

Pathomorphological Studies on Natural Cases of Marek's Disease in Japanese Quails (*Coturnix coturnix japonica*)

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ABSTRACT

The incidence of natural outbreaks of Marek's disease in a Japanese quail flock was investigated on the basis of mortality rate, clinical signs, cytology, PCR, gross and histopathology. The mortality rate was 2 percent over a period of 20 weeks. The death started from 16 weeks onwards and continued upto 35 weeks with the highest incidence was recorded during 25–30 weeks. Cytology smears prepared from feather follicles, livers and kidneys showed infiltration of pleomorphic lymphocytes. Grossly, there was severe diffuse enlargement of organs like hepatomegaly, splenomegaly, nephromegaly along with ovarian lesions. Focal lymphomas in these organs were also noticed in few cases. Microscopically, there were pleomorphic lymphoblastic infiltration in liver, spleen, kidney, ovary and sciatic nerve tissues noticed. The final confirmation was done by Polymerase Chain Reaction (PCR). This is the first report on Marek's disease in Japanese quails in Namakkal region which is the hub of laying chicken in Tamil Nadu.

Keywords: Marek's disease, cytology, Japanese quails, lymphomas, PCR

Marek's disease (MD) is a lymphoproliferative disease caused by herpes virus and affects chicken, other domesticated and wild birds. It is characterized by multiple T-cell lymphoma formation in visceral organs and peripheral nerves (Calnek and Witter, 1991). Clinical signs observed for MD vary from mild depression followed by ataxia and paralysis, skin nodular lesions, stunting and mortality (Santin *et al.*, 2006). Though Japanese quails are hardy in nature, susceptibility to experimental infection with the JM strain of MD virus was recorded (Crucilo *et al.*, 2010). Compared with chicken, the MD features in quails were low intensity and low incidence of lymphoproliferation in the nervous system (Pradhan *et al.*, 1985). There are only a few reports available on the natural outbreaks of MD in Japanese quails (Imai *et al.*, 1990). However, there is no report on Marek's

disease in Japanese quails in Namakkal district which is the hub of laying chicken in Tamil Nadu. Hence, the present study deals with the occurrence of MD in Japanese quails raised in Namakkal district where MD has not yet been recorded so far.

MATERIALS AND METHODS

Three to five Japanese quail carcasses were brought regularly over a period of 20 weeks to the Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal, Tamil Nadu with the history of continuous mortality. Farm visit was made to record the clinical signs by ailing birds, method of rearing and mortality rate.

Detailed necropsy was conducted and gross lesions were noted. Feather follicle smear, livers

and kidneys impression smears were prepared and subjected to Giemsa and Leishman staining for cytological diagnosis. The organs showing lesions were collected in 10% neutral buffered formalin for histopathology. The paraffin embedded blocks were cut at 5 µm thickness and the tissue sections were stained with Haematoxylin and Eosin (H&E).

Tissue DNA extraction kit (Nucleo-pore, India) was used for extraction and purification of genomic DNA from tissues. Pooled samples of feather follicle, liver, spleen, kidney and ovary amples were used for DNA extraction. A 20ml PCR was carried out for amplifying Meq gene of MDV-1 genome produced a 1081 bp fragment by employing forward primer 5'-GGC ACG GTA CAG GTG TAA AGA G-3' and reverse primer 5'-GCA TAG ACG ATG TGC TGC TGA G-3' as per Gong *et al.* (2013). Master mix was used for PCR with following cyclic conditions: 94°C for 2 min and 35 cycles of 94°C for 1 min; 65°C for 1 min; 72°C for 1 min and final extension of 72°C for 10 min.

RESULTS AND DISCUSSION

The ailing Japanese quails did not exhibit much clinical signs except general weakness, impaired growth rate and torticollis. Similar types of symptoms were also recorded by earlier authors⁷. The mortality started from 16 weeks onwards and continued upto 35 weeks with regular mortality of 2 per cent over a period of 20 weeks. The highest incidence was recorded during 25 to 30 weeks.

On necropsy, liver showed diffuse enlargement and occupied the entire abdominal area in most of the cases. In few cases, there were grayish white numerous tiny focal lymphomas scattered over the hepatic parenchyma (Fig. 1). Spleen also exhibited severe enlargement upto ten times than the normal and to the size of small lemon with or without focal lymphomas (Fig. 2). Both kidneys revealed diffuse enlargement with multiple nodular glistening grayish white foci of tumours (Fig. 3). There were no observable gross

lesions in lung, proventriculus, intestine and nervous tissue. These findings were in agreement with the earlier report of Nagarajan *et al.* (2013) who also recorded the natural cases and was contradict with the findings in experimental infection with the JM strain of MDV where all organs showed lesions.

Feather follicle cytology revealed the presence of large lymphoid aggregates composed of pleomorphic lymphocytes and tumourous lymphoblasts (Fig. 4). This cytology picture is very well exemplified that the feather follicle was the possible source of infection. Impression smears from liver and kidney stained with Giemsa and Leishman stain showed the presence of pleomorphic lymphocytes, anisocytosis, anisokaryosis and lymphoblasts with mitotic figures.

Histological examination of various organs showed infiltration of heterogeneous population of pleomorphic lymphoid cells. The focal form resembled hyperplastic nodules which were clearly demarcated from the normal hepatocytes (Fig. 5). In diffuse form, mild to moderate infiltration of neoplastic lymphocytes in the sinusoidal space and around the perivascular area along with degenerative changes of adjacent hepatocytes.

In spleen, the difference between splenic corpuscles and their germinal centers was completely lost due to severe infiltration of pleomorphic lymphocytes. Marked thickening of the splenic vessels along with neoplastic lymphocytic infiltration around the blood vessels were also observed. Kidney sections revealed diffuse infiltration of pleomorphic lymphocytes in the interstitial space (Fig. 6) which replaced the renal parenchyma and leaving only few renal tubules and glomeruli. Though there were no observable gross lesions in sciatic nerve, microscopic picture showed pleomorphic lymphocytic infiltration in few cases. It was further confirmed in PCR by targeting Meq gene of MDV-1 genome produced a 1081 bp fragment (Fig. 7).



Fig. 1: Liver showing numerous tiny grayish white lymphomas scattered over the hepatic parenchyma



Fig. 2: Spleen exhibiting severe enlargement due to diffuse lymphoid infiltration

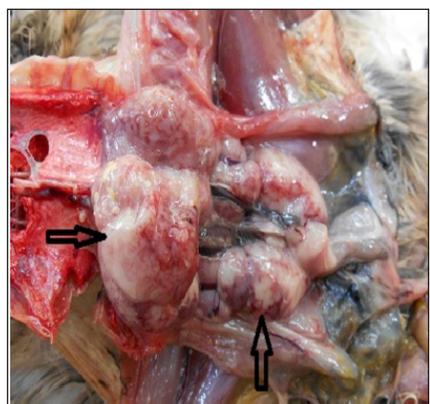


Fig. 3: Kidneys showing severe enlargement with lymphomas

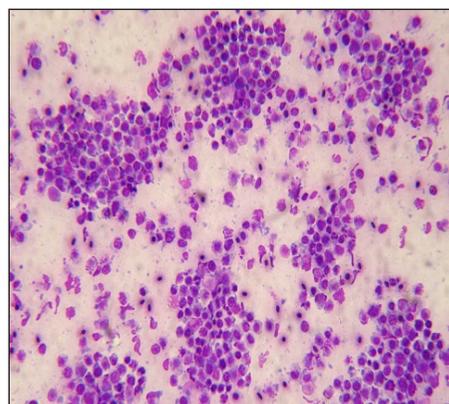


Fig. 4: Cytology of feather follicle smears revealing numerous pleomorphic lymphoblastic infiltration (Giemsa × 100)

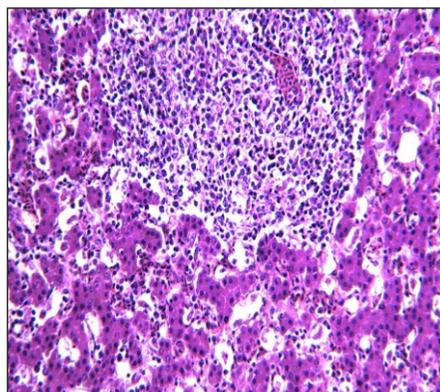


Fig. 5: Liver showing focal infiltration of pleomorphic lymphocytes and demarcated from the normal hepatic tissue (H&E × 400)

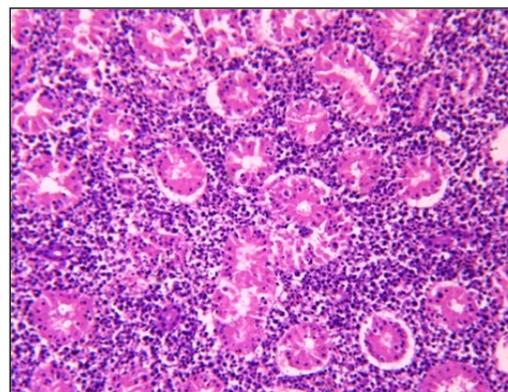


Fig. 6: Kidney exhibiting severe infiltration of pleomorphic lymphocytes in the interstitial space and replacement of normal renal tubules (H&E × 400)

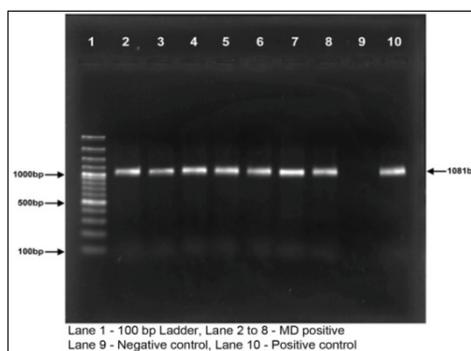


Fig. 7: Electrophoresis of PCR product showing positivity for Marek's disease while targeting Meq gene at 1081 bp

Compared with chicken, the MD features in quails were low intensity and low incidence of lymphoproliferation in the nervous system (Pradhan *et al.*, 1985). It was evidenced by the normal gross appearance of sciatic nerve and pleomorphic lymphoid infiltration in the nervous tissues of very few cases by histopathology. The possible source of infection of the Japanese quails could be a spread from commercial layers reared in nearby farms as the MD virus spread mainly through aerosol infection from feather dust particles.

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