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Outbreak of Marek's Disease in Chicken Flocks with Uncommon Pattern of Gross Lesions#

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Abstract

Marek's disease is one of the highly contagious and oncogenic diseases of poultry that remains as a threat to the fast-growing poultry sector, even after proper vaccination. Generally, the disease is identified incidentally during the routine postmortem examination as nerve enlargement or as gross tumor lesions. The present article discusses the outbreak of Marek's disease in three vaccinated commercial layer flocks with high mortality and subtle gross nodular lesions. Three to five-month-old, commercial layer chickens from three different farms were submitted for post mortem at Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Kerala with the history of weight loss and mortality rate of about 25 per cent. On postmortem examination, the carcasses revealed severe emaciation and atrophied to mildly enlarged visceral organs in the majority of cases except in three birds with small white nodules. Microscopical examination revealed, varying degrees of pleomorphic lymphoid cell proliferation in the liver, spleen, kidney, ovary, pancreas and thyroid. Polymerase chain reaction targeting *gB* gene confirmed the presence of Marek's disease virus (MDV) in birds, with and without gross tumors. The birds from farms of different flock size showed similar high mortality rate but mostly without gross tumour lesions of MD. These findings emphasise the importance of histopathological examination in routine postmortem of chickens to identify diseases with gross nonspecific lesions.

Keywords: Marek's disease, layer chicken, non-nodular lesions, emaciation, atrophy of liver

Marek's disease (MD) is a highly contagious, oncogenic and immunosuppressive disease of chicken. The disease was first reported in 1907 from Hungary in paralysed roosters,

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followed by reports of several outbreaks in 1960s (Ramasamy *et al*., 1989). The disease was brought under control in 1970s after the development of live vaccine from herpes virus of turkey (HVT). However, re-emergence of MD outbreak in vaccinated flocks were being reported from different corners of the world. Marek's disease virus belongs to the genus, Mardivirus of subfamily alphaherpes virinae. The genus has three species of which the species Gallid alphaherpes virus 2, previously known as serotype 1 of MDV, with a linear, double stranded DNA genome of 160-180 kbp is the pathogenic one (Poonam *et al*., 2017).

Domestic chickens are considered the natural host of MDV. Clinical signs of MD are most commonly seen at 4 to 18 weeks of age, but older birds can also develop the disease. Diffuse or nodular tumour formation in visceral organs or feather follicles and enlargement of nerves are the characteristic lesions of the disease (Tambiev *et al*., 2021). In this article, we report an outbreak of MD in commercial layer chickens from three farms with high mortality but subtle characteristic gross lesions.

Materials and methods

Commercial layer chickens aged three to five months with a history of emaciation and death, submitted to the department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad, Kerala for postmortem examination during the period from October 2021 to January 2022 formed the material for the study. The birds were brought from three different farms in the district of Wayanad, Kerala, but it was learned that all these birds were procured from the same egger nursery at 45 days of age and reared in cages. The birds were vaccinated against MD, Newcastle disease and infectious bursal disease. The flock size in the farms was 4000, 300, and 500 and the birds from the farms are designated hereafter as flock one, two and three, respectively. The clinical history reported from all the three farms was similar such as unusual death of birds starting from about three months of age with severe loss of weight, droopiness, reluctance to stand up and walk, development of anorexia within a few days of initial weakness, and death. Mortality was 1000 birds (25%), 60 birds (20%) and 120 (24%) in flock one, two, and three respectively at the reporting time.

Detailed postmortem of the birds was conducted, lesions recorded and samples collected in 10 per cent neutral buffered formalin for histopathological examination and in ice and kept at $^{-}20^{o}$C till processing for molecular diagnosis. Samples were also collected aseptically for bacterial culture. Intestinal scrapings were collected and examined under a microscope for the presence of coccidian life stages. The samples for histopathology were processed by routine method, sections taken at 5µ thickness and were stained with haematoxylin and eosin (Suvarna *et al*., 2018).

Extraction of DNA from pooled visceral organs was carried out using conventional phenol-chloroform method. Total RNA was extracted using TRI Reagent® (SIGMA Life science, USA) and complementary DNA strand was synthesised using Revert Aid H Minus First Strand cDNA Synthesis kit (Thermo Scientific, USA). The samples were screened (Table 1) for the presence of viruses targeting glycoprotein

Table 1. Details of PCR primers used for screening of MDV, ALV, REV and CIAV

Sl No	Primer Name	Sequence 5'-3'	Target gene/region	Product size (bp)
1	MDVF	GTGGAAAGAGGTGACTGAAATG	*gB*	491
	MDVR	AGAAATTGGAGCATGGCGA		
2	ALVF	CTRCARCTGYTAGGYTCCCAGT	*env*	229
	ALVR	GYCAYCACTGTCGCCTRTCCG		
3	REVF	CATACTGGAGCCAATGGTT	*5'LTR*	291
	REVR	AATGTTGTACCGAAGTACT		
4	CIAF	ATGCACGGGAACGGCGGAC	*VP2*	651
	CIAR	TCACACTATACGTACCGGGG		

B (*gB*) gene of MDV (Jwander *et al*., 2012), *env* gene of avian leukosis virus (ALV) (Mohammadi *et al*., 2008), *5'LTR* of reticuloendotheliosis virus (REV) (Davidson *et al*., 1995) and *VP2* gene of chicken infectious anaemia virus (CIAV) (Wani *et al*., 2013). The PCR products were visualized and photographed in a gel documentation system under UV illumination. Nucleic acid extracted from vaccines and confirmed positive cases kept in department were used as positive control. A no template control was used as negative control to rule out contamination in the reactions.

Culture examination for infectious microorganisms was performed in brain heart infusion agar plates by incubating at 37°C for 24-48 hours aerobically. Colonies developed were subcultured twice on brain heart infusion agar plates to obtain a pure culture of bacteria. The pure culture was used for assessing growth on selective media such as MacConkey agar. Further identification of bacteria was done by biochemical tests (Carter, 1990; Markey *et al*., 2013).

Results and discussion

Five bird carcasses from flock one were submitted for postmortem examination on 1st week of October 2021. The carcasses were found underweighted (less than 350g) with severely atrophied pectoral muscles and prominent keel bone (Fig.1). Liver size was small in a bird (Fig.2), normal to slightly enlarged in three birds, while only one bird exhibited moderately enlarged liver with multifocal white areas (Fig.3). Kidney was dark red coloured in two birds while pale white in three birds. Spleen was small or normal sized in three birds and mildly enlarged in two birds. Ovaries were atrophied and greyish white in colour in all the birds. Deposit of caseous yellowish material on thoracic air sacs was noticed in one bird. Other gross lesions observed were congestion and edema of the lungs, red mottling of pancreas in one bird, and ulcers in proventricular mucosa (Fig.4) in two birds. Coccidia was detected microscopically in the intestinal scrapings of two birds.

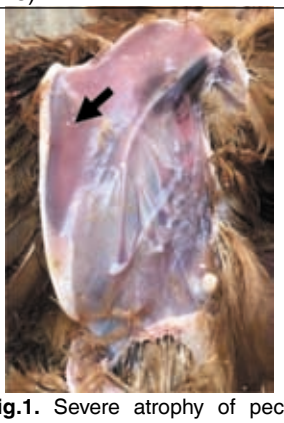

Fig.1. Severe atrophy of pectoral muscles (arrow) with prominent keel

Fig.2. Mild atrophy of liver (arrow)

Fig.3. Hepatomegaly with multifocal white areas (arrow)

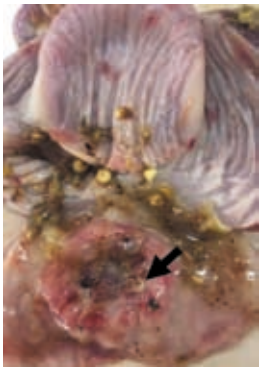

Fig.4. Ulcer in proventricular mucosa (arrow)

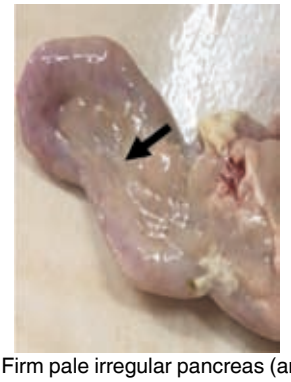

Fig.5. Firm pale irregular pancreas (arrow) adhered diffusely to intestinal wall

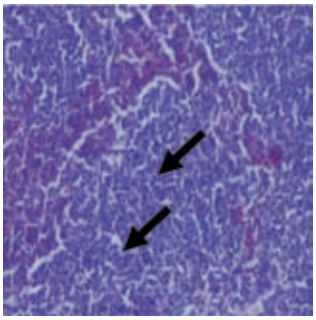

Fig.6. Diffuse infiltrate of pleomorphic lymphoid cells in the hepatic parenchyma (arrow) 400X

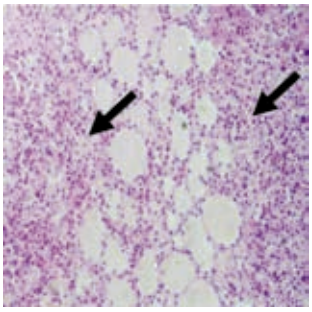

Fig.7. Lymphoid cells infiltrating deeply into thyroid parenchyma which compress the normal thyroid follicles (arrow) 400X

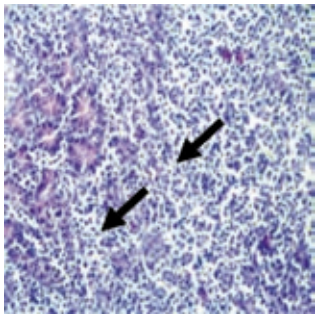

Fig.8. Diffuse pleomorphic lymphoid cell population replace and efface the pancreatic acini (arrow) 400X

Birds from flock two presented in the second week of October, 2021 were also underweighted (less than 350g) with severe atrophy of pectoral muscle. Liver was moderately enlarged with diffuse fibrinous perihepatitis in two birds. Pancreas was pale and found diffusely and closely adherent to the duodenal serosa (Fig.5) in one bird.

Birds from flock 3 were presented twice for postmortem, the first lot in the last week of December 2021 (105 days age) and second on January 2022 (118 days age). All the birds were underweighted and the gross lesions in the first lot (3 birds) included severe atrophy of pectoral muscle (3/3 birds), atrophy of liver (2/3 birds) and multifocal white nodule in liver (1/3). Coccidia was detected in all the cases. While in the second lot, postmortem revealed severe emaciation and multifocal pale white areas in the liver.

Histologically, variable degree of proliferation of pleomorphic lymphoid cells were observed in visceral organs. In the atrophic liver, hepatocyte size was reduced. Proliferating lymphoid cells were replacing the hepatocytes in larger livers with white areas (Fig.6). In thyroid, lymphoid cell proliferation was limited to the capsule in most of the cases but infiltrated parenchyma in one bird in flock 1 (Fig.7) while in the pancreas, parenchyma was infiltrated in varying degree in most of the birds (Fig.8). Pancreas revealed diffuse fibrosis along with the pleomorphic lymphoid cell proliferation. Varying degree of lymphoid cell proliferation was evident in other visceral organs such as lung, kidney, spleen, proventriculus with ulceration, intestinal wall and ovary.

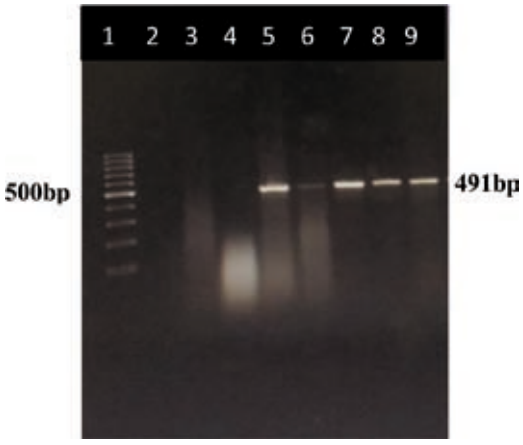

Fig.9. Agarose gel positive for MDV
Lane 1: 100 bp DNA Ladder
Lane 2: No template control
Lane 3-8: Samples
Lane 9: Positive control

Marek's disease virus (Fig.9) was detected by PCR in the birds from all three flocks, while ALV, CAV, and REV could not be detected in any of these cases. Sequence analysis of positive amplicon of MDV was done in NCBI BLAST. On BLAST analysis, MDV isolate showed 99.23 per cent similarity to Chinese isolate Gallid alphaherpes virus 2 strain HNLC503. *Escherichia coli* was isolated from birds with perihepatitis.

From the histopathology and molecular findings, the disease was diagnosed as MD with concurrent coccidiosis and/or *E.coli* infection. Clinical history of chronicity and emaciation were highly pronounced in the present case. Earlier, Biggs (2001) reported nonspecific signs

such as inappetence, reduced weight gain and diarrhea in chronic MDV infection. Finally, death occurs due to starvation and dehydration.

Heavy mortality was observed in all the three flocks. Heavy mortality in virulent and very virulent MD were reported earlier (Kannaki and Gowthaman, 2020) but has rarely been found in farms with low flock size. Characteristic gross lesions of MD are nerve thickening, diffuse or nodular tumour lesions in visceral organs or feather follicles (Das *et al*., 2018; Bertzbach *et al*., 2020). But in the present study nerve thickening or feather follicular lesions were not seen. Visceral tumours were rare while atrophy of visceral organs was seen in many birds. In such cases, with heavy mortality and lesions of *E.coli* infection or coccidiosis may lead to misdiagnosis unless histopathology is not performed. Marek's disease can be confirmed by the presence of pleomorphic lymphoid cells along with molecular diagnosis (Balasubramaniam *et al*., 2017; Singh *et al*., 2012). Mild hepatomegaly, splenomegaly, renomegaly, ovarian atrophy were earlier reported in MD (Stamilla *et al*., 2020). Enlargement and lymphoproliferative lesion of pancreas was reported by Haridy *et al*. (2019) in Japanese silkie fowl in MDV- ALV (A-E sub groups) coinfection. But no such viral coinfection could be detected in the present study. Nair *et al*. (2020) observed infiltration of lymphoid cells deeply into the thyroid parenchyma and expansion of the interfollicular spaces. But in this study, most of the infiltrations were limited to thyroid capsule.

Presence of coccidiosis and *E.coli* infection might be secondary due to the immunosuppressive effect of the virus (Gimeno and Schat, 2018; Ates *et al*., 2020; Singh and Mukherjee, 2018). Often, virus induced immunosuppression and concurrent infection with immunosuppressive agents were difficult to diagnose and control.

The absence of visible nodular lesions in many of the carcasses may be because of the vaccination effect (Rogers *et al*., 2008) or the early phase of the disease. As the chicks were procured from the same nursery, there is a chance of common source of infection. All these birds were from a particular commercial layer breed. Increased susceptibility to MDV in the particular layer breed need to be further studied.

Conclusion

Marek's disease is a cause of high mortality in vaccinated flocks. Caution must be taken in diagnosing the condition with gross lesions alone in routine postmortem examination. The evolution of the virus and the efficacy of current vaccines need to be evaluated to prevent further loss to the poultry farming community.

Conflict of interest

The authors declare that they have no conflict of interest.

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