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Pathology and diagnosis of infectious bursal disease in chicken

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Abstract
The case was seen in the age group of 5th week with a mortality of 48% in a period of ten days to post infection which attain peak mortality on its 5th day and decline gradually on the study. The most typical signs exhibited by the affected flocks were acute onset of depression, dullness, dehydration, ruffled feathers on back on neck, praying position on ground and anorexia. On necropsy most of the carcasses showed dark discoloration of thigh and pectoral musculature with paint brush hemorrhages on the breast and thigh muscles. Microscopic examination of bursa revealed complete lymphoid depletion leading to formation of cysts filled with heterophils and exudates and extensive hemorrhages in interfolicular tissue. Confirmatory diagnosis of virus was done by RT-PCR.

Keywords: Pathology and diagnosis, bursal disease, chicken

Introduction
Amongst the viral diseases of poultry, Infectious Bursal Disease has been considered as one of the most important diseases causing great economic loss to poultry industry. It is also known as Gumboro disease, infectious avian nephrosis and infectious bursitis by different scholars. IBD is an acute, contagious viral disease of chicken (Shabbir *et al.*, 2013) [13] population. The virus not only affects domesticated birds but also wild poultry throughout the world by mainly targeting the immune system causing immunosuppression (Rehman *et al.*, 2016) [11] of chicken. IBD has a global impact on poultry industry, however various control/preventive measures are put in place to reduce the losses. Chickens of the age group of 3-6 weeks are most prone to clinical disease. Successful infections followed by an incubation period of 3-4 days lead to a clinical disease which is characterized by high morbidity, mortality and marked immunosuppression of the affected ones (Mohanty *et al.*, 1971) [8]. The affected ones become depressed, become reluctant to move, and show ruffled feathers with white watery diarrhoea, trembling, severe prostration followed by death. The characteristic gross lesions of the disease include dehydration of muscles might be due to watery diarrhoea with ecchymotic hemorrhages, Bursa of Fabricius becomes enlarged and shows pale yellow discoloration with creamy exudate. Pin point hemorrhages on the pectoral and thigh musculature are usually prominent (Ingrao *et al.*, 2013) [4]. Confirmatory diagnosis of IBDV is most commonly performed by serology using Enzyme linked immunosorbent assay (ELISA), Agar gel precipitation test (AGPT), Virus neutralization test (VNT) of bursal sections and molecular techniques like reverse transcriptase polymerase chain reaction (RT-PCR) have frequently used to detect viruses from the field samples (Gohm *et al.*, 2000; Mathivanan *et al.*, 2004) [6, 7].

Materials and Methods
The present case study was conducted in vizag Andhra Pradesh from an outbreak in layer of 5th week flock located in the Anakapalle district of Andhra Pradesh with a capacity of 20,000 number of birds which were well maintained under intensive system. In the study samples were collected from dead carcass of layer flock presented for post mortem. The birds presented to post mortem were examined grossly and representative tissue samples were aseptically collected from the proventriculus, caecal tonsils, spleen, liver, bursa of Fabricius, lungs, and trachea in sterile container containing PBS and also another sterile container fixed in 10% buffered formalin for histopathological examination as per procedure of Luna, 1968 are send

to the Department of Pathology of College of Veterinary Science, Assam Agricultural University, Khanapara as soon as possible immediately by transportation. Suspected cases were further screened for confirmatory diagnosis by molecular test using RT-PCR as per the recommendations by Nandhakumar *et al.*, 2020 [10].

Results

On examination of affected flock revealed that birds show clinically ruffled feathers on neck region, depression, dullness, dehydration, anorexia, and almost all the affected found to be abnormally fallen down like praying position. The mortality reached 48% with a record of peak mortality on the 5th day of post infection which later decline gradually. On post mortem examination showed showed darked discoloration of thigh and pectoral musculature. Paint brush hemorrhages on the breast and thigh muscles (Fig 1.C) and in some cases large patches of hemorrhages on thigh muscles was observed and the lesions were more suggestive of IBD. The most striking gross pathological findings in almost all suspected cases were recorded in Bursa of Fabricius (Fig 1.A). However, swelling was obvious in almost all findings. Most of the carcasses were showing congestion and hemorrhages on the mucosa of proventriculus, while in some cases hemorrhages could be recorded at the junction of proventriculus and gizzard (Fig 1.B). microscopic examination of bursa of Fabricius revealed congestion, complete lymphoid depletion in the follicles leading to formation of cysts filled with necrotic debris, heterophils, hemorrhages in the interfolicular tissue and increase in interfollicular space (Figs. 2.A). In some cases, bursa section showing complete lymphoid depletion in the follicles leading to formation of vacuolation inside the follicles and increased fibroplasia of fibrous connective tissues. The suspected positive samples by gross examination were then processed for molecular detection by Reverse Transcriptase-Polymerase Chain Reaction (RTPCR) using the oligonucleotide primers targeting VP2 gene of IBD and were found positive for IBD virus with amplification product of 604 bp.

Fig 1: A Photographs showing enlarged, swollen bursa with caseous mass formation and hemorrhagic bursal mucosa.

Fig 1B: Shows hemorrhage at the junction of proventriculus and gizzard

Fig 1C: Showing hemorrhages on thigh muscles

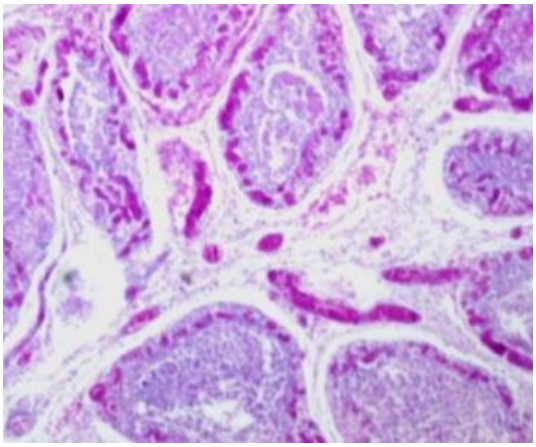

Fig 2A: Bursa sections showing haemorrhages, complete lymphoid depletion in the follicles leading to formation of cysts filled with exudates in the interfolicular tissue H&E x 100

Discussion

The clinical signs like dullness, depression, anorexia, ruffled feathers and whitish watery diarrhoea recorded during the present investigation are in agreement with the findings of Butcher and Miles (2012) [2]; Rashid *et al.,* (2013) [12]; Bhutia and Singh (2017) [1] and Momin and Singh (2018) [9]. The post-mortem findings of the present study included darked discoloration of thigh and breast muscles with frequent paint brush hemorrhages in some cases, which supports the reports of several workers (Islam and Samad, 2004 [5]; Singh, 2008; Sultana *et al.,* 2008 [14]; Bhutia and Singh, 2017 [1] and Momin and Singh, 2018) [9]. The microscopic changes of bursa of Fabricius in present study included complete lymphoid depletion in the follicles leading to formation of cysts in the medulla region filled with necrotic debris, heterophils and diffuse interfollicular hemorrhages. In some cases, areas of oedema with severe heterophillic and lymphocytic infiltration in the interfolicular connective were also recorded. These findings are in the line of earlier observations of several workers (Dutta *et al.,* 2007; Bhutia and Singh 2017; Momin and Singh, 2018) [3, 1, 9].

Conclusion

The most typical signs exhibited by the affected flocks were acute onset of depression, dullness, dehydration, ruffled feathers on back on neck, praying position on ground and anorexia. On necropsy most of the carcasses infected with IBD were dehydrated due to watery diarrhoea and showed darkened discoloration of thigh and pectoral musculature. Paint brush hemorrhages on the breast and thigh muscles were observed. The most striking microscopic changes included complete lymphoid depletion in the bursal follicles leading to formation of cysts filled with necrotic debris, heterophils, diffuse hemorrhages in follicles and inter-follicular spaces and increase in interfollicular space. IBDV which was confirmed by detection of VP2 gene of IBDV in tissues like cloacal bursa and spleen by RT-PCR.

Conflict of interest: None

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