RESEARCH ARTICLE

Pathological and Molecular Study of Infectious Bursal Disease in Commercial Chicken

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Abstract

A total of 100 Poultry carcasses suspected for infectious bursal disease were collected from 20 poultry farms and conducted postmortem at the Department of Veterinary Pathology, NTR College of Veterinary science, Gannavaram (Andra Pradesh , India) . Sick birds showed ruffled feathers, loss of appetite, whitish diarrhea and soiled vent. On postmortem examination, grossly bursa of Fabricius was swollen, enlarged with mucous exudate in lumen and haemorrhages were noticed on the mucosa. Paint brush haemorrhages were noticed on thigh muscles and at the junction of gizzard and proventriculus. Kidneys were swollen and ureters were filled with urate crystals. Histopathology of bursa of Fabricius revealed shrinkage of follicles, severe lymphoid depletion, necrosis, lymphocytolysis and cyst formation in follicles. Diagnosis of the disease was confirmed by targeting the VP2 gene of infectious bursal disease virus (IBDV) with a reverse transcriptase polymerase chain reaction which revealed an amplicon size of 743 bp. Based on gross and histopathological lesions and molecular findings disease was confirmed as infectious bursal disease.

Key words: Gross lesions, Histopathology, Infectious bursal disease, Poultry, Diagnosis

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Introduction

Infectious bursal disease (IBD) is an acute, highly contagious disease that results in mortality and immunosuppression of young chicken (Spackman *et al*., 2018). The consequences of immunosuppression associated with IBDV are vaccination failure and susceptibility of chickens to opportunistic pathogens (Fan *et al.,* 2020).

It is an economically important disease, caused by birna virus belongs to genus Avibirnavirus. The family Birnaviridae is a non-enveloped virus that contains a bi-segmented, double-stranded RNA genome (Muller and Nitschke, 1987). In the field, the emergence of new viruses with new properties are due to the high mutation rate of the RNA-polymerase in these viruses which generates a genetic diversification, hence the new viruses persist in immune populations. Up to now two serotypes of IBDV have been identified. However, only serotype I viruses are naturally pathogenic to chickens (Jackwood and Saif, 1987). The IBDV genome is divided into segments A and B, segment A is about 3.4 kb and B is about 2.8 kb. The large segment A encodes 4 viral proteins, the two capsid proteins, VP2 (48 kDa) and VP3 (32–35 kDa), the viral protease VP4 (24 kDa), and a nonstructural protein VP5 (17–21 kDa), while the smaller segment B encodes VP1 (90 kDa), an RNA-dependent RNA polymerase. Among five proteins of IBDV, the VP2 is the major host-protective antigen that induces serotype-neutralizing antibodies (Fahey *et al.,*1989). IBDV affects 3-6 week old chicks, once a rearing site has been contaminated horizontal transmission of virus occurs between flocks through ingestion of feed and water contaminated with infectious feces.

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Even though IBDV was isolated in 1957, still now different virulent strains with different virulence, emerged successively and spreading disease all over the world and posing new challenges in prevention and control of this disease (Withers *et al.,* 2005 and Thai *et al.,* 2022). In India, the IBD also reported from various regions due to emergence of novel very virulent strains in chicken which were already vaccinated with classical of intermediate types of vaccines (Akram *et al.,* 2020, Deorao *et al.,* 2021 and Agnihotri *et al.,* 2022).

Keeping this in view, the current study was designed to assess the different clinical signs, gross and histopathology, and molecular diagnosis of infectious bursal disease in commercial chicken.

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Materials and Methods

A total of 100 moribund birds were collected from the 20 poultry flocks (5 birds from each flock) in Andhra Pradesh (India). The history of affected birds was collected from the poultry farmers. Clinical symptoms were noted. After the postmortem examination of the dead birds, tissue samples were collected in 10% formalin for histopathology and on ice and stored at-80°c for molecular studies.

The tissues were processed using paraffin embedding technique for preparation of sections and stained with haematoxylin and eosin (H&E) for observing microscopic changes. Pooled bursal tissue sample were taken from 20 poultry flocks and were screened for the presence of IBDV by reverse transcriptase PCR (RT-PCR) for VP2 gene. The RNA from bursal tissues was extracted as per the manufacturer's protocol, using the "RNeasy Mini Kit" (Qiagen). The eluted RNA was preserved at -80°C until further use. One-step cDNA synthesis was carried out using cDNA synthesis kit (Thermoscientific, USA). Briefly,9 µL total RNA extract from each tissue sample was added with 1 µL of random hexamer primer, incubated at 65°C for 5 min and immediately chilled on ice. Four µL of reaction buffer, 2 µL of 10 mmol dNTP mix, 2 µL of DMSO, 1 µL of Ribolock RNase inhibitor and 1 µL of Revert Aid H Minus M-MuLV reverse transcriptase enzyme were added to the mixture and incubated for five min at 25°C followed by 60 min at 42°C. The reaction was terminated by heating at 70°C for 5 min, cooled on ice and stored at -70°C until used. To amplify a 743 bp region of VP2 hyper variable region, the primers 743-FP-(5'GCCCAGAGTCTACACCAT-3') and 743- RP-(5'-CCCGGATTATGTCTTTGA-3') (Jackwood and Sommer-Wagner, 2005 and Raja *et al.,* 2018) were used. The amplification was carried out in 25 µL reaction mixture consisting of 12.5 µL of 2x Mastermix (Quiagen),1 µL of DMSO, 1 µL of each primer (10 picomoles), 7µL of nuclease free water and 2.5 µL of cDNA from each tissue sample. The amplification was carried out in a thermal-cycler (Eppendorf) with initial denaturation at 94 ^{o}C for 2 min, followed by 30 cycles of denaturation at 94 ^{o}C for 1.5 min, annealing at 53 ^{o}C for 1 min, elongation at 72 ^{o}C for 1 min and a final extension at 72 ^{o}C for 7 min. Negative control was included in all the PCR reactions. Vaccine strain (intermediate strain) was used as positive control. Finally, the amplified PCR products were detected by electrophoresis on 1.5% agarose.

Results and Discussion

In this study, from each flocks 5 poultry carcasses were taken (total 20x5=100) as representative samples. These birds include the commercial layers and broilers. Morbidity was as high as 50-60% and the mortality rate ranges from 12–20% in a flock. Chicken with 3 to 5 wks age were most commonly affected.

Clinical signs

In sick birds, symptoms observed before death were dullness, depression, watery diarrhea and dehydration. Other clinical signs like ruffled feathers, anorexia, trembling, gasping, prostration, disinclination to move, soiled vents and vent picking were also observed. These signs were similar to those described in the early outbreaks of IBD by Pandey (2021), Dey *et al.* (2019) and Omer and Khalafalla (2022).

Gross pathology

Grossly the carcasses of died birds were dehydrated, there were petechial haemorrhages in the leg and thigh muscles (Fig.1), on the mucosa of the proventriculus and at the junction of proventriculus and gizzard (Fig.2). Bursa Fabricius was swollen, edematous and haemorrhagic in nature (Fig.3). Mucosal surface was covered by thick mucus andedema and haemorrhages were noticed (Fig.4). Duodenum showed congestion and increased mucous was noticed all over the intestine. Caecal tonsils showed necrosis. The liver was swollen and congested. In few birds spleenic congestion and spleenomegaly was also noticed.The kidneys were swollen and ureters contained urate deposits. The gross lesions were in accordance with earlier reports (Dey *et al*., 2019 and Nanda kumar *et al*., 2021 and Raja *et al*., 2018)

Histopathology

The histological changes observed in the bursa of Fabricius were hyperaemia, edema and infiltration of heterophils and lymphocytes in early stages and followed by degeneration and necrosis of lymphocytes. Mild to moderate depletion of lymphocytes due to lymphoid necrosis both in cortex and medulla were observed (fig. 5). There was interfollicular edema and infiltration of heterophils and erythrocytes. In advanced stages lymphoid follicles in bursa had lymphocytolysis and occasional areas of coagulative necrosis forming cystic cavities in medullary areas of follicles (Fig. 6). There was hyperplasia of inter follicular spaces with reticuloendothelial cells and fibroblastic stroma (Fig. 7). Spleen follicles showed congestion, depletion of lymphocytes and occasional necrosis with reticuloendothelial cell hyperplasia. In caecal tonsils, necrosis of lymphoid cells was observed (Fig. 8). Mild lymphoid depletion was noticed in thymus (Fig. 9). These above lesions were specific for IBD disease and also observed by various workers (Raja *et al*., 2018: Prabhu *et al*., 2020, and Hou *et al*., 2022) which supported the present observations.

Kidneys revealed moderate congestion and aggregation of lymphoid cells in interstitial spaces with focal haemorrhages, tubular degeneration (Fig.10) and necrosis of glomeruli. Microscopically haemorrhages were observed in proventriculus mucosa and thigh muscles (Fig.11&12). These histopathological findings are in accordance with other virulent IBDV outbreaks studied by Prabhu *et al.*(2020), Eterradosi and saif (2020) and Pandey *et al*.(2021).

Molecular study

For molecular confirmation of IBDV in bursal tissues, the VP2 gene was selected as it plays important role in antigenicity,

cell tropism, virulence and apoptosis and induces serotype-neutralizing antibodies (Jackwood *et al.,* 2008 and Wu *et al*.,2020). cDNA of pooled Bursal tissue samples were positive with VP2 gene specific primers by producing an amplicon size of 743 bp (Fig.13) approximately which confirms the IBD virus. Out of 20 poultry flock's bursa samples, 17 flocks (85%) were found positive for IBDV. Among these 20 positive flocks, 15 flocks were (75%) vaccinated. These results were supported by previous studies of Raja *et al*. (2018), Nandakumar *et al*. (2021), Shinde *et al*. (2021). Reporting of re-emergence of virulent IBD viruses from the different parts of world and India helps in understanding the complexity of disease process, diagnosis and control measures of disease.

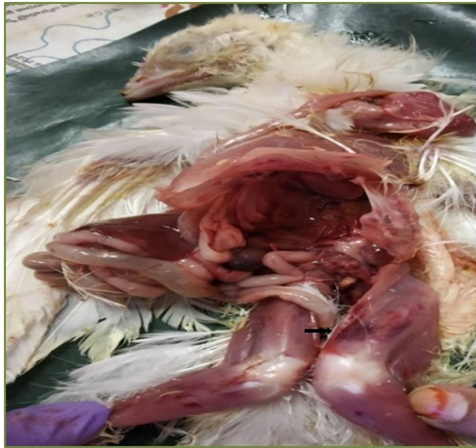

Fig. 1: Bird showing haemorrhages on thigh muscles

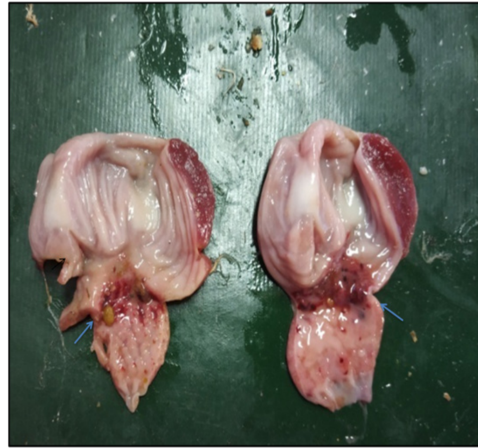

Fig. 2: Haemorrhages at the junction of proventriculus and gizzard

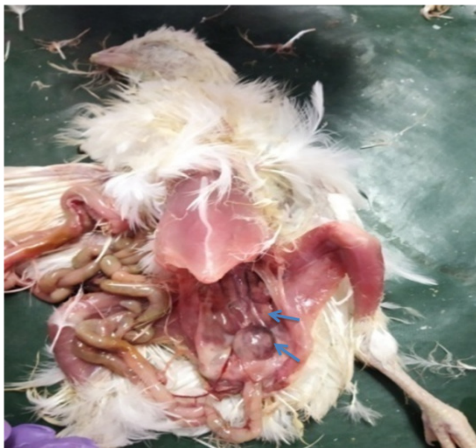

Fig. 3: Bird showing enlarged, haemorrhagic bursa and swollen kidneys

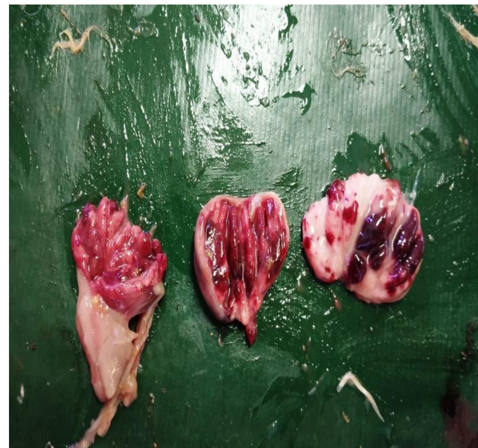

Fig. 4: Enlarged, edematous and haemorrhagic bursa

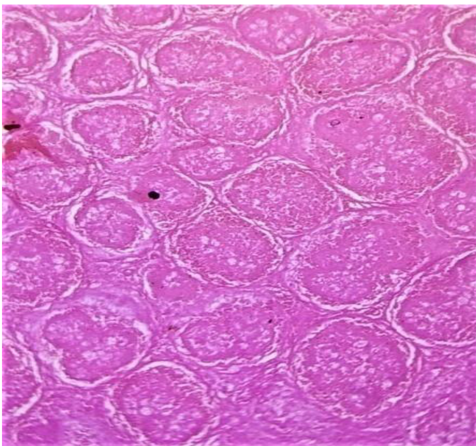

Fig. 5: Bursa showing lymphoid depletion and coagulative necrosis E X 100

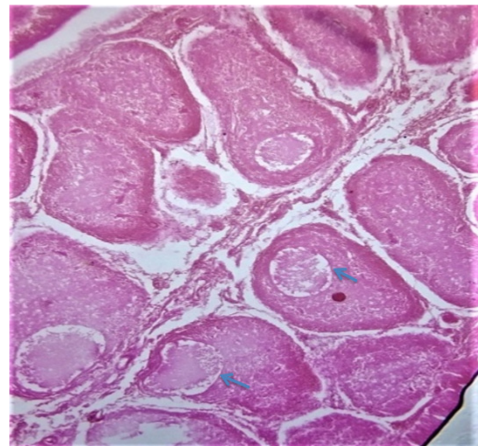

Fig. 6: Bursa showing lymphocytolysis and cystic spaces HE X 100

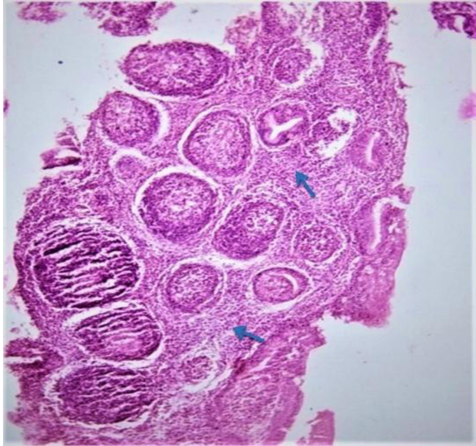

Fig. 7: Bursa showing interfollicular fibrosis HE X 100

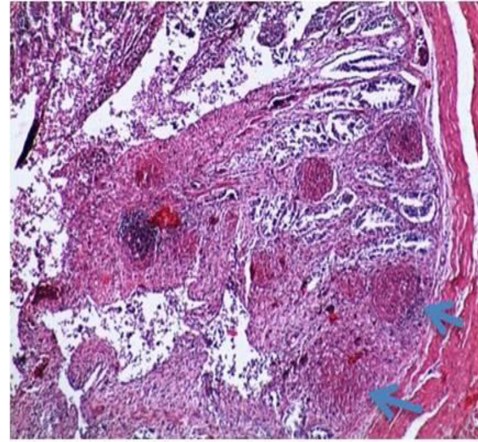

Fig. 8: Caecum showing lymphocytolysis HEX 100

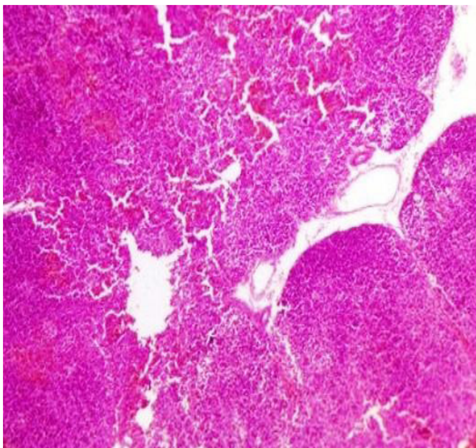

Fig. 9: Thymus showing lymphoid depletion HE X 100

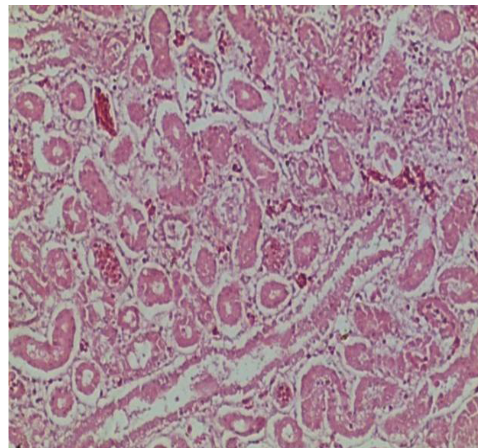

Fig. 10: Kindey showing degenerative changes HEX 100

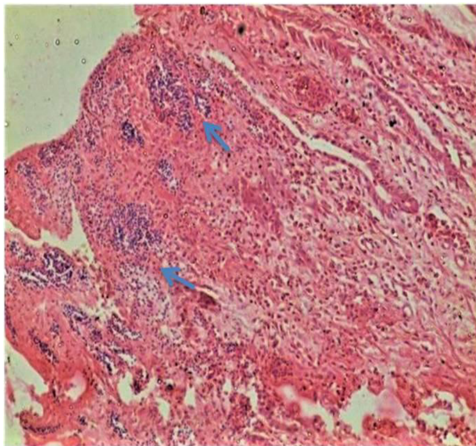

Fig. 11: Proventricular mucosa showing haemorrhages HE 100X

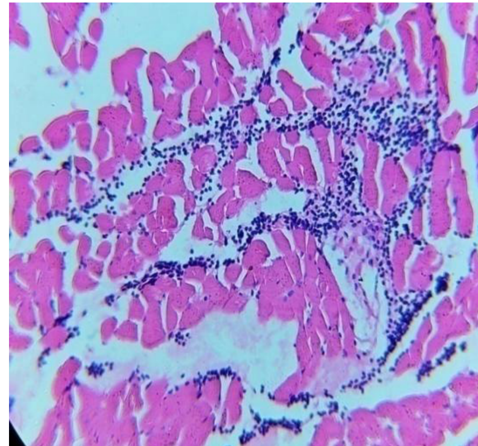

Fig. 12: Thigh muscle showing haemorrhages HE X100

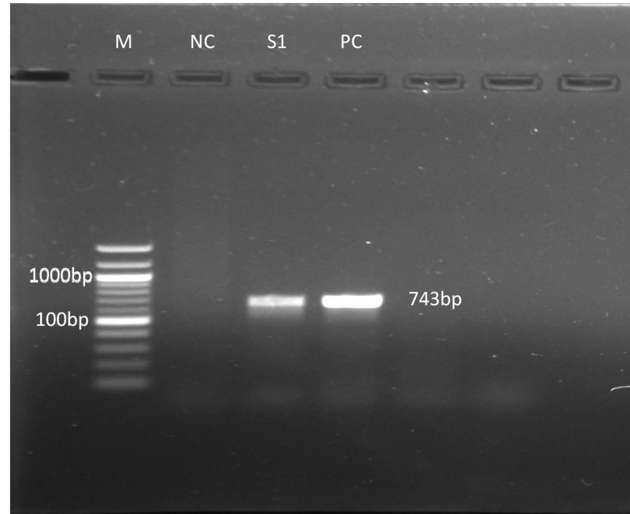

Fig. 13: Agar gel electrophoresis of amplified PCR products of VP2 gene (743 bp)
M-100 bp ladder, NC- Negative control, S1-sample, PC-Positive control

Conclusion

Based on clinical, pathological and molecular findings, the disease is confirmed as virulent Infectious Bursal Disease. From the present study, it is concluded that despite advancement of molecular biology and virology with development of new vaccines, IBD is still prevalent in many organized poultry farms.

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