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**Sneha Sagarika**

Department of Veterinary  
Pathology, College of Veterinary  
Science & Animal Husbandry,  
OUAT, Bhubaneswar, Orissa,  
India

**Dehiprasanna Das**

Department of Veterinary  
Pathology, College of Veterinary  
Science & Animal Husbandry,  
OUAT, Bhubaneswar, Orissa,  
India

**Susen Kumar Panda**

Department of Veterinary  
Pathology, College of Veterinary  
Science & Animal Husbandry,  
OUAT, Bhubaneswar, Orissa,  
India

**Supriya Das**

Department of Veterinary  
Pathology, College of Veterinary  
Science & Animal Husbandry,  
OUAT, Bhubaneswar, Orissa,  
India

**Basanti Jena**

Department of ARGO, College of  
Veterinary Science & Animal  
Husbandry, OUAT,  
Bhubaneswar, Orissa, India

**Rajesh Kumar Sahu**

Department of Veterinary Public  
Health & Epidemiology, College  
of Veterinary Science,  
Rajendranagar, Hyderabad,  
Telangana, India

**Correspondence****Sneha Sagarika**

Department of Veterinary  
Pathology, College of Veterinary  
Science & Animal Husbandry,  
OUAT, Bhubaneswar, Orissa,  
India

## Avian leukosis in chickens: A Clinico-pathological survey

**Sneha Sagarika, Debiprasanna Das, Susen Kumar Panda, Supriya Das, Basanti Jena and Rajesh Kumar Sahu**

### Abstract

A clinico-pathological survey of avian leukosis in naturally infected chickens was carried out in the present study organized poultry farms basing on clinical signs, gross, cytological and histopathological lesions in suspected tumour cases. Clinical signs of the affected chickens included emaciation, anorexia, decreased breeding potential and egg production. Necropsy revealed variable sized grey to yellow tumour like lesions on visceral organs such as liver, spleen, lungs, heart, kidney and ovaries. The lesions were more pronounced in liver characterised by nodular, miliary and diffuse type tumorous growths. Cytologically, impression smears revealed uniform sized lymphoblastic cells with thin rim of finely granular cytoplasm and a clear vesicular nucleus. Histopathologically, sections revealed extensive proliferation and infiltration of homogenous lymphoblasts cells in various organs with degree of degenerative changes. Lesions suggestive of avian leukosis were all observed in layer, mostly in Aseel breed. In one meat type bird, there was tumorous growth in the leg region.

**Keywords:** Avian leukosis, chickens, cytology, tumours, liver, histopathology, lesions

### 1. Introduction

At the beginning of 20th century, the flourishing poultry industry gave birth to a new oncogenic viral pathogen of Retroviridae family, avian leukosis virus (ALV), the major cause of mortality and huge economic loss to the developed poultry industry even until today [22]. Avian leukosis, commonly known as avian leukosis complex (ALC) is a variety of neoplastic disease conditions in chickens such as lymphoid leukosis, myeloblastosis, erythroblastosis, osteopetrosis, myxosarcomas, fibrosarcomas etc. Among these conditions, lymphoid leukosis is of the greatest incidence [11]. Avian leukosis is placed on List C of the Food and Agriculture Organization *Animal Health Yearbook 1995*, designating diseases of socio-economic and/or public health importance [15]. The world-wide spread of the newly emergent subgroup J strain of ALV, and associated mortality has generated serious concern and warrants the inclusion of avian leukosis amongst diseases significant to international trade. They were the first neoplastic diseases in any species to be shown, 100 years ago. Avian leukosis/sarcoma viruses (AL/SV) infect large segments of modern commercial poultry industry that widely expose humans on a consistent basis [20]. ALVs induce a variety of tumours, reduce productivity and induce immune suppression and other production problems in affected flock [7]. ALV infection has been regarded as one of the important causes of economic losses in the poultry industry and therefore it is essential to establish ALV free flocks. The epidemiological, pathological and molecular studies indicate that subgroup J Avian leukosis virus (ALV-J) infections are widely spread in “yellow chickens” of local breeds in China [18]. In a histopathological survey, it was reported that lymphoid leukosis was mostly observed in layer birds over 13 weeks of age and not in any of the tissues from broiler birds [1]. Earlier report revealed about 20-25% incidence of ALV induced tumours in poultry in and around Hyderabad [19]. They reported that gross examination of tumours was found to be an aid in diagnosing the type of tumours in poultry and histopathology is the best and reliable method for diagnosis of the type of tumours [19]. Researcher suggested that classical differential diagnosis of avian oncogenic viruses is based on virus isolation and histopathological examination of tumour tissues [6].

Therefore, the present study was carried out to diagnose avian leukosis in naturally infected chickens in organized poultry farms on the basis of clinico-pathological findings such as clinical signs, gross lesions, cytological and histopathological observations in suspected tumour cases in field conditions.

## 2. Materials and Methods

The birds of various age groups presented to the Department of Veterinary Pathology for necropsy examination, samples collected from various organized Government and private poultry farms in and around Bhubaneswar suspected for avian leukosis during the period from December 2014 to June 2015 were included in this study. The information pertaining to the study such as detailed history of clinical signs, mortality, age and type (meat/egg) of chickens were recorded. All the dead birds were necropsied systematically as per the standard procedure. All the organs were examined thoroughly for any tumourous growths and were recorded with reference to location, size, shape, colour, consistency etc. 169 representative tissue samples from tumourous lesions of 52 suspected cases were collected to carry out cytological and histopathological examinations. The collected organ impression smears were prepared as per the standard procedure. Then the impression smears were stained with Leishman's or Giemsa stain for cytological examination and interpretation as per standard procedure. The representative tissue samples were collected and preserved in 10% formal saline solution for histopathological studies. The formalin fixed tissues were processed by routine histological techniques. Paraffinised tissue sections were prepared with 4-5 µm thickness and stained by routine Haematoxylin and Eosin method. Stained slides were then mounted with cover slip by DPX mountant. Then slides were examined under Olympus microscope for histopathological examination and interpretation.

## 3. Results and Discussion

### 3.1 Clinical Signs

Detailed history regarding the clinical signs of some live birds suspected for avian leukosis and dead birds submitted for necropsy were collected and recorded. They had decreased breeding potential, reduced growth rates, depression, inactivity, anorexia, decreased egg production, eggs of reduced size and quality and mortality causing severe economic losses. The birds were thin and dehydrated with pale and anaemic combs and wattle. These findings are in agreement with the findings of earlier studies [3, 12].

### 3.2 Gross Lesions

A total of 52 cases suspected for avian leukosis were examined thoroughly at necropsy for the presence of gross lesions in different organs. Thirty four cases were suspected for Avian leukosis depending upon gross lesions of livers (28), spleen (16), hearts (10), kidneys (24), proventriculi (8), ovaries (3), lungs (2), trachea (1), duodenum (2) and leg muscle (1) out of total 169 no of tissue samples.

Variable sized grey to yellow obvious tumour-like nodular lesions were present in various visceral organs such as liver, spleen, kidney, heart, ovary, lungs, trachea, proventriculus, duodenum (Fig-1 to 5). The tumours were soft, smooth and glistening and the cut surface was greyish to creamy white with areas of necrosis. The lesions were found to be more pronounced in liver accompanied by nodular, miliary and diffuse pattern of tumourous growths. Spleens were grossly enlarged with miliary grayish white nodules on them or with diffuse leukosis. Heart showed distinct multiple nodular tumour masses without any gross enlargement. The tumour masses were soft and white in colour involving both epicardium and myocardium. Kidneys were greatly enlarged and pale with poorly demarcated big whitish nodules. Proventriculi were enlarged with single or multiple white

nodules on them. Ovaries were enlarged with greyish white nodules and had lobulated tumour masses having cauliflower like appearance. Affected lungs were congested with multiple lymphoid nodular tumour masses. Trachea and duodenum were slightly thickened with areas of leukosis. In only single case of meat type bird, there was a severe tumourous growth in the left leg region (Fig-6).

Thirty four (65.38%) suspected cases grossly revealed enlargement of visceral organs and presence of multiple nodular tumours on them. These findings are in agreement with the earlier reports [17, 12, 9, 19]. The nodular, miliary and diffuse forms were coinciding with the previous report [17]. Tumourous growths which was present in the leg muscle in one case was also reported earlier [19].

### 3.3 Cytology

The cytological examination of impression smears taken from tumourous growths of affected tissues revealed lymphoid cells with thin rim of finely granular cytoplasm and a clear vesicular nucleus. Marked degree of uniformity in size and appearance of neoplastic cells was observed (Fig-7). Based on cytological evaluation of impression smears, 38.46% of the cases had lesions suggestive of Avian leukosis which was also supported as per earlier reports [17, 19].

### 3.4 Histopathological Lesions

A total of 169 tissue samples from 52 suspected cases were subjected to histopathological examination. Fifteen cases were confirmed for Avian leukosis based upon histopathological lesions of livers (12), spleen (10), hearts (6), kidneys (11), proventriculi (4), ovaries (2), lungs (1) and trachea (1), duodenum (1) and leg muscle (1) out of total 169 no of tissue samples.

### 3.5 Liver

The histological sections of liver revealed diffuse and focal infiltration of uniform sized lymphoid cells and lymphoblasts predominantly resulting atrophy of hepatocytes due to compression and replacement. Individualisation of hepatocytes with variable degree of degenerative changes and necrosis was observed (Fig-8, 9). These features were confirmation for lymphoid leukosis [1, 9, 19]. In nodular type of lymphomas, the nodules were surrounded by a band of fibroblast like cells and consisted of aggregates of uniform large lymphoid cells or lymphoblasts of early developmental stages. These findings were in coincidence with the previous observation [10, 19]. Multifocal eosinophilic myeloid cells along with lymphoblasts and erythroblast proliferation and necrosis and degeneration were observed in some sections. These findings were in the same line with previous reports [3].

### 3.6 Spleen

Depletion of lymphocytes in germinal centres and clear nodule formation by neoplastic lymphoid cells in spleen were observed and similar type of lesions were reported [16], who described that depletion of lymphocytes in the thymus, bursa and spleen is an essential feature in the ALV infected birds (Fig-10).

### 3.7 Heart & Proventriculus

Diffuse infiltration of neoplastic lymphoid cells among the myofibrils of Heart and in the submucosa of proventriculi with moderate fibrous tissue proliferation were observed (Fig-11). This study was also reported earlier stated that heart and proventriculus were the sites of predilection for metastasis and diffuse infiltration of neoplastic lymphoid cells [14].

### 3.8 Ovary

Ovary sections were characterized by invasion of normal tissue by homogenous population of lymphoblasts. These lesions were according to the findings of earlier researcher [19]. Different stages of cavernous haemangiomas were recorded in ovary sections (Fig-12).

### 3.9 Kidney

Heavy infiltration of uniform lymphoid cells in lungs and kidney was observed (Fig-13) which was in agreement with statement that, metastases of lymphoid tumours in lungs and kidney [14].

### 3.10 Leg

The growth collected from leg region revealed large uniform round lymphoid cell infiltration and aggregation with thin cytoplasm and clear vesicular nucleus along with myolysis, degeneration and necrosis (Fig-14). Same things were earlier reported [5, 8].



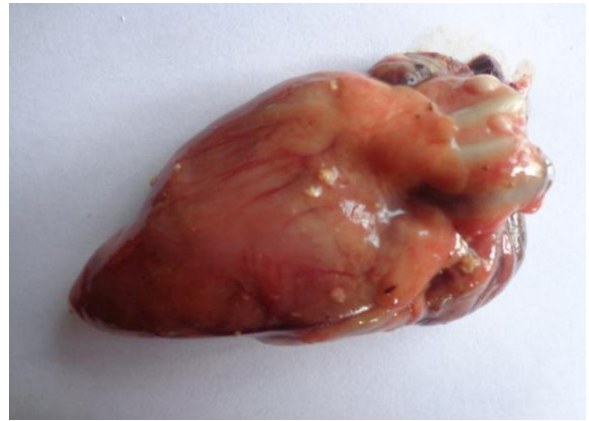
**Fig 1:** Liver with diffuse multiple white nodular growth.



**Fig 2:** Enlarged spleen with grayish white nodules.



**Fig 3:** Enlarged kidney with grayish white nodules.



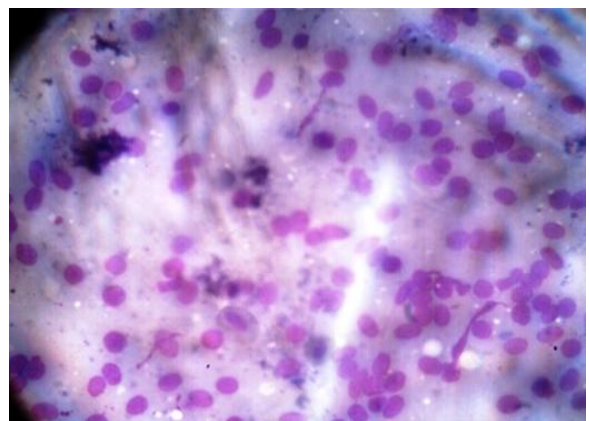
**Fig 4:** Enlarged heart with grayish white nodular growth.



**Fig 5:** Grayish white ovaries with cauliflower like appearance; appearance.

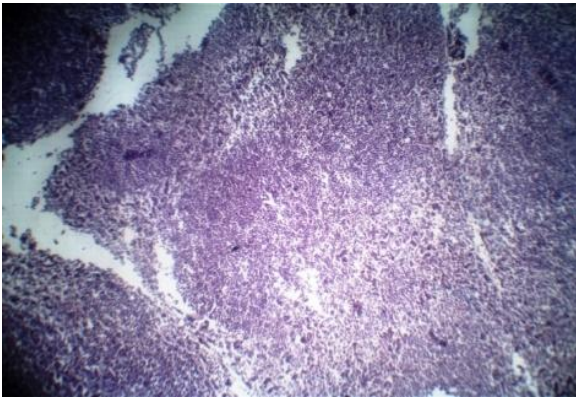


**Fig 6:** Nodular growth at leg region.

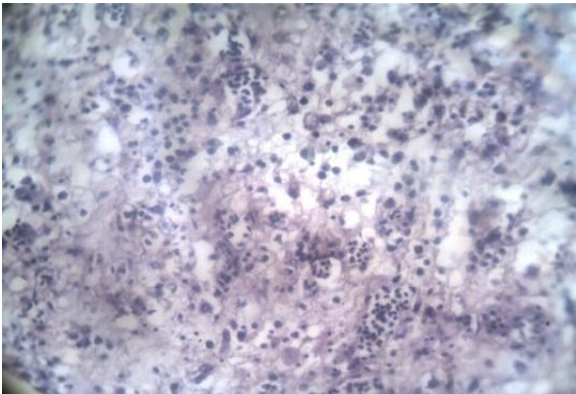


**Fig 7:** Cytological smear showing uniform neoplastic cells Geimsa stain  $\times 100$ .

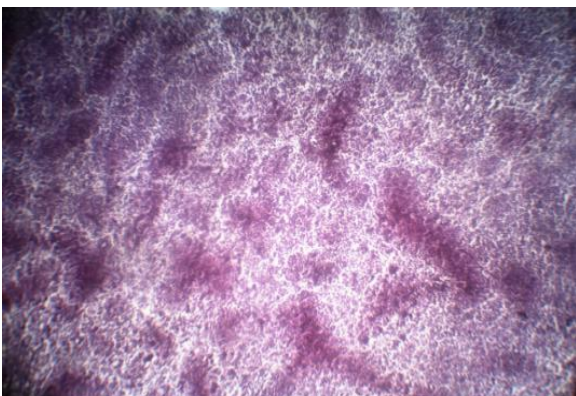




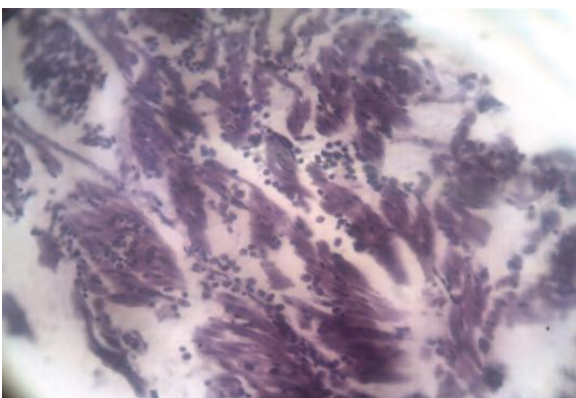
**Fig 8:** Individuation of hepatocytes with diffuse proliferation of lymphoblasts in liver H&E×10.



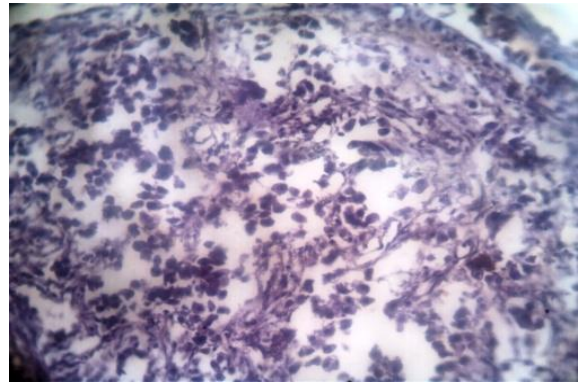
**Fig 9:** Hepatocytes degeneration, necrosis and infiltration of plasma cells, lymphoblasts in liver H&E×40.



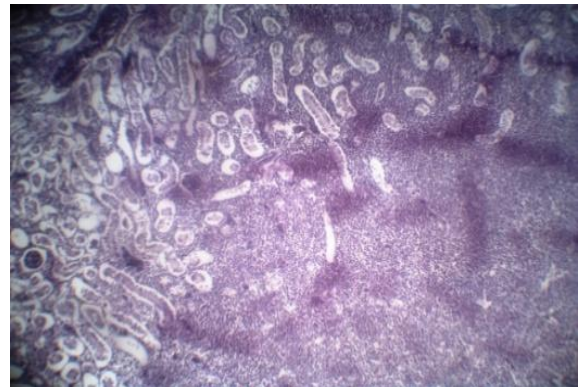
**Fig 10:** Spleen infiltrated with uniform sized lymphoblasts H&E×10.



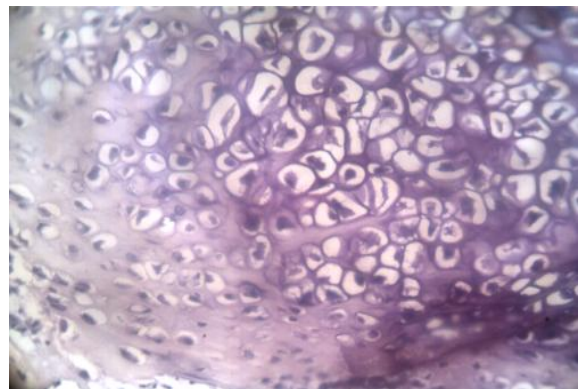
**Fig 11:** Myocardial oedema with scanty infiltration of uniform lymphoblasts H&E×40.



**Fig 12:** Ovarian parenchyma infiltrated with uniform sized lymphoblasts H&E×40.



**Fig 13:** Uniform lymphoblasts infiltration in kidney H&E×10.



**Fig14:** Osteofibroma. H&E×40.

#### 4. Conclusion

From the present study, it can be concluded that despite implementation of several eradication programmes and development of breeding lines of chickens resistant to avian leukosis by several breeding companies have been an enormous success, but were not suitable for eradication of ALV infection on a commercial scale. It is still prevalent in many organized poultry farms. The incidence is highest in female egg type birds of age group 18- 30weeks. Affected birds had decreased breeding potential, reduced growth rates, in-appetence, depression and inactivity. Necropsy examination along with cytological evaluation of tumourous lesions in various organs of suspected neoplastic cases was found to be an aid in diagnosing avian leukosis which can give necessary preliminary information in the field condition. Histopathological examination is still found to be one of the best and reliable methods for diagnosis and differential diagnosis of avian leukosis from other neoplastic diseases in poultry.

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