

*Short Communication***Incidence of Multiple Lymphoproliferative Diseases among Poultry Layer Birds in an Organized Farm in Central Kerala****Devi, S. S.^{*}, Nisha, N., Priya, P. M.¹ and Vijayan, N.**

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Abstract

Ten birds of six months of age were taken as representative samples of an organized layer farm in Thrissur district of central Kerala having flock strength of 500, where unusual mortality (> 60%) was reported. All the birds belonged to the same batch and were vaccinated against commonly prevalent conditions like Marek's disease and New castle disease. The dead birds were subjected to detailed postmortem examination in the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur. On necropsy, six birds showed lymphoproliferative type of lesions in various visceral organs. The lesions consisted of either gross enlargement of the organs or focal white nodular masses on the surfaces which extended deep into the parenchymatous tissue. The tissues were subjected to histopathological and molecular investigations. With Hematoxylin and Eosin staining, the different tissues revealed lymphoid infiltration consisting of mixed population of lymphocytes, lymphoblasts and reticulo endothelial cells. Polymerase Chain Reaction (PCR) was employed for the isolation and identification of the etiological agent and the results confirmed that four birds out of six, had co-existence of all the three lymphoproliferative disease conditions namely, Marek's Disease, Avian Leukosis and Reticuloendotheliosis.

Key words: Lymphoproliferative Diseases, Reticuloendotheliosis

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Introduction

The three main lymphoproliferative diseases in poultry are Marek's Disease, Lymphoid leukosis and Reticuloendotheliosis (Payne and Venugopal, 2000). All these conditions are equally significant in terms of production loss, poor performance and mortality. Marek's disease (Calnek and Witter, 1997) is caused

by cell associated Herpes virus and the acute form of the disease is characterised by formation of lymphomas and the composition of lymphomas is complex, consisting of a mixture of small to medium lymphocytes, lymphoblasts, primitive reticulum cells, rarely plasma cells & macrophages (type A, B & C lesions). Both T & B cells are present with predominance of T cells (Ross, 1999). Lymphoid leukosis is neoplastic in nature and is caused by retro virus. Tumours consist of aggregates of large lymphoid cells / lymphoblasts with poorly defined cytoplasmic membrane, basophilic cytoplasm, vesicular nucleus with margination and clumping with one or more acidophilic nuclei (De Boer, 1987). Reticuloendotheliosis is caused by retro viruses, morphologically, immunologically and structurally different from leukosis / sarcoma group of viruses (Bagust, 1993). Histologic changes include infiltration and proliferation of large immature lympho reticular cells, described as cells of reticuloendothelial system (Okoye *et al.*, 1993 and Mark *et al.*, 1998).

Materials and Methods

Carcasses of ten birds of six months of age were made the subjects of study. The birds represented an organized layer farm in Thrissur district of central Kerala where unusual mortality was reported. All the birds belonged to same batch and were vaccinated against commonly prevalent conditions like Marek's disease and New castle disease. The carcasses of birds were subjected to detailed post mortem examination. Gross lesions were recorded and impression smears from the lesions were examined with Leishman's stain. Representative tissue samples of various visceral organs were collected in 10 percent neutral buffered formalin for histopathological studies. Paraffin blocks were prepared from the fixed tissues and sections of 4 micron thickness were taken. The sections were subjected to routine Hematoxylin and Eosin staining (Luna L. G. 1968). Molecular techniques were also tried for confirmative diagnosis and polymerase chain reaction (PCR) was employed for the isolation and identification of the etiological agents (Aly *et al.*, 1993).

Results

All the carcasses were seen highly emaciated with prominent wedge shaped keel bones. On gross examination of the carcasses, six birds showed lesions of lymphoproliferative nature, almost all the visceral organs like liver, spleen, heart, lungs, kidneys and ovaries revealed either gross enlargement or creamy white nodular masses (Fig. 1). None of the birds showed involvement of peripheral nerves or skin. Leishman stained impression smears from the nodular masses showed numerous lymphoid series of cells. Microscopic examination of the Hematoxylin and Eosin stained tissue sections revealed mixed infiltrating lymphoid population comprising small and medium lymphocytes, lymphoblasts and

lymphoreticular cells (Fig. 2). The molecular diagnostic PCR assay was carried out targeting specific *meq* gene of MD, *pol* + *GP* gene of AL and 5' prime *LPR* gene of REV.

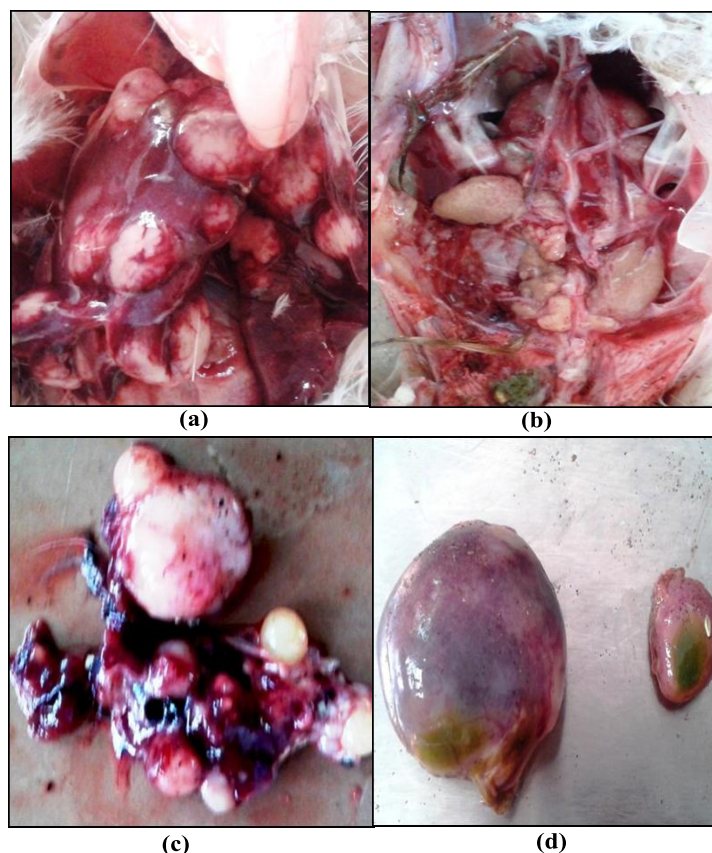


Fig.1: (a) Liver showing numerous lymphoproliferative nodules, (b) Enlarged and swollen kidneys, (c) Ovary showing lymphoproliferative lesions, (d) Enlarged spleen with focal whitish areas

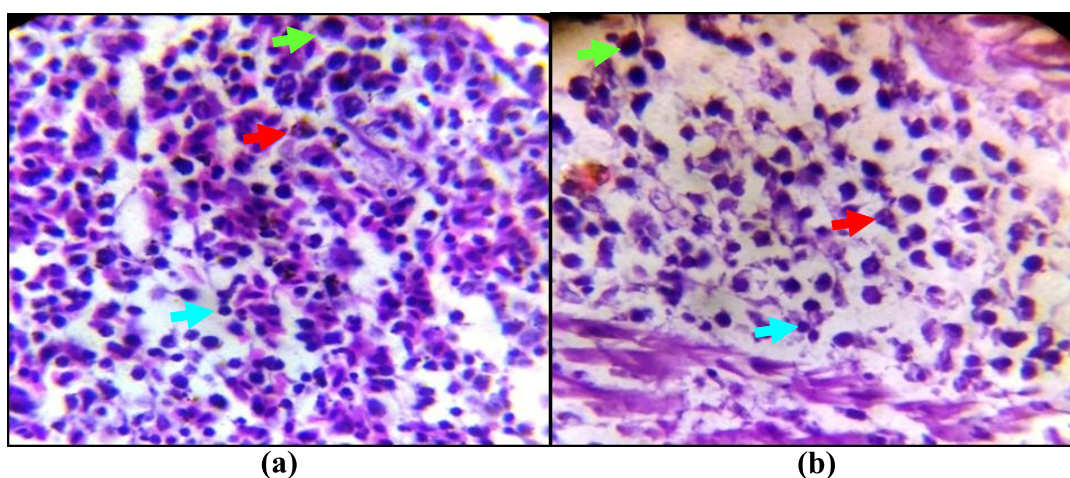


Fig.2: Liver (a) and Heart (b): Mixed infiltration by lymphoblasts (green arrow), reticulum cells (red arrow) and small round lymphocytes (blue arrow) (H&E x 400)

The expected amplicon of 1060bp, 1300bp and 291bp specific for each gene of MD, AL and REV was amplified from the DNA of tissues. Results are as follows (Table 1&2 and Fig. 3, 4, 5).

Table 1: Results of PCR Assay

	Reticulo Endotheliosis (RE) alone	Marek's Disease(MD) alone	Avian leukosis (AL) alone	AL+ MD	MD+RE	AL+ RE
No. of birds positive	Nil	1	Nil	2	3	4

Table 2: Results of PCR Assay

	MD+ AL+ RE
No.of birds positive	4

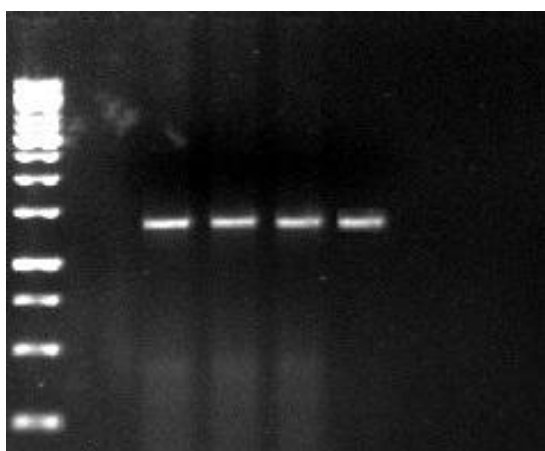


Fig.3: PCR result for Avian Leukosis
Lane 1.DNA ladder 1kb,
Lane 2,3,4,5 Positive(1300bp)

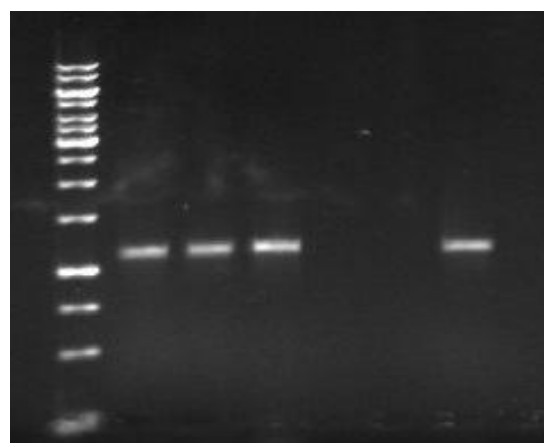


Fig.4: PCR result for Marek's Disease
Lane 1.DNA ladder 1kb ,
Lane 1,2,3,6 Positive(1060bp)

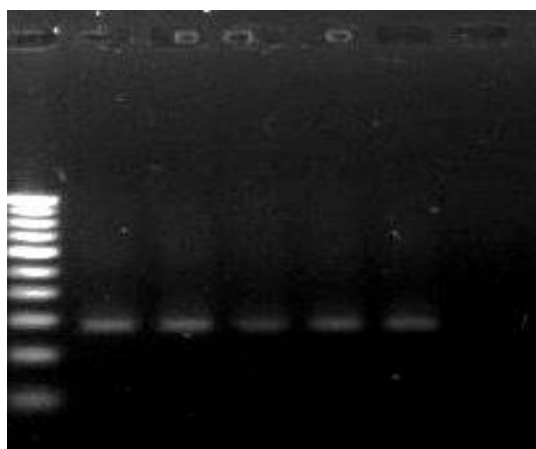


Fig.5: PCR result for Reticuloendotheliosis
Lane 1.DNA ladder 100bp ; Lane 1,2,3,4,5 Positive(291bp)

Discussion

From the results of the study, majority of the birds in the flock showed co existence of all the three conditions. Though Marek's disease is described as the most common lympho-proliferative disease of chicken by earlier workers, the present study identified RE as the most common condition in the flock. Histopathologically, there was pleomorphic infiltration by heterogenous lymphoid cells and the possibility to diagnose the condition as Type A Marek's disease was ruled out by molecular technique which brought into light the coexistence of all the three lympho-proliferative diseases in the same bird. The study also showed the re-emergence of lympho-proliferative diseases in poultry flocks of central Kerala. The incidence of MD among vaccinated flock was suggestive of either vaccination failure or the immune compromised status of the flock. RE though regarded as a non ubiquitous lympho-proliferative condition (Payne, 1992), the flock under this study revealed a greater incidence of RE than the other two conditions.

Conclusion

From the study, it can be concluded that mixed infection with viruses causing lympho-proliferation can coexist in the same bird and PCR is a diagnostic tool for molecular confirmation of these diseases.

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