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Detection of Lymphoid Leukosis virus infected chicken by testing for group specific antigen (P27) in ELISA

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Abstract

A poultry farmer in Jeelakarragudem, of West Godavari District reported death of adult Rajasri birds of 16-18 weeks of age on every alternative day since 7 days @ 1-2 birds per day without any noticeable clinical signs except for anorexia, swollen abdomen. When examined by necropsy, the liver was greatly enlarged, pale with multiple, variable sized greyish-white, soft, smooth, raised, glistening tumours on the surface of the liver, occupying the entire abdominal cavity based on which it was diagnosed as Lymphoid Leukosis. So the cloacal samples of remaining birds were collected to check for infection and checked for p27 antigen by ELISA in commercially available ELISA kit and found the incidence rate @ 54.55%.

Keywords: Lymphoid Leukosis, Rajasri birds, ELISA

Introduction

Lymphoid Leukosis (LL) is the tumour producing viral disease of chicken caused by Alpharetrovirus^[3]. The Lymphoid Leukosis is the most common manifestation of the avian leukosis/sarcoma group of viruses, produces a variety of neoplastic diseases, including erythroblastosis, myelocytomatosis, myeloblastosis and others. Not all infected birds will develop tumours. Infection can occur horizontally from bird to bird by direct or indirect contact, or vertically from an infected hen to her eggs as virus is shed into the albumin of the egg. In addition, vertical transmission may occur from virus incorporated in the DNA of a germ cell. Viremia in the hen is strongly associated with the transmission of virus congenitally^[3]. ALV are classified into five subgroups (A, B, C, D and J) based on their host range, viral envelope interference and cross-neutralization patterns. ALV subgroups A and B are more commonly associated with lymphoid leucosis^[7]. Enzyme immunoassays like Enzyme Linked Immuno-Sorbent Assay (ELISA) have proven efficacious in the detection of both leukosis antibody and antigen^[8]. Commonly p27, an antigen common to all subgroups of Avian Leukosis Virus (ALV), including endogenous viruses is detected by ELISA.

The present study aims for the detection of Lymphoid leukosis in chicken by ELISA for group specific p27 viral antigen.

Materials and Methods

Diagnostic analysis of lymphoid leukosis by Necropsy examination

Necropsy examination was performed on the dead birds for diagnosis of the disease based on gross lesions.

Cloacal swabs collection and preparation

A total of 44 birds' cloacal samples were collected from the poultry farm in the diluent provided for ELISA and later stored at -20 °C, until subsequent use and further analysis.

Determination of antibodies against Lymphoid Leukosis viral antigen (p27)

The cloacal samples were used to determine the prevalence of Lymphoid Leukosis by using a commercially available ELISA kit (IDEXX Antigen kit, USA), according to the manufacturer's instructions. The CUT OFF value was calculated based on the optical density (OD) values according to the following formula: CUT OFF= the average OD 450 of negative controls + 0.15. To ensure validity, the average OD 450 of negative controls was ≤ 0.10 ; and the average OD 450 value of positive was ≥ 1.00 . The results were interpreted as negative if the OD 450 value of sample was $<$ CUT OFF and considered positive if the OD 450 value of

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sample was \geq CUT OFF. Generally, the CUT-OFF values for positive titre in ELISA are ≥ 396 .

Results and Discussion

Necropsy examination

The visual examination revealed depressed and poor body condition of chickens with weakness, retarded growth, dehydration, emaciation, enlarged abdomen, pale violet comb and wattle (Fig.1). The gross lesions revealed the enlarged, pale liver and variable sized greyish-white, soft, smooth, raised, glistening, diffused nodular tumour lesions (0.5 cm diameter) with perihepatitis (Fig.2). Cut surface of the nodule has creamy whitish areas of necrosis. The other organs like heart and spleen also showed similar lesions (Fig.3). These lesions are similar to the lesions described by previous authors [1, 4-6, 10]. Differential diagnosis of lymphoid leukosis from Marek's disease based on gross lesions is little difficult and in this case there is no enlargement of peripheral nerves which is pathognomonic in Marek's disease thus making diagnosis easy.



Fig 3: Nodular tumour like growths on the heart of affected chicken with Lymphoid Leukosis.

Elisa for p27

Out of the 44 samples screened for Lymphoid leukosis, only 24 samples were found positive for p27 antigen (Fig.4). The prevalence% of the Lymphoid leukosis is 54.55% in the poultry farm. These results are in accordance with the results of many other authors [2, 9].



Fig 1: Emaciated carcass of a bird died due to Lymphoid Leukosis.



Fig 2: Diffusely enlarged liver with nodular tumour like growths on surface of liver.

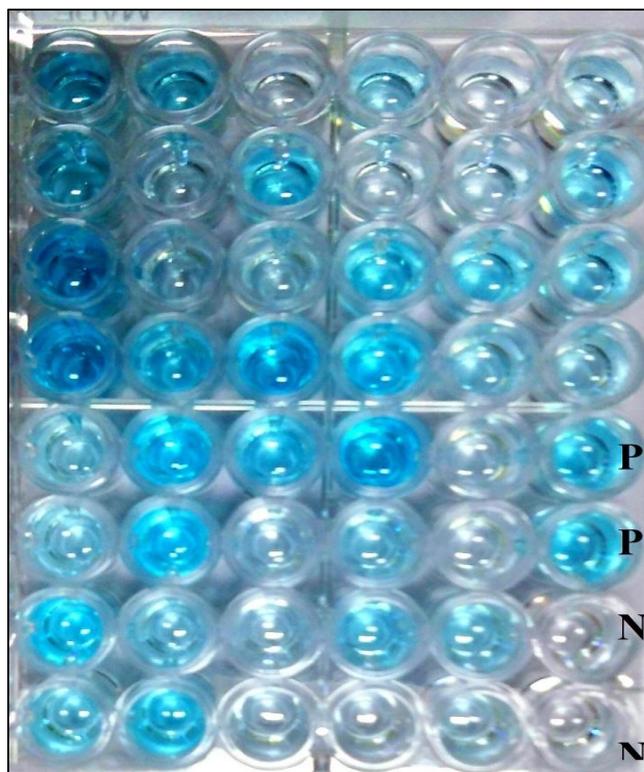


Fig 4: Elisa plate showing blue coloured wells indicating positive for Lymphoid Leukosis and colourless wells indicate negative. P indicates positive control well; N indicates negative control wells.

Conclusion

Based on the study it can be concluded that, the Rajasri birds seems to be more prone to lymphoid leukosis and hence appropriate eradication programmes should be followed to control and prevent the spread of infection in the farm.

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