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Pathological alterations and molecular identification of Marek's disease in chicken

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Abstract

Marek's Disease (MD) in chickens, caused by the oncogenic avian herpesvirus known as Marek's Disease Virus (MDV), poses significant challenges to the poultry industry. This study was aimed at the molecular detection of MDV in afflicted chickens focussing on the gross and histopathological changes. The diagnosis was confirmed by cytological analysis of touch impression smear of the visceral organs with tumour like lesions. Employing polymerase chain reaction (PCR) technique, the presence of the virus was detected in various tissues. Pathological examination revealed characteristic lesions such as lymphoproliferative tumours in various organs including the spleen, liver, kidney, proventriculus, heart and ovary. On microscopic examination, visceral organs revealed densely populated parenchyma with infiltration of pleomorphic lymphoid cells. Out of 70 samples, 17 cases tested positive for MD. The findings provide valuable insights into the occurrence, pathology and diagnosis of MD in chickens, contributing to better disease management strategies and control measures of this economically important poultry disease.

Keywords: *Marek`s disease, cytology, lymphocytes, methyl green-pyronine*

The rapid rise in demand for poultry products has resulted in massive increase in the number and size of poultry flocks in India. However, poultry farmers are also facing numerous challenges due to disease outbreaks, particularly neoplastic disease conditions, which result in significant production losses even when vaccinations are administered to day-old chickens at the hatchery.

Marek's Disease is a neuropathic and lymphoproliferative disease that mostly affects domestic chickens, but can also affect turkeys and quails to a lesser degree. The causal agent is a cell-associated, highly contagious oncogenic herpesvirus, as reported by Calnek *et al.* (1985), Hennig *et al.* (2003) and Schat and Nair (2008), and remains a significant concern for the poultry industry. The economic impact of MDV derives from both direct losses brought on by the disease and death of chickens (loss of egg production) and indirect costs resulting from the industry's widespread use of vaccinations and control measures (Rozins *et al.,* 2019). The infection primarily affects all visceral organs, including the skin and destroys lymphocytes, which suppresses the immune system, leading to secondary infections and

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increased mortality. Within the context of carcinogenesis, both transformed T- lymphocytes and the stromal cells in the tumour microenvironment, regulate tumour growth and therapeutic responses (Mantovani and Sica, 2010).

This study was aimed to explore the occurrence of MD in and around Thrissur by molecular detection of oncogenic MDV and understand the pathology of the disease by cytology and histopathological techniques. Morphological diagnosis of the disease was done using cytological and histopathological techniques and etiological diagnosis was done using PCR.

Material and methods

Collection of the samples

The carcasses of 70 birds with tumour like lesions in the visceral organs formed the material for the present study. The samples were collected from the birds brought for post mortem to the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur during December 2022 to January 2024. Detailed history including the age, sex, clinical presentation and vaccination status were collected. The frequency distribution of these parameters was noted. The location of the tumours and their distribution were noted. Size, colour and consistency of the tumours were also recorded. Impression smears from fresh tumour tissues were collected for cytological examination, air dried and fixed in methanol for cytological analysis. The impression smears were fixed in methanol and stained with Giemsa stain for cytological examination (Viraraghavan and Nair, 1965). Cytological features of pleomorphism, with lymphoid cells of various types and in varying stages of maturation as well as Methyl-green pyronine (MGP) staining were used for the differential diagnosis of MD from lymphoid leucosis (LL). Leukosis is composed of uniform lymphoblast cells and MD is comprised of pleomorphic population of cells from small lymphocytes to large lymphoblasts. The cytoplasm of blast cells being pyroninophilic, red stained cells will be seen in LL, whereas more of methyl green stained cells will be seen in MD (Suvarna *et al*., 2018).

Fresh tissue samples of spleen, liver, kidney, heart, lung, proventriculus and ovary with lesions suggestive of MD were collected. For molecular detection, pooled tissue samples were kept at -20°C in sterile tubes. The tissue samples were processed for DNA extraction using DNA synthesis kit (Origin, India) according to the manufacturer's instructions. The transcribed DNA was subjected to PCR using specific primers targeting the *meq* gene of MDV (Priya, 2015).

Histopathology

The organs with visible gross tumour lesions were removed and examined. The tissues were subjected to routine histopathological processing and staining. The stained sections were observed under light microscope and the results were recorded (Suvarna *et al*., 2018).

Results and discussion

Gross lesions

During postmortem examination, the gross lesions observed were emaciation with pronounced keel bone and atrophied muscles. The ovary in most cases were extremely enlarged with irregular greyish-white firm cauliflower like growth. The kidneys were pale with multi focal greyish-white nodules on all lobes. Enlarged liver with presence of multi focal greyish-white nodules and splenomegaly with multiple white circular nodules were noticed. Proventriculus showed thickened glands with presence of haemorrhages in the mucosa. Heart was misshapen with presence of focal greyish-white nodules (Fig 1). These observations were in accordance with the findings of Blakey *et al.* (2018) and Reddy *et al.* (2021). Examining the carcasses also revealed noticeable abnormalities in the keel bone, which had a distinctly twisted structure. Large lymphoid tumours were observed in majority of the visceral organs, including the liver, spleen, kidney, ovary, heart, proventriculus, lung and intestine.

Cytological examination

The tumorous growths in the liver kidney, heart, spleen was examined cytologically and the results showed pleomorphic small to large lymphocytes and lymphoblasts with large, round, vesicular nuclei and prominent nucleoli with a thin border of basophilic cytoplasm (Fig. 2). These findings were also described by Reddy *et al.* (2021). Cytological analysis of the ovary, lung, intestine and heart also revealed presence of pleomorphic lymphoid cells with heterochromatic nuclei. These results correlate with the studies of Biggs (1975) and Swathi *et al*. (2012).

Further characterisation by methyl green pyronin staining revealed that pleomorphic infiltration of lymphoid cells, could be well differentiated by the presence of light blue-green cells, stained by methyl green and the occasional presence of lymphoblasts could be differentiated by the red staining of pyronine. Notably, all examined visceral organs, including the liver, spleen, lung, kidney and proventriculus displayed a consistent predominance of light blue-green coloured cells (Fig.3).

Histopathology

On microscopic examination, the H and E-stained sections revealed diffuse proliferation of pleomorphic lymphoid cells in between cardiomyocytes with lysis of the myofibers. There was diffuse infiltration of atypical lymphocytes in the interstitium of the kidney and the kidney tubular epithelium showed degenerative to necrotic changes. Diffuse and severe lymphoid cell infiltration in the parenchyma of the liver was observed and the surrounding

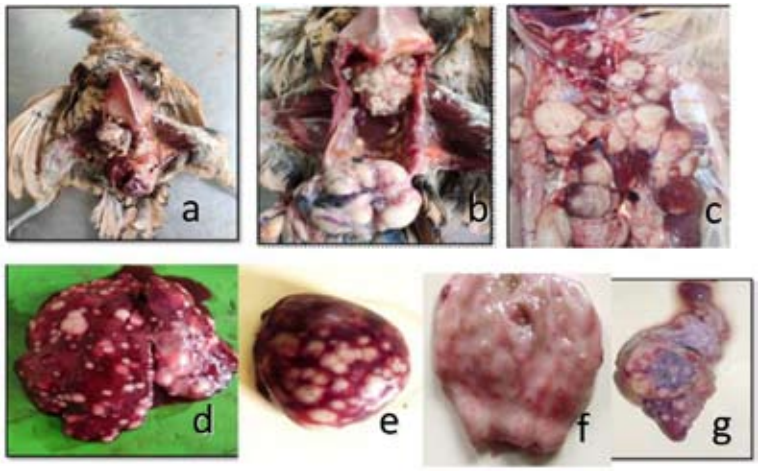

Fig. 1- Gross pathological lesions in MD- **a**- emaciated carcass with pronounced keel bone; enlarged visceral organs (**b**-ovary; **c**-kidney; **d**-liver; **e**-spleen; **f**-proventriculus; **g**-heart) with nodular, white tumours

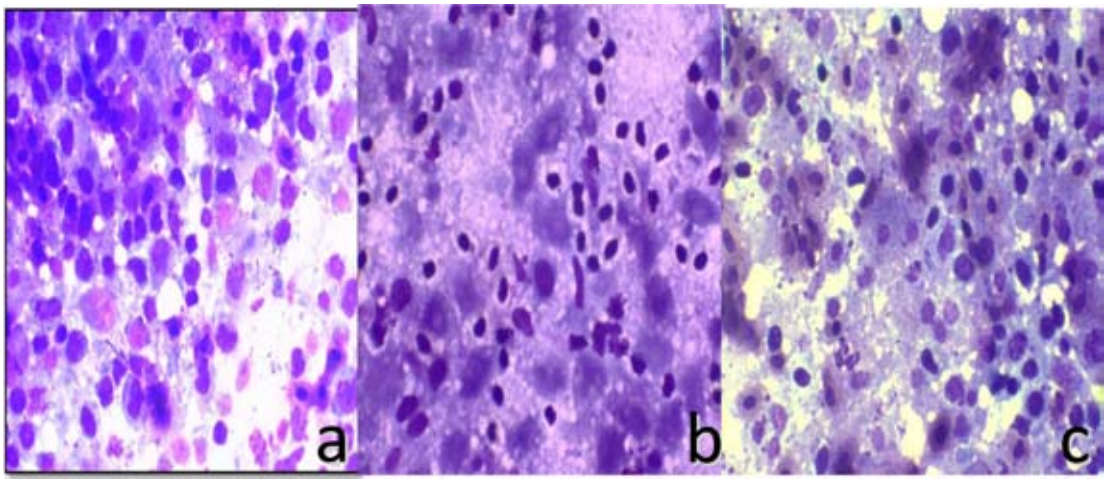

Fig. 2- Cytology of visceral organs (**a**-liver; **b**-kidney; **c**-heart) showing pleomorphic lymphoid cells- Giemsa stain X1000

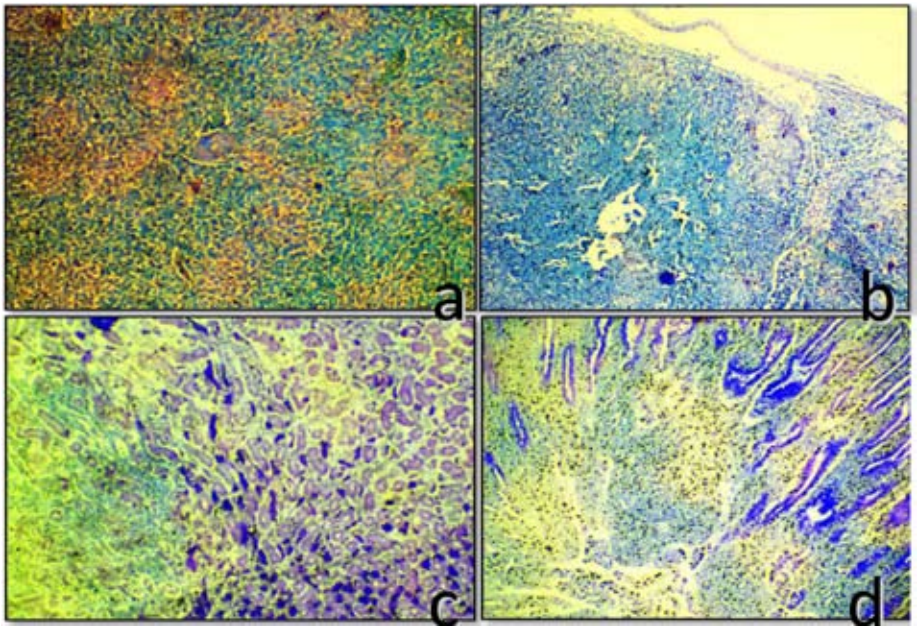

Fig. 3- Cytology of visceral organs (**a**-liver; **b**-kidney; **c**-heart) showing blue-green stained pleomorphic lymphoid cells MGP X1000

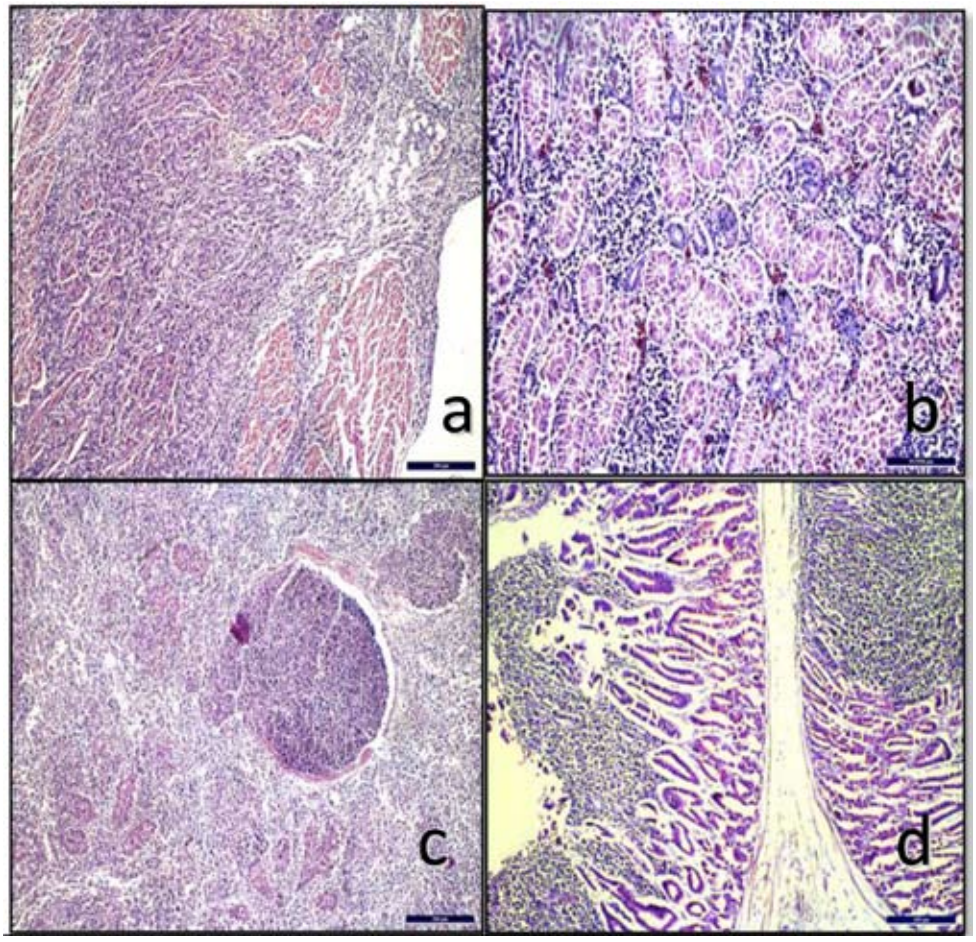

Fig. 4 Histopathology of visceral organs (**a**-heart; **b**-kidney; **c**-liver; **d**-proventriculus) showing pleomorphic lymphoid cells, disrupting the parenchyma – H&E X100

hepatic cords were disrupted and atrophied. Additionally, intense lymphoid infiltration causing mild to moderate degeneration of the glandular cells was observed in proventriculus (Fig. 4).

The microscopic lesions observed in this study was extensive infiltration of pleomorphic lymphocytes and lymphoblasts and similar lesions were also observed by Hayajenh *et al.* (2021) and Shchebentovska *et al.* (2021). Additionally, microscopic evaluation of the ovary, lung, intestine and heart revealed focal presence of pleomorphic lymphoid cells, along with marked aggregation of lymphoid cells, inducing structural disarrangement in these organs. Witter and Burmster (1979) and Biggs (1975) both documented the pleomorphism of lymphoid series, which served as the basis for the histological diagnosis of MD in the current investigation.

Molecular confirmation of MD by PCR

The molecular confirmation was done by the presence of the expected amplicon size of 1060 bp for MD when visualised after agarose gel electrophoresis (Fig.5). Out of the total 70 cases, 17 cases were positive for MD, indicating an occurrence positivity of 24 per cent.

The results of the present study, indicated an occurrence of MD in around 24 per cent of poultry in our area, despite the routine vaccination practice. The PCR amplicons of 1060 bp conclusive for oncogenic MD was used to confirm the presence of MD infection in the birds, as per Priya (2015). This necessitates the need for looking into the efficacy of vaccination and also to look into the possible emergence of new field strains of the virus. But the results have to be confirmed by using higher sample size and from different regions in the state/ country.

Conclusion

This study documents the recent occurrence of Marek's Disease in and around Thrissur. Early detection by using PCR and differential diagnosis of Marek's disease in chicken from other neoplastic diseases could be achieved by the use of cytological and histological techniques. Continuing work is needed to fully understand the prevalence, pathogenesis and pathology of this disease, so that effective control measures can be adopted to curb this economically important poultry disease.

Acknowledgement

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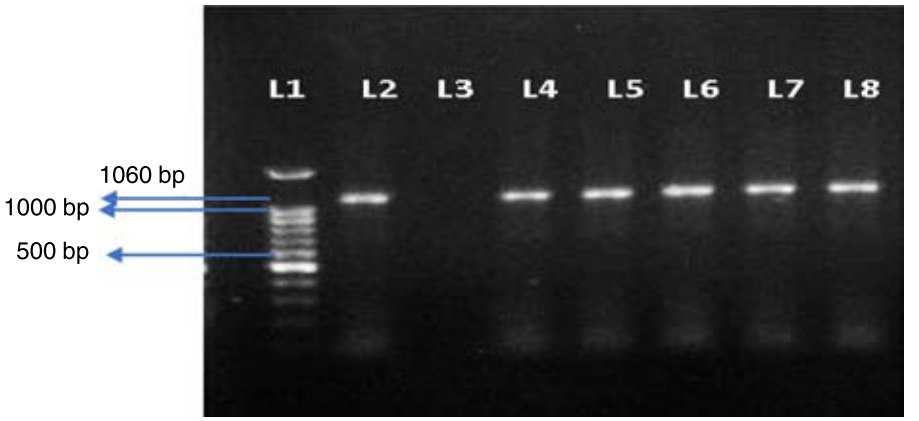

Fig. 5. PCR for *meq* gene of MDV
Lane 1: DNA molecular weight marker (100bp) Lane 2: Positive control DNA
Lane 3: Negative control Lane 4, 5, 6, 7, 8: Positive clinical samples (1060bp)

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Conflict of interest

The authors declare that they have no conflict of interest.

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