MedCrave
Step into the World of Research

International Clinical Pathology Journal

Research Article

Open Access

CrossMark

Scoring of bursal lesions in commercial broiler chickens infected with field IBD virus at Sylhet region of Bangladesh

Abstract

The current study was conducted to evaluate the pathogenicity and pathology of infectious bursal disease virus in commercial chickens. A total of 45 broiler bursa were collected from 9 commercial flocks of different age (18-29 days) of different areas at sylhet region. During necropsy chickens and Bursa of Fabricius (BF) were weighed and examined for any gross lesion and processed for histological investigations. Bursa body weight ratio and bursal lesion scoring were made to evaluate pathogenicity of the virus. The results shows that the weight of bursa increased effectively according to the body weight. According to the evaluation system bursa were scored as excellent, average, medium and bad. In post mortem examination there was severe hemorrhage present on BF, breast, thigh muscle and also at the junction between proventriculus and gizzard. Gelatinous and atrophied bursa was found. Bursal lesion score were done based on histopathological investigations and highest bursalleison score was 4.6±0.40. Bursa were characterized by atrophy, necrosis, fibroplasia, lymphoid depletion, cyst formation and infiltration of inflammatory cells. In conclusion, the bursa characteristics bursal index/bursa-body weight ratio, gross lesion and bursalleison scoring are practical for any field investigations to evaluate the pathogenicity of Infectious Bursal Diseases virus (IBDV).

Keywords: bursa-body weight ratio, pathology and bursal lesion scoring, atrophy, bursal

Volume 8 Issue 1 - 2020

Most Farzana Aktar, Monira Noor, Abu Hena Mustafa Kamal, Md. Masudur Rahman
Sylhet Agricultural University, Bangladesh

Correspondence: Md. Masudur Rahman, Department of Pathology, Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Sylhet 3100, Bangladesh.
Email rahmanmm.dpp@sau.ac.bd

Received: March 04, 2020 | **Published:** April 06,2020

Introduction

Infectious bursal disease (IBD) is one of the killer diseases of poultry in Bangladesh. The causative agent of this highly contagious, immunosuppressive viral disease is infectious bursal disease virus (IBDV), a bisegmented double stranded RNA virus belonging to the family *Birnaviridae*.[1,2] There are two distinct serotypes of IBDV, which can be differentiated by cross-neutralization assays.[3] Serotype 1 viruses are pathogenic for chickens but individual strains differ remarkably in their virulence. Serotype 2 strains isolated from fowl, turkeys and ducks[3] are nonpathogenic for chickens. The pathogenic serotype 1 IBDV isolates are subdivided into classical virulent (cv) or very virulent (vv) and antigenic variant strains. Infectious bursal disease virus (IBDV) affects the bursa of Fabricius (BF) of chickens due to a lytic infection of proliferating lymphocytes of the B-cell lineage.[4–6]

Classical IBDV strains cause a strong inflammatory response in the BF, and chickens infected with virulent classical IBDV show clinical signs.[7,5] In contrast, variant IBDV strains result in atrophy of the BF in the absence of any inflammatory changes.[8] The subclinical disease caused by variant IBDVs may barely be noticed and sometimes only an increase in the incidence of respiratory diseases might be observed.[8,9] More dramatic are vvIBDVswhich cause a fast depletion of the B lymphocytes in the BF that is also associated with petechial hemorrhage in the muscles and a hemorrhagic BF.[10] All three pathotypes of IBDV induce immunosuppression leading to an increase in the opportunity for secondary pathogens to invade the host and cause multisystemic diseases.[11–13] There is less number of poultry farms in Sylhet comparing with other region of Bangladesh. Now-a-days farmers are getting interested to set poultry farms in Sylhet region as industry. The prevalence of IBD at Sylhet region studied by MR Islam et al,[14] and Badruzzaman et al,[15] were 24.96% and 22% respectively. Therefore, considering all the above facts, this research work was undertaken to study the pathology caused by field IBD viruses in chickensand to determine the pathogenecity of field IBD viruses based on scoring of bursal lesions.

Materials and methods

A total of 45 (forty five) IBD affected broiler chickens were collected from 9 (nine) different commercial broiler farmslocated at different areas of Sylhet region in Bangladesh where IBD outbreaks occurred and diagnosed by necropsy. Necropsy was performed as per standard procedure described elsewhere[16] and the gross lesions were recorded. From necropsied chickens45 (forty five) bursa were collected and 5 (five) bursa were selected from each flock for pathological study. The weights of affected chickens were taken before necropsy. The calculation of Bursal Index (BI) was obtained by the formula: weight of BF /body weight x 100.[17] At necropsy, the BF was collected and preserved in 10% formalin for histopathological studies. The fixed tissue samples were further processed, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) as per previously described method.[18] Bursal lesion scores were made as per method described previously[19] on a scale 0-6. Scores for the tissue alterations were 0=no alteration; 1=cells with pyknotic nuclei in the medullary region of the lymphoid follicles and discrete dissociation of the interstitial connective tissue, or lymphoid rarefaction, 2=hypotrophy of medullary and cortical regions of the lymphoid follicle due to the degeneration of lymphoepithelial cells, and/or lymphocytes and/or

macrophage- or plasmocyte-type cell afflux, associated to a discrete dissociation and infiltration of inflammatory cells in the interstitial connective tissue. 3=lymphoid depletion in the cortical and medullary zones and/or intense degeneration of lymphoepithelial cells in the lymphoid follicle, associated to a discrete edema and interstitial infiltration of inflammatory cells, besides roughening and/or discrete epithelial invagination and perivascular lymphoid hyperplasia at the septum of the conjunctive tissue; 4=degeneration of cells from the medullary and cortical regions, with moderate infiltration of inflammatory cells in the lymphoid follicle, interstitial fibroplasia and many foci of epithelial invagination; 5=medullary necrosis with cyst formation, intense granulocyte infiltration in the parenchyma and/or necrosis with hemorrhage in the intersticium or lymphoid follicles and/or lymphoid follicles with epithelial invagination or fibrosed and intense interstitial fibroplasia; 6=absence of lymphoid follicles due to hemorrhage, necrosis, atrophy or fibrosis. Data were analyzed for statistical significance using an unpaired two-tailed Student's *t*-test. A *p*-value <0.05 was considered as significant.

Results

Bursal index

Bursal Index (BI) of 9 flocks were determined and these were 0.113±0.0004, 0.109±0.0015, 0.164±0.0084, 0.205±0.0054, 0.158±0.0097, 0.271±0.167, 0.109±0.0003, 0.281±0.0267, 0.113±0.0152 in flock A, B, C, D, E, F, G, H and I, respectively (Table-1). There was positive level of significanceamong the flocks (p<0.001).Bursal weight with regard to the body weight of respective chicken in different flocks showed a correlation coefficient of r2=0.71. This positive and significant correlation explainedthatthe weight of the bursa increased effectively according to the body weight (Figure 1).

Table 1 Bursal Index of IBDV infected commercial broiler chickens under field condition

Flock no	Age (day)	Bi % (mean±se)	Scoring	P-value	Level of significance
A	25	0.113±0.0004	Bad		
B	29	0.109±0.0015	Bad		
C	21	0.164±0.0084	Medium		
D	25	0.205±0.0054	Average		
E	24	0.158 ±0.0097	Medium	<.0001	significant
F	20	0.271 ±0.167	Excellent		
G	28	0.109 ±0.0003	Bad		
H	18	0.281 ±0.0267	Excellent		
I	29	0.113 ±0.0152	Bad		

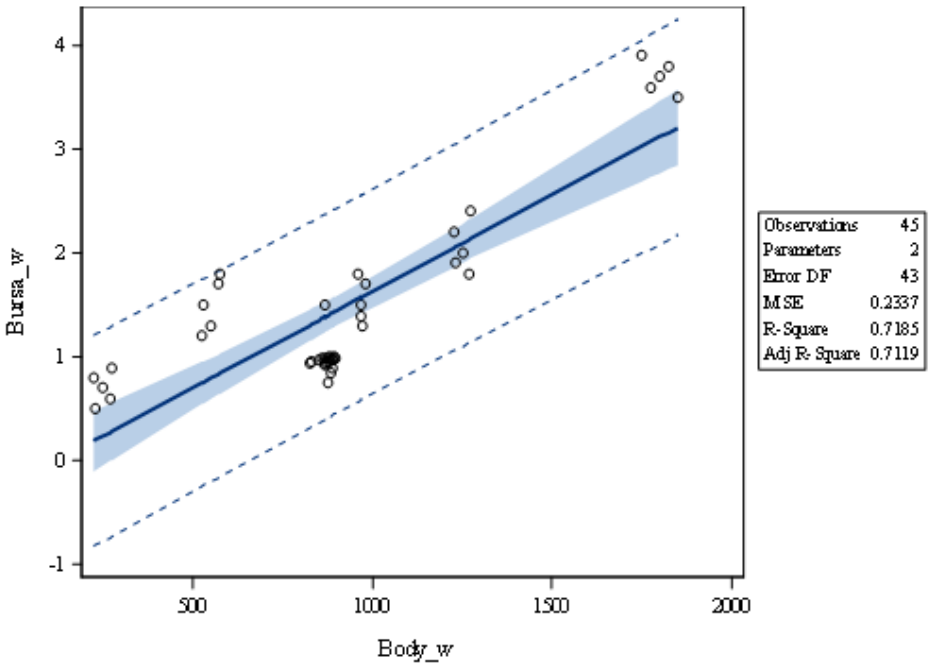

Figure 1 Correlation between body weight and bursal weight in commercial broiler chickens infected with field IBDV.

Pathological study

In post-mortem examinations gross pathological changes were noticed in various organs. The major leisons were found in the BF which included edematous swelling of the bursa (18 days), hemorrhagic bursa (21 days) (Figure 2A), gelatinous leison covering serosal surface of bursa (20days) (Figure 2B) and atrophied bursa (28 day) (Figure 2C). Gross lesions in other organs included hemorrhage at the junction between proventriculus& gizzard and also onthe breast and thigh muscles (Figure 2D).

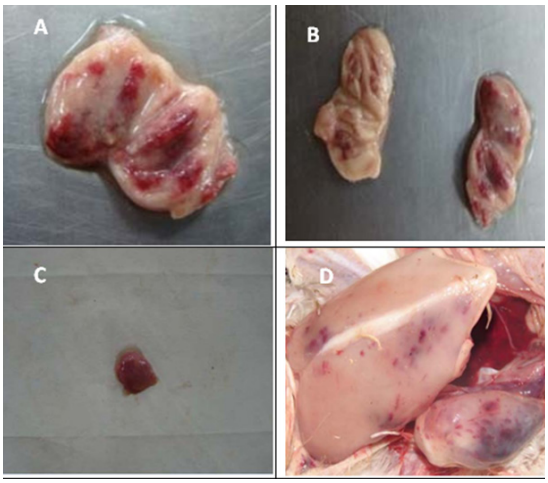

Figure 2 Pathology in field IBDV infected broiler chickens. A: Hemorrhage in bursa (21 days), B: Gelatinous leison present in bursa (20 days), C: Atrophied bursa (28 days) and D: Hemorrhage present in breast muscle (22 days).

Bursal lesion score

To determine the pathogenicity of field IBD viruses, histopathological study of BF were performed. Based on the histopathological study, bursal lesion scores of IBD affected commercial chickens were made as described in the methodology and leison scores are shown in Table 2 & Figure 3. Five chickens (n=5) were considered at each sampling occasion and p-values were calculated among IBD affected chickens. Scoring of bursal lesions was done according to Al-Mayah & Abu Tabeekh[19] based on 0-6 scale (1-6). According to Table 2, average bursal lesion scores varied from 4.6 to 1.2 among different flocks.

Table 2 Bursal lesion scores of IBDV infected commercial broiler chickens under field condition

Flock No	Age (days)	Lesion score of individual chicken	Average lesion score (Mean± SE)	Level of significance (p-value)
A	25	4,4,4,5,5	4.4± 0.244	
B	29	4,4,5,5,5	4.6± 0.244	
C	21	2,2,2,3,3	2.4± 0.24	<.0001
D	25	1,1,2,2,2	1.6±0.244	
E	24	2,2,2,2,2,	2± 0	
F	20	1,1,1,1,2,	1.2± 0.20	
G	28	4,4,4,5,6	4.6± 0.40	
H	18	1,1,1,1,2	1.2±0.20	
I	29	4,4,5,5,5	4.6±0.244	

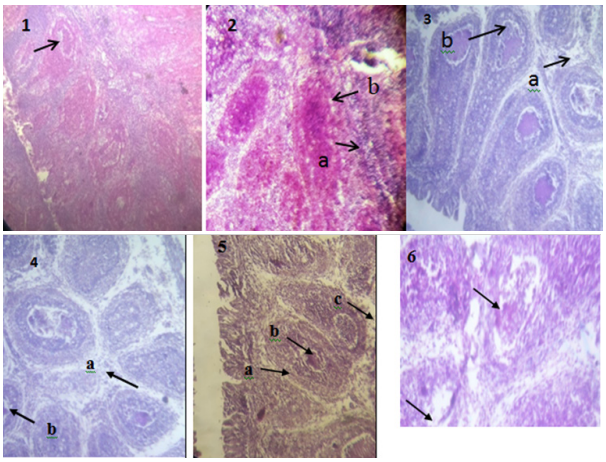

Figure 3 Bursal lesion scoring in field IBDV infected broiler chickens. Score -1: arrow showed an area of slight lymphoid depletion (H& E X10), Score-2: a) infiltration of inflammatory cells in the interstitial connective tissue b) fatty degeneration in the bursal follicle (H&EX20), Score-3: (a) Edematous area between bursal follicle (b) depletion of lymphoid cell (H&EX20), Score-4: a) Interistitial fibroplasia b) moderate infiltration of inflammatory cells (H&E X20), Score-5: a) intense interistitial fibroplasia b) hemorrhage in the lymphoid follicle c) necrotic changes. (H & E X20) and Score-6: arrow showed absence of lymphoid follicle due to hemorrhage (H & E X10).

Discussion

The study was designed to determine the pathogenecity of field IBD viruses based on scoring of bursal lesions and the pathology of bursa of chickensinfected withfield IBD virus. For this a total of 45 infected bursal samples were collected from 9 different farms at Sylhet region. Histological slideswere preparedfrom BF of affected broiler chickens from each flock and based on the histopathological study, bursal lesion scores of IBDV affected chickens were made. The gross lesions observed in this study are consistent with the previous reports.[20,21] Bursal index has been considered as the vital factor in determining the pathogenicity of IBDV as there is a proportional relationship between bursa-body weight ratio and the pathogenicity of the virus.[22,23] In the present study, there was a significant differencein bursa-body weight ratios among IBDVinfected commercial broiler chickens. In our study,average bursal indexes were recorded as 0.113±0.0004 (Flock-A), 0.109±0.0015 (Flock-B), 0.164±0.0084 (Flock-c), 0.205±0.0054 (Flock-D), 0.158±0.0097 (Flock-E), 0.271±0.167 (Flock-F), 0.109±0.0003 (Flock-G), 0.281±0.0267 (Flock-H) and 0.113±0.0152 (Flock-I) (Table 1). Our results were correlated withprevious findings where Bursa-body weight ratios were recorded as 1.99±0.15 in naïve; 2.98±0.09, 3.19±0.03, 2.44±0.34, 2.45±0.11, 2.24±0.41 2.38±0.55 in vaccinated; and 2.45±0 in IBD affected chickens respectively and significant differences in bursa-body weight ratios existed in naïve and vaccinated chickens and IBD affected chickens.[24] In the present study positive and significant correlation (r=0.71)between body weight and bursal weight in commercial chickens was recordedindicating the weight of the bursa increased effectively according to the body weight. The findings were correlated with the previous findings where correlation coefficient between body weight and bursal weight was reported as r =0.51.[25] The histopathological examination of the bursa of Fabricius confirmed the occurrence of IBD because very typical microscopic alterations wererecorded in infected chickens.[26] The intensity of microscopic lesions in the bursa of Fabricius may also be quantified to evaluate the level pathogenecity of field IBD virus. In the present study, the histological lesions in the bursa of Fabricius were scored from 0 to 6

scales (Table 2). Our findings were correlated to the previous findings where bursa of IBDV infected chickens showed significantly higher lesion score (3.33±0.33) compared to naïve and vaccinated chickens.[24]

In this study, the histopathological lesion scores of commercial broiler chickens infected with field IBD virus were reported. The distribution of the observed lesions was variable. They were characterized by pyknotic nuclei in the medullary region of the lymphoid follicle, slight lymphoid depletion, infiltration of inflammatory cells in the interstitial connective tissue, degenerative alterations, edema, interstitial fibroplasia, medullary necrosis with cyst formation, fibrosis, absence of lymphoid follicle due to hemorrhage and other types of alterations in the lymphoid follicles (Figure 3). The results of bursal lesion score correlated withprevious findings.[19,24,27–29]

Conclusion

Infectious Bursal Disease (IBD) is an acute, highly contagious viral disease of chickens often known as Gumboro disease. In the current study, we investigated the pathogenicity and pathology of IBDV in broiler chickens under field condition. Determining bursa- body weight ratio, pathology and bursal lesion scoring might be implicated to evaluate the pathogenicity of IBDV in broiler chickens under field condition to identify the severity of the problem in real time to take necessary steps to control the disease.

Acknowledgments

The research was supported by the Department of Pathology, Sylhet Agricultural University, Sylhet 3100, Bangladesh. Authors are thankful to the Department of Anatomy and Histology, Sylhet Agricultural University,Sylhet 3100,Bangladesh for their cordial cooperation to prepare histological slides.

Conflicts of interest

The authors declare that no conflict of interest exists.

References

1. Dobos P, Hill BJ, Hallett R, et al. Biophysical and biochemical characterization of five animal viruses with bisegmented double–stranded RNA genomes. *J Virol* .1979;(32):593–605.
2. Leong JC, Brown D, Dobos P, et al. *Birnaviridae*. In: MHV Regenmortel, CM Fauquet, DHL Bishop, et al. *Virus Taxonomy Classification and Nomenclature of Viruses.* 2000. USA: Academic press; 2000. p. 481–490.
3. McFerran JB, McNulty MS, McKillop ER, et al. Isolation and serological studies with infectious bursal disease viruses from fowl, turkey and duck:demonstration of a second serotype. *Avian Pathol*. 1980;9(3):395–40.
4. Hirai K, Calnek BW. In vitro replication of infectious bursal disease virus in stablished lymphoid cell lines and chicken B lymphocytes. *Infect and Immun.* 1979;(2):964–970.
5. Käufer I, Weiss E. Significance of bursa of Fabricius as target organ in infectious bursal disease of chickens. *Infect and Immun.*1980;27(2):364–367.
6. Müller H. Replication of infectious bursal disease virus in lymphoid cells. *Arch of Virology.*1986;(87):191–203.
7. Cosgrove AS. An apparently new disease of chickens – Avian Nephrosis. *Avian Dis*. 1962;(6):385–389.
8. Rosenberger JK, Cloud SS. Isolation and characterization of variant infectious bursal disease viruses. *J American Vet Med Asso.*1986;(189):35.
9. Saif YM. *Infectious bursal disease virus type*. In: Proceedings of the 19th National Meeting on Poultry Health and Condemnations Ocean Citypp. 1984; p. 105–107.
10. Van den Berg TP, Meulemans G. Acute infectious bursal disease in poultry:protection afforded by maternally derived antibodies and interference with live vaccination. *Avian Pathol*. 1991;20(3):409–421.
11. Fadly AM, Winter field RW, Olander HJ. Role of the bursa of Fabricius in the pathogenicity of inclusion body hepatitis and infectious bursal disease viruses. *Avian Dis*. 1976;20(3):467–477.
12. Pejkovski C, Davelaar FG, Kouwenhoven B. Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. *Avian Pathol.*1979;8(1):95–106.
13. Moradian A, Thorsen J, Julian RJ. Single and combined infections of specific–pathogen–free chickens with infectious bursal disease virus and an intestinal isolate of reovirus. *Avian Dis.* 1990;34(1):63–72.
14. Islam MR, Das BC, Hossain MR, et al. A Study on the Occurrence of Poultry Diseases in Sylhet Region of Bangladesh. *Int J Poul Sci*. 2003;(25):354–356
15. Badruzzaman ATM, Noor M, Mamun MAL et al. Prevalence of diseases in commercial chickens at Sylhet division of Bangladesh. *Int Clin Pathol J*. 2015;1(5):104–108.
16. Charlton BR. *Bacterial Diseases*. In: Avian Disease Manual. 5th edn. USA: The American Association of Avian Pathologists; 2000. p. 103–1013.
17. Bennet C. How to score bursa size in broilerchicken flocks. *University of Saskatchewan.* 2002.
18. Bancroft JD, Gamble M. *Theory and Practice of Histological Techniques*. Elsevier Health Science. 2008. p. 53–105.
19. Al–Mayah A, Tabeekh M. Histopathological Changes of Bursa Fabricius of Imported Broilers and Local Chicks Vaccinated With Two Types of Infectious Bursal Disease Vaccines. *Iraq acasci j*. 2012;6(1):80–95.
20. Singh J, Banga HS, Brar RS, et al. Histopathological and immunohisto chemical diagnosis of infectious bursal disease in poultry birds. *Vet World*. 2015;8(11):1331–1339.
21. Enurah LU, Nwosuh CI, Ehizibolo DO, et al. Gross and Histopathological Changes in Chickens Infected with Infectious Bursal Disease Virus (IBDV) in a Farm in Vom, Plateau State, Nigeria. *Asian J Res Animal and Vet Sci*. 2018;2(4):1–9.
22. Mazariegos LA, Lukert PD, Brown J. Pathogenicity and immuno–suppressive properties of infectious bursal disease "intermediate" strains. *Avian Dis*. 1990;34(1):203–208.
23. Iván J, Nagy N, Magyar A, et al. Functional restoration of the bursa of Fabricius following in ovo infectious bursal disease vaccination. *Vet ImmunImmunopathol*. 2001;79(9):235–248.
24. Murmu R, Islam MN, Juli MSB et al. Pathogenicity and immunosuppressive properties of GM–97 strain of infectious bursal disease virus in commercial broiler chickens. *J Adv Vet Anim Res.* 2014; 1(1):1–7.
25. S Sellaoui N, Alloui S, MehenaouiDjaaba. Evaluation of Size and Lesion Scores of Bursa Cloacae in Broiler Flocks in Algeria. *J World's Poult Res.* 2002;2(3):37–39.
26. Riddel C. *Avian Histopathology*. American Association of Avian Pathologists. 1st edn. University of Pennsylvania. 1987. p. 71.
27. Islam MN, Rashid SMH, Hoque MF, Juli MSB, et al. Pathogenicity of IBDV related to outbreaks in the vaccinated flocks and the causes of vaccination failure. *J InnovDevelStrat*. 2008; (2):22–30.

28. Tsukamoto K, Tanimura N, Kakita S, et al. Efficiency of three live vaccines against highly virulent strains of infectious bursal disease virus in chickens with or without maternal antibodies. *Avian Dis*.1995;(39):218–229.

29. Chakraborty P, Nath BD, Islam RM, et al. Comparative susceptibility of fayoumi, indigenous and white leghorn chicks to infectious bursal disease. *ARPN J AgriBiolo Sci.* 2010;(5):27–34.