

Original Research Article

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Histopathological and Molecular Diagnosis of Avian Reticuloendotheliosis Virus in Commercial Layer Chicken

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ABSTRACT

Keywords

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Seven dead commercial layer birds of 84 weeks age were examined to know the cause of death. The total flock size was 50,000 and birds were raised in cages from day one of age. The total mortality over a period of 10 weeks was 2.1 per cent. The birds appeared dull and depressed. At necropsy, ovary, pancreas revealed swollen and greyish nodular growth, firm in consistency and smooth when cut. Histopathology of ovary, liver, heart and brain corroborated with the gross lesions. Reticuloendotheliosis virus (REV) was confirmed by conducting polymerase chain reaction (PCR) (Mitra *et al.*, 2013).

Introduction

Reticuloendotheliosis virus (REV) is an immunosuppressive and neoplastic condition affecting chickens caused by gamma retro virus belongs to retroviridae family (Buchen – Osmond, 2004) and transmitted by a horizontal route by direct contact between birds, indirectly by some insect vectors like mosquitos and also by a vertical route by eggs (Motha *et al.*, 1987). The clinical disease associated with REV is acute reticular cell neoplasia, chronic lymphomas and an immunosuppressive runting disease (Crespo *et al.*, 2002). Various reports explained that REV

as contaminant of Marek's disease and Fowl pox vaccines which resulted in delayed growth, feather abnormalities, anemia and leg paralysis (Wei *et al.*, 2012). The present paper describes the histopathological and molecular diagnosis of avian reticuloendotheliosis virus in commercial layer chicken raised entirely in cages.

Materials and Methods

Seven dead commercial layer birds of 84 weeks of age were examined to know the cause of death with a case history of dull, off feed, pale comb and 6 per cent loss in

production with 2.1 per cent mortality over a period of 10 weeks. The total flock size was 50,000 and all the birds were raised in cages from day one of age.

The persistent mortality (0.14% per week) was recorded from 52 to 74 weeks of age and it increased at the age of 75 weeks from 0.14 per cent to 0.21 per cent per week. The total mortality over a period of 33 weeks (52 to 84 weeks) was 5.46 per cent.

A detailed necropsy was conducted on dead birds and gross lesions were recorded. The tissue samples from different portions of liver, ovary, pancreas and heart were collected in 10 per cent formalin, processed and sections were stained with haematoxylin and eosin.

DNA extraction and PCR

Suspected liver, spleen and kidney samples were collected in dry ice for PCR confirmation (Gong *et al.*, 2013). DNA was extracted by DNeasy blood and tissue kit as per manufacturer’s instruction.

The obtained DNA was stored at -20°C until for further analysis. Then polymerase chain reaction was carried out by using previously reported primer set for REV as shown in Table 1.

The PCR reactions was carried out in final volume of 25µl which include volume of

12.5µl of master mix(2 X), 1µl of forward and reverse primer each(10 pmol/µl), 7.5µl of deionized water and 3µl of extracted DNA and the above mixture of materials was subjected to PCR in a thermal cycler (Eppendorff) as per the procedure of Gong *et al.*, (2013).

The analysis of PCR product was carried out in 1.5 per cent agarose gel stained with ethidium bromide (0.5µg/ml) and documented under Gel documentation system.

Results and Discussion

Gross pathology

The affected birds appeared dull, depressed and leg paralysis. At necropsy, liver revealed moderate enlargement. Pancreas showed greyish white nodular growth on the surface (Fig.1). Ovary showed cauliflower like greyish white nodular growth (Fig.2).

Histopathology

Histopathology study of reticuloendotheliosis suspected liver showed diffuse severe reticuloendothelial cell (RE) infiltration in hepatic parenchyma (Fig.3). In ovary, diffuse severe reticuloendothelial cell infiltration in ovarian stroma (Fig.4).

In heart patchy moderate reticuloendothelial cell infiltration in cardiac muscle (Fig.5). In brain perivascular cuffing (Fig.6) and gliosis were seen (Fig.7).

Table.1 Primer used for detection of REV viruses

Virus	Primer	Sequence	Gene and Size	Reference
REV	Forward Reverse	TCG ATT GCG GTA GCT CCA C - Renv-1 CCA TCG AGAGTG ACA TTG C - Renv -2	gp73, 642bp	Singh <i>et al.</i> , 2003



Fig. 1. Chicken - Pancreas - Greyish white nodular growth on surface



Fig-2 - Chicken - Ovary - Cauliflower like greyish white nodular growth

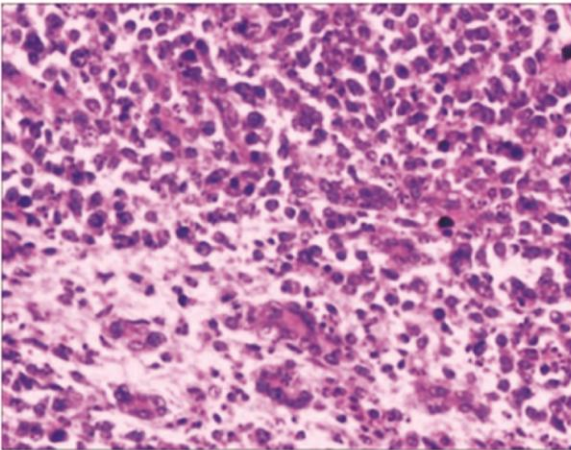


Fig. 3. Chicken - Liver - Diffuse severe reticuloendothelial cell infiltration in hepatic parenchyma - 400X

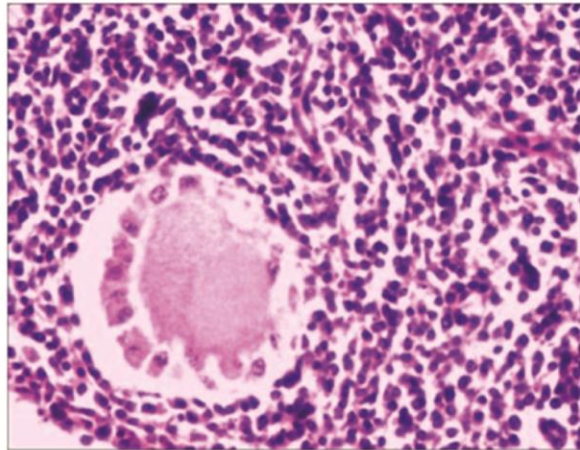


Fig- 4 - Chicken - Ovary - Diffuse severe reticuloendothelial cell infiltration in ovarian stroma - 400 X

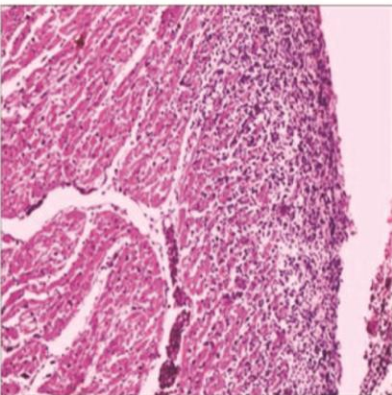


Fig- 5. Chicken - Heart - Patchy moderate reticuloendothelial cell infiltration in cardiac muscle- 100 X

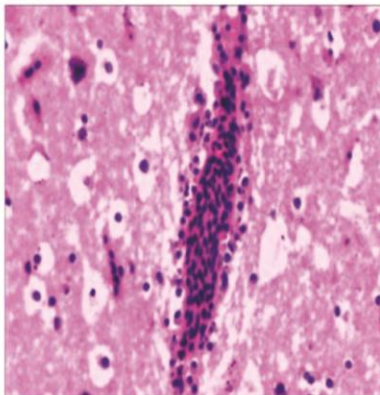


Fig -6 . Chicken - Brain - Perivascular cuffing - 400 X

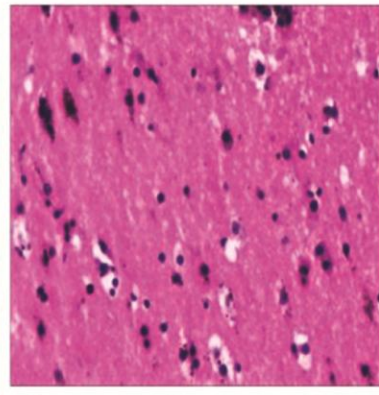
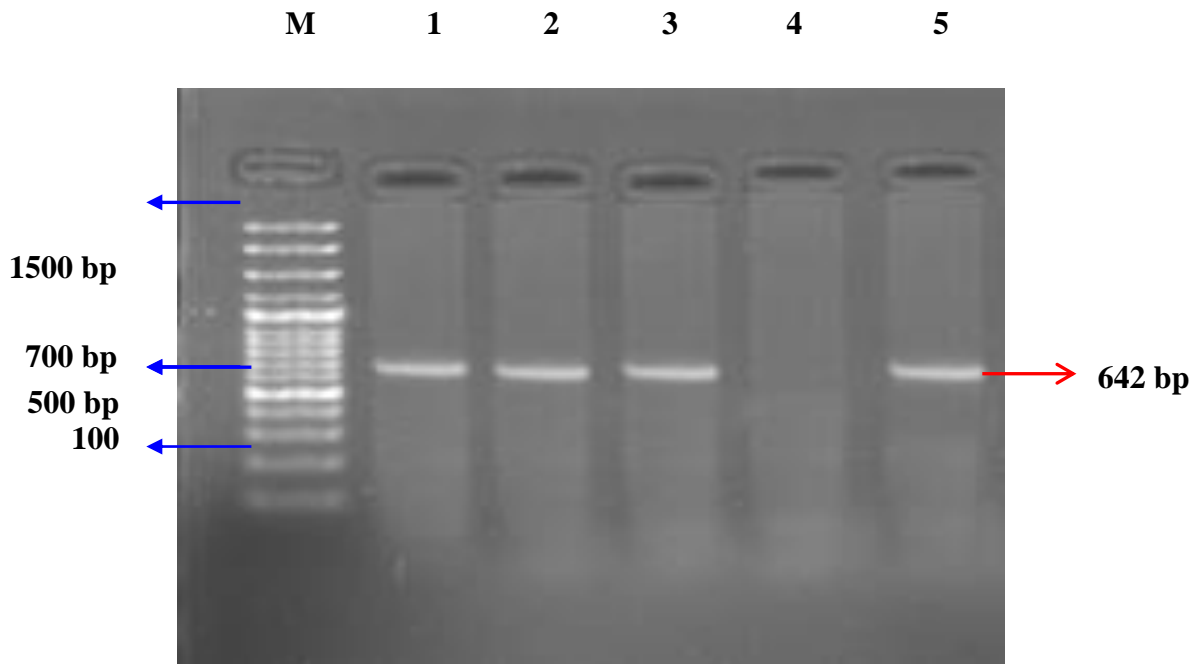


Fig -7 . Chicken - Brain - Gliosis - 400 X

Fig.8 M-100 bp ladder, 1(liver), 2 (kidney) and 3 (spleen) Positive samples with a band at 642 bp specific for REV, 4- Negative control and 5 – Positive control



PCR

PCR was conducted at Department of Veterinary Microbiology, Veterinary College and Research Institute, Orathanadu- 614 625 for 3 field samples taken from suspected cases and REV was confirmed.

Reticuloendotheliosis was diagnosed in commercial Layer chicken of 84 weeks age. Clinically birds showed 6 per cent less egg production, dull, off feed and pale comb with 5.46 per cent mortality over a period of 33 weeks. Liver sections revealed diffuse severe RE cell infiltration in liver parenchyma.

Diffuse severe RE cell infiltration in ovarian stroma, patchy moderate RE cell infiltration in cardiac muscle and perivascular cuffing, gliosis in brain were noticed. The histopathological changes of liver, ovary, heart and brain observed in this study agreed

with the findings of earlier workers (Ponnusamy *et al.*, 2018). The PCR test was highly useful for detection of REV virus (Gopal *et al.*, 2012; Ahmed *et al.*, 2018).

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