ACUTE INFECTIOUS BURSAL DISEASE IN CHICKENS: PATHOLOGICAL OBSERVATION AND VIRUS ISOLATION

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Summary

Pathological and virological investigations were conducted on suspected outbreaks of infectious bursal disease (IBD) in a broiler farm and five pullet-raising poultry farms of Mymensingh and Tangail districts of Bangladesh. About 80 to 100 percent chicks were affected at the age of 26 to 45 days and mortality varied from 20 to 30 percent in broilers and 40 to 80 percent in layer chicks. Signs, symptoms, gross and microscopic lesions were typical of acute IBD. Several isolates of virus could be obtained by embryo inoculation and the virus was diagnosed as infectious bursal disease virus (IBDV) by agar gel immunodiffusion test (AGID). The virus isolate belonged to the very virulent pathotype of IBDV causing 100 percent mortality in three weeks old chicks on experimental infection.
(**Key Words**: Infectious Bursal Disease, Pathology, Chickens, Bangladesh)

Introduction

Infectious bursal disease (IBD), popularly known as Gumboro disease, is a contagious disease of young chickens caused by a dsRNA virus belonging to the family Birnaviridae. Since the first description of the disease (Cosgrove, 1962) infectious bursal disease virus (IBDV) drew the attention of avian virologists mostly because of its profound immunosuppressive effects (Allan et al., 1972). In mid 1980s several antigenic variants of IBDV appeared in the USA (Snyder et al., 1988). This was followed by the emergence of a very virulent pathotype of IBDV (vvIBDV) in western Europe, which caused an acute disease with very high mortality reaching up to 70% in natural infection and 100% in experimental infection (van den Berg et al., 1991). Within a couple of years the vvIBDV spread across Asia (Nunoya et al., 1992). The poultry farms of Bangladesh have been experiencing the outbreaks of a disease resembling acute IBD since 1992. The present paper reports on the pathology of naturally occurring IBD in Bangladesh and isolation and identification of the virus.

Materials and Methods

Collection of samples and pathological study

Three subsequent outbreaks of suspected IBD in a broiler farm and five outbreaks in one White Leghorn and four Fayoumi pullet-raising farms of Mymensingh and Tangail districts of Bangladesh during the year 1993 were investigated. History, clinical signs and symptoms of the disease were recorded. Representative samples of dead birds were selected from each outbreak for pathological and virological study.

A thorough postmortem examination was conducted on each bird. Gross lesions were recorded and pieces of tissues from various organs were preserved in 10% neutral buffered formalin for histopathological study. A part of the bursa of Fabricius was collected aseptically and stored at −20℃ in 50% sterile buffered glycerol for virus isolation. For histopathology, the fixed tissues were processed for paraffin embedding, sectioned and stained with haematoxylin and eosin following standard procedures.

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Isolation of virus

Preserved samples of the bursa of Fabricius were washed with sterile phosphate buffered saline (PBS) and homogenised to make a 10-20% (w/v) suspension in PBS. The suspensions were clarified by low speed centrifugation and treated for 30 minutes at room temperature with gentamycin @ 500 μg/ml. Each of the suspensions was inoculated in five 10-day old embryonated chicken eggs through the chorio-allantoic membrane (CAM) route of inoculation. The embryos were examined daily for mortality by candling and those died within 24 hours of inoculation were discarded. The embryos which died in the next seven days were opened aseptically and the CAMs and embryos were collected and preserved at −20°C. All the surviving embryos were killed by chilling on day 8 post inoculation (p.i.) and examined for the presence of any lesions.

Four of the isolates, thus obtained, were subjected to a second passage in embryonated chicken eggs as above to confirm their embryo pathogenicity. On the basis of the consistent pattern of embryo mortality and embryo lesions, one of the isolates namely BD-6 was selected for further study.

Pathogenicity of the isolate BD-6 was tested by chick inoculation in a preliminary trial. A total of eight White Leghorn chicks were hatched in the laboratory and raised in cages in relative isolation. At three weeks of age five chicks were inoculated intramuscularly with the BD-6 isolate @ one ml of 20% (w/v) embryo homogenate per bird. The remaining three chicks were kept separately as uninoculated controls. The chicks were observed closely for seven days for the appearance of any sign of illness. The birds which died were subjected to routine postmortem examination. The bursa of Fabricius obtained from the dead birds were homogenised and inoculated in chicken embryos as above for reisolation of the virus.

Identification of the virus

Identity of the virus isolate BD-6 was confirmed by an agar gel immunodiffusion (AGID) test against a known reference antiserum to the classical serotype 1 of IBDV. The known reference antiserum and reference antigen were obtained from the Central Disease Investigation Laboratory, Department of Livestock Services, Dhaka. The BD-6 test antigen was prepared as a 50% (w/v) suspension of the bursa of Fabricius obtained from four five-week old chicks which were killed four days after oral infection with the BD-6 isolate. Similar suspension was also prepared from non-infected chicks to be used as negative control antigen. The test was performed in a plastic petri dish containing agar gel (0.9% agarose in phosphate buffered saline containing 0.1% sodium azide as preservative). Wells of 5 mm diameter were cut 3 mm apart in a daisy pattern. The reference antiserum was placed in the central well and the test antigen, reference antigen and negative control antigen were placed in the peripheral wells. The plates were examined for precipitation lines at 24 and 48 hours against reflected light.

Results

History and clinical manifestations

The size of the affected flocks was between 200 to 250 birds in layer chicks and 600 birds in broilers. The chicks were vaccinated against Marek's disease and Newcastle disease. The birds were affected at the age between 26 and 45 days. About 80 to 100% chicks were affected in both broiler and layer type birds. Mortality in broilers varied from 20 to 30% while that was 40% in White Leghorn chicks and 75 to 80% in Fayoumi chicks (table 1). The recovered birds remained stunted. Concurrent coccidiosis and aspergillosis were noticed in one and two farms respectively.

The clinical signs and symptoms included anorexia, depression, ruffled feathers, diarrhoea and death. Mortality reached a peak within two to three days of onset and then declined but continued for a week or so.

Postmortem findings

At necropsy, the carcasses were found either in good flesh or highly dehydrated and emaciated. Congestion of the subcutaneous blood vessels was frequently seen. Petechial and ecchymotic haemorrhages and congestion were often observed in the leg and breast muscles. In most cases the bursa of Fabricius was swollen and oedematous with creamy or yellowish discoloration. Gelatinous exudate was frequently found in the serosa of the bursa. Congestion and haemorrhages on the serosal and mucosal surfaces of the bursa were also noticed in few cases. Occasionally, the bursa became atrophied and whitish or creamy, which sometimes contained cheesy mass in the lumen. The spleen became swollen and mottled in some cases but atrophied and pale in others. The thymus was found necrosed in most cases. Few chicks had swollen and pale kidneys. The liver in some cases had congestion and pale areas. Haemorrhages in the caecal tonsils and at the junction between proventriculus and gizzard were seen in one case.

TABLE 1. CHARACTERISTICS OF OUTBREAKS AND RESULTS OF VIRUS ISOLATION FROM THE BURSA OF FABRICIUS BY EMBRYO INOCULATION

Sample No.	Age and breed of birds	Mortality in flock	Appearance of bursa	Results of embryo inoculation			
				No. inoculated	No. died	Died on	Embryo lesion
BD-3	37 d old White Leghorn	40%	atrophied, creamy	5	0	–	+
BD-4	33 d old Fayoumi	80%	swollen, oedematous	5	0	–	+
BD-5	-do-	-do-	swollen, cheesy core	5	0	–	–
BD-6	-do-	-do-	swollen, cheesy core	5	3	2 dpi	+
BD-7	35 d old broiler	30%	atrophied, necrotic	5	0	–	–
BD-8	45 d old Fayoumi	75%	haemorrhagic	4*	1	7 dpi	+
BD-9	-do-	-do-	swollen, oedematous	4*	2	2 dpi	+
BD-10	35 d old broiler	30%	atrophied	4*	2	7 dpi	+
BD-11	26 d old broiler	25%	swollen, oedematous	5	0	–	–
BD-12	-do-	-do-	atrophied	4*	1	7 dpi	+
BD-14	42 d old Fayoumi	80%	swollen, oedematous	4*	1	7 dpi	+
BD-15	42 d old broiler	20%	very atrophied	5	0	–	–

* One embryo from each of these groups died at 24 hour p.i. which were discarded.
dpi - day post inoculation.

Histopathology

Severe depletion of lymphocytes was the main pathological lesion in the bursa of Fabricius. The areas of lymphoid depletion in the follicles were either haemorrhagic (figure 1) or contained eosinophilic homogenous tissue debris with many karyorrhectic nuclei of lymphocytes (figure 2). In few cases the depleted lymphocytes were replaced by proliferating reticuloendothelial cells. Heterophilic infiltration around the follicles was also noticed. Almost complete depletion of lymphocytes resulting in atrophy of follicles, fibroplasia around the follicles and cystic spaces within the follicles

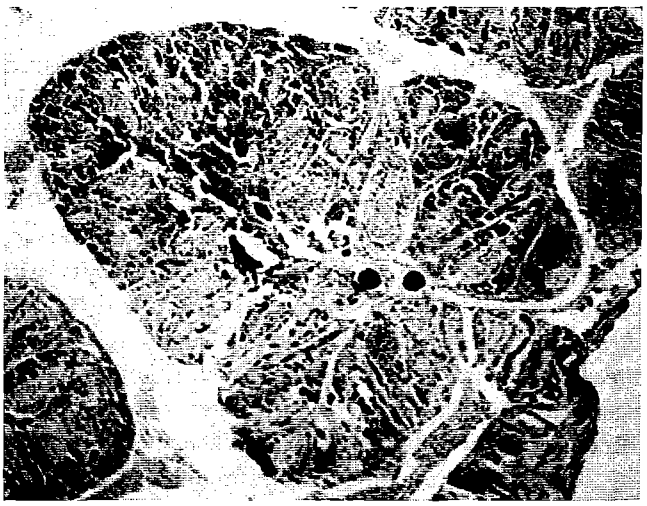

Figure 1. Severe depletion of lymphocytes and extensive haemorrhage (dark areas) in the bursa of Fabricius of a layer chick died of infectious bursal disease. (H & E stain, × 62).

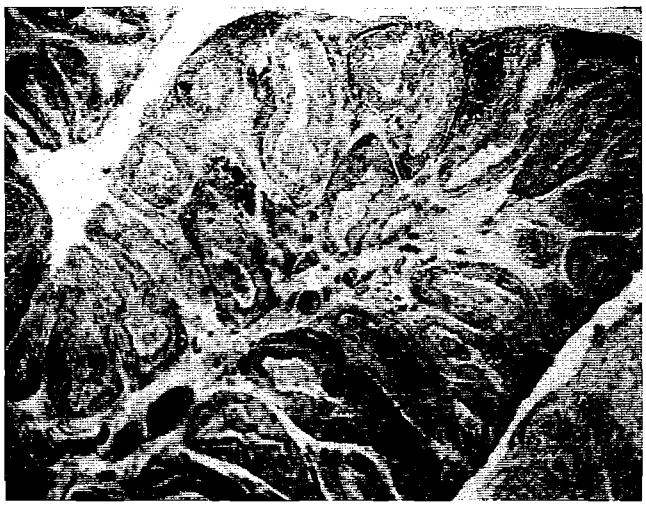

Figure 2. Lymphoid depletion and necrotic tissue debris in the follicles of the bursa of Fabricius of a layer chick died of infectious bursal disease. (H & E stain, × 62).

(figure 3) were noticed in few cases, particularly in the broilers.

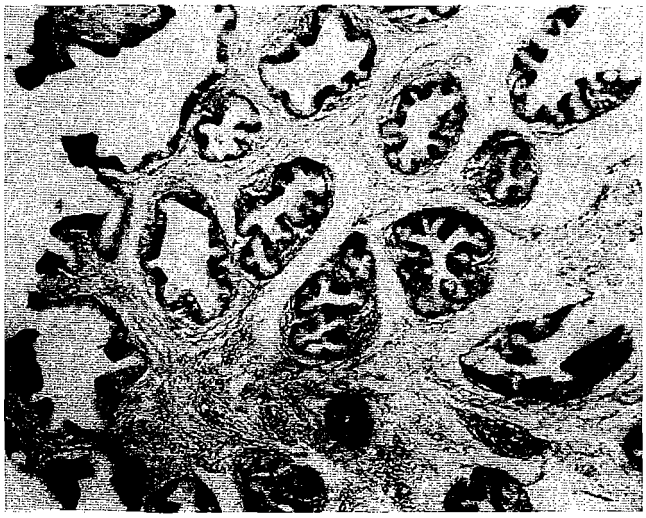

Figure 3. Complete depletion of lymphocytes with cystic follicular atrophy and fibroplasia in the bursa of Fabricius of a broiler chick died of infectious bursal disease. (H & E stain, × 62).

Lymphoid depletion of various degree was observed in the spleen, this was occasionally associated with congestion, haemorrhage, and thickening of the artery wall with fibrinoid degeneration. Moderate to severe lymphoid depletion was seen in the thymus and caecal tonsils.

In the liver the central veins were congested. Fatty change and focal or diffuse coagulation necrosis of hepatocytes were also observed in few cases. Nephrotic lesions including degeneration, dissociation and coagulation necrosis of tubular epithelial cells were occasionally seen in the kidney.

Virus isolation and identification

Virus isolation was attempted from 12 samples of antibiotic-treated homogenised bursa of Fabricius. The results are presented in table 1. Six samples caused embryo mortality on either day 2 or 7 p.i. Severe congestion and haemorrhages were seen in dead embryos, particularly in the breast muscles, heart, liver, along the feather tracts of the skin and occasionally in the cerebral regions and toe joints. Most of the surviving embryos, belonging to the groups where some mortalities were observed, exhibited some lesions. Moreover, two samples (BD-3 and BD-4), which did not cause embryo mortality, induced some lesions. The remaining four samples did not kill embryo and neither did induce any lesion. Four selected positive samples, namely BD-6, BD-9, BD-12 and BD-14, were passaged once again in chicken embryos. All of these except BD-12 caused embryo mortality on day 2 and 3 p.i. in the second passage.

The finally selected isolate BD-6 was tested for its pathogenicity in three weeks old chicks. All the inoculated chicks became ill on day 2 or 3 p.i. with depression, anorexia and slight diarrhoea. All of them died on day 3 or 4 p.i. At necropsy, a large amount of serofibrinous fluid was found at the site of intramuscular inoculation in the leg, around the cervical region and in the serosa of the bursa. The bursa was swollen and the spleen was pale in colour. Uninoculated control birds remained healthy throughout the observation period of seven days. Suspensions of the homogenised bursa of Fabricius obtained from the infected birds killed chicken embryos on day 2 or 3 p.i. following inoculation through the CAM route. Embryo lesions were similar to those observed earlier with the original sample.

In AGID test the BD-6 antigen gave positive reaction against the known reference antiserum.

Discussion

This study was conducted on eight outbreaks of suspected IBD. Clinical manifestations and gross and histopathological lesions were studied. Virus was isolated from representative samples, a selected isolate was tested for its pathogenicity in chicks and identity of the isolate was confirmed by AGID test.

Chicks aged between 26 and 45 days were affected with 20-30% mortality in broilers and 40-80% mortality in layer chicks. This observation of high mortality, particularly in layer chicks, is similar to those reported in other countries where very virulent pathotype of IBDV had emerged (van den Berg et al., 1991; Nunoya et al., 1992). Relatively lower mortality in broiler chicks was also observed by van den Berg et al. (1991) and Nunoya et al. (1992). Among the layer chicks, apparently Fayoumi breed seemed to have higher mortality (75 to 80%) as compared to that in White Leghorn breed (40%). However, any conclusion on breed differences among the layer type chicks can not be drawn from the present study as only a single outbreak of IBD in White Leghorn chicks was investigated. Moreover, there also might be variation in the virulence of virus strains involved.

The clinical manifestations, gross and histopathological lesions observed in the present study are in general similar to those documented earlier and reviewed by Okoye

(1984) and Lukert and Hitchner (1984). However, there were some variations in the type and magnitude of lesions from one sample to another. The bursa of Fabricius was atrophied in some cases while swollen and oedematous or haemorrhagic in others. Similarly, the spleen was swollen and mottled in some cases but pale and atrophied in others. These variations might have been due to the differences in the duration of illness prior to death in individual birds. Microscopic lesions also varied. The presence of oedema, haemorrhage and congestion along with lymphoid depletion was common in layer chicks, while almost complete lymphoid depletion leading to follicular atrophy and fibroplasia was more frequent in broilers. The latter might be indicative of a relatively longer course of the disease. The degree of reticuloendothelial cell proliferation and heterophil infiltration also varied from sample to sample, the reason of which could not be explained readily.

Six out of 12 samples of bursal tissue homogenate caused embryo mortality and two more samples induced embryo lesions without mortality. The embryo lesions were similar to those usually observed with IBDV (Lukert and Hitchner, 1984). The remaining four samples which did not kill embryos or induce any lesions could not be ruled out as negative for IBDV as subsequent blind passages were not conducted. Moreover, gross and histopathological lesions observed in these samples were typical of IBDV infection.

The isolate BD-6 was found to be highly pathogenic for three weeks old chicks during a preliminary experimental trial. All the inoculated chicks became ill by day 2 or 3 p.i. and died between day 3 and 4 p.i. The very short incubation period might have been due to the intramuscular inoculation of the virus in a massive dose. However, 100% mortality within 24 to 48 hours of the onset of the illness suggests that the isolate belongs to the very virulent pathotype of IBDV. One hundred percent mortality in chicks following experimental infection with vvIBDV was also reported by van den Berg et al. (1991). The virus was reisolated in the embryos from the experimentally infected chicks. The detailed pathology following experimental oral infection of five-week old chicks with BD-6 isolate has been studied in another experiment, the result of which will be presented in a separate communication.

The identity of the BD-6 isolate was further confirmed by AGID test where it gave positive reaction against a known reference antiserum to the classical serotype 1 IBDV.

Acknowledgements

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