

## Comparative histomorphology of non-affected and affected lymphoid organs by Newcastle disease in broiler chicken

Md. Najmul Hassan Parvez<sup>1\*</sup>, Md. Aktharul Alam<sup>1</sup>, Md. Royhan Gofur<sup>2</sup> and Khadija Al Ferdous<sup>1</sup>

<sup>1</sup>Department of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

<sup>2</sup>Department of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi, Bangladesh

### Abstract

**Context:** Newcastle disease (ND) remains a constant threat to the poultry industry and a limiting disease for poultry producers worldwide including Bangladesh.

**Objectives:** The study was conducted to compare histomorphology of non-affected and affected lymphoid organs by Newcastle disease in broiler chicken (Cobb 500) in small scale commercial poultry farms at northern part of Bangladesh

**Materials and Methods:** A total of ten farms having 300-700 birds (broiler chicken) have been selected for the present study where total number of birds was 5000. Lymphoid organs (spleen, thymus, cecal tonsil and bursa of Fabricius) of both healthy and suspected ND affected broiler chicken were studied. Organs were cleaned with normal saline solution and the changes were recorded. Immediately after necropsy, organs were fixed in 10% formalin, then processed and studied microscopically using Hematoxylin and Eosin staining.

**Results:** The prevalence and mortality were recorded as 47% and 34.12% respectively and mortality was 1.52 times higher in non-vaccinated birds. Marked depression, prostration, drooping wings, muscle tremor, greenish white diarrhea, edema of the head, inactive, weak, accompanied by the nervous signs like twisted necks (torticollis) with high mortality were the major clinical signs. The necropsic changes were swollen and mottled spleen, enlargement of cecal tonsil with hemorrhage, swollen bursa, thymic enlargement etc. Necrosis and lymphocytic degeneration in the spleen, nuclear pyknosis and macrophages infiltration in spleen, hemorrhagic ceacal tonsils and congestion in the mucosa of the tonsil, lymphoid depletion, lymphocytolysis, glandular transformation of epithelium of bursa, medullary lymphocytolysis in thymus were the common histopathological changes in broiler chicken affected with Newcastle disease.

**Conclusion:** Newcastle disease is still among the most prevalent diseases of chicken in the study area. Clinicopathologic findings will provide some bases for ND diagnosis. However, vaccination, improved management and bio-security are important for sustainable and profitable poultry farming in Bangladesh.

**Keywords:** Newcastle disease, lymphoid organs, histomorphology, broiler chicken

### Introduction

Newcastle Disease (ND) in domestic poultry is a focus for concern throughout much of the world's agricultural community because of severe economic losses that have occurred from illness, death, and reduced egg production following infection with pathogenic or disease causing strains. ND is a highly contagious and fatal disease of domestic poultry, caged birds and wild birds, caused by a haemagglutinating type-1 avian paramyxovirus belonging to genus rubulavirus. It has a worldwide distribution, although it has been eradicated from some countries. However, the reported chronic occurrence of ND virus infection in psittacine and wild birds (Collins, *et al.*, 1994) raises the possibility of the disease being introduced at any time by wild birds to the local and commercial chickens, even in countries free of the disease. The disease can be confused with highly pathogenic avian influenza, fowl cholera, infectious bursitis and infectious laryngotracheitis which show similar clinical signs (Ojok, 1993). It has been considered as one of the important viral diseases of poultry worldwide. ND and highly pathogenic avian influenza, these two poultry diseases are considered to be sufficiently serious to be included in List A of the Office International des Epizooties (OIE, 1996). It is an acute highly contagious viral disease of various species of birds of all age groups (Alexander, 2000) characterized by respiratory, digestive and nervous signs (Mishra *et al.*, 2000). The virulence of ND strains varies greatly with the host, but breed or genetic stock does not appear to have a significant affection on the susceptibility of chickens to the disease (Huang *et al.*, 2003). It causes considerable

economic losses to the poultry industry due to stress, high mortality, morbidity, decreased egg production and hatchability throughout the world (Alexander, 2000).

ND has been recognized as one of the major problems of the large and small scale poultry industries in Bangladesh (Islam *et al.*, 1998; Rahman and Samad, 2003) and it causes up to 40-60% of total mortality among poultry population in Bangladesh (Talha *et al.*, 2001). The transmission of NDV occurs through newly introduced birds, selling or giving away sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing (Tu *et al.*, 1998). Due to its worldwide occurrence, ND drew the attention of research workers for an effective control program. Vaccination along with strict bio-security measures are considered as effective means for its control. Therefore, the practice of vaccination became a routine work throughout the world (Rahman *et al.*, 2002). Vaccination as a means to prevent the disease is routinely practiced in Bangladesh (Samad, 2000). However, outbreaks of ND have been occurring frequently due to failure of vaccination resulted from high level of maternal antibody titre in young chickens which neutralizes vaccine virus and heterogeneous level of antibody titer in adult birds (Rahman *et al.*, 2002). Unfortunately, the prognosis for this disease is poor, with nearly a 100 percent mortality rate in chickens, once infected (Alexander, 2000).

ND virus usually enters the body via the respiratory system and/ or the intestinal tract. Following multiplication, it spread rapidly into the bloodstream where the virus is carried by the blood to different organs including lymphoid organs (Alexander,

\* Corresponding author: nhparvez76@gmail.com

1988). Histo-pathological changes found in ND virus infection may vary greatly. Among changes, depletion of lymphoid tissues and haemorrhagic and necrotic lesions in organs occur in most descriptions of natural and experimental infections (Levy *et al.*, 1975; Mohammadamin and Qubih, 2011). Correct disease diagnosis, and knowledge about the frequency of occurrence of any disease in an area in a given period (prevalence), is a pre requisite for instituting effective and reliable disease control and prevention measures (Ojok, 1993).

As the gross lesions and organs affected in birds infected with ND virus are dependent on the strain and pathotypes of the infecting virus, in addition to the host and other factors that affect the severity of the disease (Saif *et al.*, 2003), the comparative study between ND affected and non-affected broiler chicken is vital as to confirm ND diagnosis, describe and document the lesions, especially in lymphoid organs in broiler chicken, based on necropsy and histopathology technique. Therefore the present study was designed with an aim to investigate the histomorphology of lymphoid organs of ND affected and non-affected broiler chicken in northern part of Bangladesh.

## Materials and Methods

### Animals

A total of ten farms having 300-700 birds (broiler chicken; Cobb 500) in different Thana of Dinajpur district of Bangladesh have been selected for the present study where total number of birds was 5000.

### Necropsy

A representative number (n=15 from each farm) of broiler chickens was killed by cervical subluxation for gross and microscopic examination. Immediately after killings, the chickens were examined for the gross lesions reported by earlier authors (Alexander, 2000; Mishra *et al.*, 2000; Cattoli *et al.*, 2011; Jaganathan *et al.*, 2015) and then the lymphoid organs (spleen, thymus, cecal tonsil and bursa of Fabricius) were collected and washed in normal saline solution and the changes were recorded. Immediately after gross examination and necropsy, organs were fixed in 10% formalin.

### Histopathology

After fixation, the selected samples were processed in the laboratory following standard histological method (Gridley, 1960) and embedded on paraffin. The paraffin sections were then cut at 6  $\mu$ m thickness using microtome machine (Mu 509, Euromex, Japan). After cutting, the sections were floated on luke-warm water in a floatation bath at 37°C for stretching, then the sections were attached on cleaned glass slides using Mayer's egg albumin and dried on a hot plate of slide warmer boxes. The sections were then stained with Hematoxylin and Eosin stain (Gridley, 1960) for histomorphological study.

### Statistical analysis

Data were analyzed by student's *t*-test with the help of SPSS version 20. The values for prevalence and mortality were represented as percentage (%).

## Results and Discussion

Gross and histopathological observations of lymphoid organs (spleen, thymus, cecal tonsil and bursa of Fabricius) of healthy and Newcastle disease affected broiler chickens (Cobb 500) in

small scale commercial poultry farms at northern part (Dinajpur district) of Bangladesh were studied and different clinical, gross and histopathological features were recorded and compared between those of healthy and ND affected broiler chickens.

### Clinical findings

The signs of healthy (Fig. 1a) and ND affected (Fig. 1b-d) birds were recorded. The clinical signs of the birds affected with ND varied from farm to farm and were chicken with marked depression (Fig. 1d), prostration, drooping wings, muscle tremor, greenish white diarrhea, edema of the head, inactive, weak, accompanied by the nervous signs like twisted necks (torticollis) (Fig. 1c) with high mortality reaching 100%. Sometime complete inability to make sound. Major clinical findings, we observed in this study, are almost similar as reported by earlier authors (Samad, 2000; Mishra *et al.*, 2000; Alexander, 2000).

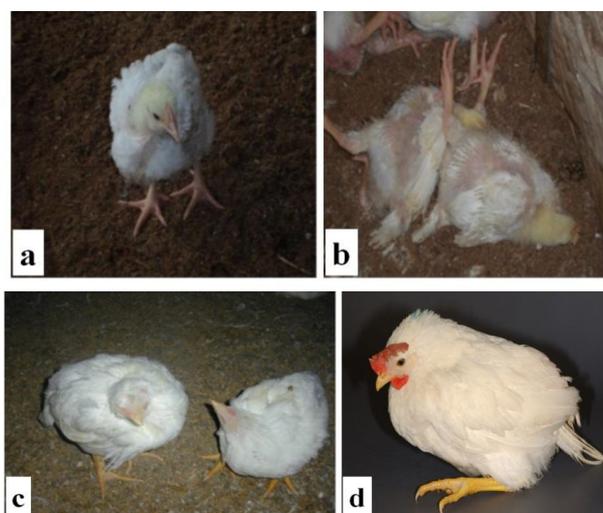


Fig. 1. a) Non-affected bird; b) affected birds with ND; c) torticollis due to CNS infection by ND; d) the bird stands on its hock joints, a sign of generalized depression

### Status of prevalence and mortality of the disease

The study revealed the status of prevalence and mortality of Newcastle diseases in broiler chickens at northern part of Bangladesh. A total of 5000 birds from ten farms were examined during the study period from which 2350 birds of four farms were found infected with ND. The Prevalence of ND was in 47% and the rate of mortality was 34.12%. The rate of mortality was significantly ( $p < 0.05$ ) lower in vaccinated flock (27.92%) than in non-vaccinated flock (42.25%) (Table 1).

Prevalence of ND at different commercial broiler farms in northern part of Bangladesh was 47% (Table 1) which is almost similar as reported by Rahman *et al.* (2012). They studied the prevalence of ND in different districts of Bangladesh and reported that the prevalence of NDV in Dhaka, Gazipur, Mymensingh and Bogra districts were 50.0%, 45.0%, 25.0%, and 32.5%, respectively with an average 32.5% in broiler chicken. Our result also supports the findings in abroad reported by different authors like El-Yuguda *et al.* (2007) stated that 46% prevalence in Borno state of Nigeria and Khan *et al.* (2011) stated that 33% prevalence in Faisalabad, Pakistan.

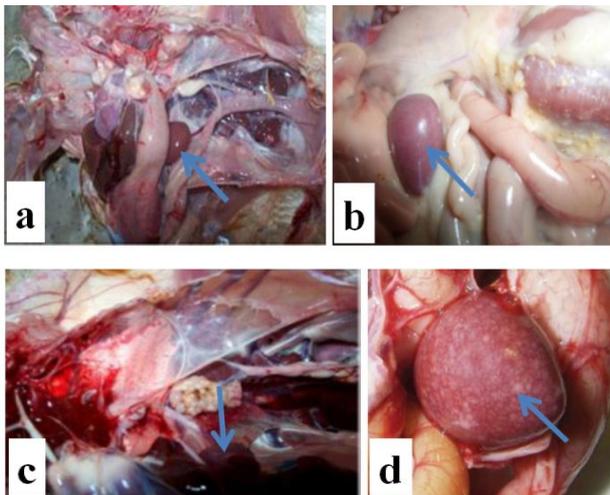
Hossain *et al.* (2010) reported that ND still occurs in Bangladesh every year in the form of epidemic and appears to cause up to 40-60% of the total mortality in poultry population creating one of the major problems in the development of poultry industry in Bangladesh. The mortality rate (34.12%) found in the present study somewhat below the range reported by Hossain *et al.* (2010). Khan *et al.* (2011) stated that 54% mortality in Faisalabad, Pakistan. In case of non-vaccinated birds mortality is higher (42.5%) comparatively than vaccinated birds (27.92%) due to NDV. The rate of mortality was 1.52 times higher in non-vaccinated birds. Result is similar with Barman *et al.* (2010) stated that the rate of mortality was 1.5 times higher in non-vaccinated birds compared to vaccinated birds for NDV.

**Table 1.** Status of prevalence and mortality of ND in the studied area of Bangladesh

No. of birds studied	No. of birds affected	Prevalence (%)	No. of birds affected	No. of birds died	Vaccination status	Mortality (%)
5000	2350	47	1000	425	Given	42.50
			1350	377	Not given	27.92
		Total	2350	802	-	34.12

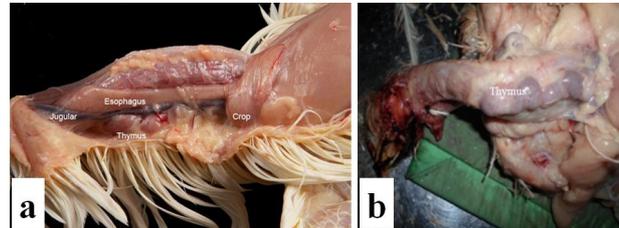
### Necropsy findings

There was brownish red colored spleen (Fig.2a) in the healthy chicken whereas remarkable swollen spleen with white sub-capsular necrotic foci in the spleen (Fig. 2b) of broiler chicken affected with ND has been found in maximum cases in necropsy of present study. This type of foci has been observed in all ages of broiler chicken those were affected by ND. The spleen became slightly blackish in a color (Fig. 2c) in few ND affected birds. But such kind high swollen didn't occur in case of early ages of ND affected birds. Spleen was found in few cases as mottled with visible necrotic foci (Fig. 2d). The gross pathological lesions found in this study are similar with the findings of Cattoli *et al.* (2011) who stated that swollen and mottled spleen with blackish in color and necrotic foci was the most common in case of NDV affected birds. Our findings are also similar to the observations of Banerje *et al.* (1994) noted swollen spleen with necrotic foci in the sub capsular area in the same ND affected bird.



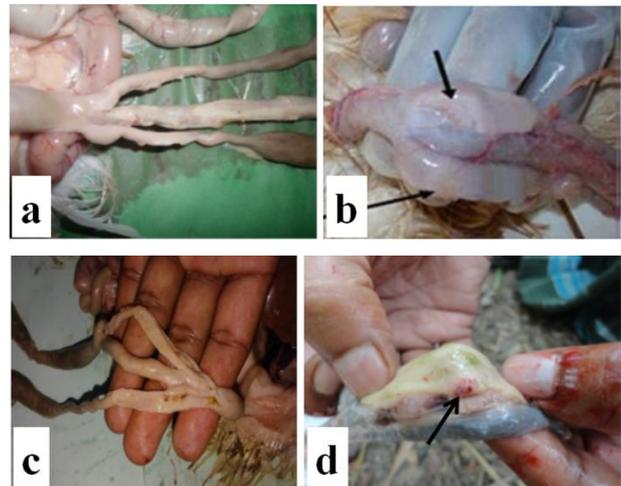
**Fig. 2.** a) Non-affected spleen (arrow); b) swollen spleen with white necrotic foci (arrow); c) blackish spleen of affected bird (arrow); d) mottled spleen with necrotic foci (arrow)

The paired thymus of the chicken consisted of a series of separate, pale red or yellowish irregular lobes. In the adult, there were three to eight such lobes, of varying size and shape (Fig. 3a), extending along each jugular vein as far as the thyroid gland. Enlargement of thymus (Fig. 3b) was the most common and remarkable findings in broiler chicken affected with ND virus, is similar to the study of Jaganathan *et al.* (2015) who also observed the thymic enlargement occurred in NDV affected birds.



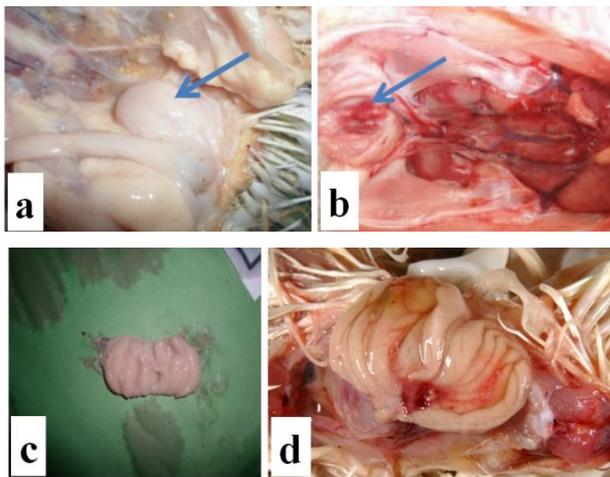
**Fig. 3.** Thymus of a) non-affected and b) ND affected broiler chicken

Cecal tonsils were found in the proximal one third of the paired tubular cecum, which laid along each side of the large intestine (Fig. 4a). They were broad tubular in shape. The shape of the cecal tonsils of broiler was similar to the report of Getty (1975) in chicken. Enlargement of ceca/cecal tonsils (Fig. 4b) was highly remarkable in case of adult ND affected birds compared to normal ceca (Fig. 4a). In some cases bilateral enlargement (Fig. 4b) of cecal tonsils observed in young chicken. Mishra *et al.* (2000) and Okoye *et al.* (2000) reported the enlargement of cecal tonsils in ND affected birds. In addition to the enlargement, hemorrhage (Fig. 4d) was also observed in the cecal tonsils in ND affected broiler chicken whereas no hemorrhage was observed in normal caecal mucosa (Fig. 4c). Although the Newcastle disease does not have lesions pathognomonic to it, typical lesions are proventricular haemorrhage, most commonly seen in the inner surface near the junction with the ventriculus, and in the caecal tonsils (Mishra *et al.*, 2000; Okoye *et al.*, 2000). We also found the hemorrhage at the inner surface of caecal tonsils. Greenish diarrhea which is also similar with the findings of Alexander (2000).



**Fig. 4.** a) Non-affected cecal tonsil; b) bilateral enlargement (arrow) of cecal tonsils of affected bird; c) mucosa of non-affected cecal tonsil; d) hemorrhage (arrow) in mucosa of cecal tonsil of ND affected bird

The bursa of Fabricius of broiler chicken is a single lympho-epithelial organ which is peculiar to birds. The organ appeared as a dorsal median diverticulum of the proctodeum, being smooth and globular in shape and yellowish white in color (Fig. 5a). There was a remarkable swollen (Fig. 5b) in bursa of Fabricius has been occurred in adult birds of 30 days of age but not clearly visible in case of early ages of broiler chicken affected with ND virus. Color has also been changed from yellowish to slightly reddish (Fig. 5b) when affected by ND virus. When open, hemorrhage and yellowish necrotic debris (Fig. 5d) like substance has been observed in ND affected birds whereas the mucosa of healthy birds was clear (Fig. 5c). Our findings are supported by the observation of Nkiruka (2014) reported that the color of bursa of Fabricius of the broiler chicken becomes reddish due to ND.



**Fig. 5.** Bursa of Fabricius (arrow) of a) non-affected and b) ND affected broiler chicken and c) inner layer of bursa of non-affected broiler and d) inner layer of bursa of ND affected broiler chicken with hemorrhage and debris

#### Histological features of lymphoid organs of healthy broiler chicken

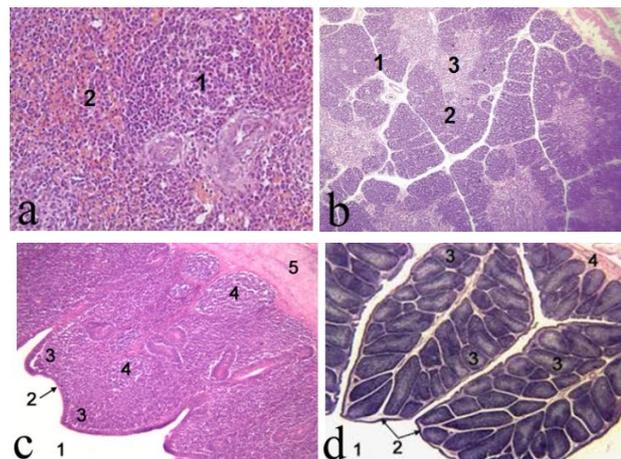
The spleen of the broiler chickens was covered by a thick fibrous capsule with very few trabeculae. The parenchyma consisted of red and white pulps. The red pulps were less distinct and these were scatteredly distributed within the white pulp (Fig. 6a). The white pulp was composed of network of reticular cells and reticular fibers within which small, medium and large sized lymphocytes and plasma cells were diffusely distributed. It contained sheathed arteries and lymphatic nodules. The red pulp of the spleen was formed from venous sinuses and anastomosing cord of reticular cells, macrophages, lymphocytes and blood cells. The network of the splenic tissue was consisted of a network of reticular cells and fibers. Histological structure of spleen of the present study in broiler is similar as described by different authors (Bacha and Wood, 1990; Dellmann and Eurell, 1998).

Thymus was enclosed by a thin connective tissue capsule. Numerous fine septa of connective tissue originated from the capsule and divided the organ into incompletely separated lobules. Each lobule organized into a peripheral cortex and a central medulla. The cortex stained more deeply basophilic than that of medulla (Fig. 6b). Inside the medulla pale stained

diffuse Hassall's corpuscles were found, which were arranged in a concentric formation. The histological architecture of the thymus in the present study is similar to the findings of Bacha and Wood (1990); Dellmann and Eurell (1998) and Karim *et al.* (2005).

Cecal tonsils were composed of four histological layers i.e. tunica mucosa, submucosa, muscularis and serosa. The mucosal folds were well developed. Their lining epithelium was simple columnar epithelium. The bases of the mucosal folds were thick and the apexes were pointed or rounded. Diffuse lymphoid tissue and lymphoid nodules were observed in the mucosa and submucosa (Fig. 6c) in chickens of the present study. This report is in agreement with Bacha and Wood (1990) and Dellmann and Eurell (1998).

The bursa of Fabricius was consisted of long thick mucosal folds (plicae) which were projected into the lumen and lined by the pseudostratified columnar epithelium. Each plicae consisted of mainly of large number of polyhedral, prominent elongated and square shaped follicles which were closely packed together and separated little bit with very small amounts of connective tissue (Fig. 6d). Each bursal follicle was composed a peripheral cortex and a central medulla. The histological features of bursa in the present study are similar to the findings of Bacha and Wood (1990); Dellmann and Eurell (1998) and Tarek *et al.* (2012).



**Fig. 6.** Microscopic structure of lymphoid organs of healthy broiler chicken. a) spleen; 1- white pulp, 2- red pulp; b) thymus; 1- septum, 2- cortex, 3- medulla; c) cecal tonsil, 1- lumen, 2- mucosa, 3- lamina propria, 4- lymphatic nodule, 5- muscularis externa; d) bursa of Fabricius, 1- lumen, 2- mucosal epithelium, 3- follicle, 4- muscularis (H and E,  $\times 10$ )

#### Histopathological findings of lymphatic organs of ND affected broiler chicken

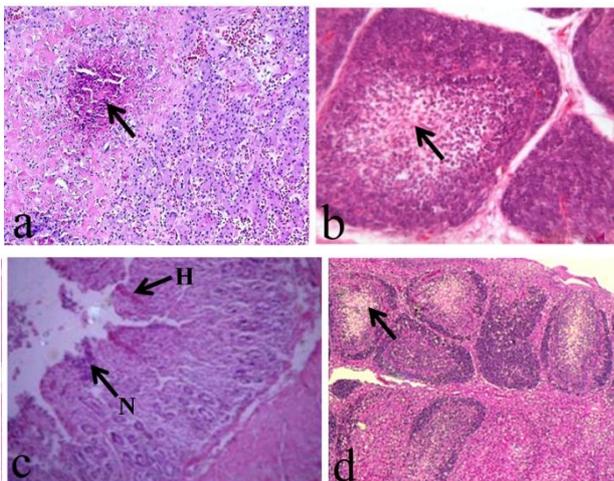
In the present study histopathological lesions in the spleen were multifocal areas of necrosis (Fig. 7a) which is similar to the observation of Cattoliet *al.* (2011). The swollen, necrosis and lymphocytes degeneration in the spleen, nuclear pyknosis and macrophages infiltration in the spleen tissue has also been observed in the present study which is similar to the findings of Banerje *et al.* (1994) reported the principal alterations observed as lymphocytic meningo-encephalitis and myelitis as well as splenic swollen lymphoid necrosis and hemorrhages. Our

findings also concurred with the findings of Kon-Ogura *et al.* (1993) and Malik and Emmerson (2002).

Thymic medullary lymphocytolysis (Fig. 7b) has been found in the ND affected birds which is similar with the findings of Jaganathan *et al.* (2015) where noted that medullary lymphocytolysis is most common in the thymus of ND affected chickens and it is also correlated with the report of Kianizadehet *et al.* (2002).

Hemorrhagic ceecal tonsils (Fig. 7c), congestion in the mucosa of the tonsil, necrosis and sloughing off of the ceecal tissue of broiler chicken (Fig. 7c) has been observed in present study. Similar results were also reported by Patissonet *et al.* (2001) and Kianizadehet *et al.* (2002).

Present study revealed that bursa of Fabricius of ND affected birds were showing lymphoid depletion, lymphocytolysis (Fig. 7d), glandular transformation of epithelium which is similar to the findings of Jaganathan *et al.* (2015) that are follicles showing scattered lymphoid depletion in the cortex and medulla and mild interfollicular fibrosis with glandular transformation of epithelium and also correlated with the findings of Kianizadeh *et al.* (2002).



**Fig 7.** Histopathologic structure of lymphoid organs of ND affected broiler chicken. a) necrotic spleen (arrowhead); b) thymus with medullary lymphocytolysis (arrowhead); c) caecal tonsil with haemorrhage (H) and necrosis (N); d) bursa of Fabricius showing lymphoid depletion and lymphocytolysis (arrowhead) (H and E,  $\times 10$ )

## Conclusions

Newcastle disease is still one of the most endemic and fatal poultry diseases that constraint chicken production in the northern part of Bangladesh. Despite the availability of vaccines for ND, poultry farmers are continue to experience of losses due to mortalities, drop of weight gain, diagnostic and control costs. Mortality has been occurred 1.52 times higher due to ND in the non-vaccinated birds. However, vaccination must be an important consideration for farm owners that will increase the profitability of farmers by reducing the risks of ND as vaccine reduces the mortality of birds. Proper diagnosis of the disease, improved management and bio-security must have to be ensured for the profitable and sustainable broiler farming especially at small scale level in Bangladesh.

## Author's contribution

Authors have no conflict of interest to report.

## References

- Alexander DJ. Newcastle disease and other avian paramy-xoviruses. *Rev. Sci. Tech.* 2000; 9:443-462.
- Alexander DJ. Newcastle disease diagnosis. pp.147-160. In: Newcastle disease. DJ Alexander, ed.; Kluwer Academic Publishers, Boston. 1988.
- Bacha WJ and Wood LM. *Color Atlas of Veterinary Histology.* Lea & Febiger. Philadelphia, USA. 1990. pp.189-191.
- Banerjee M, Reed WM, Fitzgerald SD and Panigraphy B. Neurotropic velogenic Newcastle disease in comorants in Michigan: pathology and virus characterization. *Avian Dis.* 1994; 38(4): 873-8.
- Barman LR, Flensburg MF, Permin A, Madsen M and Islam MR. A controlled study to assess the effects of vaccination against Newcastle disease in village chickens. *Bangladesh Vet.* 2010; 27(2): 56 – 61.
- Cattoli G, Susta L, Terregino C and Brown C. Newcastle disease: a review of field recognition and current methods of laboratory detection. *J. Vet. Diagnos. Invest.* 2011; XX(X): 1–20. DOI: 10.1177/1040638711407887
- Dellmann HD and Eurell JA. *A Textbook of Veterinary Histology.* 5<sup>th</sup> ed., Williams and Wilkins, A Waverly Company, Philadelphia, USA. 1998. pp. 226-235.
- El-Younguda AD, Ngulde IS, Abubakar MB and Baba SS. Village chicken health, management and production indices in selected villages of Borno state, Nigeria. *Family Poultry.* 2007; 17 (1&2): 41-48. <http://www.fao.org/ag/againfo/themes/en/infpd/newsletters.html>.
- Getty R. Sisson and Grossman's the Anatomy of the Domestic Animals. 5<sup>th</sup> ed. Vol. 2. WB Saunders Co. Philadelphia, London. 1975. pp. 2010-2018.
- Gridley MF. *Manual of Histologic and Special Staining Technique.* 2<sup>nd</sup> ed., McGraw-Hill Book Company. USA. 1960. pp. 28-31, 82-83.
- Hossain KMM, Ali MY and Yamato I. Antibody levels against Newcastle disease virus in chickens in Rajshahi and surrounding districts of Bangladesh. *Int. J. Biol.* 2010; Vol. 2, No. 2; [www.ccsenet.org/ijb](http://www.ccsenet.org/ijb)
- Huang Z, Chy K, Panda S and Samal SK. Newcastle disease virus v protein is associated with viral pathogenesis and functions as an alpha interferon antagonist. *J. Virology.* 2003; 77: 8676-8685.
- Islam MR, Khan M, Das PM and Bari ASM. Poultry diseases diagnosed at necropsy in 1997 and 1998 in the Department of Pathology of Bangladesh Agricultural University, Mymensingh. Proceedings of 5th BSVR Annual Scientific Conference held on 3-4 December, 1998 at Bangladesh Agricultural University, Mymensingh.
- Jaganathan S, Ooi PT, Phang LY, Allaudin ZNB, Yip LS, Choo PY, Lim BK, Lemiere S and AudonnetJC. Observation of risk factors, clinical manifestations and genetic characterization of recent Newcastle disease virus outbreak in West Malaysia. *BMC Vet. Res.* 2015; 11:219; DOI: 10.1186/s12917-015-0537-z
- Karim MR, Khan MZI and Haque Z. The dynamics of immunocompetent cells in the major lymphoid organs and mucosa associated lymphoid tissues of chemotherapy treated chickens. *J. Bangladesh Soc. Agric. Sci. Tech.* 2005; 2: 5-8.
- Khan MY, Arshad M and Hussain I. Epidemiology of Newcastle disease in rural poultry in Faisalabad, Pakistan. *Int. J. Agric. Biol.* 2011; 13: 491–497.

- Kianizadeh M, Aini I and Gholami GR. A comparative study on histopathologic effects of Iranian Newcastle disease virus isolates. *Arc.Razi Institute*. 2002; 54: 17-29.
- Kon-Ogura T, Kon Y, Onuma M, Kondo T, Hashimoto Y and Sugimura M. Distribution of T cell subsets in chicken lymphoid tissues. *J.Vet. Med. Sci.* 1993; 55(1):59-66.
- Levy R, Spira G and Zakay-Rones Z. Newcastle disease virus pathogenesis in the respiratory tract of local or systemic immunized chickens. *Avian Dis.* 1975; 19: 700-706.
- Malik NS and Emmerson PT. Molecular cloning and nucleotide sequencing of Newcastle disease virus. In: D.J. Alexandar (Ed.). *Newcastle Disease*. Kluwer Academic Publisher, Boston, 2002. pp. 79- 97.
- Mishra S, Kataria JM, Verma KC and Sah RI. Response of chickens to infection with Newcastle disease virus isolated from guinea fowl. *Tropic. Anim. Heal. Prod.* 2000; 32: 277-284.
- Mohammadamin OG and Qubih TS. Histopathology of virulent Newcastle disease virus in immune broiler chickens treated with IMBO. *Iraqi J. Vet. Sci.* 2011; 25(1): 9-13.
- Nkiruka OO. Evaluation of factors limiting turkey production in Enugu state and comparison of pathology associated with Newcastle disease in turkeys and chickens. PhD thesis. Department of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. 2014.
- OIE (Office International des Epizooties). Newcastle disease. In *Manual of standards for diagnostic tests and vaccines*, 3rd Ed. OIE, Paris. 1996. pp. 161-169.
- Ojok L. Diseases as important factors affecting increased poultry production in Uganda. *Der Tropenlandwirt*. 1993; 94: 37- 44.
- Okoye JOA, Agu AO, Chineme CN and Echeonwu GON. Pathological characterization in chicken of a velogenic Newcastle disease virus isolated from Guinea fowl. *Rev. Elev. Med. Vet. Pay.* 2000; 53: 325-330.
- Patisson M, McQuain LI and Morrison T. The P protein and the nonstructural 38K and 29K proteins of Newcastle disease virus are derived from the same open reading frame. *Virology*. 2001; 164: 256-264.
- Rahman MA and Samad MA. Pattern of occurrence of single and concurrent disease associated with mortality in commercial chickens in Bangladesh. *Bangladesh J. Vet. Med.* 2003; 1: 15-20.
- Rahman MM, Bari ASM, Giasuddin M, Islam MR, Alam J, Sil GC and Rahman MM. Evaluation of maternal and humoral immunity against Newcastle disease virus in chicken. *Int. J. Poultry Sci.* 2002; 1(5): 161-163.
- Rahman MS, Rabbani MG, Uddin MJ, Chakrabarty A and Her M. Prevalence of Avian Influenza and Newcastle disease viruses using rapid antigen detection kit in poultry in some areas of Bangladesh. *Arc. Clin. Microb.* 2012; Vol. 3 No. 1(3); doi: 10.3823/248; <http://www.imedpub.com>
- Saif YM, Barnes HJ, Glisson JR, FadlyAM, McDougald LR and Swayne DE. *Diseases of Poultry*. 11<sup>th</sup> ed., Blackwell Publishing, USA. 2003. pp. 66–78.
- Samad MA. An overview of livestock research reports published during the twentieth century in Bangladesh. *Bangladesh Vet. J.* 2000; 34: 53-149.
- Talha AFS, Hossain MM, Chowdhury EH, Bari ASM, Islam MR and Das PM. Poultry diseases occurring in Mymensing district of Bangladesh. *Bangladesh Vet.* 2001; 18: 20-23.
- Tarek K, Mohamed M, Omar B and Hassina B. Morpho-histological study of the thymus of broiler chickens during post-hatching age. *Int. J. Poultry Sci.* 2012; 11 (1): 78-80.
- Tu P, Eskola J and Lassila O. Ontogeny of the chicken lymphoid system. In *Avian Immunology* (ed. M. E. Rose, L. N. Payne & B. M. Freeman), Brot. Poultry Sci. Ltd. Edinburgh, Scotland. 1998. pp. 45-62.
- Collins, M.S., Strong., Alexander, D.J. (1994): Evaluation of the molecular basis of pathogenicity of the variant Newcastle disease virus termed "pigeon PVM-1 viruses". *Arch. Virol.* 134, 403-411.