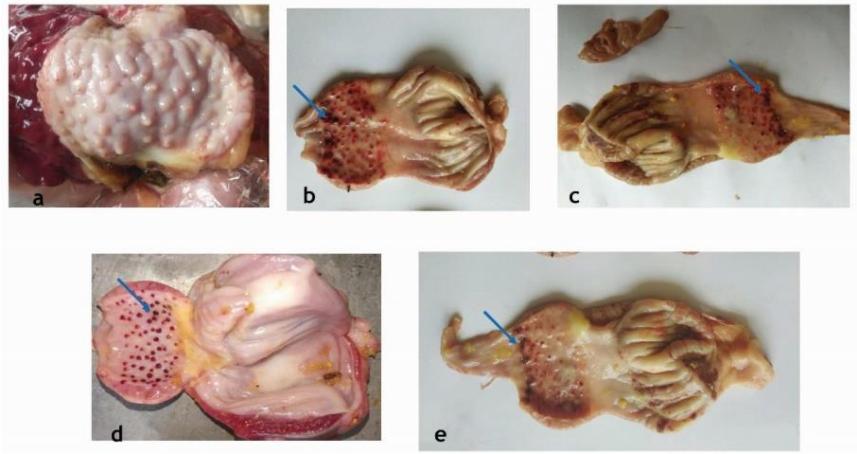

Figure 4. (a) 40-day-old bird showing normal appearance of proventriculus, (b) 50-day-old bird showing hemorrhages at the tip of the proventriculur glands (arrow), (c) 42-day-old bird showing pin point hemorrhage in the proventriculur gland (arrow), (d) 8-week-old bird showing pin point hemorrhage at the tip of the proventriculur gland (arrow) and (e) 45-day-old bird showing pin point hemorrhage at the tip of the proventriculur gland (arrow).

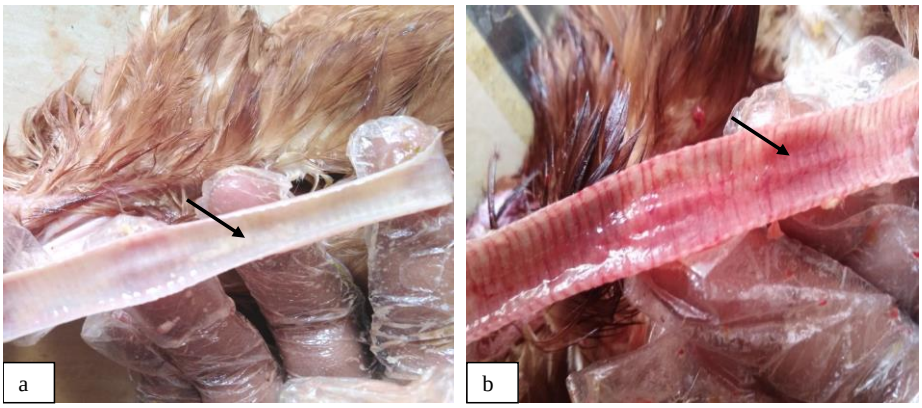

Figure 5. (a) 42-day-old bird showing normal appearance of trachea and (b) 9-week-old bird showing hemorrhages in the trachea (arrow).

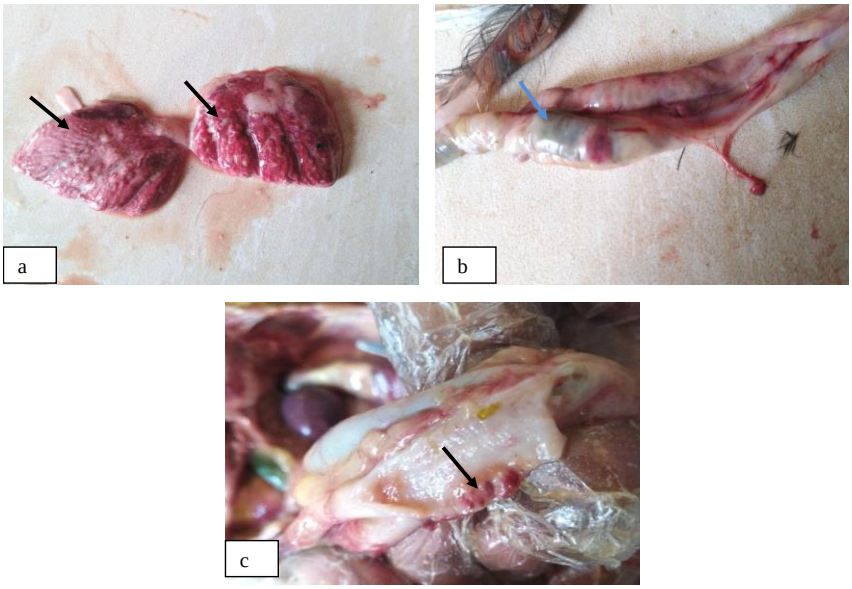

Figure 6. (a) 32-day-old bird showing congestion and consolidation of lung (b) 12-week-old bird showing button like ulcers in the caeca (intestine) (arrow) and (c) 10-week-old bird showing inflammation and hemorrhage of the caecal tonsil (arrow).

3.3. Histopathological lesions of ND

Histopathological examination of the section of proventriculus of ND affected bird of 14-week-old showing infiltration of inflammatory cells in the lumen and sloughing of glandular epithelial cells (Figure 7), 16-week-old bird of the section of cecal tonsils showing hemorrhage in the lamina propria and associated with lymphatic nodules (Figure 8a), and hemorrhage with infiltration of inflammatory cell in cecal tonsil (Figure 8b), and 9-week-old bird of the section of proventriculus showing congestion surrounding the lobule (Figure 9a), necrosis and infiltration of inflammatory cells (Figure 9b) and hyperemia on glandular epithelial cells of proventriculur gland (arrow) (Figure 9c).

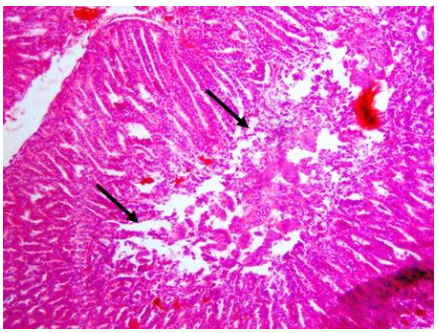

Figure 7. The section of proventriculus of 14-week-old bird showing infiltration of inflammatory cells in the lumen and sloughing of glandular epithelial cells of proventriculur gland (arrow) (H & E, 10X).

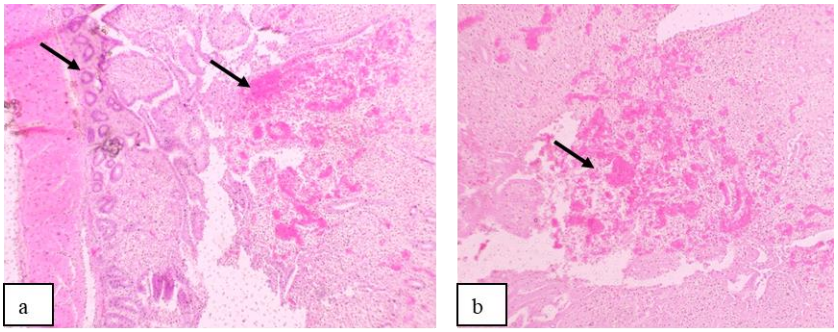

Figure 8. (a) The section of cecal tonsil of 16-week-old bird showing hemorrhage in the lamina propria and associated with lymphatic nodules (arrow) and (b) showing hemorrhage and infiltration of inflammatory cells (arrow) (H & E, 4X).

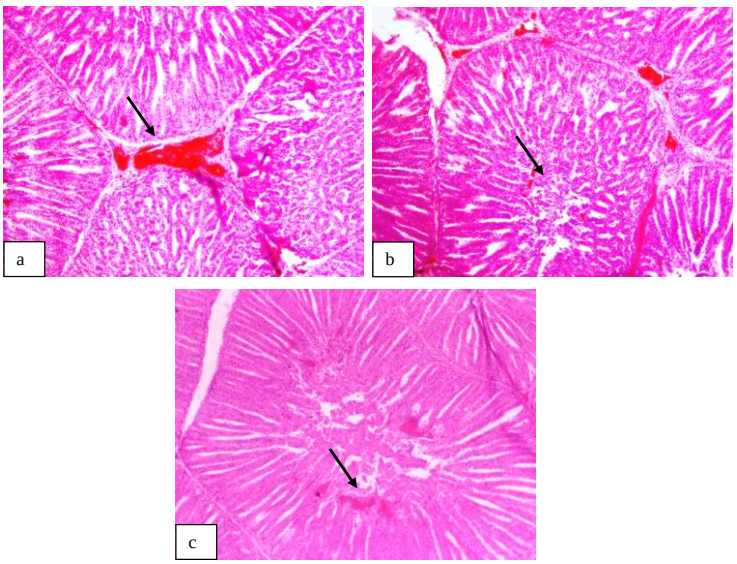

Figure 9. (a) The section of 9-week-old bird proventriculus showing congestion surrounding the lobule (arrow), (b) presence of necrosis and infiltration of inflammatory cells (arrow) and (c) hyperemia on glandular epithelial cells of proventriculur gland (arrow) (H & E, 4X & 10X).

4. Discussion

The Newcastle disease is highly contagious in poultry, with mortality and morbidity, causing heavy economic impact on food security in developing countries, like Bangladesh (Triosanti *et al*., 2018). The main goal of the current investigation was to find out the prevalence, clinical signs; gross and histopathological lesions of ND affected birds in commercial farms of Bogura district. The overall prevalence of Newcastle disease was 14.20% in apparently healthy and 26.65% of different tissues of sick and dead birds at study area. The prevalence of ND in apparently healthy chickens was lower than in sick and dead birds, suggesting that while the disease may not show obvious clinical signs in healthy birds, they can still harbor the virus and potentially contribute to the spread. In contrast, a higher prevalence was found in sick and deceased chickens, which is expected since ND typically causes severe symptoms in infected poultry, leading to higher morbidity and mortality. Additionally, tissue-specific infection rates were observed in the cecal tonsils and proventriculus of sick and dead chickens. The proventriculus exhibited a higher rate of infection compared to the cecal tonsils, which could point to a more significant role of the proventriculus in the progression of the disease. This result is relatively close to the reports of Mamun *et al*. (2019) who reported 11.78% prevalence of ND in poultry at Kishoregonj district, Talukdar *et al*. (2017) who reported 14.20% prevalence in Dhaka. However, the findings of Uddin *et al*. (2010); Meher and Allan (2020) who reported 8.92% and 9.83% prevalence, were considerably lower than that of the present study. The differences among these results may be due to epidemiological factors, such as seasonal variations, geographical location, age and breed of the birds, as well as the commercial chickens' immunity.

The prevalence of NDV was highest at the age of (5-36) weeks of age and lowest at the age of (0-40) days old. This suggests that chickens within the age group of 5 to 36 weeks are more susceptible to NDV, likely due to a combination of factors such as immune system development, exposure to the virus, and potential management practices on farms. In contrast, younger birds under 40 days old showed lower prevalence, which may be attributed to maternal immunity transferred through eggs or the fact that these birds may not yet be fully exposed to the virus. On the other hand ND was highly associated with the age of birds, and aged birds had a relatively higher chance of having ND than relatively younger birds (Sumona *et al*., 2020; Sarker *et al*., 2021).

The prevalence of NDV is highest 56% in summer, 18.5% in winter season and lowest 11.5% in rainy season. We found that ND occurred more frequently in summer and winter seasons than in any other seasons, which suggests that chickens are more prone to ND during the summer and winter seasons. This seasonal variation suggests that environmental factors, such as temperature, humidity, and farm management practices, may influence the spread of NDV. Higher temperatures and possibly lower humidity during the summer could create more favorable conditions for the virus to thrive and spread among poultry populations. In contrast, the rainy season, characterized by cooler temperatures and potentially higher humidity, may limit the virus's spread or reduce its impact, possibly due to changes in farm management or reduced exposure to environmental stressors. It's possible that the immune system can't function properly during the hottest and coldest months due to heat and cold stress (Kokolus *et al*., 2014; Messmer *et al*., 2014). The seroprevalence of ND was higher in the rainy season and lower in the summer season for both broiler and layer chickens (Akther and Hassan, 2022).

The clinical signs observed, such as depression, weakness, off-feed, greenish watery diarrhea, dehydration, ruffled feathers, sneezing, and vent picking. These symptoms are characteristic of Newcastle Disease and serve as primary indicators of infection. Other signs, such as ruffled feathers, sneezing, and vent picking, were observed in varying proportions of the infected birds, suggesting they are less consistent but still relevant in diagnosing the disease. The frequency of these additional symptoms highlights the variability of clinical manifestations in NDV-infected poultry, reinforcing the importance of considering a range of signs when diagnosing and managing the disease. The findings of the present study are consistent with the findings of Nooruzzaman *et al*. (2022); Getabalew *et al*. (2019) and Abdisa and Tagesu (2017). Clinical signs of ND like depression, lethargy, ruffled fur, swelling of the eyelid and tissues of the head and torticollis, tracheal rales were also mentioned by Indi Dharmayanti *et al.* (2024) and Mazlan *et al.* (2017).

Apparently unwell and deceased birds were subjected to necropsy and histological examination in order to describe the disease. The observed pathological lesions were pin point hemorrhages in the proventriculur gland, hemorrhages in the trachea, congestion and consolidation of lungs, button like ulcer in the caeca and inflammation with hemorrhage in cecal tonsils. Sloughing of glandular epithelial cells, hemorrhage with infiltration of inflammatory cells in the lumen, congestion, necrosis and hyperemia on glandular epithelial cells of proventriculur gland. Hemorrhage in the lamina propria and infiltration of inflammatory cells in cecal tonsils. Among the various postmortem observations, the most frequently observed lesion was pinpoint hemorrhage at the tip of the proventricular gland. Additionally, hemorrhages were noted in the trachea, alongside congestion and consolidation of the lungs, indicating severe respiratory involvement. The presence of button-like ulcers in the caeca and inflammation with hemorrhages in the cecal tonsils were also common findings. These lesions are

characteristic of Newcastle Disease and reflect the systemic nature of the infection, affecting multiple organ systems. The findings are support with the findings of others (Abdisa and Tagesu, 2017; Das *et al*., 2018; Sedeik *et al*., 2019; Dzogbema *et al*., 2021; Nooruzzaman *et al*., 2022). The observed lesions and histopathological changes in infected birds serve as crucial diagnostic markers for effective disease identification and management. Understanding these aspects is vital for developing targeted prevention and control measures, improving biosecurity practices, and ensuring the long-term growth and sustainability of the poultry sector in the region (Rashid *et al*., 2018; Islam *et al*., 2019; Trisha *et al*., 2021; Alam *et al*., 2021).

5. Conclusions

The prevalence of Newcastle disease was higher in sick and dead birds compared to apparently healthy birds. The disease was most prevalent in older birds and during the summer season. Key clinical signs included depression, ruffled feathers, greenish watery diarrhea, and reduced feed intake. Common gross lesions observed were pinpoint hemorrhages in the proventricular gland, tracheal hemorrhages, and button-like ulcers in the caeca. Histological findings revealed inflammatory cell infiltration, epithelial sloughing, and congestion in the proventricular gland and cecal tonsils.

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Data availability

All related data are within the manuscript.

Conflict of interest

None to declare.

Authors' contribution

Nazmun Nusrat Farhana collected samples and data, conducted the experiments, and wrote the initial draft of the manuscript. Rashida Khaton designed the experiment, supervised the study, and corrected the manuscript. All authors have read and approved the final manuscript.

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