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Histopathological and molecular diagnosis of lymphoid leucosis in commercial layer chicken

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Abstract
Fifteen dead commercial layer birds of 45 weeks age were examined to know the cause of death. The total flock size was 25,000 and birds were raised in cages from day one of age. The total mortality over a period of 15 weeks was 15%. The birds appeared weak and emaciated. At necropsy, liver, spleen, kidney revealed enlargement, mottling, greyish spots/foci with tumour nodules. The tumours were firm in consistency and smooth when cut. Histopathology of liver and kidney were corroborated the gross lesions. Lymphoid leucosis (LL) was confirmed by conducting polymerase chain reaction (PCR).

Keywords: Layer chicken, lymphoid leucosis, PCR, histopathology

Introduction
Avian leucosis viruses (ALVs) are placed in the alpha retrovirus genus of the family Retroviridae and divided into six subgroups based on viral envelope antigens. Subgroups A, B, C, D and J are classified as exogenous viruses and E is endogenous. ALV A-D can induce B cell lymphomas and ALV-J causes myeloid leucosis. ALV is a lymphoproliferative disease of chickens affecting primarily the bursa of Fabricius transforming the B-type lymphocytes (Sathish *et al.*, 2015) [5]. It produces a monomorphic lymphoid cell infiltrations or tumours in visceral organs.
The ALVs cause erythroid, lymphoid and myeloid leucosis and a variety of other tumours like fibrosarcomas, haemangiomas, nephroblastomas and osteopetrosis. Of the neoplasms included in the leucosis/sarcoma group, lymphoid leucosis (LL) was until recently the commonest form, occurring mainly in the layer type birds. Losses from these disease are of significant economic importance. The present paper describes the histopathological and molecular diagnosis of lymphoid leucosis in commercial layer chicken raised entirely in cages.

Materials and Methods
Fifteen dead commercial layer birds of 45 weeks of age were examined to know the cause of death with a case history of conjunctivitis, off feed, pale comb and 4% loss in production with 15% mortality over a period of 15 weeks. The total flock size was 25000 and all the birds were raised in cages from day one of age. The persistent mortality (0.60% per week) was recorded from 31 to 35 weeks of age and it increased at the age of 36 weeks from 0.60 % to 1.20% per week. The total mortality over a period of 15 weeks (31 to 45 weeks) was 15%.
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. Proviral DNA extraction from the suspected liver samples was done by DNeasy blood and tissue kit (M/s Qiagen, Germany) and the extraction procedure was followed as per the manufacturer's instruction. Proviral DNA for *pol-env* glycoprotein (*gp* 85) gene of ALV-A (LL) (Amplicon size 1154 bp) was obtained and stored at -20˚C until for further analysis. Then, polymerase chain reaction was carried out by using previously reported primer set for LL as shown in Table 1.

Table 1: Primer used for detection of ALV- A (LL)

Virus	Primer	Sequence	Gene and Size	Reference
ALV-A (LL)	Forward Reverse	F 5'- CCG GAG TGG CTC GCG AGA TGG-3' R 5 -GCC TATCCG CTG TCACCACTG-3'	*Pol-env* *gp85* 1154 bp	Barathidasan (2014)

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 proviral 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) [2]. 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 weak and emaciated musculature At necropsy, liver revealed diffuse enlargement with greyish white soft smooth glistening nodules of 1 to 5 mm diameter on the surface (Fig.1). Kidney showed enlargement, congestion or mottling with indistinct greyish white foci (1-3 mm) on the surface (Fig.2). The gross changes of liver and kidney observed in this study agreed with the findings of earlier workers (Soujanya *et al*., 2019 and Ravikumar *et al*.,2019) [6, 3].

Histopathology

Histopathology study of Lymphoid leucosis (LL) suspected liver showed diffuse severe monomorphic lymphoid cell (MLC) infiltration in hepatic parenchyma (Fig.3). In kidney multifocal severe MLC infiltrating the renal parenchyma were noticed (Fig .4).

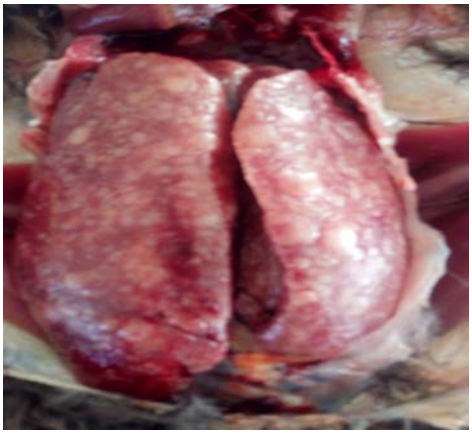


Fig 1: Chicken- LL Liver showing diffuse enlargement with greyish white soft smooth glistening nodules of 1 to 5 mm diameter on the surface

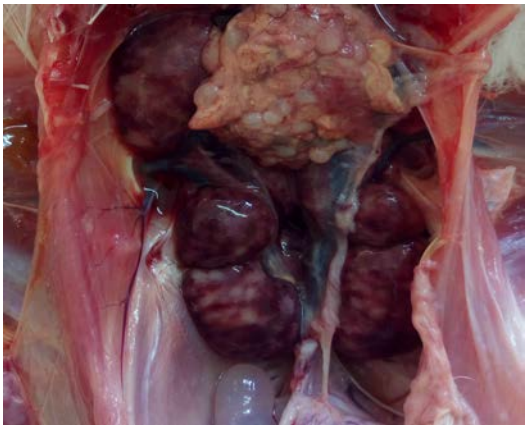


Fig 2: Chicken- LL – Kidney showing enlargement, congestion or mottling with indistinct greyish white foci (1-3 mm) on the surface

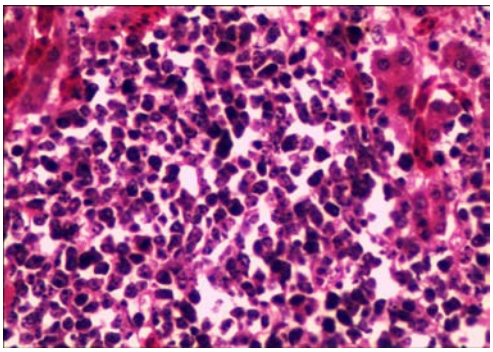


Fig 3: Chicken- LL – Liver - Diffuse severe MLC infiltration in hepatic parenchyma- 400X

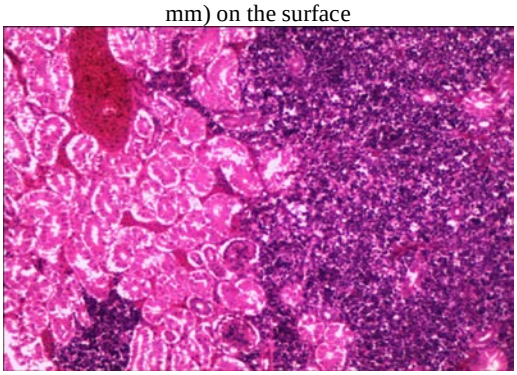
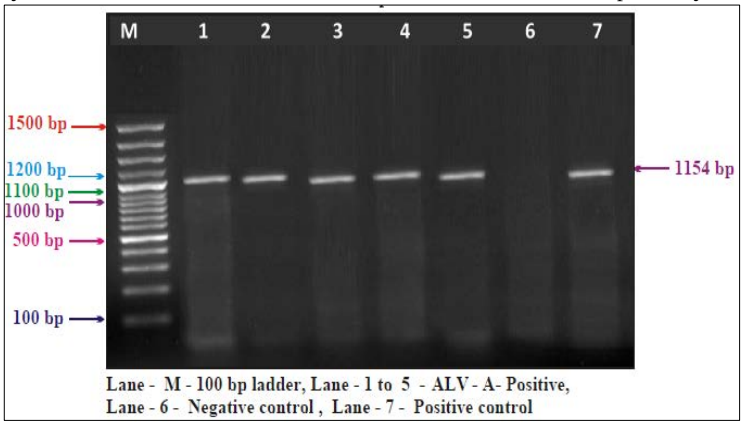


Fig 4: Chicken - LL - Kidney - Multifocal severe MLC infiltration in renal parenchyma -100X



Lane - M - 100 bp ladder, Lane - 1 to 5 - ALV - A- Positive,
Lane - 6 - Negative control , Lane - 7 - Positive control

PCR

PCR was conducted at Department of Veterinary Microbiology, Veterinary College and Research Institute, Orathanadu- 614 625 for 5 field samples taken from suspected cases LL and LL was confirmed (Fig 5). Lymphoid leucosis (LL) was diagnosed in commercial layer chicken of 45 weeks age. Clinically birds showed 4% production loss, weak, debilitated with 15 percent mortality over a period of 15 weeks. Histopathologically liver showed diffuse severe monomorphic lymphoid cell (MLC) infiltration in hepatic parenchyma. In kidney multifocal severe MLC infiltrating the renal parenchyma were noticed. The histopathological changes of liver and kidney observed in this study agreed with the findings of earlier workers (Sagarika *et al.*, 2017 and Soujanya *et al.*,2019) [4, 6]. The PCR test was highly useful for detection of LL virus (Barathidasan, 2014) [1].

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