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Pathological changes and molecular detection of infectious bronchitis and concurrent infection with infectious bursal disease in poultry#

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Abstract

The current study was conducted with the objective of determining the cause for a significant increase in mortality rates in poultry farms situated in and around Thrissur. The study material consisted of 100 birds aged between two and four months, which were brought for postmortem to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy. The birds with a history of respiratory illness were included in the study. On detailed postmortem examination, catarrhal or caseous exudate in the trachea/nasal passages, pulmonary congestion, swollen and pale kidneys, splenomegaly with congestion were noticed in cases suspected for infectious bronchitis (IB) infection alone. Enlarged and congested kidneys; enlarged bursa with cheesy material inside the lumen and splenomegaly with subcapsular haemorrhages were noticed in carcasses suspected for concurrent infection with infectious bursal disease (IBD). On microscopic examination, bursa of IB infection alone revealed densely populated lymphocytes with necrotic changes in the medulla and bursa of concurrent infection with IBD revealed extensive haemorrhage in the cortical region and large cyst in the medulla of the plicae. The cases were confirmed using a reverse transcriptase polymerase chain reaction (RT-PCR). Out of 100 samples, 33 cases tested positive for IB. Upon further investigation of these 33 samples, 25 samples were

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found to be concurrently infected with IBD. The condition was confirmed as infectious bronchitis with concurrent infection with infectious bursal disease based on both pathological findings and molecular analysis.

Keywords*: Pathology, Infectious bronchitis, concurrent infection, molecular detection*

Infectious bronchitis virus (IBV) presents significant financial and biosecurity challenges to the commercial poultry farming industry. Infectious bronchitis virus is the causative agent of a multisystemic infection affecting the respiratory, reproductive and renal systems, with clinical signs resembling those of various viral and bacterial diseases observed in poultry. Avian infectious bronchitis (IB) is a rapidly spreading, acute upper respiratory tract illness that affects birds of all age groups and poses a substantial economic risk to the poultry industry. Morbidity is consistent at 100 per cent; however, mortality rates can range from 0 to 82 per cent (Cavanagh and Naqi, 2003; Jackwood, 2012).

Infectious bronchitis virus is an enveloped, single-stranded, positive-sense RNA virus belonging to the *Coronaviridae* family within the *Gammacorona virus* genus. Its genetic material consists of two untranslated regions (UTRs) situated at both the *5'* and *3'* ends of the viral genome (Ziebuhr *et al.,* 2000). Infectious bursal disease (IBD) is a highly contagious disease caused by infectious bursal disease virus (IBDV), part of the *Avibirna virus* genus within the *Birnaviridae* family. The IBD is marked by immunosuppression and mortality usually ranging from three to six weeks (Singh *et al.,* 2015). The infection mainly targets the bursa of Fabricius and results in immunosuppression due to destruction of lymphocytes.

The presence of IB and sub-clinical IBD in broiler chicks of four to five days old has been documented by Krithiga *et al*. (2019). They highlighted that IBD could lead to a suppression of the immune system, increasing the vulnerability of the birds to additional infections like infectious bronchitis. Co-infection of both IB and IBD is relatively common in chickens, and one of the contributing factors to such outbreaks is believed to be the presence of low levels of maternal antibodies in the young chicks. The current study describes the pathological changes and molecular detection of IB as single infection as well as in concurrent infections with IBD.

Material and methods

The poultry carcasses obtained from commercial poultry farms and backyard poultry rearing units situated in and around Thrissur district of Kerala during the period from March 2022 to May 2023 formed the study material. Around 20-25 samples/ farm were received from commercial poultry farms, while approximately two to three samples/ unit were from backyard poultry units. The birds were in the age group of two to four months and majority of them were Gramasree birds. Detailed postmortem examination was conducted and the gross lesions were recorded. Tissue samples were collected from lung, liver, spleen, kidney and bursa of Fabricius of individual cases showed lesions suggestive of IB and IBD.

Tissues collected from organs with observable gross lesions were subjected to histopathological examination. These tissues were processed through routine manual procedures, including dehydration, clearing, and embedding in paraffin for analysis. Tissues were cut into sections with a thickness of 5 µM using a rotary microtome and subsequently stained with Hematoxylin and Eosin using standard staining procedures. The prepared sections were mounted using DPX mountant solution and covered with cover slips to facilitate microscopic examination (Suvarna *et al*., 2018).

The pooled tissue samples were stored at -20°C in sterile tubes containing RNA later for molecular studies. The pooled tissue samples were processed for RNA extraction using TRIzol reagent. The RNA was reverse transcribed to cDNA by using commercial cDNA synthesis kit (Thermo Scientific, United Sates) according to the manufacturer's instructions. The transcribed cDNA was subjected to Reverse-transcriptase PCR (RT-PCR) using specific primers targeting the *5'UTR* of IBV as per Callison *et al*. (2006) and *VP2* gene of IBDV according to Singh *et al*. (2015).

The PCR product was visualized by subjecting it to electrophoresis in one percent agarose gel, using 1X TAE buffer (Thermo Scientific). The gel was visualized and the results were documented in a gel documentation system (Biorad).

Results and discussion

Gross lesions

During postmortem examination, the gross lesions observed in cases of single infection with IB were catarrhal or caseous exudate in the trachea/nasal passages, pulmonary congestion (Fig.1), swollen, pale kidneys (Fig. 2) and splenomegaly with congestion. The gross lesions recorded in cases with concurrent infection of IBD were enlarged and congested kidneys (Fig. 3), enlarged bursa with cheesy material inside the lumen (Fig. 4) and splenomegaly with subcapsular haemorrhage. On gross examination of carcasses with IB infection alone, tracheal lesions closely resembled those reported by Najafi *et al.* (2017), showing minimal catarrhal exudate in the tracheal lumen and mild mucosal hyperemia. In IB infection alone cases, lungs showed edema, congestion, and pneumonic changes, sharing similarities with the findings of Jackwood and de Wit (2013) who observed pneumonia in regions adjacent to the large bronchi. The tracheal epithelium being the predilection site for the organism to multiply, the trachea and lung tissue undergo pathological changes. The swollen, pale kidneys and bursa with no gross lesions observed in this study in IB infection alone were also documented in earlier reports (Jackwood and de Wit, 2013). Paleness of kidney could be attributed to vascular damage or blockage due to the inflammatory response, leading to swelling and a reduction in blood supply. The gross lesions observed in the bursa of Fabricius and spleen during concurrent infection with IBD was consistent

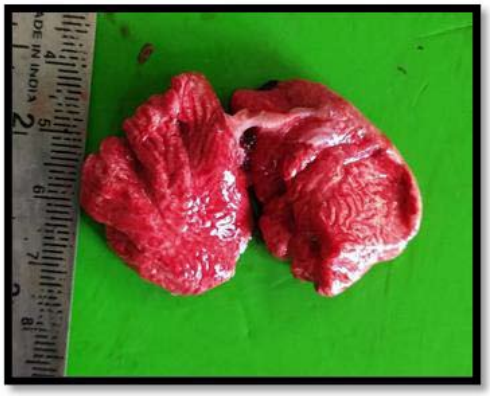

Fig. 1. Lungs – Edematous and congested with pneumonic changes near large bronchi

Fig. 2. Kidneys - Pale and swollen. Bursa with no gross lesions

Fig. 3. Kidneys - Enlarged and congested

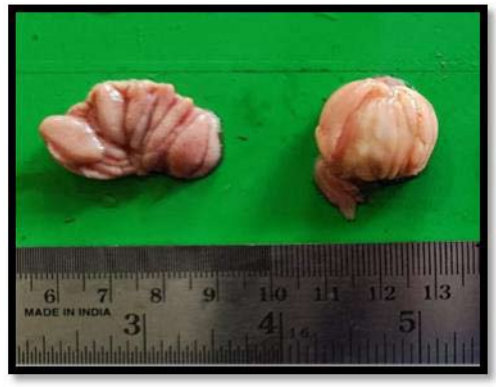

Fig. 4. Bursa - Enlarged with cheesy material inside the lumen

with previous findings (Islam and Samad, 2004). These reports documented the bursa as enlarged, edematous and haemorrhagic, with the cut surface displaying slimy to gelatinous materials. Spleen exhibited characteristic features of haemorrhage and swelling.

Histopathology

On microscopical examination, bursa of Fabricius in IB infection alone revealed densely populated lymphocytes with some necrotic changes in the medulla of the follicle (Fig. 5). The bursa of Fabricius in concurrent infection with IBD showed extensive haemorrhage present throughout cortical region of bursal plicae (Fig. 6) and large cyst were present in medullary region (Fig. 7). The microscopic examination of IB infected cases revealed the following histopathological changes such as desquamation of epithelial cells along with mononuclear cell infiltration along with congestion and haemorrhages in the sub-mucosal layer (Fig. 8) of the trachea; congestion and haemorrhages along with infiltration of lymphoid cells in the interstitium (Fig. 9) of the lungs; focal to diffuse haemorrhages with infiltration of inflammatory cells in the interstitium of the kidneys (Fig. 10) and diffuse ecchymosis with lymphoid depletion in the spleen (Fig. 11). Histopathological examination of the trachea in cases of IB infection alone demonstrated similarities with the findings of Chousalkar *et al.* (2007) and Jackwood and de Wit (2013) who reported loss of cilia, sloughing of epithelial cells and minor infiltration of heterophils and lymphocytes in the lumen. Furthermore, a substantial infiltration of lymphoid cells was observed in the lamina propria. This could be attributed to the ability of IBV to inflict damage on various types of epithelial cells within the respiratory tract, as elucidated by Raj and Jones

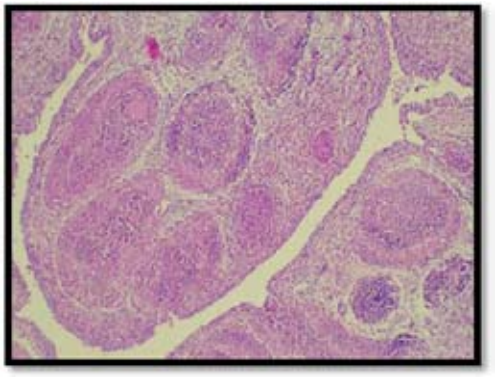

Fig.5. Bursa of Fabricius – Densily populated lymphocytes with some necrotic changes in the medulla (H&E x100)

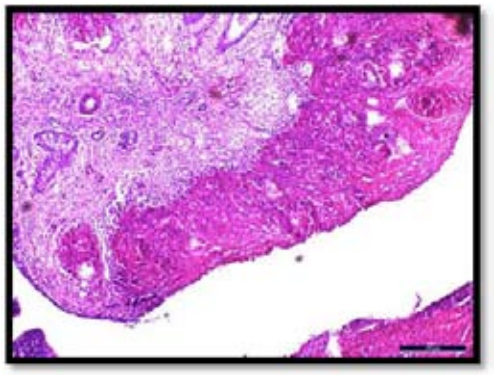

Fig.6. Bursa of Fabricius – Extensive haemorrhage throughout the cortical region of the plicae (H&E x400)

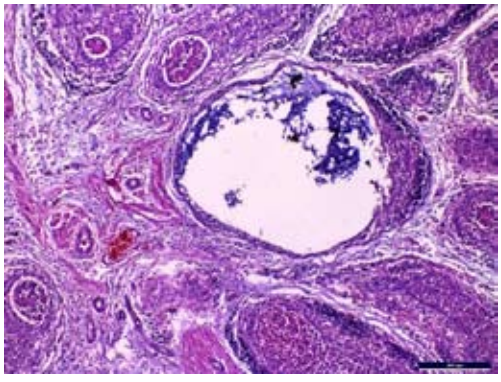

Fig. 7. Bursa of Fabricius – Large cyst in the medulla (H&E x400)

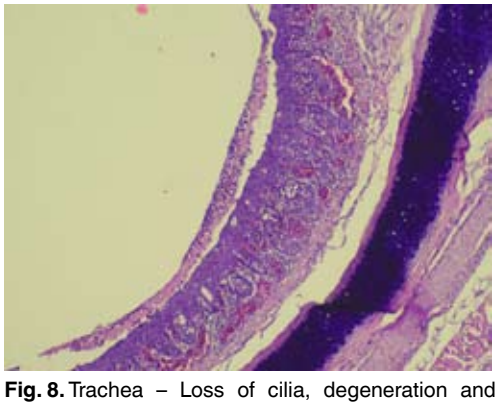

Fig. 8. Trachea – Loss of cilia, degeneration and shedding of ciliated epithelial cells with mucus (H&E x100)

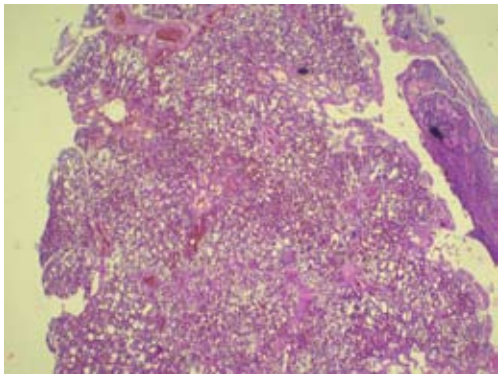

Fig. 9. Lungs - Congestion and haemorrhages along with infiltration of lymphoid cells in the interstitium (H&E x40)

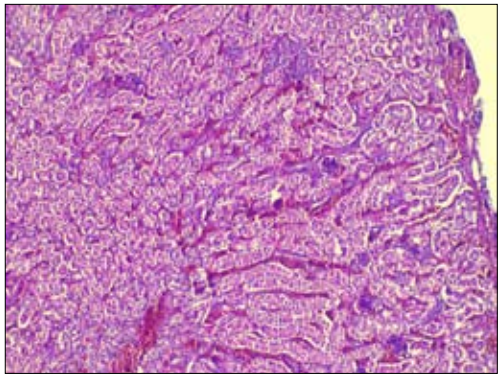

Fig.10. Kidney - Focal to diffuse haemorrhages with infiltration of inflammatory cells in the interstitium (H&E x40)

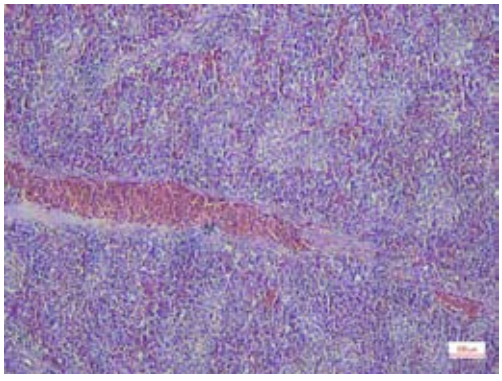

Fig. 11. Spleen - Diffuse ecchymosis with lymphoid depletion (H&E x100)

(1997) and Boltz *et al*. (2004). Congestion and haemorrhages along with infiltration of lymphoid cells noticed in the submucosal layer of the trachea were in alignment with Kotani *et al.* (2000), who noted significant hyperemia and edema in the submucosa. This might be due to respiratory epithelial cells encouraging particular IgA (immunoglobulin A) synthesis, resulting in tracheal congestion and haemorrhage to eradicate the virus as explained by Kothlow *et al.* (2008). The observations in kidney in cases of IB infection alone were consistent with the findings of Najimudeen *et al.* (2022). Their study identified necrotic changes in the tubules, hyper eosinophilic cellular debris and thickening of the interstitial connective tissue due to haemorrhages and mononuclear infiltrations in the interstitium. The ability of infectious bronchitis virus to replicate within the renal tubules and ducts may explain the increased occurrence of renal lesions, as observed in the study conducted by Abdel-Moneim *et al.* (2005).

Extensive haemorrhages in the bursal parenchyma were identified as microscopic lesions observed during concurrent infection with IBD in the bursa of Fabricius. The findings were consistent with the observations made by Gurel *et al*. (2003). Their study documented extensive haemorrhages in both intrafollicular and interfollicular areas, as well as infiltration of heterophils and histiocytes, along with the presence of necrotic foci in the medullary areas of the lymphoid follicles. The cystic changes in the bursal follicles indicating a depletion of the lymphocytes were in line with the results of Guvenc *et al.* (2004). Additionally, they noted the accumulation of heterophils in the interstitial tissue and observed necrotic changes in the medullary zone of the bursal follicle. The bursa of Fabricius is the primary organ affected during IBD infection culminating in significant loss of the lymphoid tissue resulting in immunosuppression.

Molecular confirmation of IB and IBD by RT-PCR

Virus isolation and identification is the gold standard test for diagnosis of IBV and IBDV, which is tedious and time consuming. The molecular confirmation was done by the presence of the expected amplicon size of 143 bp for IB (Fig. 12 - Representative image) and amplicon size of 480 bp for IBD (Fig. 13 - Representative image), when visualized on

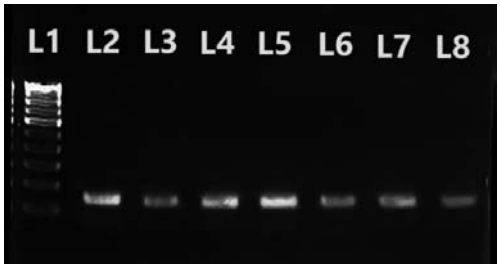

Fig. 12. Agarose gel electrophoresis showing positive PCR product of 143 bp of IBV
L1 : Molecular weight marker 100bp
L2 : Positive control (Vaccine from Nobilis Intervet, India)
L3-L8: IB positive samples

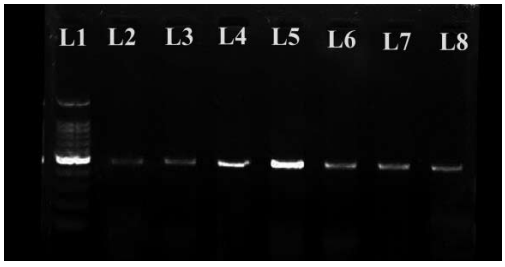

Fig. 13. Agarose gel electrophoresis showing positive PCR product of 480 bp for *VP2* gene of IBDV
L1 : Molecular weight marker 100bp
L5 : Positive control (from Infectious bursal disease vaccine-Ventri Biologicals, Vaccine division, India)
L2-L4 &L6-L8: IBD positive samples

one percent agarose gel. A total of 33 cases were positive for IB out of hundred samples. On further examination of 33 samples, 25 samples were positive for concurrent infection with IBD.

As per Kwon *et al.* (1993), molecular detection methods like RT-PCR will give a rapid and accurate result with high sensitivity. Callison *et al.* (2006) found that using RT-PCR to target the 5'UTR of IBV was effective in detecting all IBV strains due to the conserved nature of this region. The VP2 gene has exhibited a notable level of specificity for IBDV, and researchers have utilized this specificity to detect IBDV in field samples through RT-PCR, as demonstrated by Awandkar *et al.* (2018).

The report provides an account of the gross pathological features, histopathological alterations observed in the bursa of Fabricius and molecular detection methodology employed for detecting IB and IBD in poultry.

Careful consideration of the simultaneous presence of IB and IBD was essential due to the immunosuppressive property of IBD, which could increase the susceptibility to IB infection. Otherwise, it could be interpreted that IB might play a role in compromising the immune system, potentially contributing to the onset of other infections**.**

Conclusion

Infectious bronchitis will lead to production failures and the presence of IB along with IBD will add to the loss with escalated mortality due to the immunosuppressive nature of IBDV making the birds amenable to other viral diseases and secondary bacterial infections. The timely detection of the diseases will enable the clinician to adopt effective methods to combat the disease, thereby preventing further mortality in the flock and loss to the farmers.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflict of interest.

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