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## Occurrence of avian reticuloendotheliosis virus in commercial layer chicken

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### Abstract

Ten dead commercial layer birds of 74 weeks age were examined to know the cause of death. The total flock size was 40,000 and birds were raised in cages from day one of age. The total mortality over a period of 23 weeks was 6.2%. The birds appeared weak and emaciated. At necropsy liver and kidney revealed mild enlargement and greyish tumorous foci and nodular growth on surface and were firm in consistency and smooth when cut. Histopathology of liver and kidney were corroborated the gross lesions. Reticuloendotheliosis virus (REV) was confirmed by conducting polymerase chain reaction (PCR) (Ravikumar *et al.*, 2019).

**Keywords:** Layer chicken, reticuloendotheliosis virus, PCR, histopathology

### 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) [6]. The clinical disease associated with REV is acute reticular cell neoplasia, chronic lymphomas and an immunosuppressive runting disease (Crespo *et al.*, 2002) [3]. 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) [10]. The present paper describes the occurrence of avian reticuloendotheliosis virus in commercial layer chicken raised entirely in cages.

### Materials and Methods

Ten dead commercial layer birds of 74 weeks of age were examined to know the cause of death with a case history of weak, emaciated and 4% loss in production with 6.2% mortality over a period of 23 weeks. The total flock size was 40,000 and all the birds were raised in cages from day one of age. The persistent mortality (0.2% per week) was recorded from 52 to 66 weeks of age and it increased at the age of 67 weeks from 0.2 % to 0.4% per week. The total mortality over a period of 23 weeks (52 to 74 weeks) was 6.2%.

A detailed necropsy was conducted on dead birds and gross lesions were recorded. The tissue samples from different portions of liver and kidney were collected in 10 % formalin, processed and sections were stained with haemotoxylin and eosin.

### Proviral DNA extraction and PCR

Suspected liver and kidney samples were collected in dry ice for PCR confirmation (Gong *et al.*, 2013) [4]. Proviral DNA was extracted by DNeasy blood and tissue kit as per manufacture instruction. The obtained proviral 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.

**Table 1:** Primer used for detection of REV viruses

Viruses	Primer	Sequence	Gene and Size	Reference
REV	Forward	TCG ATT GCG GTA GCT CCA C - Renv-1	gp73,642bp	Singh <i>et al.</i> , 2003 [9]
	Reverse	CCA TCG AGAGTG ACA TTG C - Renv -2		

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The PCR reactions was carried out in final volume of 25  $\mu$ l which include volume of 12.5  $\mu$ l of master mix (2 X), 1  $\mu$ l of forward and reverse primer each (10 pmol/ $\mu$ l), 7.5  $\mu$ l of deionized water and 3 $\mu$ l 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) [4]. The analysis of PCR product was carried out in 1.5 per cent agarosegel stained with ethidium bromide (0.5 $\mu$ g/ml) and documented under Gel documentation system.

## Results and Discussion

### Gross pathology

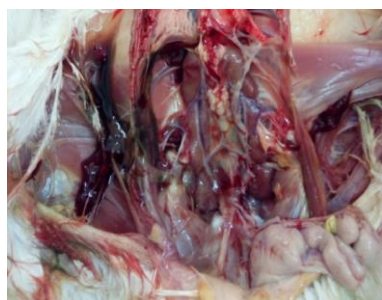
The affected birds appeared weak and emaciated. At necropsy liver revealed mild enlargement, greyish tumourous foci with nodules on the surface (Fig.1). Kidney showed enlargement, greyish white nodular foci on the surface (Fig.2).

### Histopathology:

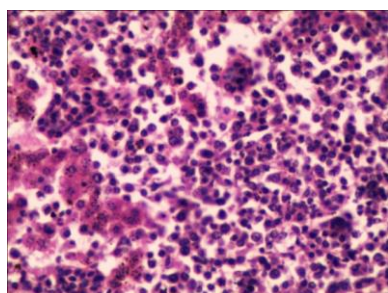
Histopathology study of Reticuloendotheliosis suspected liver showed diffuse severe reticuloendothelial cell (RE) infiltration in hepatic parenchyma (Fig.3). In kidney multifocal moderate reticuloendothelial cell infiltration in renal parenchyma were seen (Fig.4).



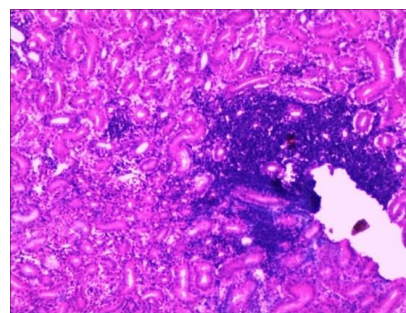
**Fig 1:** Chicken- REV- Liver showing mild enlargement, greyish tumourous foci with nodules on the surface



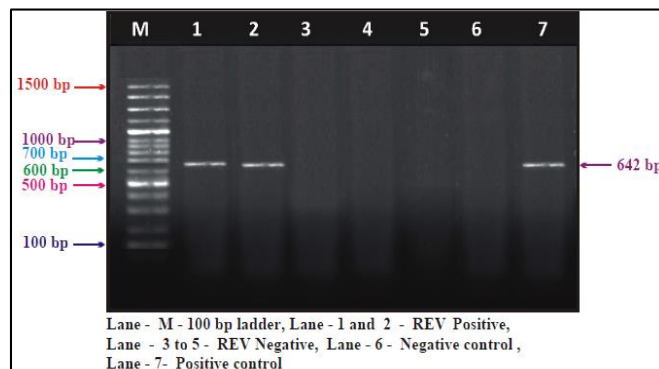
**Fig 2:** Chicken- REV – Kidney showing enlargement, greyish white nodular foci on the surface



**Fig 3:** Chicken- REV – Liver - diffuse severe reticuloendothelial cell (RE) infiltration in hepatic parenchyma - 400X



**Fig 4:** Chicken- REV – Kidney - multifocal moderate reticuloendothelial cell infiltration in renal parenchyma -100X



**Fig 5:** PCR: PCR was conducted at Department of Veterinary Microbiology, Veterinary College and Research Institute, Orathanadu- 614 625 for 5 field samples taken from Suspected case of REV and REV was conformed (Fig.5.)

Reticuloendotheliosis was diagnosed in commercial Layer chicken of 74 weeks age. Clinically birds showed 4% egg production loss, weak and emaciated with 6.2 percent mortality over a period of 23 weeks. Liver sections revealed diffuse severe RE cell infiltration in liver parenchyma. Multifocal moderate RE cell infiltration in renal parenchyma were noticed. The histopathological changes of liver and kidney observed in this study agreed with the findings of earlier workers (Ponnusamy *et al.*, 2018) [7]. The PCR test was highly useful for detection of REV virus (Gopal *et al.* 2012; Ahmed *et al.*, 2018) [5, 1]

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