

Pathological Investigation Of Infectious Bursal Disease (IBD) In Broiler At Dinajpur District

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Abstract: The current research conducted with 8 broiler farms with sum of 3250 birds of various age group from four different upazila like Sadar, Birol, Birgonj and Kaharol were suspected for Infectious Bursal Disease (IBD). On the basis of detail about farm history, clinical signs and postmortem investigation of infected chicks, the prevalence of IBD was 11.11%, 10.4%, 9.06% and 9.11% in Sadar, Birol, Birgonj and Kaharol upazila respectively, with an overall prevalence 10.03% at Dinajpur District. The prevalence of IBD in broiler chickens was the highest (13.13%) at 4th week of age and the lowest (5.6%) at 6th week of age. No broiler chick was identified as positive for IBD in their first two weeks of age. The highest mortality was observed at Kaharol upazila (5.11%) and the lowest (3.2%) at Birol upazila, with total mortality rate 4.27%. The necropsy findings of infected chicks revealed haemorrhages on thigh and Brest muscles; enlarged, edematous, hyperemic and haemorrhagic bursa of fabricious followed by atrophy. In some cases, kidneys were found swollen. Severe lymphoid depletion and reactive cells infiltration in the interfollicular space were found in histopathological studies. Therefore, it was concluded that susceptibility of chicks to IBD is influenced by its age. Ruffled feather, depression, whitish diarrhoea with haemorrhagic muscles and inflamed, edematous, hyperemic bursa of fabricious is attributable to infectious bursal disease (IBD).

Keywords: Histopathology, Broiler, Infectious Bursal Disease (IBD), Prevalence at Dinajpur.

Date of Submission: 12-10-2018

Date of acceptance: 27-10-2018

I. Introduction

Poultry plays a pivotal role in bridging the protein gap of animal origin in Bangladesh. Protein is the most important constituent of human's food. Poultry meat and eggs are two major sources of animal protein. Poultry meat and eggs provide approximately 38% total animal protein in the country [1]. It is estimated that the share of poultry in the animal protein of human diet increased from 14% in 1977 to 23% in 1987 and in further estimated to 30% in 1995 [2]. The poultry sector is also an integral part of the farming system in Bangladesh. The number of poultry grew at an annual rate of 6.7 percent over the period 1990-97. About 50,000 poultry farms and 26,000 duck farms have already been established in private sector in addition to the government farms [3].

The major constraints in poultry farming are the outbreak of several devastating diseases causing economic loss and discouraging poultry rearing [4]. Among the various diseases, infectious bursal disease (IBD) popularly known as Gumboro disease is the number one killer disease of chickens. It is a major poultry pathogen in the poultry industry [5]. The first outbreak of IBD occurred at the end of 1992 [6] [7] [8] and has become a major problem in the poultry industry, causing up to 80% mortality in the field outbreaks [7] [9]. One of the earliest signs of infection in a flock is picking of their own vent. Other signs included depression, anorexia, soiled vent feathers, whitish watery diarrhoea, ruffled feathers, trembling, severe prostration and finally death [10]. The disease is characterized mainly by severe damage of the bursa of fabricius (BF) followed by immunosuppression [11] [12]. There are frequent occurrences of this disease, reported by the farm-owners, even when the flocks have been vaccinated against the disease [13]. IBD is economically important for the poultry industry in function of the immune depression that it causes [14]. The primary target organ for IBDV is the bursa of Fabricius [12]. IBDV affects the actively dividing B-lymphocytes bearing cell surface IgM [15] [16] developing the severe morphological alteration of bursa of Fabricius [12] and producing a profound immunosuppression [17].

The immunosuppression prevents the birds from optimally responding to vaccine and ultimately leads to increase the incidence of numerous concurrent infections including Marek's disease [18], Newcastle disease [19] coccidiosis, infectious bronchitis [20], hemorrhagic-aplastic anemia and gangrenous dermatitis [21], infectious laryngotracheitis [22] inclusion body hepatitis, reovirus [23] chicken anemia agent, salmonellosis, colibacillosis *Mycoplasma synoviae* [24] and *Eimeria tenella* [25]. One of the significant components of the control of the disease is its vaccination which if improved may help in lowering the incidence of the disease in poultry [26]. However, there is no such study in Dinajpur district which is one of the major belts in Bangladesh. Considering the above facts, current study was undertaken a). To investigate the overall prevalence and mortality rate of disease in broiler encountered at Dinajpur district b). To study the clinical findings of Infectious Bursal Disease (IBD) of the affected flock at Dinajpur district c). To study the prevalence of IBD in relation to age of birds and d). To study the gross and histopathological changes of different organs developed due to Infectious Bursal Disease.

II. Materials And Methods

2.1 Sample collection and Processing

From the selected farms all dead as well as live sick chickens were collected with detailed particular of the outbreaks of IBD including farm location, history, age, breed, total number of birds and affected birds in farm, intervals between the batches, vaccine schedule, daily mortality and total mortality and clinical signs of affected birds were also recorded. In each case sampling was done following standard sampling methods and send to the laboratory. Different organ like liver, bursa of fabricious, breast and thigh muscle, kidney were collected during necropsy for further study. 10% neutral buffered formalin, Xylene, Hematoxylin and Eosin stain. PBS, Distilled water etc. were used for necropsy and histopathology of collected samples. All the diagnostic works were carried under the Laboratory of Department of Pathology & Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU). Clinical diagnosis and in some cases necropsy examinations were carried out at the place of sampling whereas histopathology of all samples were done in the laboratory.

2.2 Preparation of Harris' Hematoxylin Solution and other Chemicals

Hematoxylin crystals-5g, Alcohol (100%)-50 ml, Ammonium or potassium alum-100 g, Distill water-1L, Mercuric oxide (red)-2.5 g. Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

For 1% stock alcoholic eosin taking Eosin Y, water soluble- 1g, Distilled water- 20 ml, 95% alcohol- 80 ml and finally Eosin was dissolved in water and then 80 ml of 95% alcohol was added. For working eosin solution- Eosin stock solution- 1 part, Alcohol, 80%- 3 parts then 0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

2.3 Necropsy Examination of Suspected Birds

The necropsy was done on the selected birds taken from suspected flocks. At necropsy, gross changes were observed and recorded carefully by systemic dissection based on routine necropsy examination. The lesion containing tissues and organs were also collected and preserved in 10% neutral buffered formalin for the histopathology.

2.4 Histopathological Study

During necropsy, Bursa of Fabricius was collected, preserved in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin according to standard method [27].

2.5 Processing of Tissue and Sectioning

The tissues were properly trimmed into a thin section to obtain a good cross section of the tissue and washed under running tap water for overnight to remove the fixative. Dehydrated in ascending grades of alcohol to prevent shrinkage of cells using 50%, 70%, 80%, 90% alcohol, and three changes in absolute alcohol, for 1hr in each. Then cleaned in two changes in chloroform to remove alcohol, 1.5hr in each. After that tissues were embedded in molted paraffin wax at 56-60°C for two changes, 1.5hr in each. Paraffin blocks containing tissue pieces were made using templates and molted paraffin. Then the tissues were sectioned with a microtome at 5-6µm thickness. The sections were allowed to spread on lukewarm water bath (40-45°C) and taken on a glass

slide. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides containing sections were air dried and stored in cool place until staining.

2.6 Routine Hematoxylin and Eosin staining

Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 minutes in each). Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 minutes in each; 95% alcohol for 2 minutes; 80% alcohol for 2 minutes; 70% alcohol for 2 minutes) and distilled water for 5 minutes. The tissues were stained with Harris' hematoxylin for 10 minutes. Then sections were washed in running tap water for 10-15 minutes and staining was differentiated in acid alcohol (1 part HCl and 99 parts 70% alcohol), 2-4 dips. The tissue sections were then washed in tap water for 5 minutes and dipped in ammonia water (2-4 times) until sections became bright blue and stained with eosin for 1 minute and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 minutes in each). The stained sections were then cleaned by 3 changes in xylene, 5 minutes in each and finally the sections were mounted with cover slip using DPX. The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

2.7 Statistical Study

In this study the mortality rate was calculated by the following statistical formula-

$$\text{Mortality rate (\%)} = \frac{\text{Deaths occurring during a given time period}}{\text{Birds Population during the same time period}} \times 100$$

Prevalence of disease was calculated by the following statistical formula-

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

III. Results And Discussion

3.1 Mortality and Prevalence Status

Table-1 and Fig. 1 showed the mortality and prevalence of IBD at different region of Dinajpur district whereas Table-2 and Fig. 2 showed the prevalence of IBD at different age group. A total of 3250 birds were examined during the study period from which 326 birds (10.03%) are found infected with IBD. The mortality rate is 4.27%. No case was found in first two weeks of age.

3.2 Necropsy Examination Result

For the conformation of Infectious bursal disease, the pathological lesions of different parts of the body were examined mainly on bursa of fabricious and thigh muscle. During necropsy examination the most frequent gross lesions of IBD were haemorrhages in the breast muscle and thigh muscles (Fig 3 and 4). The main changes, enlarged and haemorrhagic bursa of fabricious (Fig 5) were found in primary stage. The bursal folds become edematous, haemorrhagic and abnormally thick with accumulation of exudates and The junction of proventriculus and gizzard showed haemorrhagic lesions also appeared. In some cases, kidneys were swollen.

3.3 Result of Histopathological examination

Section of the bursa of fabricious showed loss of normal corticomedullary architecture of bursa and in most follicles severe lymphoid depletion was observed (Fig 6 and 7). Reactive cells infiltration by heterophils and macrophages in the interfollicular space (Fig 8).

3.4 Discussion

A total of 3250 of the 326 affected broiler chicks were diagnosed as IBD (Table 3) and observed clinical signs were morbidity, high mortality, watery or whitish diarrhoea, vent picking, unsteady gait, ruffled feathers and sudden death which correspond with the findings of Lukert and Saif (2003) [28]; Islam and Samad (2004) [29].

Overall prevalence of IBD in broiler chickens was 10.03% and 11.11%, 10.4%, 9.06% and 9.11% prevalence in Sadar, Birol, Birgonj and Kaharol upazila of Dinajpur district respectively (Table 1). The highest prevalence was found in Sadar and lowest was found in kaharol upazila (Table 1). These results support to the reports of Hossain *et al.*, (2010) [30] who reported 12.23% prevalence of IBD in broiler at Rajshahi district. These results support with the reports of some others. Khan *et al.*, (2009) [31] stated that 7.75% prevalence in Peshawar. Rahman *et al.*, (2010) [32] reported 26.75% prevalence of IBD in Dinajpur district. These differences among the results of present and earlier study could not be easily explained, but might be attributed to epidemiological factors, such as weather, seasonal variations, geographical location, differences in sample collection technique and data collection.

On the basis of age group, the prevalence of IBD was 7.33%, 13.13%, 11.57% and 5.6% at the age of 3rd, 4th, 5th and 6th week of age (Table 2) respectively. The prevalence of IBD in broiler chickens was the highest (13.13%) at 4th week of age and the lowest (5.6%) at 6th week of age. While no case was found in first two weeks of age and the broilers of four weeks of old were highly susceptible to IBD. Similar reports have been described by Khan *et al.*, (2009) [31] who reported that susceptibility of chickens to IBD is influenced by their age reaching a peak at 4 weeks of age. Rajaonarison *et al.*, (2006) [33] who observed the highest prevalence of IBD in broiler during the 3rd to 5th week of age. The highest mortality (5.11%) was found in kaharol upazila and 3.2% was found in Birol upazila (Table 1) which support the finding of Mohanty *et al.*, (1971) [34] and Islam and Samad (2004) [29].

In this observation, the gross pathological lesions were hemorrhages in the breast muscles (Fig 3) and thigh (Fig 4); enlarged, edematous, hyperemic and haemorrhagic bursa of fabricious (Fig 5). In some cases, kidneys were found swollen. These findings support earlier observation of Paul (2004) [35]; Richard and Miles (2004) [36] and Rajaonarison *et al.*, (2006) [33] who reported that at necropsy the gross pathological lesions were dehydrated and darkened carcass, hemorrhages were present on pectoral, leg and thigh muscles. Histopathological study revealed the finding as severe lymphoid depletion (Fig 6 & 7), reactive cells infiltration by heterophils and macrophages in the interfollicular space (Fig 8). These lesions were in agreement with those described by Hoque *et al.*, (2001) [37].

IV. Figures And Tables

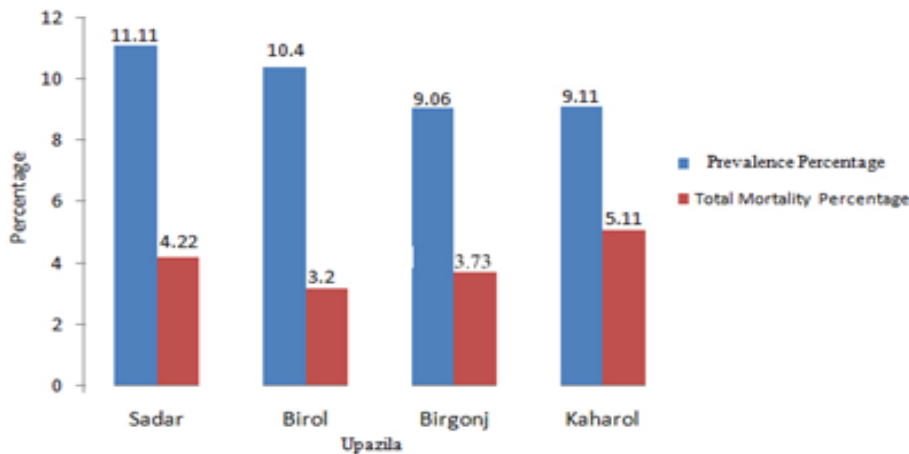


Figure 1. Percentage wise distribution of disease in four upazila of Dinajpur

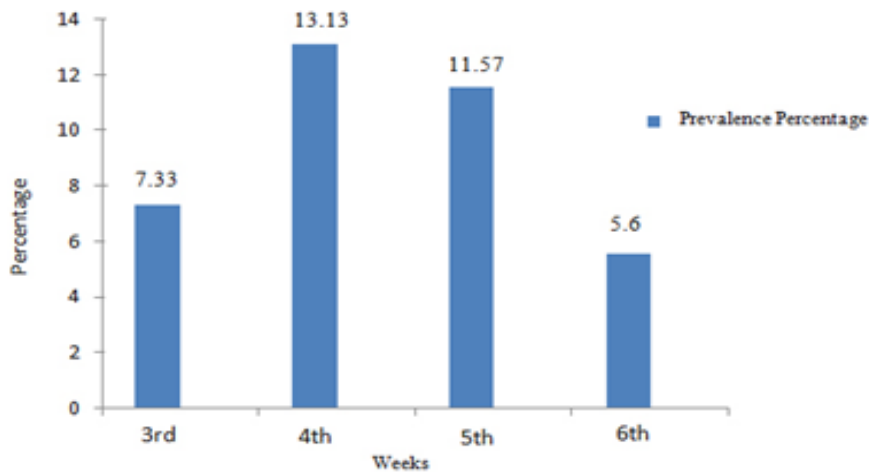


Figure 2. Age wise distribution of infectious bursal disease in four upazila, Dinajpur



Figure 3. Haemorrhage in the breast muscle



Figure 4. Haemorrhage in the thigh muscles



Figure 5. Haemorrhagic Bursa

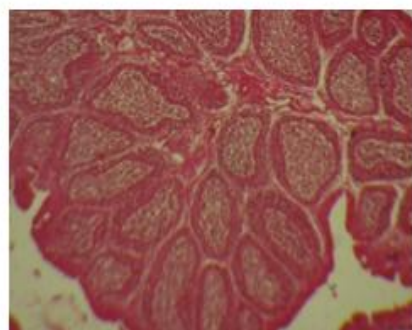


Figure 6. Lymphoid depletion

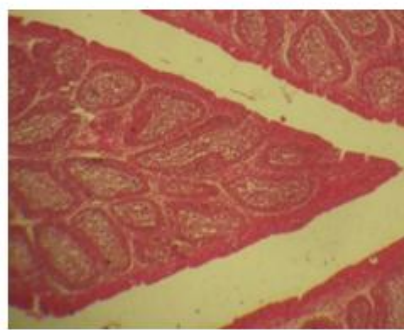


Figure 7. Lymphoid depletion

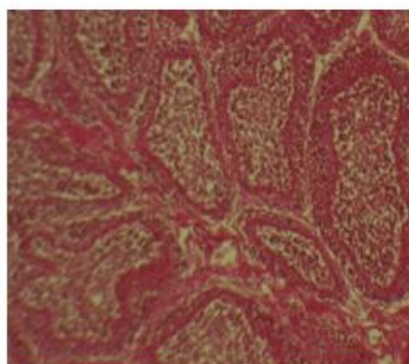


Figure 8. Reactive cells infiltration by heterophils

Table-1: Prevalence and mortality rate of IBD in Broiler chicks at different Upazila of Dinajpur

Name of Upazila	No. of Farm Visited	No. of Birds observed	No. of infected birds	No. of Dead Birds	No. of Birds Necropsy Done	Mortality (%)	Prevalence (%)
Sadar	3	1350	150	57	7	4.22	11.11
Birol	1	250	26	8	3	3.2	10.4
Birgonj	2	750	68	28	5	3.73	9.06
Kaharol	2	900	82	46	6	5.11	9.11
Total	8	3250	326	139	21	4.27	10.03

Table-2: Prevalence of IBD in Broiler chicks at different age group

Age of Birds(Weeks)	No. of Birds observed	No. of infected birds	Prevalence (%)
3 rd	900	66	7.33
4 th	1150	151	13.13
5 th	700	81	11.57
6 th	500	28	5.6
Total	3250	326	10.03

V. Conclusion

Current study shows marked depression, unsteady gait, ruffled feathers, whitish diarrhoea, atrophy of bursa of fabricius and sudden death is attributable to Infectious Bursal Disease virus (IBDV). IBD pathologically characterized and identified by necropsy and histopathological examination. The prevalence is very high at the age of 4th but low in 6th age of chick. Average prevalence and mortality of IBD at Dinajpur district is 10.03% and 4.27% respectively. The occurrence of IBD outbreaks in broiler farms as observed in this study indicates not only due to lack of immunization plan but also poor management system, resulting heavy economic loss. Scheduled vaccination, along with good management practices need to control of infectious bursal disease (IBD)

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Dr. Md. Nahid Hossain, " Pathological Investigation Of Infectious Bursal Disease (IBD) In Broiler At Dinajpur District." *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)* 11.10 (2018): 73-79.