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Pathology and molecular diagnosis of infectious bursal disease in an organized farm

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Abstract

The present investigation was conducted to find out the cause of huge mortality in University Poultry and Duck Farm, Mannuthy. About sixty poultry carcasses presented to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy formed the materials for the study. On detailed postmortem examination, enlargement of bursa of Fabricius with presence of mucous exudate in lumen, haemorrhages in thigh muscles and swollen kidneys were noticed. On microscopical examination, bursa of Fabricius revealed severe lymphoid depletion, shrinkage of follicles and accumulation of necrotic debris in follicles. Lesions were suggestive of infectious bursal disease. Etiological confirmation of the cases were done with a reverse transcriptase polymerase chain reaction (RT-PCR) targeting the VP2 gene of Infectious bursal disease virus (IBDV), which revealed an amplicon size of 480 bp. Molecular and pathological findings with bursal lesions confirmed the disease as infectious bursal disease.

Keywords: Infectious bursal disease, RT-PCR, vaccine failure

Introduction

Poultry farming is an important sector in livestock production and a good reliable source of income to farmers in rural areas. There are several viral, bacterial, parasitic, and managerial diseases of poultry that cause direct financial loss to farmers. Among these, infectious bursal disease (IBD) ranks high (Singh *et al.*, 2015) [14].

Infectious bursal disease is a highly contagious disease of young chicken caused by infectious bursal disease virus (IBDV), characterized by immunosuppression and mortality generally at 3 to 6 weeks of age (Singh *et al.*, 2014) [13]. Infectious bursal disease virus is stable in environment, virus remains viable for at least 6 months in dry litter and more than 1 year in unused dry chicken houses and very resistant to inactivation by various chemical disinfectants (Edgar and cho, 1976) [6]. There is no alternative without vaccination to prevent IBD or Gumboro disease (Lukert and Saif, 1997) [10]. However, outbreaks in vaccinated chicken flocks have been reported in many parts of the world. The present work is carried out to find out the etiology of the huge mortality associated with poultry in an organized farm.

Materials and method

Postmortem and sample collection

About sixty poultry carcasses presented to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy from the University Poultry and Duck Farm, Mannuthy with the history of high mortality in the farm after administration of 28th day booster vaccination of infectious bursal disease formed the materials of the study. During postmortem examination gross lesions in all organs including bursa of Fabricius, thigh muscles and kidneys were noticed and recorded. Tissues showing lesions were collected in 10% neutral buffered formalin for histopathological examination and sterile tubes containing RNA later and stored at -20° C for molecular detection of the virus.

Molecular detection

RNA extraction AND cDNA synthesis

Viral RNA was extracted using Trizol method (sigma) according to manufacturer's protocol. The extracted RNA was treated with dimethylsulphoxide (DMSO) before synthesis of cDNA. One part molecular biology grade DMSO was added to four parts of IBDV RNA (OIE terrestrial manual, 2016) [11].

The mixture was treated at 98 °C for 5 minutes and snap chilled on ice. Complementary DNA (cDNA) was synthesized from the this DMSO treated RNA using random hexamers utilizing RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) as per the manufacturer's protocol.

The primers used in the study for the detection of infectious bursal disease were selected as per Singh *et al.* (2014) [13] and RT-PCR (Biorad) was carried out with minor modifications. The first strand cDNA was synthesized using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific). The PCR conditions were standardized as per the details given in the Table 1. The PCR product was detected by electrophoresis in one per cent agarose gel in 1 X TAE buffer (Thermoscientific). The gel was visualized and the results were documented in a gel documentation system (Biorad).

Table 1: PCR conditions for the amplification of infectious bursal disease virus.

Steps		Temperature	Time
Initial denaturation		95 °C	5 min
34 cycles	Denaturation	95 °C	30 sec.
	Annealing	59.5 °C	60 sec
	Extension	72 °C	30 sec
Final extension		72 °C	30 sec.
Hold		4 °C	10 min.

Histopathology

The tissues collected from organs showing gross lesions were subjected to histopathological examination. The tissues were dehydrated, cleared and embedded in paraffin by routine manual processing. Tissues were cut at 4-6 µm thickness with Rotary microtome, and were stained with Haematoxylin and Eosin by routine staining procedure. These were mounted with DPX mountant solution and covered with cover slips for microscopical examination.

Results

Gross pathology

On detailed postmortem, the carcasses revealed enlarged bursa of Fabricius (Fig. 1) with presence of mucous exudates in the lumen, widespread thigh muscle hemorrhages (Fig. 2) and pulmonary congestion with consolidation in some areas. Kidneys were enlarged and congested and in some cases haemorrhages were noticed at proventriculus-gizzard junction and pin-point haemorrhages were noticed in the mucosa of caecal tonsils.

Molecular confirmation of infectious bursal disease

Pooled samples from the dead birds were screened by PCR and were positive for the predicted amplicon size of 480 bp in 1.5 per cent agarose gel for IBD (Fig. 3).

Histopathology

On microscopical examination, bursa of Fabricius revealed severe lymphoid depletion, shrinkage of follicles, accumulation of necrotic debris in follicles (Fig. 4) and interfollicular haemorrhages. Interfollicular proliferation of fibrous connective tissue (Fig. 5) and sloughing of bursal epithelium (Fig. 6) were evident in most of the follicles. Skeletal muscles revealed haemorrhages between the muscle fibers. Kidneys revealed vacuolar degeneration of the tubules, glomerular shrinkage and haemorrhages.

Molecular and pathological findings with bursal lesions confirmed the disease as infectious bursal disease and outbreaks in the vaccinated flocks were might be due to vaccination failure, which can be attributed to different factors.

Discussion

The present report describes the pathological features and molecular detection of infectious bursal disease in an organized farm. The PCR amplicons of 480 bp corresponding Singh *et al.* (2014) [13] and Akkara *et al.* (2018) [11].

Grossly, bursa of Fabricius were enlarged or swollen, with presence of mucoid exudates were similar to earlier reports (Bhutia *et al.*, 2017) [12]. Varying degrees of thigh muscle haemorrhages seen in the cases were similar to those described by previous researchers (Sultana *et al.*, 2008, Islam and Samad, 2004) [15, 8]. Some of the birds showed haemorrhages in proventriculus-gizzard junction. In some cases, kidneys were enlarged, congested and swollen with prominent tubules, which is attributed to impaired excretion of urates from the ureters by the swollen bursa.

On histopathological examination, bursa of Fabricius revealed lymphoid depletion of follicles, follicular cysts filled with necrotic debris, shrinkage of follicles and interfollicular connective tissue proliferation. Haemorrhages were noticed in interfollicular tissues in some cases. These findings are similar to the observations made by Zeleke *et al.* (2005) [16] and Dutta *et al.* (2007) [5]. Kidneys revealed vacuolar tubular degeneration, glomerular shrinkage and haemorrhages which is in accordance with earlier report (Singh *et al.*, 2014) [13].

The apparent inability to control IBDV infections sometimes through vaccination may be due to improper administration of vaccine virus, antigenic differences among the viruses (Rosenberger *et al.*, 1987) [12], insufficient potency of the live attenuated vaccine virus (Ismail and Saif, 1991) [9] and the interference between the residual maternally derived antibodies and the vaccine virus (Etteradossi, 2001) [7].

A vaccination failure occurs when, following vaccine administration, the birds are unable to develop adequate antibody titer levels and/or are susceptible to a field disease outbreak (Butcher and Miles, 1994) [3]. According to De Herdt *et al.*, (2005) [4] ELISA could be an effective tool in IBDV vaccination timing for assessing effective immunity development. The present report describes the pathological features of IBD and emphasize the need to take sufficient care before, during and after vaccination for development of sufficient immunity to the disease.

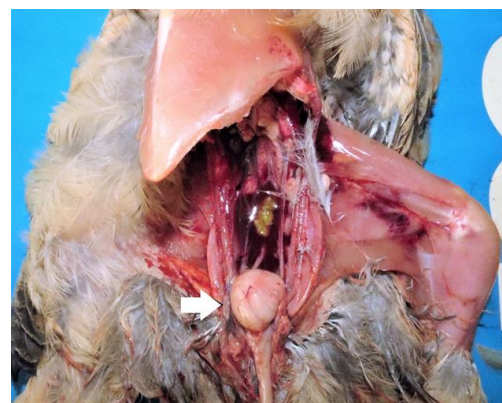


Fig 1: Enlarged bursa of Fabricius (arrow)



Fig 2: Widespread haemorrhages in thigh muscle

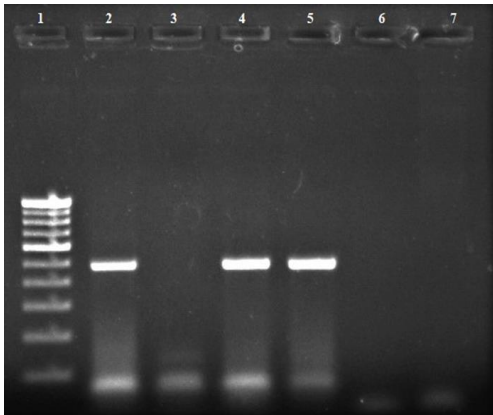


Fig 3: Agarose gel electrophoresis of RT-PCR amplified products of infectious bursal disease virus (representation) Lane 1: 100 bp ladder Lane 2: Positive control Lane 3: Negative control Lane 4, 5: Positive samples Lane 6&7: Negative samples

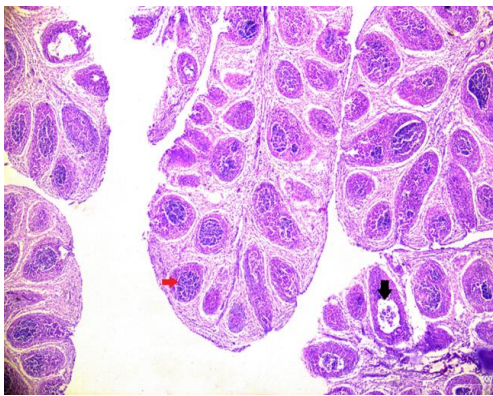


Fig 4: Bursa of Fabricius- Severe lymphoid depletion (black arrow) and severe degenerative necrotic lesion (red arrow) with inflammatory cell infiltration (H&E x400)

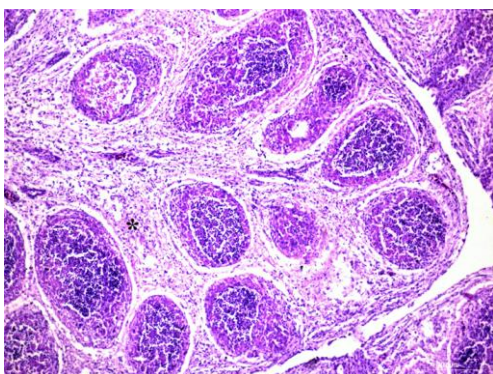


Fig 5: Bursa of Fabricius - Interfollicular connective tissue proliferation (asterisk) (H&E x200)

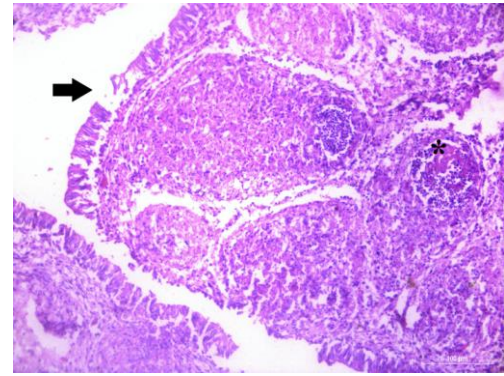


Fig 6: Bursa of Fabricius- Sloughing of bursal epithelium (arrow), intrafollicular edema and influx of heterophils (asterisk) (H&E x200)

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