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RESEARCH ARTICLE

MOLECULAR DIAGNOSIS OF INFECTIOUS BURSAL DISEASE OUTBREAK IN CHICKENS IN AND AROUND SHILLONG, MEGHALAYA

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ABSTRACT

A study was conducted during the period from August, 2015 to April, 2016 to survey the occurrence of viral diseases in chicken in and around Shillong, Meghalaya, to study the pathology and finally to diagnose them by using common molecular techniques. A total of 370 dead and sick birds were collected from different organized and unorganized poultry farms in and around Shillong, Meghalaya. Of these, 109 cases (i.e. 29.46%) were diagnosed as viral diseases. Out of 75 chickens suspected for IBD based on the clinical history, signs, gross and histopathology, 48 cases (12.97%) could be confirmed by RT-PCR using a primer of length 643 bp targeting the VP2 gene. Most of the cases were seen in age group of 3-6 (47.92%) weeks, followed by 6-9 (20.83%), 1-3 (18.75%) and 9-12 (12.50%). The morbidity and mortality rates recorded during the study period ranged from 3.5 - 5.4% and 38.5 - 52.6% respectively. The characteristic signs recorded during the study included dullness, depression, anorexia, ruffled feathers and yellowish white or greenish yellow diarrhoea. Most of the birds were disinclined to move and pecked at their vents. On post-mortem, most of the birds showed darkened discolouration of thigh and breast muscles with paint brush like haemorrhages. Bursa were enlarged and swollen with accumulation of thick mucoid, creamy or bloody exudates. Some birds showed congestion and haemorrhages at the junction of bursa and proventriculus. Microscopic examination of bursa revealed complete lymphoid depletion, formation of cyst filed with necrotic debris, heterophils and diffused haemorrhages. For confirmatory diagnosis, virus detection was done by RT-PCR.

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INTRODUCTION

Infectious bursal diseases (IBD) is a highly contagious acute viral disease of 3-6 weeks old birds which causes immunosuppression by damaging bursa of Fabricius and impairs the growth of young chickens resulting to significant economic losses in the poultry industry (Lukert, 1997). It is caused by infectious bursal diseases virus (IBDV), a non-enveloped double stranded RNA (dsRNA) virus belonging to family Birnaviridae (Jackwood *et al*., 1984). Chicken and turkey are the natural hosts of virus and all breeds of chicken are affected. Mortality due to IBD ranges from 1 to 40% (Kurade *et al*., 2000; Saif *et al*., 2000). It occurs all around the year (Babiker *et al*., 2008), but more in winter season followed by rainy, summer and spring seasons (Jindal *et al*., 2004; Sultana *et al*., 2008).

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Incubation period of IBD ranges from 2 to 4 days. The disease is manifested by dehydration, trembling, ruffled feathers, anorexia, vent pecking, depression and whitish loose diarrhea (Butcher, 2012). The bursa of Fabricius is the primary target organ of the virus. Post-mortem examination of the birds usually shows enlarged and swollen bursa with accumulation of thick mucoid, creamy or bloody exudates. Petechial haemorrhages on the mucosal surface of bursa with blood clots may be seen in severe case (Younus, 1996; Zeleke *et al*., 2005). Most cases show darkened discolouration of thigh and breast muscles with paint brush like haemorrhages. Some birds use to have congestion and haemorrhages at the junction of bursa and proventriculus. Microscopic changes include diffuse haemorrhages in bursal follicle with focal areas of lympho-follicular necrosis, both in cortex and medullary area. Cystic degeneration, lymphoid depletion and areas of edema may also be present. Proliferation of connective tissue, follicular atrophy, severe heterophillic and lymphocytic infiltration in the interfolicular connective are also recorded (Dutta *et al*., 2007; Samanta *et al*., 2008).

Diagnosis is mainly based on clinical history, signs, gross and microscopic changes. For confirmatory diagnosis serological test like AGPT and ELISA can be used for detecting IBDV antigen and antibody (Gaba, 2004). Beside this RT-PCR and nucleic acid hybridization are used for the detection and differentiation of various IBD viruses (Lin *et al*., 1994; Kataria *et al*., 2001). Both organized and unorganized poultry farms in and around Shillong, Meghalaya were visited regularly during the study period from August, 2015 to April, 2016 and the morbidity, mortality, age of affection of various diseases were recorded. To assess the age-wise variations in the incidence of the diseases, the birds were grouped as 1-3, 3-6, 6-9, 9-12 and above 12 weeks old. In case of mortality/outbreak of diseases in the poultry population, the clinical signs exhibited by the individual bird during illness were recorded in details according to the description of the respective poultry farm's owner or attendant. In addition, sometimes some sick/moribund birds were kept under careful observation with feed and water *ad libitum* till death to record the detailed clinical signs along with other abnormalities.

Detailed post-mortem examination of all the dead birds was performed and gross tissue changes were recorded carefully. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, brain, feather follicles, etc.) showing lesions were carefully collected in 10% formaldehyde solution for histopathological examination. These were processed and stained with Mayer's hematoxylin and eosin (Bancroft., 1980). The diagnosis of the disease was made mainly basing on the clinical signs, characteristic gross and microscopic changes. For confirmatory diagnosis, virus detection was done by RT-PCR, which was performed as per the procedure described by OIE manual (2012) (OIE, 2012) with modification. In the present study, the maximum cases of IBD was recorded in 3-6 weeks old birds (47.92%), which is in support of earlier record[1] as well as the report of the workers (Mor *et al*., 2010) who found maximum cases (52.80%) in 21-30 days old birds followed by (33.13%) in 31-40 days old birds in Haryana. The younger chicks of 1-3 weeks as well as 6-9 weeks old were also found affected during the investigation, which is in conformity with the earlier reports (Fadley, 1983; Okoye, 1981).

The disease was found to occur all around the year and the same was reported by previous workers (Dey *et al*., 2009). The percent morbidity varied from 3.5-5.4%, while percent mortality varied between 38.5-52.6% during the period under study, which is nearly similar to the previous reports (Kurade *et al*., 2000; Saif *et al*., 2000; Dey *et al*., 2009). The low morbidity and mortality rates recorded during this present study might be due to regular vaccination of the chicks and proper managemental practices. The clinical signs like dullness, depression, anorexia, ruffled feathers and yellowish white or greenish yellow diarrhea recorded during the present investigation are in agreement with the earlier findings (Islam, 2004; Butcher, 2012; Rashid *et al*., 2013). Most of the birds were disinclined to move and pecked at their vents and pericloacal feathers were stained with urates as similarly described by workers (Cosgrove, 1962; Landgraf *et al*., 1967). The post-mortem findings of the present study included haemorrhages and darkened discoloration of thigh and breast muscles in most cases (Fig.1), which supports the findings of several workers (Lukert, 1997; Sultana *et al*., 2008; Islam, 2004; Singh, 2008).

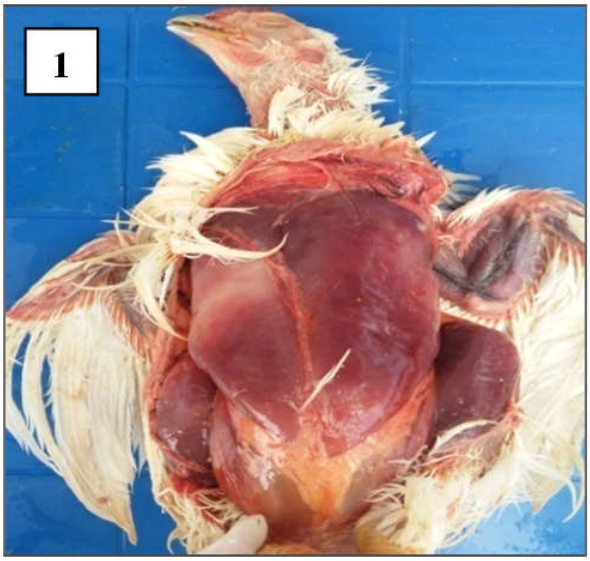

Fig.1. IBD affected bird showing haemorrhages on breast and thigh muscles

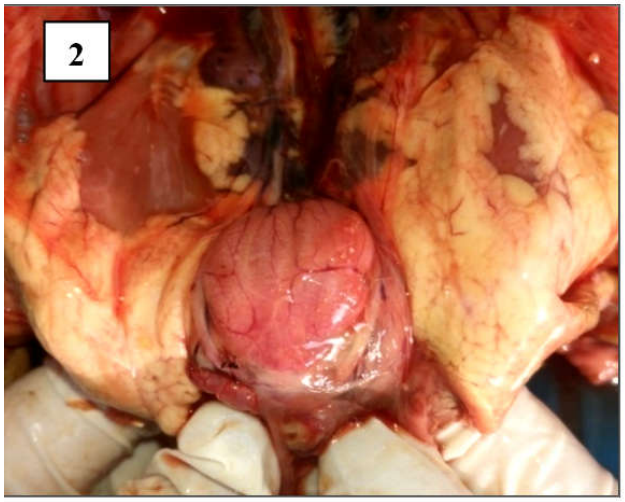

Fig.2. IBD affected bird showing enlarged, congested and swollen bursa

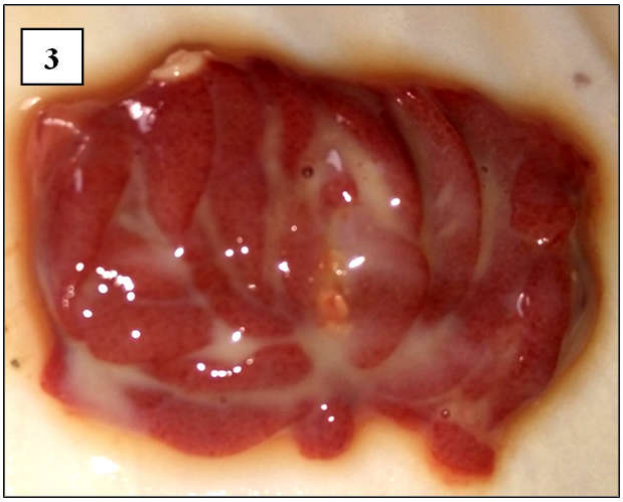

Fig. 3. Enlarged and swollen bursa with accumulation of thick creamy exudates

In most of the cases, bursa was congested, enlarged and swollen (Fig.2) with accumulation of thick creamy (Fig.3) or cheesy exudates, while in some cases; there were presence of gelatinous exudates around bursa.

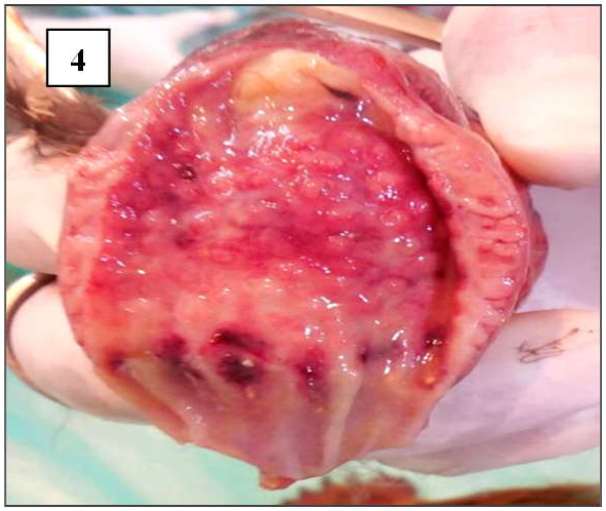

Fig.4. IBD affected bursa showing congestion and haemorrhages at junction of proventriculus and gizzard

These findings are in agreement with the previous reports (Sultana *et al*., 2008; Younus, 1996; Zeleke *et al*., 2005; Dutta *et al*., 2007). Congestion and enlargement of liver were also noticed in few cases; however, these findings were not consistent. Spleens in most of the cases were enlarged, mottled and very often small grey foci uniformly dispersed on the surface. Most of the birds showed congestion and haemorrhages on the mucosa of proventriculus, while some cases revealed congestion and haemorrhages at the junction of proventriculus and gizzard (Fig.4). These gross lesions of liver, spleen and proventriculus recorded during this present study are found almost similar to those described by previous researchers (Dutta *et al*., 2007; Islam, 2004). In most of the cases, the kidneys were congested, enlarged and swollen (Fig.5), which might be due to deposition of urates caused by the enlarged bursa. Similar observations have been reported by some workers (Dutta *et al*., 2007; Islam, 2004).

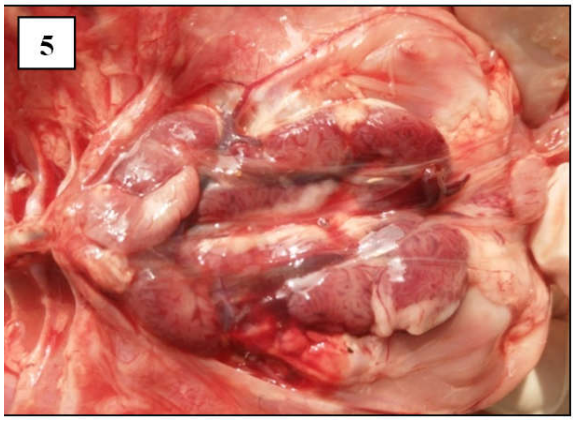

Fig.5. Congested, enlarged & swollen kidneys with prominent tubules

Thymus in most cases was found to be enlarged, congested and haemorrhagic, which might be due involvement of virulent form of IBDV and secondary infections. Microscopically, the bursa of Fabricius showed congestion, complete lymphoid depletion in the follicles leading to formation of cysts (Fig.6) filled with necrotic debris, heterophils and haemorrhages in the interfolicular tissue. In few cases, areas of exudates, necrotic debris with severe heterophillic and lymphocytic infiltration in the bursal lumen were also recorded.

These findings are in the line of earlier observations of several workers (Lukert, 1997; Younus, 1996; Zeleke *et al*., 2005; Dutta *et al*., 2007; Samanta *et al*., 2008). In most of the cases, the spleen showed depletion of lymphocytes, congestion and focal or diffused areas of haemorrhage, which are in support of the previous reports (Dutta *et al*., 2007). The kidney lesions of tubular epithelium degeneration and congestion in the interstitium are supported by the findings of workers (Dutta *et al*., 2007). There were congestion, degeneration of hepatocytes and lymphoid aggregations in portal areas in the liver sections. Lymphoid depletion in caecal tonsils recorded during the present study period supports the findings of earlier researchers (Uddin *et al*., 2010) who observed significant reduction of lymphocytes in caecal tonsils, proventriculus, duodenum, jejunum, ileum and caecum. Severe congestion in parabronchial area of lungs and microscopic changes of liver which showed congestion, degeneration of hepatocytes and lymphoid aggregations in portal areas might be due to involvement of virulent form of IBDV and secondary infections.

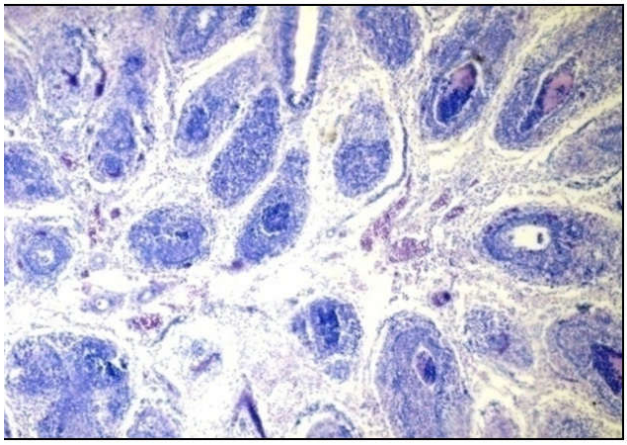

Fig.6. Bursa showing lymphoid depletion, cyst formation and congestion (H & E, 10x)

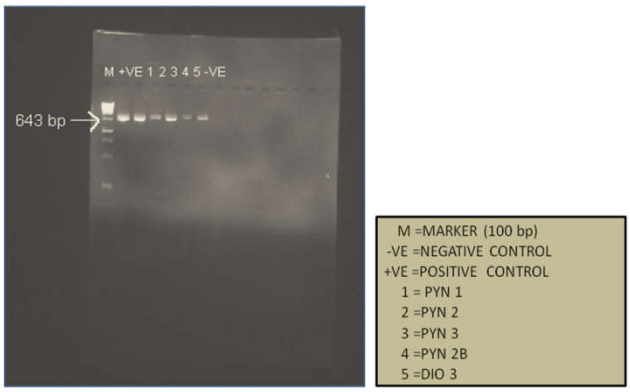

Fig. 7. 1.5% Agarose gel electrophoresis stained with Ethidium bromide showing the PCR products (643 bp) of IBD virus in tissue samples

The disease was clinically diagnosed on the basis of clinical history from the responsible persons of the farms, recorded clinical signs and gross and microscopic lesions of affected chickens. RT-PCR, a nucleic acid based detection test, was used as confirmatory diagnosis for the detection of IBD viral genome (Fig.7). Tissue samples comprising of bursa, spleen, thymus and liver from a total of 75 clinically IBD suspected cases were tested for detection of the F gene.

Out of 75 IBD suspected cases, 48 (64%) cases were found positive. Similar diagnostic techniques have also been performed by several workers (Zahoor *et al*., 2010; Islam *et al*., 2011; Barathidasa *et al*., 2013). The present RT-PCR positive results (64%) is lower than that of earlier workers (Fatima *et al*., 2013) who could detect 81 (95.29%) samples positive out of 85 bursal samples, which might be due improper clinical diagnosis of IBD suspected cases.

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