Short Communication

Concurrent Infection of Infectious Bronchitis (IB) and Sub-Clinical Infectious Bursal Disease (IBD) in an Organised Poultry Farm

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Abstract

Four carcasses of 4-5 days old commercial broiler chicks were submitted for post-mortem examination to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. The owner reported 60% mortality in that flock. On necropsy examination, chalky deposits on the pericardium, liver and in mesonephric tubules of the kidneys were observed. Histopathological examination revealed focal pneumonia and congestion in lungs, uric acid pericarditis, fatty degeneration with capsular thickening in liver, degeneration and uric acid crystals in the renal tubules. Spleen and bursa of Fabricius revealed lymphoid depletion. Reverse-transcriptase polymerase chain reaction (RT-PCR) carried out with the pooled tissue samples gave positive results for both Infectious Bronchitis (IB) and Infectious Bursal Disease (IBD). The nephrogenic form of IB would have resulted in nephrosis, uric acid pericarditis and perihepatitis. Though the Bursa of Fabricius did not reveal any striking gross pathology, the histopathological changes and positive PCR results for IBD suggest a sub-clinical infectious bursal disease virus (IBDV) infection.

Key words: Broiler, IB, IBD, Lymphocytolysis, Nephrosis, RT-PCR

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Introduction

Infectious bronchitis (IB) and Infectious bursal disease (IBD) are important viral infections causing economic loss to the farmers. Infectious bronchitis in chicken is caused by Avian corona virus. Though Infectious bronchitis virus (IBV) mainly infect the respiratory tract, there are some highly nephropathogenic strains causing about 30 per cent mortality in younger chicks as reported by Balasubramaniam *et al.* (2012).

Infectious bursal disease is a highly contagious viral disease of poultry affecting immature chicken resulting in lymphoid cell depletion in the bursa of Fabricius leading to immunosuppression. It is caused by Avi birna virus. Infectious bursal disease virus (IBDV) infects primarily B lymphocytes. Chickens of less than three weeks of age are affected with sub-clinical disease resulting in bursal atrophy and severe immunosuppression making the birds susceptible to other viral and bacterial infections. Lukart and Saif (2003) noted that sub-clinical infections occur in chicks, having less maternal antibodies against the disease. Sub-clinical IBDV infections are generally recorded during post-mortem and histopathological examination. The sub-clinical infections are economically significant as they result in poor weight gain and high susceptibility to other concurrent infections. In spite of the advice rendered to farmers and breeders regarding the importance of periodic vaccination, outbreaks are reported in the poultry population. The present investigation reports a case of concurrent IB and sub-clinical IBD in an organised poultry farm.

Materials and Methods

Four carcasses of 4-5-day old broiler chicks which were submitted for necropsy examination to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, formed the study material. The cases were from an organised broiler poultry farm and a mortality of 60 per cent was reported.

Necropsy was conducted and the gross lesions were recorded. Tissue samples such as heart, lung, liver, kidneys and bursa were collected in 10 per cent neutral buffered formalin for histopathology. Lymphoid organs namely spleen and bursa of Fabricius; and internal organs were collected as pooled samples for molecular diagnosis. Tissues were processed using standard procedures and the sections were stained with haematoxylin and eosin according to Luna (1972). The pooled tissue samples (liver, lung, spleen, and bursa of Fabricius) were processed and subjected for RNA extraction using Trizol method. The RNA was reverse transcribed to cDNA by using commercial cDNA synthesis kit (Thermo scientific, United Sates) according to the manufacturer's instructions and subsequently subjected to PCR using specific primers targeting the 5'UTR of IBV as per Callison *et al.* (2006) and VP2 gene of IBDV as proclaimed by Singh *et al.* (2014). The thermal cycling conditions were 95 °C for 10 min or initial denaturation followed by 35 cycles of 95 °C for 45 sec for denaturation, 53 °C for 1 min for annealing, 72 °C for 1 min for extension and a final extension of 72 °C for 10 min in case of IBV and for IBDV, the PCR cycle conditions standardized were 95 °C for 5 min, followed by 34 cycles of 95 °C for 30 sec, 59.5 °C for 1 min, 72 °C for 30 sec and a final extension at 72 °C for 6 min. The PCR products were detected by agarose gel electrophoresis in one per cent agarose gel in Tris acetate EDTA buffer (1X) and the agarose gel was visualized in a gel documentation system. The sequences of the primers were depicted below-

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IBV

F: 5' GCTTTTGAGCCTAGCGTT 3'

R: 5'GCCATGTTGTCACTGTCTATT 3'

IBDV

F: 5' ACAGGCCCAGAGTCTACACCATAA 3'

R: 5' ATCCTGTTGCCACTCTTTCGTAGG 3'

Results and Discussion

Gross Examination

Grossly, pulmonary congestion and chalky deposits were observed over the pericardium and liver. The kidneys were pale, enlarged, prominent, distended mesonephric tubules with urate deposits (Fig. 1). The bursa of Fabricius appeared slightly enlarged and edematous. The other organs were apparently normal.

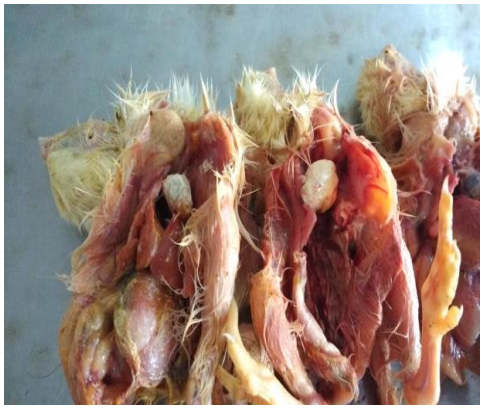

Fig. 1: Gross- Visceral gout- presence of chalky white deposits on the internal organs

Histopathological Examination

Histopathological examination of the tissue samples revealed focal pneumonia and congestion in lungs, uric acid pericarditis and myocarditis, fatty degeneration with capsular thickening in liver, degeneration and uric acid crystals in the renal tubules and lymphoid cells depletion in spleen and bursa of Fabricius (Fig. 2).

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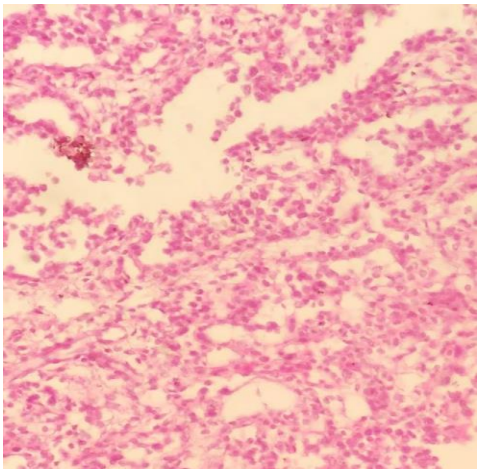

Fig. 2: Histopathology- Lymphoid cell depletion in bursa of Fabricius. H& E X100

Reverse-transcriptase PCR (RT-PCR)

In RT-PCR followed by agarose gel electrophoresis, amplicons size of 149 bp specific to IBV (Fig. 3) and 480 bp specific to IBDV (Fig. 4) were documented.

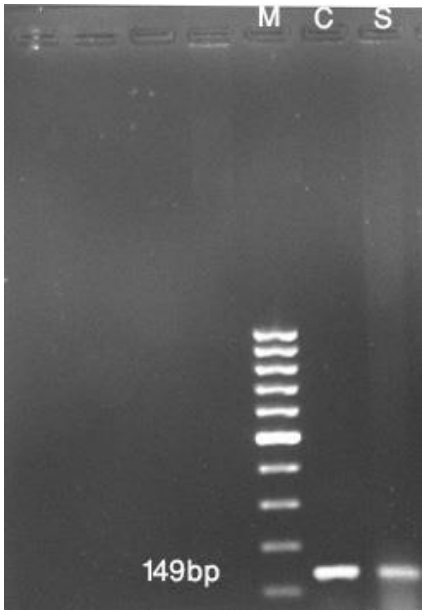

Fig. 3: Agarose gel electrophoresis showing positive PCR product of 149 bp IBV 5'UTR

M: Molecular weight marker 100bp
C: Positive control (Vaccine from Nobilis Intervet, India)
S: IB positive sample

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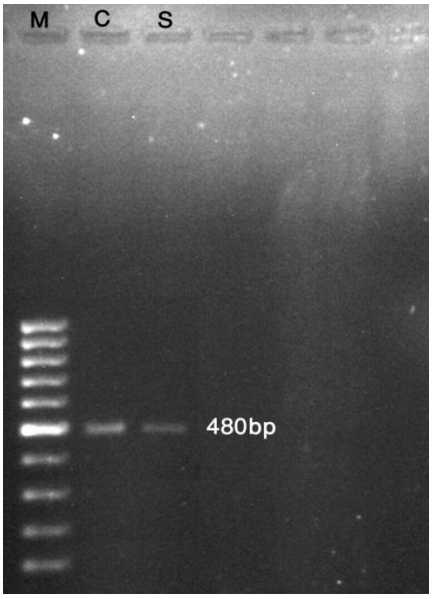

Fig. 4: Agarose gel electrophoresis showing positive PCR product of 480 bp for VP 2 gene IBDV

M: Molecular weight marker 100bp

C: Positive control (from Infectious bursal disease vaccine-Ventri Biologicals, Vaccine division, India)

S: IBD positive sample

Concurrent infection of IB and IBD is not uncommon among chicken. A mortality of 60 per cent with the gross lesions such as pneumonia, uric acid pericarditis, nephrosis and slightly enlarged bursa of Fabricius gave the impression that the infection could be of multiple etiology. The post-mortem lesions in lungs and uric acid deposits in internal organs along with nephrosis suggested a disease affecting the respiratory and urogenital system in younger birds leading to a presumptive diagnosis of IB as noted by Balasubramaniam *et al.* (2012). Moreover, field outbreaks of nephropathogenic form of IB have also been frequently reported in India as recorded by Bayry *et al.* (2005). However, the slightly enlarged and edematous appearance of the bursa of Fabricius and high mortality in young chicks in the early stages had been recorded by Homer *et al*. (1992) in sub-clinical IBD. The birds were also not vaccinated against IBD and IB as they were less than a week old and in-ovo or day 0 vaccination against IBD is not being practised in India. Subsequent histopathological and molecular examinations confirmed the presence of IB and IBD in the affected flock. The immunosuppression due to IBD would have resulted due to the damage to bursa of Fabricius, as opined by Kegne and Chanie (2014), might have made the chicks susceptible to IB.

The golden standard for diagnosis of IBV and IBDV is virus isolation and identification, which is tedious and time consuming. Molecular detection methods like RT-PCR are found to be highly sensitive and will give a rapid and accurate result. It will enable the clinician to follow effective strategies to combat the infection, thereby preventing further mortality in the flock.

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Conclusion

The sub-clinical infection in chicks suggests that they should have acquired the infection due to a poor level of maternal antibodies. As we are not aware about the immunological status of the breeder flock, it is presumed to be one of the reasons for the outbreak. Hence, it is very much essential to maintain an adequate antibody titre against IBD in the breeder flock and implement strict bio-security measures so as to prevent heavy economic loss to the farmer.

Acknowledgement

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