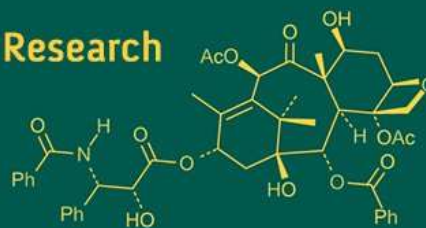
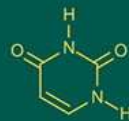
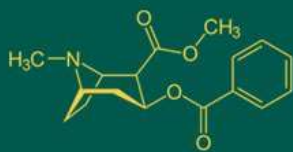


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Pathological and molecular diagnosis of Marek's disease in Kamrupa chickens

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Abstract

Marek's disease (MD) is an economically important lymphoproliferative disease of poultry caused by *Gallid alphaherpesvirus 2*. The present study investigated a suspected outbreak of MD in a flock of 12-week-old Kamrupa dual-purpose chickens through gross pathology, histopathology and molecular confirmation. Ten birds were subjected to postmortem examination. Gross lesions included emaciation with prominent keel bones, atrophied breast muscles, hepatomegaly with diffuse white nodular lesions, marked splenomegaly, pale nodular kidneys, thymic atrophy and haemorrhagic lesions in the proventriculus and gizzard. Histopathological examination revealed diffuse proliferation of pleomorphic lymphoid cells in the liver and spleen with loss of normal tissue architecture, while kidneys and proventriculus showed lymphomatous and lymphoproliferative lesions. For molecular confirmation, DNA extracted from representative tissues was subjected to PCR targeting the *Meq* gene. Amplification of the expected 400 bp product confirmed the presence of Marek's disease virus (MDV). Based on characteristic gross and microscopic lesions along with PCR confirmation, the outbreak was diagnosed as MD.

Keywords: Marek's disease, *Gallid alphaherpesvirus 2*, Lymphoproliferative lesions, *Meq* gene, PCR

Introduction

Poultry industry is one the fastest growing sectors and a major contributor to the national economy of India [3]. The re-emergence of certain viral diseases causes significant economic losses to this industry. MD is one such important re-emerging disease affecting nervous and lymphoid tissues along with other visceral organs [8, 1]. It is a contagious, viral oncogenic lymphoproliferative disease of poultry caused by *Gallid alphaherpesvirus 2* of the family *Herpesviridae* and among the three recognised serotypes it is the only tumour inducing serotype [4]. The present study aimed to investigate a suspected case of MD in poultry through gross and histopathological examination and molecular confirmation by detection of the viral genome.

Materials and Methods

Ten 12-weeks-old Kamrupa dual-purpose chickens were presented to the Poultry Disease Research and Consultancy, AFVU, Khanapara, Guwahati-22, for post-mortem examination following complains of a sudden increase in mortality of birds in the flock. The birds were reared under a deep litter system and had been vaccinated against MD.

A detailed postmortem examination was done and gross pathological lesions were documented. Representative tissue samples like liver and spleen were collected in 10% formalin for routine histopathological procedure and stained with H&E stain as given by [2]. For DNA extraction and Polymerase Chain Reaction (PCR) representative tissue samples were collected and stored at -20 °C. DNA was extracted by DNeasy blood and tissue kit as per manufacturer's protocol. PCR targeting the *Meq* gene was performed using a published primer as shown in Table 1. For conducting PCR, a 25 µl reaction mixture were prepared with a 2 µl of extracted DNA template, 8.5 µl of nuclease free water, 12.5µl of Master mix, 1 µl of forward and reverse primer (10 pmol/µl). The reaction mixture was subjected to amplification in a thermocycler (Applied Bio-system, USA) as per the reaction condition given by [6].

1.5% agarose gel was prepared with 1X TAE buffer stained with ethidium bromide (0.5µg/ml) for analysis of amplified

PCR product and visualized under gel documentation system.

Table 1: Primer sequence used for detection of MD virus

Primer	Sequence	Product size
Forward	5'-GGA-TCG-CCC-ACC-ACG-ATT-ACT-ACC-3'	400 bp
Reverse	5'-ACT-GCC-TCA-CAC-AAC-CTC-ATC-TCC-3'	

Results and Discussion

Gross pathology

External examination of the carcass showed shrunken and pale combs and wattles, prominent keel bones and atrophied (Fig. 1) and pale breast muscles with distended abdomens. A postmortem examination of the birds revealed hepatomegaly, pale and friable, with greyish-white diffuse nodular lesions (Fig. 2). The spleen was enlarged approximately three to four times, occupied a considerable portion of the abdominal cavity (Fig. 3), had a soft consistency and the cut surface revealed diffuse white nodular lesions. The proventriculus exhibits multifocal haemorrhages and ulcers; the gizzard also revealed haemorrhages. The thymus was markedly atrophied and showed congestion with white nodular lesions. Kidneys were also pale and had nodular lesions. The lesions observed in this study were consistent with the findings reported by [5, 7, 8, 1].



Fig 1: Carcass showing a prominent keel bone and atrophied breast muscles



Fig 2: Enlarged liver showing diffuse white nodular lesions



Fig 3: Spleen showing splenomegaly with diffuse white nodular lesions, occupying portion of the abdominal cavity

Histopathology

Microscopic examination of the hepatic parenchyma exhibited diffuse proliferation of pleomorphic lymphoid cells, predominantly lymphocytes and lymphoblasts, resulting in atrophy of adjacent hepatocytes and loss of normal hepatic architecture (Fig. 4). The splenic parenchyma showed diffuse proliferation of pleomorphic lymphoid cells, predominantly lymphocytes and lymphoblasts, with loss of normal splenic architecture (Fig. 5). In the kidneys, proliferation and infiltration of lymphoid cells within the parenchyma formed nodular lymphomatous lesions. The proventriculus showed aggregates of pleomorphic lymphoid cells forming lymphoproliferative lesions. These observations were comparable to the histopathological findings described by [7, 1, 9].

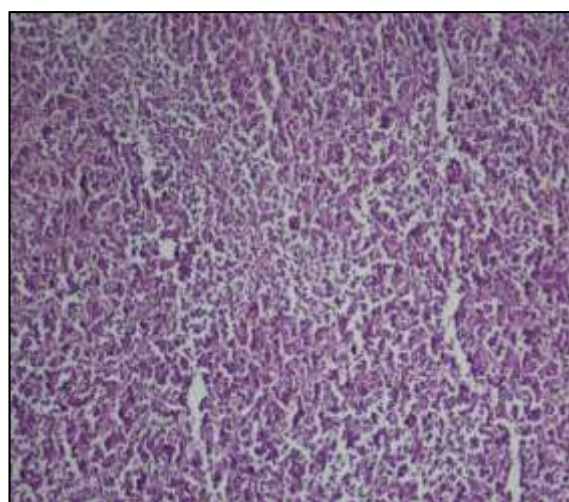


Fig 4: Photomicrograph of liver showing diffuse proliferation of pleomorphic lymphoid cells (H&E, 10X)

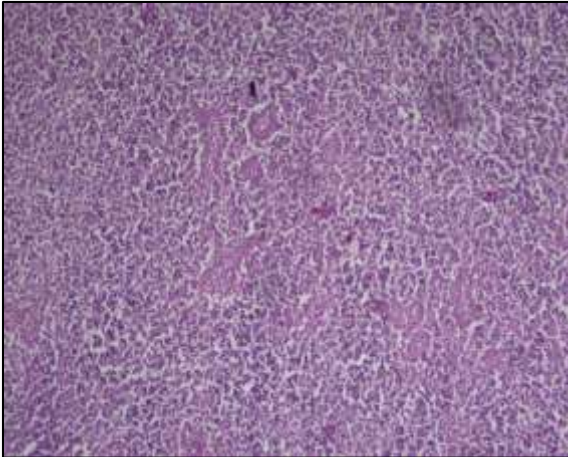


Fig 5: Photomicrograph of spleen showing lymphoproliferative changes with loss of normal splenic architecture (H&E, 10X)

PCR

MD viral DNA extracted from the representative liver and spleen samples was successfully amplified using primers specific to the *Meq* gene (400 bp). The expected PCR product was observed in the samples and positive control, with no amplification in the negative control (Fig. 6).

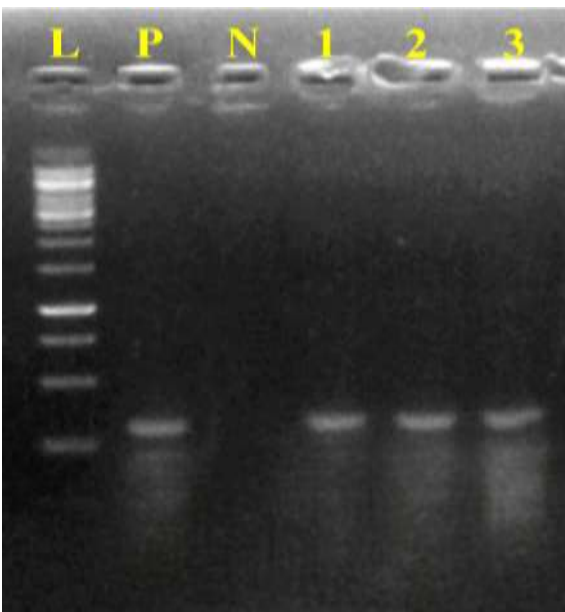


Fig 6: Electrophoresis analysis of PCR product of MD *Meq* gene (L-ladder: 1kb, P-Positive control, N-Negative control and 1, 2, 3-Samples)

Conclusion

Based on the characteristic gross lesions, histopathological findings and PCR amplification of the *Meq* gene, the disease was confirmed as MD in 12-week-old Kamrupa chickens. The affected birds showed lymphoproliferative lesions in the liver, spleen, kidneys and proventriculus. PCR detection of the expected 400 bp product further confirmed the presence of MDV. The study highlights the usefulness of gross pathology, histopathology and PCR for accurate diagnosis of MD in poultry.

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