CAIRO UNIVERSITY
FACULTY OF VET. MEDICINE
DEPARTMENT OF PATHOLOGY

IMMUNOLOGICAL AND PATHOLOGICAL STUDIES IN DIAGNOSIS OF VIRUSES INDUCED TUMORS (AVIAN LEUKOSIS) IN CHICKENS

Thesis presented by

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Approval Sheet

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Abstract

This study was carried out on 245 as total number of chickens of 10 flocks from different provinces. The examined cases were classified into 47 commercial layers and 198 broiler parents to study the gross and microscopical lesions of different tumors induced by avian leukosis ALV&ALV-J in broiler breeders and layers flocks. Serological tests were conducted on 245 serum samples to detect the antibodies of avian leukosis virus ALV&ALV-J. Polymerase chain reaction PCR was carried out on tissue samples to confirm the presence of DNA of ALV subgroup J. The histopathological results revealed that 4 flocks showed lymphoid leukosis tumors (2 layers and 2 broiler parents) and the results of ELISA were positive for LL. The other 6 flocks (6 broiler parents) showed myeloid leukosis induced by the novel subgroup-J of ALV and the results of ELISA were positive for ML The result of PCR test indicated positive amplification of 545 bp fragment with the extracted DNA of ALV-J for 4 flocks showed myeloid tumor lesion (4 broiler parent). This work concluded that The histopathological examination plays a crucial and decisive role in diagnosis among different types of neoplasms caused by avian leukosis virus that differentiate between myeloid &lymphoid leukosis and the application of PCR test verified the presence of DNA of ALV-J that induced the tumors of myeloid leukosis.

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List of abbreviations

ALSV = Avian leukosis Sarcoma Viruses.
ALV-J = Avian leukosis Virus Subgroup J.
ALV(A,B) = Avian leukosis Virus Subgroup A,B.
AEV = Avian Erythroblastosis Virus.
AMV = Avian Myeloblastosis Virus.
Ab = Antibodies.
Ag = Antigen.
BP = Broiler parents.
C- onc = Cellular oncogene.
CEF = Chicken embryo fibroblasts.
CF = Complement fixation test.
ELISA = Enzyme linked immunosorbant assay.
Env = Envelope.
GSA = Group specific antigen.
HPRS-103= A strain induce myeloid leukosis.
LL = Lymphoid leukosis.
L/S = Leukosis /sarcoma.
LTR = Long terminal repeat.
MDV = Marek's disease virus.
ML = Myeloid leukosis, Myelocytomatosis.
Onc = oncogene.
PCR = Polymerase chain reaction.
S/P = Sample positive.
V- onc = Viral oncogene.
VNT = Viral neutralization test.

Introduction

Introduction

Avian leukosis viruses (ALVs) are the most common naturally occurring avian retroviruses associated with neoplastic diseases and mortality of infected chickens. These viruses considered as one of the devastating causes of economical losses in poultry production. These losses are represented in loss of egg production in commercial broiler breeder flocks, and body weight suppression in meat type chickens, as well as severe economic losses due to increase mortality rate **(Gavora *et al.*, 1980).**

Lymphoid leukosis is the most common naturally occurring B- cell lymphoma caused by ALV **(Payne and Fadly, 1997).** A novel subgroup of ALV has been isolated from meat type chickens in United Kingdom designated subgroup (J) which cause myeloid leukosis or myelocytomatosis, and renal tumors **(Payne *et al.*, 1991)**. Several isolates of avian leukosis virus subgroup J have been isolated in USA from broiler breeder by **(Fadly and Smith, 1999).**

Myeloid leukosis has been emerged as a serious cause of mortality and decrease in egg production to 30% in broiler breeder flocks in Egypt and data indicated that the incidence of ALV-J was common among foreign and native breeds in Egypt **(Mona, 2000).**

A new problem appeared on surface affecting the commercial layers flocks that suffered from severe decrease in egg production and increase mortalities. Also, a characteristic morphologies of myeloid tumors was observed.

Aim of work

This work was designed to investigate the incidence, the pathological changes and serological status of avian leukosis virus in broilers and layers chicken flocks to realize the proper diagnosis.

The objectives of the present study:

1) Description of gross and microscopic lesions of different tumors of avian leukosis.

2) Using special stains in order to confirm and classify the tumors findings.

3) Serological examination of serum samples using ELISA to detect ALV antibodies in examined cases.

4) Application of Polymerase chain reaction (PCR) on tissue samples to detect DNA of Avian leukosis virus subgroup(J).

Review of Literature

Definition and classification:

Matthews (1982) mentioned that the avian retroviruses considered as a subgenus of avian type C oncoviruses.

Coffin (1992) mentioned that the viruses of the leukosis /sarcoma group were termed avian leukosis-related viruses (ALVs), which had the same physical and molecular characteristics and sharing common group specific antigen.

Regenmortel *et al.* (2000) reported that the leukosis/sarcoma (L/S) group of diseases were designated a variety of transmissible benign and malignant neoplasms of chickens caused by members that belong to the family Retroviridae.

History and Incidence:

Furth (1933) reported that the three types of tumors caused by filterable agents and designated them as lymphomatosis, myelomatosis, and endothelioma of chickens.

Jungherr (1941) stated that avian leukosis virus was previously described as visceral lymphomatosis.

Biggs (1961) reported that the visceral lymphmatosis was termed to lymphoid leukosis (LL).

Mladenov *et al.* (1967) stated that the kidney tumors were accompanied with several strains of leukosis such as group ES4 strain of erythroblastosis, MH2 strain of reticuloendothelioma and MC29 as myelocytomatosis.

Purchase and Cheville (1975) reported that the incidence of lymphoid leukosis (LL) in chickens may be reduced by the wide spread of infectious bursal disease virus because the bursa of fabricious considered as the main source of metastasis of neoplastic lymphoblasts.

Smith (1987) reported that the viruses of subgroup E avian leukosis consisted mainly of endogenous leukosis viruses of low oncogencity.

Payne *et al.* (1991) recorded that the novel subgroup of exogenous ALV, designated subgroup J (ALV-J) found in broiler breeders flocks in United Kingdom. The host range pattern of ALV-J differs from those viruses of subgroups A to G and I which provided support for placing it in a new envelope subgroup designated J.

Fadly and Smith (1999) recorded that the strains of avian leukosis virus subgroup J (ALV-J) were obtained from broiler breeder flocks in United States that exhibited myeloid leukosis.

Mona (2000) recorded that the incidence of ALV-J from broiler parent flocks in Egypt are described as myeloid leukosis ML.

Etiology

Virus subgroups:

Weiss *et al.* (1982) recognized that the avian leukosis/ sarcoma L/S viruses that affecting chickens have been divided into five subgroups (A, B, C, D, and E), according to their host range in chicken embryo fibroblasts of different genetic types. Viral envelope antigens were identified by virus and serum neutralization tests.

Coffin (1992) reported that the Leukosis/Sarcoma (L/S) group viruses have been placed in a genus termed ALV-related viruses of the family Retroviridae. This family characterized by possession of reverse transcriptase enzyme which was necessary for DNA provirus formation during viral replication.

Arshad *et al.* (1997) noted that the HPRS-103 strain induced myelocytic myeloid leukosis (ML), which had a tropism for the cells of the myeloid lineage rather than the

lymphoid lineage. This strain of ALV was designated in a new subgroup nominated as subgroup J.

Payne and Fadly (1997) reported that the avian leukosis viruses caused a variety of neoplasms with short to long latencies. They stated that the strains such as avian myeloblastosis virus (AMV), avian erythroblastosis virus (AEV) and the sarcoma virus carried specific viral oncogenes that cause rapid neoplastic transformation within a few days or weeks.

They reported that there were laboratory strains of avian leukosis /sarcoma ALVs, which lack the viral envelope *(env)* gene and their subgroup was that of the helper leukosis viruses used for their propagation

Viral Nucleic Acids:

Kung and Maihle (1987) found that the viral and cellular versions of *onc* genes are distinguished by the pre-fixes v- and c- genes respectively. The specific v-*onc* genes, with c-*onc* genes were present in acutely transforming viruses, as *erbA, erbB* in avian erythroblastosis virus (AEV), *myb* in avian myeloblastosis virus (AMV), *myc* in avian myelocytomatosis virus.

Coffin (1992) described the sequence of the structural genes of ALV consisted of *gag/pro –pol – env*. These genes encoded, respectively, the proteins of the virion group-specific

(gs) antigens and protease, RNA-dependent DNA polymerase (reverse transcriptase, or RT), and envelope glycoproteins.

The structural genes are flanked by terminal genomic sequences with gene promoter and enhancer activities, termed as long terminal repeat (LTR) regions.

Virus Replication:

Weiss *et al.* (1985) mentioned that the replication of ALV was characterized by the formation of a DNA provirus that integrated into the host cell genome. Subsequently, the proviral genes were transcribed into viral RNAs, which were translated to produce precursor and mature proteins that constitute the virion.

Strain Classification:

Beard (1980) reported that the numerous strains of ALVs were isolated from naturally occurring or experimentally induced neoplasms over many years. Many induced a predominant type of neoplasm such as lymphoid leukosis virus (LLV), avian erythroblastosis virus (AEV), avian myeloblastosis virus (AMV), and avian sarcoma virus (ASV).

The oncogenic spectrum tends to be characteristic of a particular virus strain, but often overlaps with other strains. Thus, the (RPL12) strain of ALV induces lymphoid leukosis, erythroblastosis, osteopetrosis, hemangiomas, and sarcomas; the

(BAI-A) strain of AMV induces myeloblastosis, lymphoid leukosis, osteopetrosis, hemangioma, and epithelioma.

Weiss *et al.* (1982) reported that the avian leukosis virus ALV of chickens divided into five envelope subgroups, A, B, C, D, and E on the basis of differences in their viral envelope glycoprotein, which determine the antigenicity.

Payne *et al.* (1991) reported that the strain HPRS-103 of avian leukosis virus characterized by new *env* gene. They reported that the strain HPRS-103 induced myeloid leukosis myelocytomatosis. This strain of ALV was designated in a new subgroup nominated as subgroup J.

Venugopal *et.al.* (1998) determined that the production of neutralizing antibodies or neutralization by known subgroup-specific antibodies can also be used for strain classification. Viruses within the same subgroup usually had cross-neutralize to varying extents. However, antiserum against particular isolates of subgroup J virus had no cross reactivity with other J isolates.

Pathogenesis and Epizootology:

Natural and Experimental Hosts:

Payne (1987) reported that the chickens were considered as the natural hosts for all viruses of L/S group. These viruses had not been isolated from other avian species except pheasants,

partridges, and quail. Experimentally some members of the L/S group of avian retroviruses had a wide host range and can be adapted to grow in unusual hosts.

Payne *et al.* (1992) found that the broad host range pattern of (HPRS-103 strain) differed from those of viruses of subgroups A to G and I and provided a support for placing the strain HPRS-103 of ALV-J in a new envelope subgroup, designated as subgroup J.

Payne and Fadly (1997) noted that Rous sarcoma virus RSVs had the widest host range in chickens, pheasants, guinea fowl, ducks, pigeons, Japanese quail, turkeys and rock partridges.

Transmission:

Spencer *et al.* (1980) found that infection of the cock did not influence congenital infection rate of the progeny, and the cock acted as virus carrier and source of infection to other birds.

Payne (1981) found that the exogenous ALV were transmitted both vertically and horizontally from bird to other by direct and indirect contact they reported 4 serological classes of birds in relation to ALV infection: ‹1› no viremia; no antibody (V-A-), ‹2› no viremia; with antibody (V-A+), ‹3› with viremia; with antibody (V+A+) and ‹4› with viremia; no

antibody (V+A-). The group (V-A+) included genetically susceptible birds in an infected flock mostly. The group (V+A-) transmitted ALV to high proportion of their progeny.

Payne and Bumstead (1982) found that the congenitally infected chickens were associated with virus shedding into egg albumin. They found that not all eggs had ALV in albumin give rise to infected chicks but only 1/2-1/8 of embryos were infected and however small minority of vertical infected chicks was important to maintain the infection from generation to the next.

Crittenden and Smith (1984) reported that the detection of ALV or group specific antigen in albumin or cloacal swabs was highly correlated with congenital transmission of ALV.

Fadly (1989) found that the group specific antigen GSA was stable in egg albumin for at least 63 days at 8°c. He found that the test for group specific antigen GSA was the most sensitive mean of identifying hens that transmitted exogenous ALV congenitally.

Fadly and Smith (1991) observed that the maternal antibodies against ALV delayed the ALV infection and reduced viremia and cloacal shedding of virus in progeny.

Tsukamoto *et al.* (1991) reported that the shedding of virus into egg albumin and its transmission to the embryo was consequence of virus production by albumin secreting glands of the oviduct.

Venugopal (1999) reviewed that the birds exposed to the virus at a younger age of a few weeks tend to develop tolerant viremia (V+A–S+).

On the other hand exposure of older age bird to ALV enhanced the immune status of the birds which became shedder or not shedder the virus. The outcome of post-hatch ALV-J infection by horizontal transmission differed from that of other ALV subgroups. Infection of egg-type brown Leghorn birds post-hatched leading to immune non-shedders (S-).

However, similar infections of meat-type birds produced either tolerant viraemic infection (V+A–S+) similar to congenitally infected birds or an immune shedder with a transient viraemia (V–A+S+).

Pathology

Lymphoid Leukosis

Neiman *et al.* (1980) mentioned that the infection of lymphoid leukosis virus resulted in changes in the bursal lymphoid follicles could be observed microscopically at 2 weeks age. By 7 weeks of age abnormal follicles originated from transformation of limited number of bursa cells .The transformed lymphoblastic cells proliferated and the affected follicles became engorged with uniform blast like cells with loss of distinction between cortex and medulla.

Crittenden and Kung (1984) mentioned that the transformed bursa cells of chickens infected with lymphoid leukosis were metastasized to the liver, spleen and other visceral organs through the vascular route. Metastasic tumors in the viscera usually had the same DNA fragments as bursal tumors from the same bird.

The tumors enlarged and cells burst into the vascular system and initiated metastatic foci in the visceral organs. The neoplastic transformation occurred due to the expression of the viral promoter v-gene that activated the host c-gene in B-cells resulted in neoplastic transformation. Also, it resulted in interference with normal intraclonal switch of B cell immunoglobulin production from IgM to IgG, thus, lymphoid leukosis tumor cells have IgM on their surface and not IgG.

Baba and Humphries (1985) mentioned that the infection of lymphoid leukosis virus persisted long time in bursal lymphocytes rather than in hemopoitic tissues. They found that, those B- cells of bursa of fabricious were the target cells that neoplastically transformed.

Purchase (1987) stated that fully developed lymphoid leukosis LL occurred in chickens of about 4 months of age and older. Grossly visible tumors almost involved the liver, spleen, and bursa of fabricius. Other organs often grossly involved include kidney, lung, gonad, heart, bone marrow, and mesentery. Tumors were soft, smooth, and glistening; a cut surface appeared to be grayish to creamy white and seldom has areas of necrosis. Tumor growth could be nodular, miliary, diffuse, or a combination of these forms. In the nodular form, the lymphoid tumors varied from 0.5-5 cm in diameter and might occur singly or in large numbers.

They were usually spherical but may be flattened when they were close to the surface of an organ. The miliary form, which was most obvious in the liver, consisted of numerous small nodules less than 2 mm in diameter uniformly distributed throughout the parenchyma. In the diffuse form, the organ was uniformly enlarged, slightly grayish in color, and usually very friable.

They reported in the microscopic picture that most of tumours appeared to have risen from focal nodule and often the pattern was one of coalescing foci and the parenchyma of the organ was displaced and compressed by rapidly expanding foci of lymphoid cells. The tumours composed almost exclusively of large lymphoid cells (lymphoblasts) aggregations that may vary slightly in size but were all at the same early developmental stage. They had a poorly defined cytoplasmic membrane, much basophilic cytoplasm, and a vesicular nucleus in which there were margination and clumping of the chromatin and one or more conspicuous acidophilic nucleoli. The cytoplasm of most tumour cells contains a large amount of RNA. At times there may also be a number of smaller non-neoplastic lymphocytes present which appeared to have infiltrated the tumour mass.

Bacon et al. (1989) & Fadly and Witter (1993) they observed that serotype 2 Marek's disease virus MDV enhanced the development of lymphoid leukosis in certain lines of chickens following exposure to ALV after hatching.

Mona,(1994) reviewed that the serotype 2 Marek's disease virus (MDV) had an influence on development of avian leukosis virus (ALV) and reticuloendotheliosis virus (REV) induced viremia, antibodies, and lymphomas in chickens.

She concluded that serotype 2 MDV had no effect on the developed of REV & ALV-induced viremia and antibodies but,

it enhanced the development of bursal lymphomas whether it induced by ALV or REV.

Nasser (1994) described that the pathological lesion of positive lyphomatosed chickens were varied among the three viral induced lymphoid tumor diseases (Marek's disease, lymphoid leukosis and bursal form of reticuloendotheliosis).

He found that lymphoid leukosis infected chickens revealed diffuse enlargement of liver and spleen with white mottled foci. Microscopically, the involved organs were liver, kidneys, spleen, heart, lungs, and proventriculus. The characteristic features were multicentric in origin by focal distribution of uniform cells population of large lymphoblasts surrounded by plasma cells.

Masaaki *et al.* (2004) recorded an outbreak of subcutaneous tumors associated with subgroup-A avian leukosis sarcoma virus in young layer chickens in a flock in Japan was investigated. Tumors appeared as extensive swelling in the head or wing of chickens. Two types of tumor were observed myxoma containing hyaluronic acid and neurofibroma with hyperplasia of Herbst corpuscles. Diagnosis based on detection of serum antibodies against ALV , tumors were stained by immunohistochemistry using monoclonal antibodies against subgroup A and positive reaction to primers specific for ALV subgroup A.

Myeloid Leukosis ML (Myelocytomatosis):

Mladenov *et al.* (1967) found that myelocytomas were induced by intravenous injection of strain MC29 avian leukosis virus into young chickens.

Myelocytomas was obtained after 3-11 weeks and the tumors composed of compact masses of uniform myelocytes resemble the normal myelocytes of bone marrow. The neoblastic cells characterized by large nuclei, vesicular, and usually eccentrically located with distinct nucleolus the cytoplasm was packed with acidophilic granules.

Imprint preparations of fresh tumours were stained with May-Grünwald-Giemsa, the cytoplasmic granules appeared brilliant red.

Beard (1980) described that the microscopic pattern of myeloid leukosis in liver and he stated that myelocytes crowded the sinuses, invaded the hepatic cords resulted in destroy and replacement of hepatocytes and as a principal feature; the neoplastic myelocytes formed a cohesive, organized and invasive growth in parenchymatous organs.

Payne *et al.* (1991) discovered a new member of leukosis /sarcoma group of avian reteroviruses which was called HPRS-103 strain. They recorded that the host range in several species

and tests on viral interference and neutralization resulted in HPRS-103 strain was being classified in a new enveloped subgroup of exogenous avian leukosis virus, designated J. The virus was isolated from meat-type chicken lines. The virus, of which HPRS-103 strain was the prototype, caused myeloid leukosis.

Payne *et al.* (1992) and Bai *et al.* (1995) found that the HPRS-103 strain of ALV induced myelocytomatosis in meat type chickens had taken a long latent period (about 20 weeks) due to their lackness of viral oncogene.

Payne *et al.* (1993) reported that the renal tumors were associated with the HPRS-103 strain of ALV subgroup J. They reported that the observed renal tumors were well differentiated adenoma, cystadenoma or undifferentiated tumor as nephroblastoma which was described as undifferentiated tumor of tubular epithelial cells and undifferentiated stromal cells.

Payne and Fadly (1997) reported that the virus induced myelocytomatosis had a long incubation period but shorter than lymphoid leukosis LL. They stated also that the clinical signs were similar to those of myeloblastosis and there was a skeletal growth of myelocytes which may result in abnormal protuberances of the head, thorax and shanks. They stated that

myelocytomas occurred on the surface of bones in the periosteum and near the cartilage, also in any tissue and organs of the body.

The neoplastic masses appeared dull, yellow white, soft and friable, and diffuse or nodular and often developed in flat bones of the skull, costochondral junctions of the ribs and inner sternum. They noted that tumor masses consisted of myelocytes which are similar to the normal myelocytes in the bone marrow; their nuclei were large and eccentrically located with a distinct nucleolus.

Arshad *et al.* (1997) mentioned that tissue tropism of HPRS-103 strain of subgroup J avian leukosis virus was examined using immunohistochemical technique to detect the expression of viral group specific antigen (Gag) in various tissues.

They concluded that HPRS-103 showed a lower propensity to replicate in the medullary region of the lymphoid follicles of the bursa of fabricius more than RAV-1 strain of subgroup- A avian leukosis virus.

This low bursal tropism may be a factor in why HPRS-103 did not induce lymphoid leukosis. This strain of subgroup-J replicated in blood monocytes cultures from chickens indicating a tropism for the myelomonocytic cell linage.

Payne (1998) described that the striking features of high incidence of myelocytic myeloid leukosis (myelocytomatosis) was characterized by moderate to great enlargement of liver organ in 88% of cases and by gross skeletal myelocytomas affecting the inner sternum , ribs, vertebrae and synsacrum.

Gross myeloid leukosis tumor involvement also occurred in some cases in spleen, thymus, gonads and kidneys.

The tumor of the liver characterized microscopically by extensive accumulation of immature granulated myelocytes around the portal areas and in the parenchyma. Skeletal muscles tumors consisted of solid masses of myelocytes, focal and diffuse cell tumor infiltration were present.

Renal tumors were well differentiated adenoma, cystadenoma or nephroblastoma. Myeloid leukosis in the kidney characterized microscopically by involvement of mature granualated myelocytes, but in some cases showed immature promyelocytes.

The morphology of the tumor cells can be verified in sections or smears stained with Romanowsky or May-Grünwald- Giemsa stains. The tumors composed of solid masses of uniformly differentiated mature myelocytes, whose cytoplasm was filled with acidophilic, usually round granules.

Ahmed *et al.* (1999) reported that the diagnosis of avian leukosis subgroup (J) ALV-J infection in imported broiler parent chickens based on gross pathological lesions and antibody detection to ALV-J by ELISA.

El-Gohary *et al.* (2000) recorded that the ALV-J in seven commercial brown layer farms of one breed located in two governorates. The diagnosis was based on gross and histopathological examination as well as positive ALV-J antibody detection by ELISA.

Massi *et al.* (2000) recorded that the liver lesions in broilers with ALV-J infection were mainly characterized by bile duct proliferation and diffuse heterophilic and myelocytic infiltration.

Mona, (2000) isolated the avian leukosis virus subgroup–J from imported and native breeds of broiler breeder flocks in Egypt. Diagnosis of myeloid leukosis in affected flocks was based on gross and microscopic examination of the affected tissues which were noted in liver, spleen, kidney, ovary, ribs and keel bone.

Microscopic examination of H&E stained sections revealed the presence of immature myelocytes with characteristic eosinophilic cytoplasmic granules.

Nakamura *et al.* (2000) investigated that the granulated myelocytes associated with myeloid leukosis appeared to be proliferated in the bone marrow and in the periosteum of the sternum, ribs, vertebrae, and synsacrum. The study indicated that the myelocytes could invade through the compact bones to the periosteum and ossified cartilage of trachea and larynx.

They noticed other tumors such as histiocytic sarcoma, granulosa cell tumors, pancreatic adenocarcinoma, and fibroma. Bone marrow was heavily infiltrated, pale in colour and leukemia was present. The renal tumors were well differentiated adenomas or cystadenomas.

Gingerich *et al.* (2002) recorded ALV-J in white leghorn egg layer flocks being used to produce fertile eggs for human vaccine production exhibited dramatically in low egg production and high number of non- laying birds after the onset of sexual maturity. Gross lesions of freshly dead birds necropsied revealed lacking ovarian activity approximately 60% and had lesions of bacterial bursitis or synovitis, whereas the other 40% had tumors of the viscera but not of the bursa of fabricius.

Microscopical examination of tumors revealed tissues showed typical lesions of myelocytomatosis. They suggested that hatching of day-old egg type chicks with ALV-J infected meat-type chicks in a common hatchery had contributed to cross infection.

Sultan *et al.* (2003) reported the incidence of ALV-J infection in native breeder female chickens. These chickens were crossed with infected commercial foreign boiler males.

Binrui Xu *et al.* (2004) detected avian leukosis virus subgroup-J in commercial brown egg layers in China. Diagnosis based on observations of gross lesions, histopathology, and PCR tests. The affected birds showed yellowish/ white tumors which were observed on the visceral surface of the sternum in nodular formations. The surface of some tumors had a layer of thin and brittle periosteum.

Livers were slightly swollen with mottled greyish/ white pinpoint spots under the capsule. Spleens were enlarged either slightly or may reach to several times of normal and had yellowish/ white tumor nodules in a few cases. Kidneys were markedly swollen, and some had light greyish/ white mottled tumor masses. Ovaries and oviducts of hens were undeveloped at 170 days of age. The testes of one cock were poorly developed at 70 days of age and the size was only one-eighth of the normal at the same age. A few proventriculi were swollen. The proventricular papillae were flat in 25/50 cases. No gross lesions were observed in the heart, lung, pancreas, or cerebrum.

Microscopically, 45/50 birds had ML. Tumors in bone were composed of uniform ML cells similar in appearance to normal myeloid cells. The nuclei often lay to one side of the cell

and had a marked nucleolus. The cytoplasm was filled with conspicuous spherical eosinophilic granules. Many proliferated focal tumor cells destroyed the normal structure of the bone marrow. Hepatocytes were atrophied and thin.

Tumor cells were present around veins and arteries in the liver and grew focally. In the spleen, lymphocytes decreased and tumor cells were widely present in both the red pulp and the white pulp. Tumour cells in the lungs were observed in the pneumocapillares and para-bronchial lobules. Epithelial cells of renal tubules were swollen, degenerated and separated from the basement membrane. Tumour cells in the interstitium grew focally. Tumour cells were present in the mucous membrane of the proventriculus. There were tumor cells in the lamina propria of the intestinal mucosa. Pancreatic glands were degenerated and necrotic. Many tumor cells gathered around blood vessels. Proliferated tumor cells grew focally in the ovary and oviduct. Few myeloid tumors were observed in the epicardium, and no tumor cells were seen in the testes, sciatic nerve, or skeletal muscles.

Sultan *et al.* (2004) reported that the ALV-J was investigated in two breeds of white commercial egg laying chickens raised in 7 farms suffering from low peaks in egg production, high number of culls and number of non- laying birds after sexual maturity.

Gross lesions showed non-functional ovaries and tumors in viscera and bones. Histopathological examination of tumor containing tissues and bones showed immature myelocytes proliferation with characteristic esinophilic cytoplasmic granules. They concluded that the data was interesting and need further molecular investigation and detection for antigenic variation among strains of ALV-J.

Laboratory diagnosis:

Enzyme-linked Immuno Sorbent Assay (ELISA):

Smith *et al.* (1979) recommended that ELISA test for ALV detection that it was a rapid and convenient alternative to the complement fixation CF test for identifying infected chickens in eradication programs.

Fadly *et al.* (1981) compared between ELISA and complement fixation test in detection of ALV group specific antigen GSA. They found that ELISA test had the advantage of being more sensitive and easily applied to test materials such as meconium, cloacal swabs and blood which were not suitable for complement fixation assay.

Crittenden *et al.* (1984) found that ELISA test was suitable for detection ALV group specific antigen GSA that could not be detected by complement fixation test. They found that ELISA was more sensitive 10 folds than CF test.

Tsukamoto *et al.* (1985) noted that ELISA test was more sensitive than virus neutralisation test (VNT), and considered as simple, rapid, and applicable for large scale field surveys of ALV infection.

Lee *et al.* (1986) found that monoclonal antibodies developed against p27 protein can be used in ELISA to differentiate between endogenous and exogenous avian leukosis virus infections.

Mizuno and Itohara (1986) recommended ELISA test for detection of ALV antibodies. They stated that the examination of the antibodies response from ALV-inoculated chickens revealed that detected antibodies at the same time or several weeks earlier than did virus neutralisation test (VNT).

Smith *et al.* (1986) reported that the ELISA was considered as an important and main diagnostic method for the detection of antibodies against avian leukosis/sarcoma L/S viruses.

Todd *et al.* (1993) investigated that the development of ELISA for detecting antibody specific to big liver and spleen disease was found the agreement between the results obtained by ELISA and agar gel diffusion test. Testing programs allow

more than 90% samples to be tested per ELISA plate were faster and less expensive to conduct than agar gel diffusion test and that it was well suited flock testing programs.

Venugopal *et al.* (1997) noted that ELISA is useful for the specific identification of ALV-J isolates. They detected ALV-J specific antibodies in the serum by using an antibody ELISA on plates coated with ALV-J infected chick embryo fibroblasts lysates or gp85 envelope glycoprotein of HPRS-103 produced in *Spodoptera frugipedra* cells infected with a recombinant baculovirus carrying the HPRS-103 gp85 gene.

Fuchs *et al.* (2000) developed an enzyme linked immunosorbent assay for the detection of antibody to the gp85 envelope protein of ALV-J. The test was of value for large-scale screening programs for presence of ALV-J infection.

Detection of Viral Nucleic Acid:

Weiss *et al.* (1982) used blot-hybridization analysis of viral DNA or RNA in cell extracts for detection of avian tumor viruses.

Van Woensel *et al.* (1992) used polymerase chain reaction (PCR) for ALV subgroup A to detect proviral DNA and viral RNA in various tissues from ALV infected chickens. That test

used primers selected from the second and the third variable region of gp 85 *env* gene and was specific for the members of subgroup A avian leukosis virus.

Persing *et al.* (1993) stated that the polymerase chain reaction (PCR) in addition to its multitude of uses in molecular biology was a valuable tool in the rapid and accurate diagnosis of many infectious agents.

Bai *et al.* (1995) mentioned that the (*env*) gene sequence of avian leukosis virus subgroup J was distinct from those of other subgroups. But, it showed high homology to a new subfamily of endogenous reterovirus designated EAV-HP.

Davidson *et al.* (1995) reported that the PCR was an efficient test for differentiation between MD, RE and LL in tumor bearing fowl and an effective method for differential diagnosis of tumor-bearing and immunodeficient birds.

Hauptli *et al.* (1997) used the reverse transcription RT-PCR for detection of vaccine contamination by ALV. They suggested that this system provides a rapid and specific in vitro method for the detection of ALV-RNA as a contaminant and may be applied for quality control of avian vaccines.

Smith *et al.* (1998) developed and applied that PCR tests for detection of subgroup- J ALV. They depended on that the *env* gene sequence of ALV subgroup J was distinct than other subgroups thus by using a primer (H5) designed from the 3`(U3) end of *pol* gene and amplification of endogenous elements could be avoided by using a down steam primer derived from a HPRS-103 *env* sequence.

They applied two PCR tests, the first used primer pair H5/H2 amplified a 764 bp region of all isolates but, only 5 from 12 isolates could be detected. In order to detect all ALV subgroup J virus isolates, a new PCR test was designed using a new primer H7derived from the *env* gene region. The new PCR test using H5/H7 primer pair gave 545 bp fragment on the original ALV- J isolates and the 12 antigenic variants. Also, they noted that the PCR was rapid, specific and more sensitive than the conventional diagnostic tests for ALV detection.

Pham *et al.* (1999) detected that ALV in albumen of chicken eggs using RT-PCR. They concluded that the combined use of RT-PCR and direct sequencing of the RT-PCR product provides a new approach for identifying ALV infected poultry.

Arshad *et al.* (1999) used techniques such as immunohistochemistry and in situ hybridization, capable of

detecting ALV-J antigens and nucleic acids respectively in infected tissues or tumors.

Mona Aly (2000) used PCR to test tumor DNA of affected chickens for the presence of sequences specific for ALV-J with primers specific for ALV –J as described by Smith *et al.*, 1998. DNA extracted from tumor of 4 chickens represented 4 myeloid leukosis affected flocks.

Also, she made PCR test to DNA extracted from CEF inoculated with strain ADOL- Hc 1 (ALV-J) and DNA extracted from CEF inoculated with subgroup- A ALV using primers specific to ALV-J. The result of the first two products were positive, in contrast DNA from CEF uninoculated and inoculated with subgroup-A ALV was negative for specific ALV – J.

Binrui Xu *et al.* (2004) used PCR to detect tumor DNA of affected commercial layers using primers specific for ALV - J as described by Smith *et al.*, 1998. They extracted the DNA from the affected organs showing the typical gross tumor lesions. The new PCR test using H5/H7 primer pair gave 545 bp fragment.

Material and Methods

The present work was carried out on 245 examined chickens from 10 chicken flocks from different governorates during 2003-2004. The number of samples was classified into 198 broiler parents and 47 commercial layers (Table 1). These samples were examined in the national lab for quality control of poultry production in the animal health research institute.

1. Material:

1.1. Samples:

1.1.1. Blood Samples:

A total of 245 blood samples from 2 commercial layers and 8 broiler parents flocks were examined (Table 2). Blood samples were collected and left for agglutination over night and then centrifuged at 3000 rpm /10min. to separate the serum for detection of antibodies of ALV and ALV- J by ELISA.

1.1.2. Tissue Samples:

Tissue specimens were collected from the examined chickens which divided into two parts.

1st. **First** part of tissue samples fixed in neutral buffered formalin 10% for histopathological examination.

2nd. Second part of tissue samples were collected from different organs showed tumor lesions including liver, spleen, kidneys, heart and gonads and from different sources (Table 3) were frozen at – 70°C until used for polymerase chain reaction PCR.

Table (1) showing the number of examined birds and their geographical distribution

Flock no.	Governorate	Type of flock	Age of bird	No. of examined birds
1	Giza	Broiler parent	32 weeks	23
2	Ismalia	Broiler parent	38 weeks	25
3	Behera	Broiler parent	28 weeks	20
4	Behera	Broiler parent	26 weeks	20
5	Kaliobia	Layers	36 weeks	22
6	Giza	Layers	35 weeks	25
7	Behera	Broiler parent	35 weeks	25
8	Behera	Broiler parent	30 weeks	30
9	Behera	Broiler parent	40 weeks	30
10	Dakahlia	Broiler parent	32 weeks	25

Table (2) Showing the number of blood samples collected for Serological test:

Type of chickens	No. of flocks	No. of samples
Layers	2	47
Broiler parents	8	198
Total	10	245

Table (3) Showing the number of examined flocks for DNA detection of ALV-J using PCR test:

Type of chickens	No. of examined flocks
Layers	2*
Broiler parents	4**
Total	6

* This no. represented flocks no. 5, 6.

** This no. represented flocks no. 1, 2, 9, 10.

1.2. Tests applied in the study:

1.2.1. Histopathological examination:

Tissues from liver, kidneys, heart, skeletal muscle, ovaries, bursa of fabricius, spleen, and intestine were taken from examined chickens and fixed in 10% neutral buffered formalin. The fixed specimens were then trimmed, washed, dehydrated in ascending grades of alcohols, cleared in xylene, embedded in paraffin, sectioned at 4-6μ thickness and stained with haematoxylin & eosin **Bancroft & Cook (1993).**

Moreover, special stains were used:

- Methyl green Pyronin and May – Grünwald - Gimsa

Bancroft & Cook (1993)

1.2.2. Serological examination for detection of ALV(A,B) and ALV-J antibodies in serum samples used ELISA test:

This test was applied on 245 serum samples from 10 flocks as shown in table (2).

1.2.3. Material and Equipment for ELISA:

1- Micro titer pipettes (2-20ul, and 50-200ul).
2- Multichannel pipette.
3- 1ml, 5ml, 10ml glass pipettes.
4- Graduated cylinders (50ml).
5- Laboratory grade distilled water.
6- Disposable plastic tips.
7- ELISA washer.
8- ELISA reader (96 well plate)with 650nm filter (Model SLT Sepectra)

1.2.4. ELISA kits used for detection of ALV(A,B) and ALV-J antibodies:

1.2.4. A) *A commercial ELISA kit for detection of antibodies of ALV* was purchased from IDEXX Laboratories (IDEXX Laboratories, Inc., Maine, USA).

1. ALV p27 coated plates.
2. Positive control: chicken anti-ALV in buffer with protein stabilizers preserved in sodium azide.
3. Negative control: chicken serum non- reactive to ALV in buffer with protein stabilizers preserved in sodium azide.
4. Anti-chicken horseradish peroxidase (HRPO) conjugate in tris buffer
5- Sample diluent: buffer with protein stabilizers preserved in sodium azide.
6- TMP diluent: citrate phosphate buffer contain hydrogen peroxide.
7- TMP concentrate.
8- Stop solution: 0.12% hydrofluoric acid (HF).

1.2.4. B) *A commercial ELISA kit for detection of antibodies of ALV-J* was purchased from IDEXX Laboratories (IDEXX Laboratories. Inc. Maine, USA)

1. ALV –Jgp85 coated plates.
2. Positive control: chicken anti-ALV-J in buffer with protein stabilizers preserved in sodium azide.
3. Negative control: chicken serum non- reactive to ALV-J in buffer with protein stabilizers preserved in sodium azide.
4. Anti-chicken horseradish peroxidase (HRPO) conjugate in tris buffer with protein stabilizers preserved in gentamicin.
5- Sample diluent: buffer with protein stabilizers preserved in sodium azide.
6- TMP diluent: citrate phosphate buffer containing hydrogen peroxide.
7- TMP concentrate.
8- Stop solution: 0.12% hydrofluoric acid (HF).
9- Phosphate buffer saline-tween(10x)wash concentrate.

1.3. Polymerase chain reaction test for ALV-J DNA detection:

This test was applied on tissue samples from (6) flocks of layers and broiler parents.

1.3. Material used for PCR test:

1.3.1. Digestion buffer:

1.3.1. Preparation of the digestion buffer:

(0.5 M) EDTA (pH 8) ..... 5 ml
(5 M) sodium chloride .....4 ml
(1 M) Tris (pH 8) .....2 ml

1.3.2. Phenol-Chloroform-iso Amyl Alcohol solution:

22 g of phenol crystal dissolved in 3 ml of (1M) tris (pH 8) in the microwave then after cooling (v/v) of chloroform (25 ml) were added and then add 1 ml of iso amyl alcohol.

1.3.3. Proteinase K (sigma):

100 mg / ml.

1.3.4. Tris-borate-EDTA (TBE) buffer:

This (10 x) buffer was consisted of:
Tris base .....5.4 g
Boric acid ..... 2.75 g
(0.5 M) EDTA ..... 2.0 ml
Sterile distilled water .....100 ml
Diluted TBE (10 x) as working solution was prepared as follow

100 ml of TBE buffer + 900 ml of sterile distilled water

1.3.5. Sodium Dodecyl Sulphate (SDS) (10 %):

25g of sodium dodecyl sulphate (SDS) added to 225ml of distilled water and heated to 68°C and dissolve by steering, then adjust volume to 250 ml.

1.3.6. PCR Kit, primers and positive control:

Reddy Mix PCR - Master Mix PCR reagent Kit with amplifier Taq DNA polymerase. (PCR reagents are manufactured by AB gene laboratories, Surrey, UK) (Lot No. 0311/10).

Primers: the specific sequences of nucleotides for ALV-J used in the study forward & reverse were H5/H7 respectively as

described by **(Smith *et al.*, 1998).** The sequence of the primers were: H5 (GGATGAGGTGACTAAGAAAG)

H7 (CGAACCAAAGGTAACACACG).

Positive control: positive control of ALV-J DNA (AdolHc1) was kindly supplied **by Prof. Dr. Mona Aly** Deputy of Animal Health Research Institute. Dokki

1.3.7. Equipment for PCR test:

1. Thermocycler (Biometra).
2. Electrophoresis gel (Biometra).
3. UV transilluminator (Biometra).

2. Methods:

2.1. Serological methods used for ALV antibody detection:

Commercial ELISA test for detection of ALV& ALV-J antibodies in serum samples:

The procedure recommended by the manufacturers (IDEXX, laboratories) for ALV(A,B) & ALV-J antibodies detection was applied as follow: **(Venugopal *et al.*, 1997)**

Procedures:

- Serum samples were diluted five hundreds folds (1:500) with sample diluents prior being assayed by diluting 1 µl of sample with 500 µl of sample diluents.
- 100µl of negative control was dispensed into first 2 wells, and then 100ul of positive control was dispensed into 2 following wells.
- Then 100 µl of diluted serum samples dispensed in the appropriate wells of ALV antigen pre-coated plates.
- Incubate for 30 minutes at room temperature, then wash each well with distilled water 3-5 times.
- Add 100ul of anti chicken horseradish peroxidase conjugate into each well.
- Plates were incubated for 30 min. at room temperature then washed as before.
- 100ul of TMP substrate solution into each well then plates were incubated for 15 min. at room temperature.

- 100ul of stop solution was dispensed into each well to stop the reaction. Then read by ELISA reader at 650 nm.
- **Interpretation:** The results were calculated for validity and the difference between positive control mean and negative control mean.
  The relative level of ALV (A,B) antibodies can be determined by calculating the sample to positive ratio (S/P ratio) where samples more than or equal to 0.4 should be considered positive and indicate exposure to ALV (A,B).
- The relative level of ALV-J antibodies can be determined by calculating the sample to positive ratio (S/P ratio) where samples more than or equal to 0.6 should be considered positive and indicate exposure to ALV-J.

2.2. Method for detection of DNA of ALV- J:

2.2.1. DNA extraction from tissue: (Murray & Thompson 1980)

- Add 4 ml of digestion buffer to 1 gm of minced, ground tissue and transfer to polypropylene tube with cover.
- Add 40µl proteinase K.
- Add SDS (160µl) during fast shaking.
- Incubate at 37 °C for overnight.
- Add phenol -chloroform -iso amyl alcohol 22:25:1 with tris HCl v/v. Mix by inverting tube several times till becomes homogenate. Centrifuge at 5000 rpm / 15 min.

- The aqueous phase was removed and re-extracted with phenol chloroform iso-amyl alcohol. The DNA in the supernatant was precipitated with two volumes of cold absolute ethanol alcohol, the precipitated DNA was collected by glass hook and dissolved in 0.5 ml of sterile distilled water and kept in – 20°C tell use.
-The concentration of DNA was determined by measuring absorbency at 260 nm and template concentrations were adjusted to 50 µg /ml.

2.2.2. Polymerase chain reaction (PCR):

The PCR mixture consisted of the following in a total volume 50 ul, double distilled water (40ul), dNTP (2µl), 10X buffer (5µl) , Taq DNA polymerase (0.25µl) and template DNA (1µl). two primers forward & reverse (1ul for each) Put the tubes in the thermocycler and justify the program as described by **(Smith *et al.*, 1998).**

PCR program:

PCR program was consisting of the following steps: The amplification: denaturation at 93°C for 1min., annealing at 60°C for 1min. decreasing by 1°C in each cycle and extension at 72°C for 90 seconds to 13 cycles followed by 30 cycles of 93°C for 1 min., 48°C for 1 min., 72°C for 90 sec. with final extension at 72°C for 10 min. Reactions were conducted in **Thermocycler.**

2.2.3. Gel Electrophoresis of PCR production: (Smith *et al.* 1998)

Gel electrophoresis was prepared by 1.5 gm ultra pure agarose added to 100ml TBE then melted in hot air oven. Products were stained with ethidium bromide (0.5 ul per ml of gel). Molecular size markers represented multiples of 100 bp.
A 10 µl sample of each reaction mixture was added to 2 µl of gel loading buffer (0.25% bromphenol blue dye in 25% Ficol). PCR products were loaded in 1.5% agarose after electrophoresis in 1x tris-Borate-EDTA buffer (TBE) for 2-3h at 80 volts in biometra. The stained amplified products were observed under ultra violet transilluminator.

Results of Lymphoid Leukosis:

Flock one (1)

This farm showed gross pictures for lymphoid leukosis. This flock showed high mortalities and decreased in egg performance by 60% of commercial white egg layers, gross examination was carried out for 20 chickens showed lethargy and tumours on head in 3/20. The tumours usually appeared as solitary swelling or protrusions of the integument, firm but not hard and creamy white to dull red in color. The overlying skin was wounded in some cases resulting in ulceration. Characteristically, on gross examination bursa of fabricius showed tumors in bursal folds in 2/20. Liver and spleen showed enlargement in 6/20. Also, Kidneys were markedly swollen, and some had greyish white nodular tumour masses. Ovaries and oviducts of 10/20 hens were undeveloped at 36 weeks of age. Nerves were normal and showed no pathological lesions.

Flock two (2)

The examined farm contain commercial brown egg layers and showed increased number of mortalities, decreased in egg production and increased number of culls. Twenty five birds 35 weeks of age were examined and they suffered from tumors in head and neck in 4/25. This tumour appeared as protruded swelling behind the eye lid. The gross examination showed enlargement of liver and spleen with small multiple white

nodules in 10/25 cases. Ovaries and oviducts of 10/25 hens were atrophied at 245 days of age. Skeletal muscles were normal and had no pathological lesion. Sciatic nerves had normal striation and had no swelling.

Flock three (3)

A broiler parent farm suffered from decreased in egg production at 35 weeks of age and decreased body weights. The gross examination of 25 birds showed enlargement of visceral organs. Multiple nodules observed in liver and spleen in 7/25 of cases. These nodules were grayish white color, friable in consistency and of different sizes. 11/ 25 of examined cases were suffered from atrophied ovaries and oviducts that caused delayed production. Kidneys were not enlarged and had no macroscopic nodules. Heart and skeletal muscles, sternum, ribs, bursa of fabricius and nerves were grossly normal, had no nodular formation and no pathological lesion.

Flock four (4)

A broiler parent farm suffered from high number of mortalities, decreasing in the egg performance by 70% and decreasing of hatchability. 30 cases were examined and necropsied. Visceral organs were enlarged with the appearance of small multiple nodules. 5/30 showed liver and kidneys

enlargement with multiple nodules which were white to grey in color friable in consistency.

3 /30 0f examined cases had nodule in the small intestine and skeletal muscles showed multiple nodules. The gross pictures of bursa of fabricius were normal in most of the examined birds.

Lymphoid leukosis

Liver:

Macroscopic:

The liver of infected birds with lymphoid leukosis was enlarged. The hepatic lobules showed grayish white patches intermingled with subcapsular focal haemorrhagic areas. The hepatic tumors appeared soft, smooth, and glistening. On cut section tumours showed grayish to creamy white material. The neoplastic lesions were observed in three forms the most common type was the nodular form and some cases showed the miliary or diffuse form.

In the nodular form, lymphoid tumors scattered all over the hepatic lobules in different size and shape. These nodules appeared spherical in most of examined cases but flattened lesions were also observed (Fig.1).

In the diffuse form the liver was uniformly enlarged with slightly grayish color (Fig.2). The gall bladder was distended with large amount of bile secretion.

Microscopic:

The lymphoid tumours of examined liver classified histopathologicaly into three forms nodular, miliary, and diffuse. In the nodular form, the lymphoblastic cells displaced and compressed the hepatocytes. The nodular lesions were surrounded by a band of long spindle shaped fibroblasts like cells (Fig3). In the miliary lymphoid tumors hepatic lobules showed numerous number of small size foci of aggregated lymphoblastic cells (Fig.4). In the diffuse form massive lymphoblastic cells infiltrated the hepatic lobules and replaced the most of hepatic parenchyma. The hepatocytes appeared as solitary islets inside the massive infiltration of lymphoblastic cells (Fig.5). Hepatocytes showed degenerative changes characterized by swelling and granular cytoplasm. Apoptosis of hepatocytes were observed in the form of apoptotic eosinophilic bodies contained remnants of nuclear material (Fig.6). The neoplastic cell populations characterized by large lymphoblastic cells which were homogenous in size with poorly defined cytoplasmic membrane and basophilic cytoplasm. The nuclei appeared vesicular in which margination and clumping of chromatin with the appearance of one or more obvious acidophilic nucleoli. These neoplastic cells showed mitotic figures (Fig.7). The main bile duct showed sloughing of its epithelium lining and surrounded by dense lymphoblastic infiltration and small newly formed bile ductules (Fig.8).

Kidneys:

Macroscopic:

The kidneys of infected birds with lymphoid leukosis showed diffuse enlargements with grayish white nodular lesions of varied size and shape scattered all over the renal lobes . Subcapsular focal hemorrhagic areas were seen on cut section. The tumour mass appeared soft, friable in texture and creamy in color (Fig.9).

Microscopic:

The renal tubules showed degenerative changes and intralumenal proteinacious material which characterized by deep eosinophilic structureless mass filled the tubular lumen (Fig.10).

Kidney of infected birds showed diffuse infiltrations of large lymphoid cells in between the renal tubules. The renal tubules were separated from each other by massive infiltration of lymphoblastic cells and some of them showed proliferation and hyperplasia of its epithelial lining (Fig.11). Proliferative glomerulonephritis were seen in most of cases which characterized by proliferation of mesangial, epithelial and endothelial cells. The glomerular capillary tuft occupied most of Bowman's space.

The lymphoid population of cells revealed homogenecity in size and degree of developmental stage which were mostly lymphoblastic cells (Fig.12).

These cells characterized by large cytoplasmic nuclear ratio, vesicular nuclei and prominent eosinophilic nucleolus and showed mitotic division (Fig.13). Diffuse infiltrations of lymphoblastic cell around collecting duct were also noticed (Fig.14).

Ovaries:

Macroscopic:

The ovaries of infected bird with lymphoid leukosis showed atrophied ovarian follicles. Grossly, the ovary resembled rassberry fruit in shape and size.

Microscopic:

The ovaries of infected birds with lymphoid leukosis showed massive and diffuse infiltration of large lymphoid cells allover the ovarian tissues (Fig.15). The ovaries showed atrophied ovarian tissues and undeveloped ovarian follicles. Few ovarian follicles showed developmental stages and mature graffian follicles with the appearance of primary oocytes. The lymphoblastic cells surrounded the ovarian follicles (Fig.16).

These cells characterized by large vesicular nucleus and basophilic cytoplasm. The cells were homogenous in size and shape (Fig.17).

Spleen:

Macroscopic:

The spleen of infected birds with lymphoid leukosis was greatly enlarged up to two to three folds more than the uninfected birds. The spleen appeared pale and friable in consistency. The capsular surface revealed small, multiple, grayish white nodules (Fig.18).

Microscopic:

The spleen showed diffuse infiltration of large lymphoblastic cells in the splenic pulps especially the white pulp (Fig.19). These cells were large mononuclear cells, and characterized by large vesicular nucleus with basophilic cytoplasm in homogenous shape and size. These neoplastic cells showed mitotic figures (Fig.20). The splenic blood vessels were engorged and impacted by lymphoblastic cells (Fig.21).

Skeletal Muscles:

Macroscopic:

Skeletal muscles showed multiple grayish white nodules with pale anemic color.

Microscopic:

Skeletal muscles were diffusely infiltrated by large lymphoblastic cells between the muscle bundles. These muscle bundles showed coagulative necrosis (Zenker's necrosis) which were characterized by structurless deeply eosinophilic color (Fig.22), (Fig.23).

Bursa of Fabricius:

Macroscopic:

Bursa was greatly enlarged. On cut section the bursa of fabricius showed loss of its distinct bursal pelicae (bursal folds) with great thickening of its wall. Prominent grayish white small nodules appeared on the inner surface of the bursa of fabricius (Fig.24).

Microscopic:

The histopathological picture of the bursa of fabricius revealed great enlargement of bursal lymphoid follicles (Fig.25). There was loss of distinction between cortex and medulla. The affected bursal follicles became engorged with uniformly neoplastic lymphoblast like cells. These lymphoid follicles were surrounded by thickened fibrous connective tissue (Fig.26). The affected follicles showed expansion and displacement of the adjacent normal bursal follicles. These cells showed high pyroninophilic cytoplasm with methyl green pyronin stain (Fig.27), (Fig.28).

Intestine:

Macroscopic:

The intestine showed scattered nodules on the serosal surface. These nodules were grayish white, soft in consistency and covered by connective tissue capsule (Fig.29).

Microscopic:

The nodules were in the form of circumscribed elevations attached on the intestinal wall by fibrous connective tissue and surrounded by thin capsule of fibrous connective tissue. These nodules were packed with large lymphoid cells (Fig.30)

Subcutaneous Tumors:

Macroscopic:

Tumors appeared as swelling or in the form of bulbous protrusion of the integument and most commonly observed on head and neck. These tumors had different sizes, usually solitary, and firm but not hard. The overlying skin was wounded in some cases resulting in ulceration. The tumor characterized by single nodule, sharply demarkated from the surrounding tissue. On cut section oozed creamy white mucinous exudates (Fig.31, 32, 33, 34).

Microscopic:

The tumour was identified as myxoma consisted mainly of loose embryonal connective tissue with abundant mucinous matrix. The cells were stellate or spindle shaped cells which were predominated. Short collagen fibers were observed in its matrix. (Fig. 35, 36).

Results of Myeloid Leukosis

Flock one (1)

This farm showed typical gross pictures for myeloid leukosis. This flock showed high mortalities and decreased in egg performance by 30% of broiler breeder, gross examination was carried out for 23 chickens suffered from anemia and tumours of visceral organs and can be recognized on gross examination with some degree of certainty. Characteristically, Myelocytomas occurred on the surface of bones in 3/23 with the periosteum and near cartilage which was observed at the costochondral junctions of the ribs, on the inner sternum, and pelvis. The tumours were usually nodular and multiple, with soft, friable consistency and creamy colour. The myelocytomatous infiltration, caused enlargement of the liver and spleen in 11/23 and other organs. Kidneys were markedly swollen, and some had light greyish white mottled tumour masses; in addition, 3/23 showed small multiple nodules in the breast pectoral muscles. Ovaries and oviducts of 20/23 hens were undeveloped at 228 days (32 weeks) of age. Bursa of fabricius of all cases showed no enlargement or bursal transformation. Also, nerves were normal and showed no pathological lesions.

Flock two (2)

The examined farm contained broiler parent and showed increased number of mortalities, decreased egg production and increased number of culls. Twenty five birds 270 days of age were examined. They suffered from decreased egg performance and increase in the number of culls due to undeveloped ovaries and oviducts. The gross examination showed enlargement of liver with small multiple white nodules in 10/25 of cases.
Ovaries and oviducts of 15/25 hens were atrophied at 270 days (38 weeks) of age. Skeletal muscles were normal and had no pathological lesion. Sciatic nerves had normal striation and had no swelling.

Flock three (3)

A commercial broiler parent farm suffered from delayed production until the 28 weeks (196 days) of age and decrease of weights. The gross examination of 20 birds showed enlargement of visceral organs. Multiple nodules observed in liver and spleen in 7/20 of cases. These nodules were grayish white in color, friable in consistency and of different sizes. Ten of examined cases suffered from atrophied ovaries and oviducts that caused delayed production. Kidneys were not enlarged and had no macroscopic nodules. Heart and skeletal muscles, sternum, ribs, bursa of fabricius and nerves were grossly normal, had no nodular formation and no pathological lesion.

Flock four (4)

A farm of broiler breeder, at 26 weeks of age, suffered from severe decrease egg performance by 30%. Twenty cases were examined and clinically suffered from emaciation, cyanosis of comb and ruffled feathers. After necropsy, the examined cases revealed that 14/20 had atrophied ovary and oviduct. Five cases had enlargement of liver with multiple nodules which were white in colour; spleen was enlarged with mottled appearance. Two cases showed large tumor attached to the kidney which was suspected to be nephroblastoma. The sciatic nerve showed normal striations and had no pathological changes.

Flock five (5)

A broiler parent farm suffered from high number of mortalities, decrease egg performance by 30% and decrease hatchability. Thirty cases were examined and necropsied. Visceral organs were enlarged, with small multiple nodules on its surfaces. Ten cases showed liver and kidneys enlargement with multiple nodules which were white to grey in colour friable in consistency; 15/30 of examined cases had atrophy in the ovary and oviduct.

Flock six (6)

A broiler parent farm suffered from high number of mortalities, decreasing in the egg performance by 25% and decreasing of hatchability. Twenty five cases were examined and necropsied. Visceral organs were enlarged and with the appearance of small multiple nodules on their surfaces. Fifteen cases showed liver and kidneys enlargement with multiple nodules which were white to grey in colour, and friable in consistency. Three of examined cases had myelocytomas tumor in the keel bone, small intestine and skeletal muscles showed multiple grayish white nodules. The gross picture of examined birds gave some degree of certainty to the picture of myelocytomatosis.

Kidneys:

Macroscopic picture:

The kidneys of infected birds with avian leukosis virus subgroup-J were enlarged and showed grayish white areas of varied size and shape. Subcapsular focal haemorrhagic patches were also seen (Fig.37). The kidney appeared friable with marked enlargement of its lobes in comparison with non infected birds (Fig.38).

Microscopic pictures:

The degree of pathological alterations of kidney tissue varied according to the virulence of virus and immune status of

bird. Subcapsular haemorrhages were seen in most of cases (Fig.39). Sometimes, haemorrhages were seen in the interstitial tissue. The subcapsular renal tubules showed massive degenerative changes and sloughing of some tubular epithelium. Kidneys of infected birds revealed multifocal neoplastic aggregations of immature and mature granulated myelocytes. These neoplastic masses were seen in both cortex and medulla in the form of circumscribed foci of varied size. The tumour cells were aggregated mainly around the dilated and engorged blood vessels (Fig.40). Degeneration and atrophy of renal tubules occurred adhering to the neoplastic cell masses.

The renal tubules showed degeneration with severe sloughing of its lining together with the presence of flocculated intraluminal proteinacious material (Fig.41). The renal tubules showed detachment of tubular epithelial lining and necrosis of some renal tubules. Oedema and congestion of the blood vessels and capillaries in interstitial connective tissue were also seen. Some of renal tubules showed cellular cast consisted of desquamated epithelial cells intermingled with myelocytes (Fig.42). Mineralization of some degenerated renal tubules was noticed (Fig.43).

Heart:

Macroscopic:

The thoracic cavity of infected bird with myeloid leukosis avian leukosis virus subgroup-J revealed yellowish white, soft

and friable nodular or diffuse masses. These tumour masses were seen in costochondral junctions of the ribs and the inner surface of sternum (Fig.44). The cardiac muscle of most of infected birds showed relative hypertrophy of their left side. Multiple yellowish white nodules of varied size were observed in the cardiac muscle (Fig.45).

Microscopic:

The cardiac muscle showed diffuse aggregation of granulated myelocytes in the subepicardial connective tissue (Fig.46). The nodular lesion consisted of massive aggregation of well differentiated neoplastic cells. The neoplastic cells composed of immature and mature myelocytes. The epicardium was severely infiltrated by myelocytic cells (Fig.47). The cardiac muscle fibers were separated by granulated myelocytic cells either in small or large focal manner (Fig.48). In some cases perivascular oedema with myeloid cells aggregation were seen (Fig.49). Degeneration and intermuscular oedema with myeloid cells infiltration were observed.

Liver:

Macroscopic:

The liver of infected birds with avian leukosis virus subgroup-J were greatly enlarged which occupied most of the abdominal cavity. The liver appeared friable with multiple elevated creamy nodules which coalesce together to form

patches of tumour masses were seen (Fig.50). In some cases the liver characterized by moderate to great enlargement in comparison with the non infected birds (Fig.51). Focal grayish white areas distributed all over the hepatic lobules with subcapsular focal haemorrhagic areas were seen.

Microscopic:

The hepatic lobules showed multifocal aggregations of mature and immature granulated myelocytes with frequent mitotic figures. These neoplastic cells replaced hepatocytes with relative atrophy of the surrounding cells. The spaces of disse were expanded by homogenous pale eosinophilic serous material and infiltrated with myeloid cells (Fig.52). The portal areas infiltrated by large number of proliferating myelocyets (granulated type) with hyperplasia and swelling of the bile duct epithelium. The hepatocytes around the portal areas showed degenerative changes, atrophy and disorganization of its cords (Fig.53). Hyperplasia and metaplasia of bile ducts were observed. These ducts were lined by cuboidal or columnar epithelial cells intermingled with mucous secreting cells and surrounded by granulated myelocytic cells (Fig.54).

The myeloid cells were usually observed around the veins and arteries in the portal areas as well as in the hepatic parenchyma (Fig.55). Myelocytic cells characterized by eccentrically located nuclei and had marked nucleolus with clear

mitotic figure. The cytoplasm was filled with conspicuous spherical eosinophilic granules (Fig.56).

Skeletal Muscles:

Macroscopic:

The skeletal muscles adhered to the flat bones especially the sternum, ribs and synsacrum revealed grayish white, soft and friable elevated nodules of variable sizes (Fig.57).

Microscopic:

Stained section of the pectoral muscle of the infected bird revealed coagulative necrosis (Zenker's) of muscles bundles which appeared homogenous eosinophilic structuerless masses separated by severe myelocytic cells infiltrations (Fig.58). Massive number of proliferating myelocytes were infiltrating the necrosed muscles. Longitudinal section of pectoral muscle displayed necrosis and atrophy of muscle bundles which were replaced by myelocytomas (Fig.59).

Bone and Cartilage:

Macroscopic:

Some infected birds showed the typical features of myelocytomatosis which characterized by yellowish white nodules on the visceral surface of sternum, ribs and synsacrum. The nodules were multiple creamy white with soft and friable

consistency. The surface of some tumour nodules had a layer of thin and brittle periostium (Fig.60).

Microscopic:

The examined sections of cartilaginous part of sternum revealed large numbers of proliferating mature and immature granulated myelocytes in diffuse manner around the degenerated cartilage (Fig.61, 62).

Intestine:

Macroscopic:

The intestinal mucosa revealed grayish white elevated areas scattered along the intestinal tract. Some cases showed focal necrotic areas surrounded by hyperemic zones. The intestinal lumen was filled with yellowish slimy fluid with offensive odour.

Microscopic:

The intestinal mucosa showed sloughing of epithelial lining. The intestinal villi showed myelocytic infiltrations (Fig.63). Some cases showed erosive areas in which desquamation of epithelial lining and exposure of subepithelial connective tissue as well as complete destruction of intestinal villi. The lamina propria was infiltrated with large number of mature and immature myelocytes (Fig.64). The submucosal connective tissue showed perivascular oedema and myelocytic

infiltration (Fig.65). The central lactae was infiltrated with uniformly differentiated mature myelocytes (Fig.66).

Ovary:

Macroscopic:

Most of the examined cases that suffered from decrease of egg production showed atrophy of the ovary and its follicles (Fig.67).

Microscopic:

The ovary of infected bird with myeloid leukosis showed massive diffuse and focal infiltration of myelocytic cells in the ovarian tissue (Fig.68). The ovary showed atrophied or undeveloped ovarian follicles, few ovarian follicles showed developmental stages and mature ovarian graffian follicles with primary oocyte were seen. The myelocytic cells surrounded the ovarian follicles and perivascular spaces leading to displacement of the graffian follicles (Fig.69).

These myelocytic cells showed a characteristic brilliant red colored cytoplasmic granules when stained with May - Grünwald – Gimsa stain (Fig.70).

Nephroblastoma:

Macroscopic:

Nephroblastoma considered as embryonal highly malignant tumour. This type of tumour was recorded in two examined birds infected with ALV-J. The kidneys of infected birds showed spherical well demarcated tumour mass, replacing part of the parenchyma. On cut section the tumour appeared soft friable, grayish white with haemorrhagic spots.

Microscopic:

The tumour mass was separated from apparently healthy tissue by thick fibrous connective tissue and leukocytic infiltration (Fig.71). The tumor tissue showed undifferentiated cystic renal tubules with intraluminal proteinacious cast (Fig.72). The interstitial tissue showed polymorphic stromal cells which appeared round or stellate in shape. Structures like glomeruli or metanephric precursor of glomeruli were observed (Fig.73). The renal tubule lined by cuboidal or columnar epithelium with hyperchromatic nuclei. The epithelium of the renal tubules was undifferentiated and merged with stromal cells (Fig.74).

Table (4):

Results of examined sera samples for detection ALV antibodies by using ELISA:

No.	Province	Type	Age (weeks)	No. of samples	Results		
					No. of positive	* S/P mean for positive	%
1	Giza	BP	32	23	0	-	0
2	Ismalia	BP	38	25	0	-	0
3	Behera	BP	28	20	0	-	0
4	Behera	BP	26	20	0	-	0
5	Kaliobia	L	36	22	15	**0.650**	**68**
6	Giza	L	35	25	17	**0.780**	**68**
7	Behera	BP	35	25	15	**0.560**	**60**
8	Behera	BP	30	30	21	**0.730**	**70**
9	Behera	BP	40	30	0	-	0
10	Dakahlia	BP	32	25	0	-	0
Total				**245**	**68**		**27.7**

* Sample/positive (S/P) ratio: greater than 0.4 indicated presence of ALV antibodies.

Table (5):

Results of examined sera samples for detection ALV- J antibodies by using ELISA:

No.	Province	Type	Age (weeks)	No. of samples	Results		
					No. of positive	* S/P mean for positive	%
1	Giza	BP	32	23	19	1.023	83
2	Ismalia	BP	38	25	20	0.980	80
3	Behera	BP	28	20	14	0.850	70
4	Behera	BP	26	20	15	0.905	75
5	Kaliobia	L	36	22	0	-	0
6	Giza	L	35	25	0	-	0
7	Behera	BP	35	25	0	-	0
8	Behera	BP	30	30	0	-	0
9	Behera	BP	40	30	21	0.845	70
10	Dakahlia	BP	32	25	18	0.966	72
Total				245	107		43.7

* S/P ratio greater than 0.6 indicated presence of ALV-J antibodies.

Results of serological test (ELISA):

ELISA test was applied on 245 serum samples of ten flocks from two types: commercial layers and broiler parents and from different provinces as shown in table (2). These samples were 47 serum samples of layers and 198 serum samples of broiler parents. The age was ranged from 26 up to 50 weeks of age.

- As shown in *table (4)* the results were positive for 4 flocks and negative for 6 flocks for ALV antibodies in sera by percentage ranged from 60 % (15/25) up to 70 % (21/30)
of total (68/245) 27.7% .

- As shown in *table (5)* the results were positive for 6 flocks and negative for 4 flocks for ALV-J antibodies in sera by percentage ranged from 70 % (21/30) up to 83% (19/23)
of total (17/245) 43.7%.

Table (6):

Results of PCR test for detection DNA of ALV-J in tissues :

No.	Governorate	Age	Type	PCR results
1	Giza	32	BP	+
2	Ismalia	38	BP	+
3	Behera	40	BP	+
4	Dakahlia	32	BP	+
5	Behera	35	BP	-
6	Giza	35	L	-

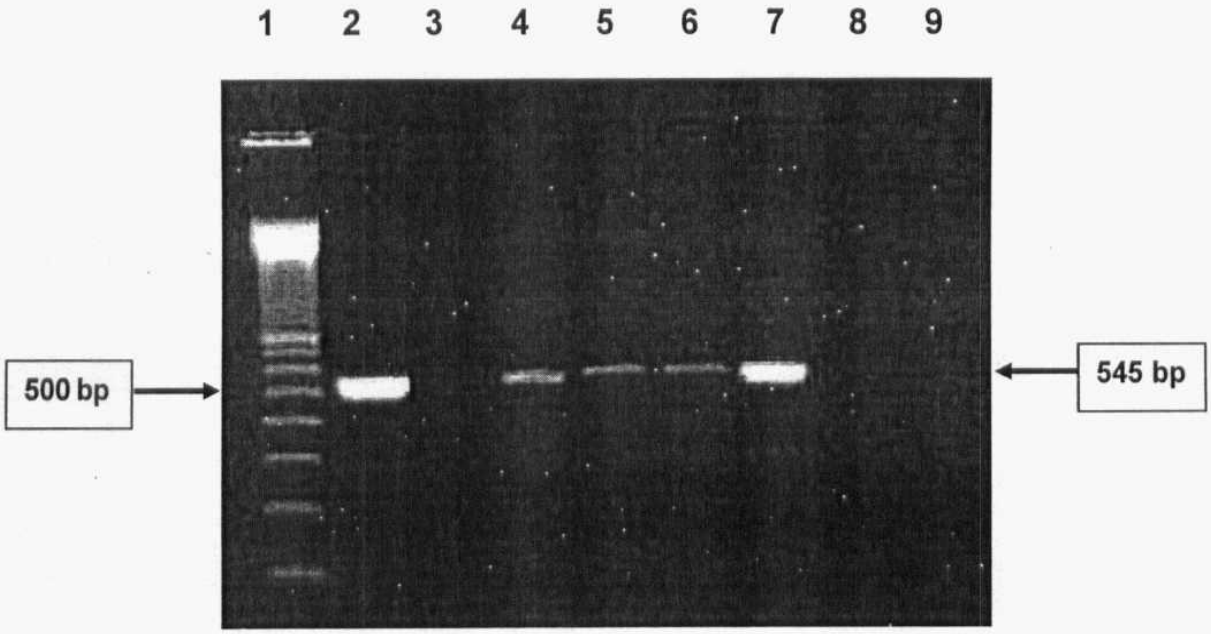

photo (1): Electrophoresis of PCR on 1.5% agarose gel stained with ethidium bromide showing:

- 100 bp molecular weight ladder (lane 1).
- Positive control amplified DNA products at 545 bp (lane 2).
- Negative control (lane 3).
- Amplification products at 545 bp fragment indicating DNA of ALV – J with H5, H7 primers (lanes 4, 5, 6 and 7).
- Samples (lanes 8, 9) indicating negative amplification.

Results of ALV-J DNA detection in tissues using PCR test:

PCR test conducted on DNA extracted from tissues showed tumor lesions from the tested flocks as shown in table (6).

The result of PCR test of 6 examined flocks indicated positive amplification of 545 bp fragment with the extracted DNA of ALV-J in lanes (4, 5, 6, and 7) for 4 flocks and negative amplification in lanes (8 and 9) for 2 flocks as shown in photo(1).

Fig (1) Lymphoid leukosis in liver showing nodular form, white to creamy colored nodules with glistening appearance.

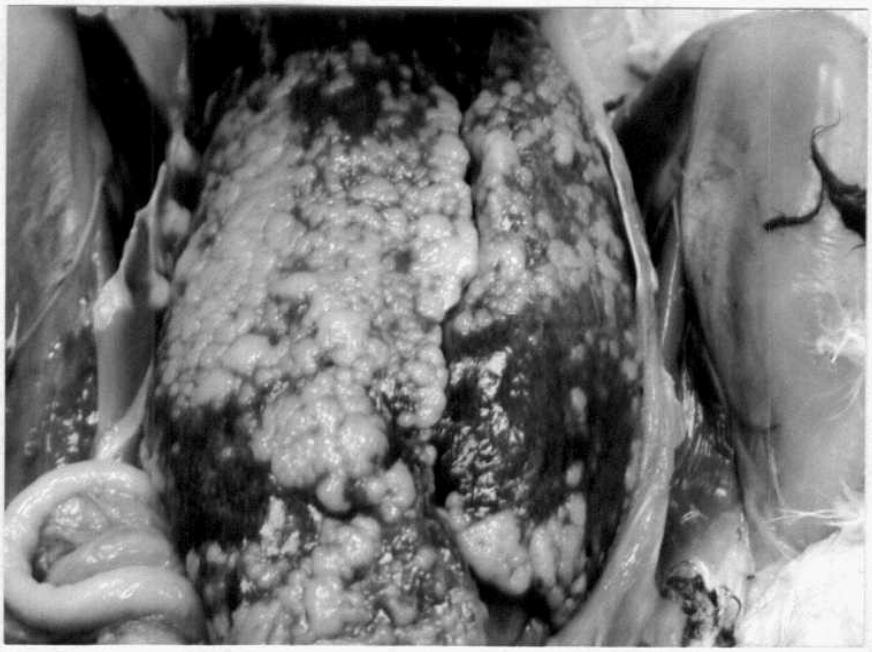

Fig (2) Lymphoid leukosis in liver showing diffuse enlargement form.

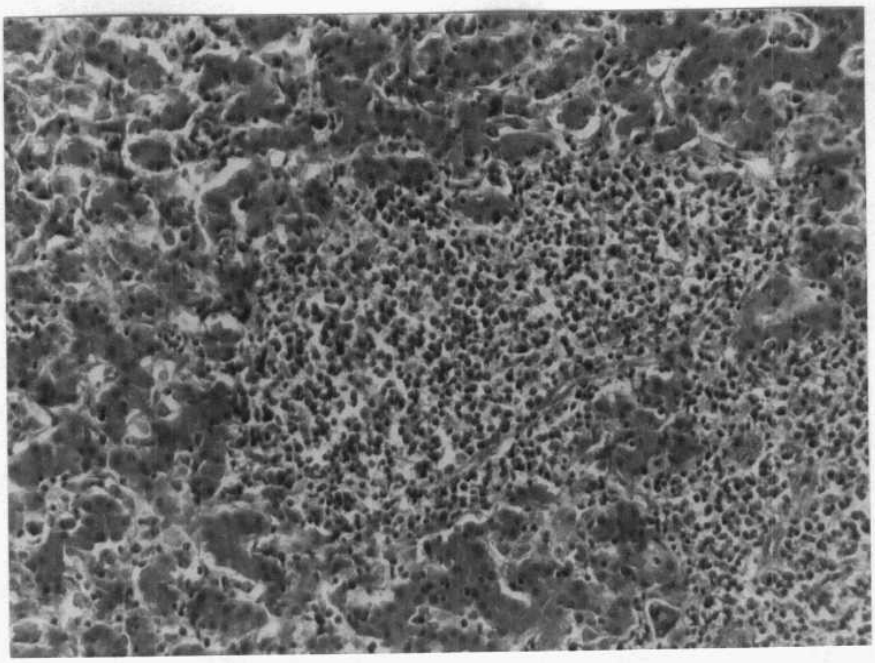

Fig (3) Lymphoid leukosis, nodular form liver showing focal infiltration of lymphoblastic cells surrounded by thin layer of long spindled shape cells (H&E x 100).

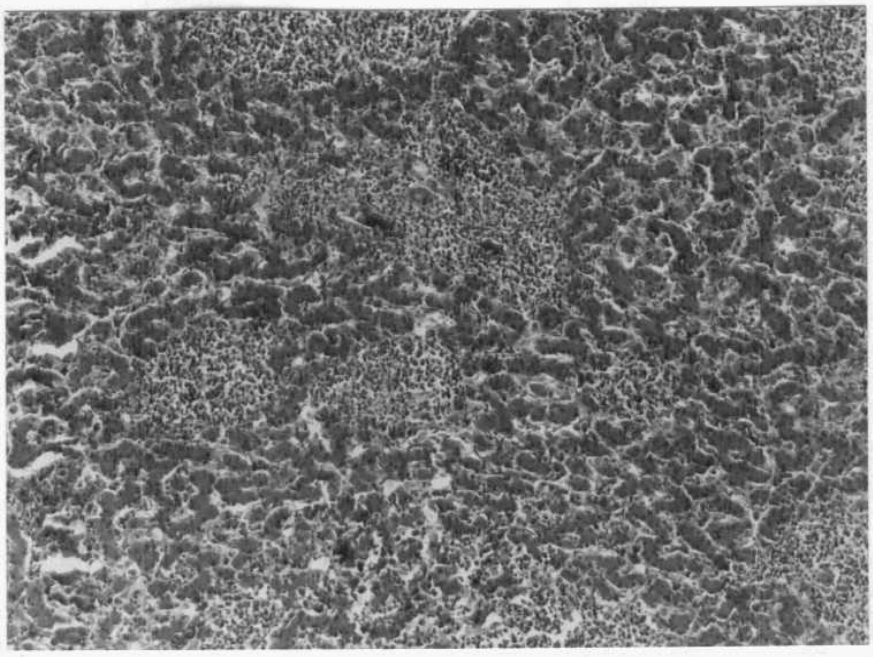

Fig (4) Lymphoid leukosis, milliary form liver showing multiple focal infiltrations of lymphoblastic cells (H&E x40).

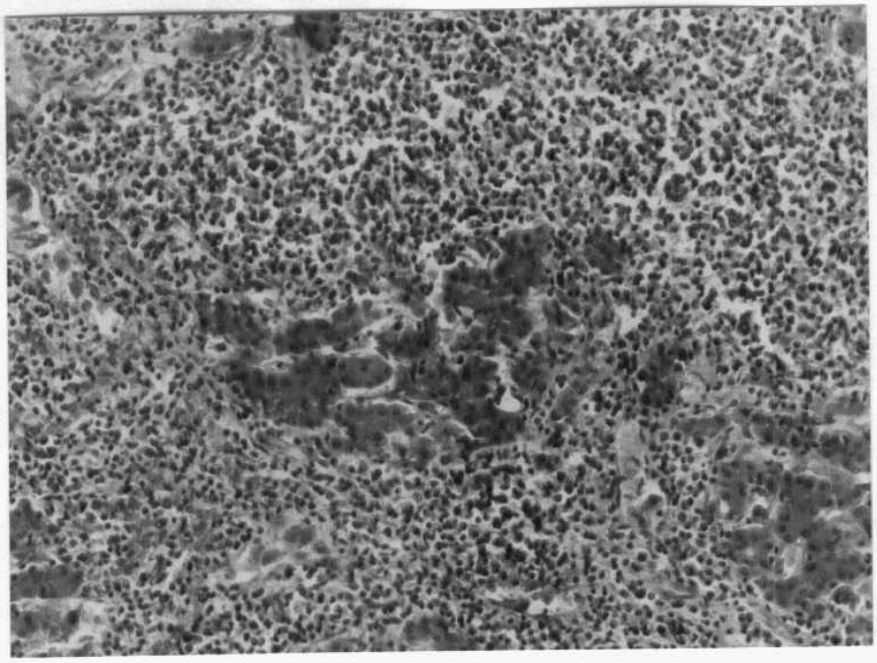

Fig. (5) Lymphoid leukosis, diffuse form liver showing diffuse infiltration of lymphoblastic cells; the hepatic cells appear as islets of hepatocytes between the infiltrating cells (H&E x100).

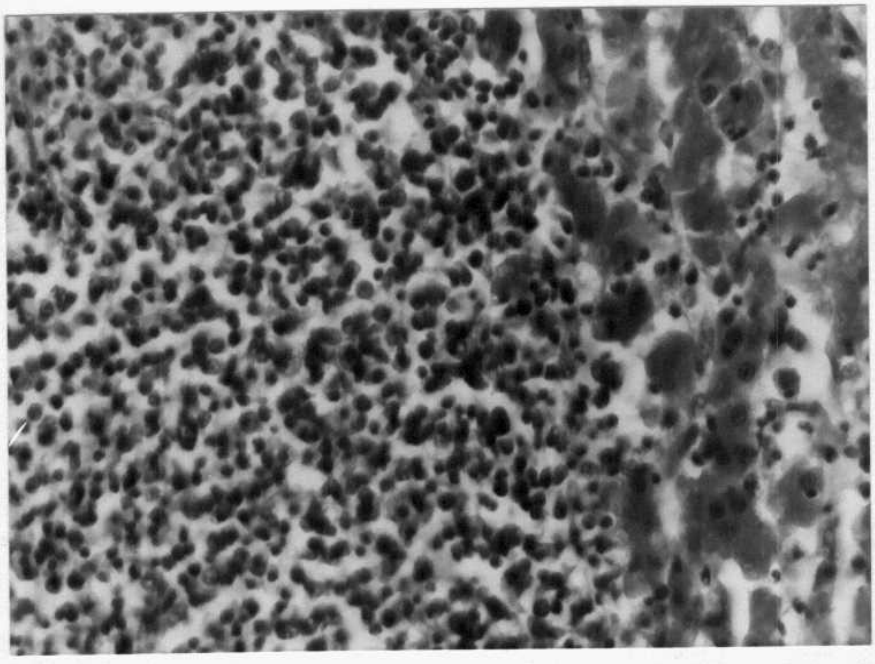

Fig. (6) Lymphoid leukosis, diffuse form liver showing lymphoblastic cells with apoptotic bodies (H&E x200).

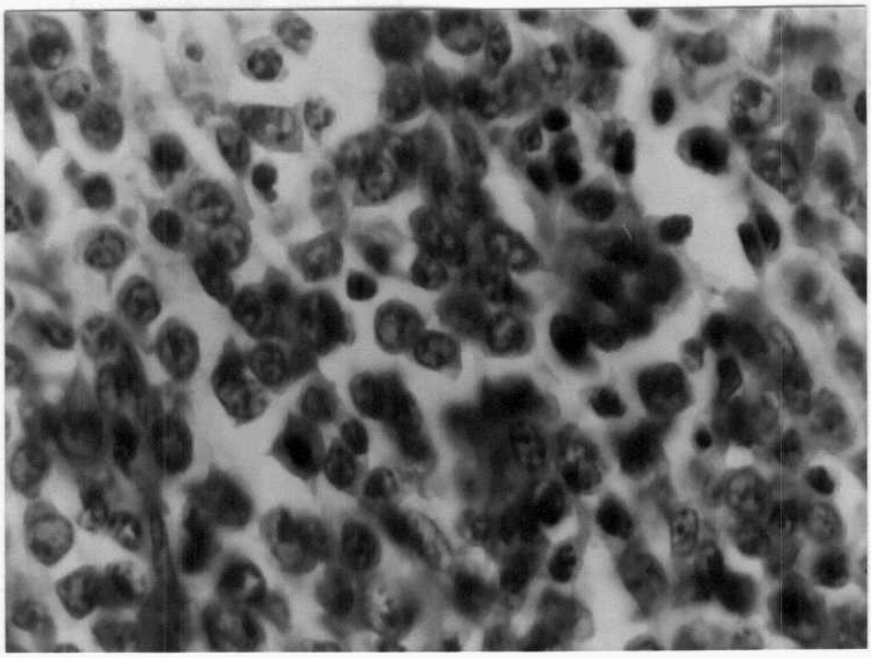

Fig (7) Lymphoid leukosis, liver showing lymphoblastic cells with basophilic cytoplasm and vesicular nucleus with prominent mitotic figures (H&E x1000).

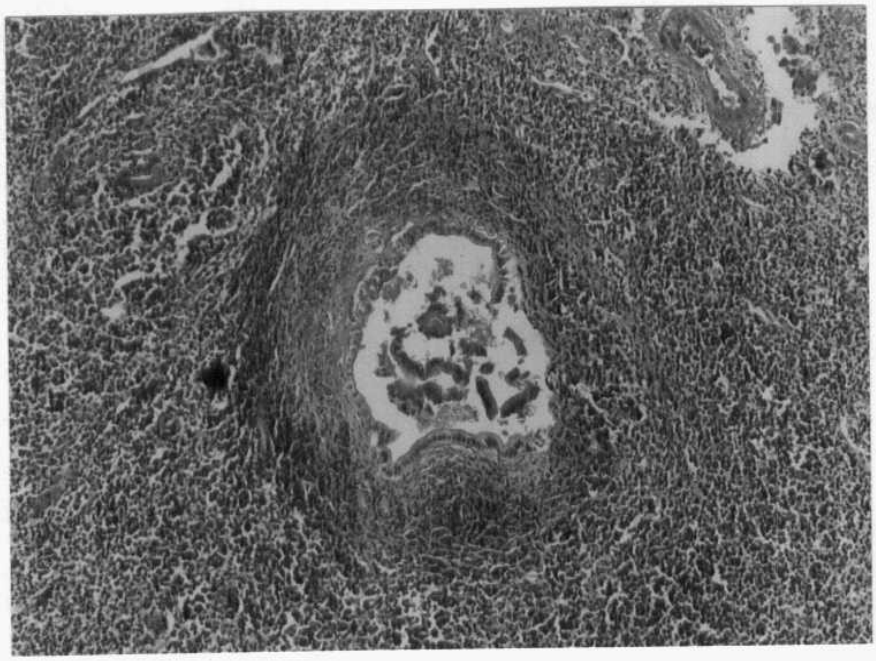

Fig (8) Lymphoid leukosis, liver showing hyperplasia of bile duct and desquamation of its lining epithelial (H&E x40).

Fig (9) Lymphoid leukosis showing enlarged kidney lobes with white creamy nodules appeared on its surface.

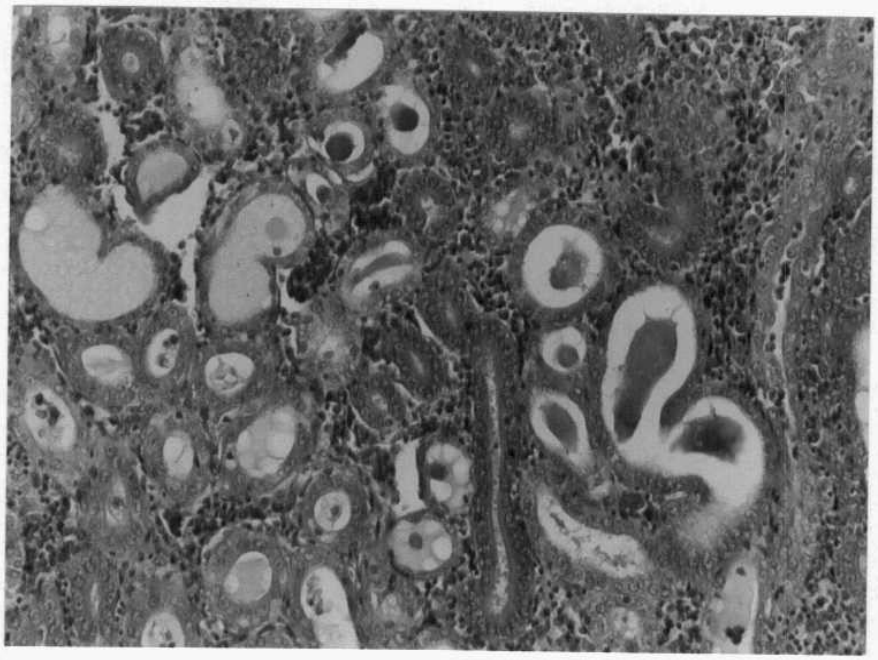

Fig (10) Lymphoid leukosis, kidney showing diffuse infiltration of Large lymphoid cells between renal tubules that containing renal casts (H&E x100).

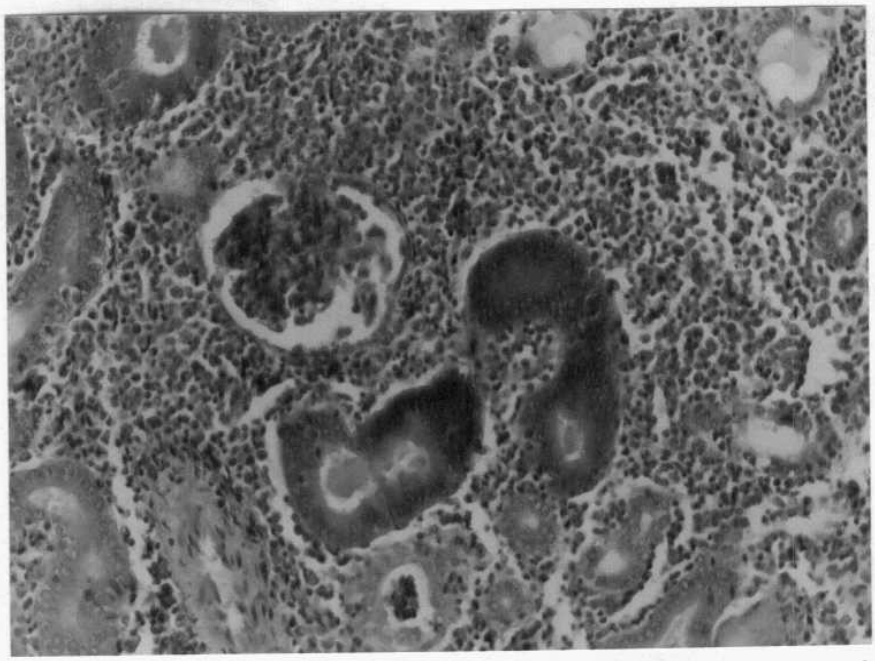

Fig (11) Lymphoid leukosis, kidney showing diffuse infiltration of lymphoblasts with hyperplasia of renal tubular epith.(H&E x200)

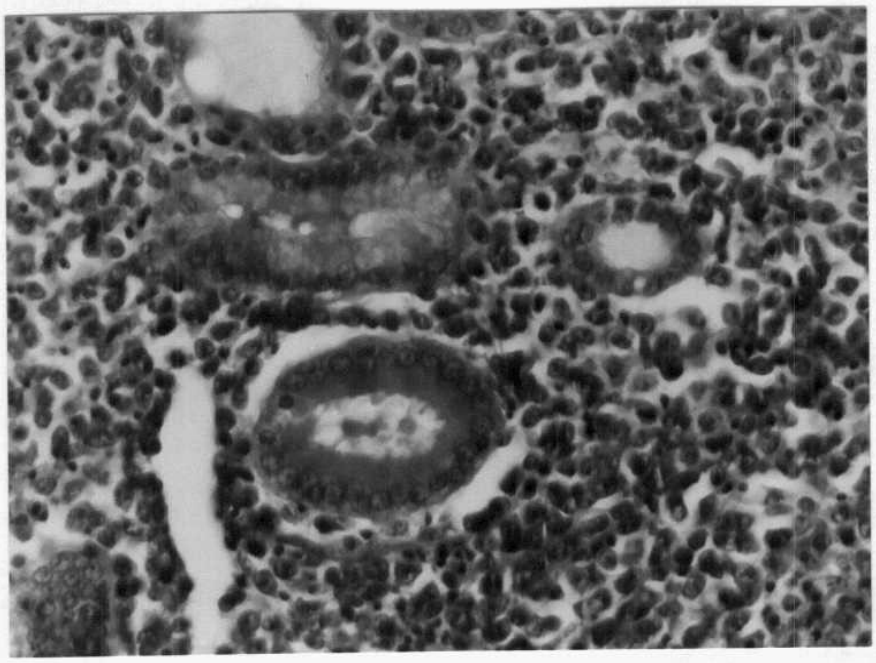

Fig (12) lymphoid leukosis, kidney showing Lymphoblasts uniform in size and morphology with vesicular nuclei, and prominent mitotic figures (H&E x400).

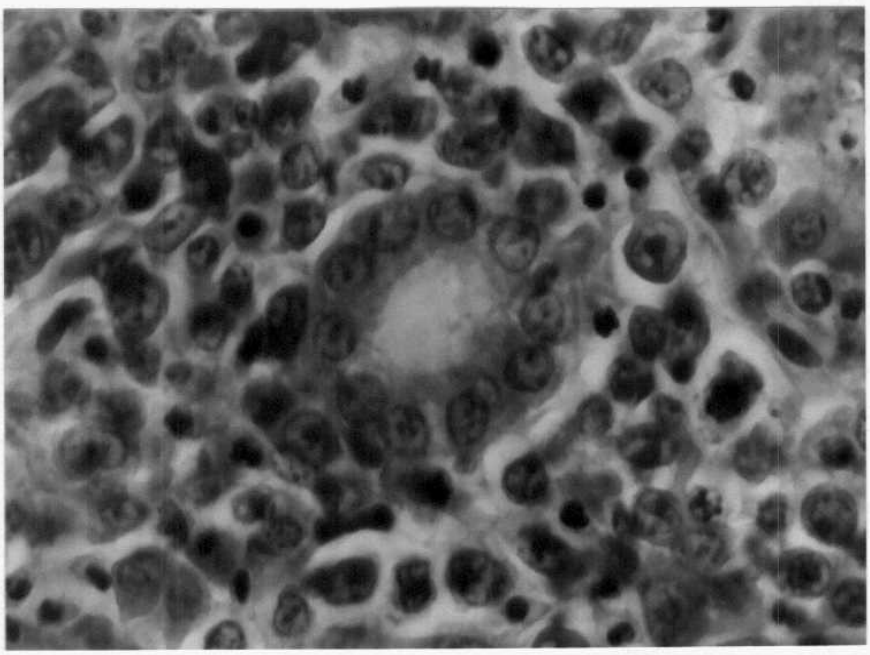

Fig (13) lymphoid leukosis, kidney showing mitotic figures in lymphoblastic cells (H&E x 1000).

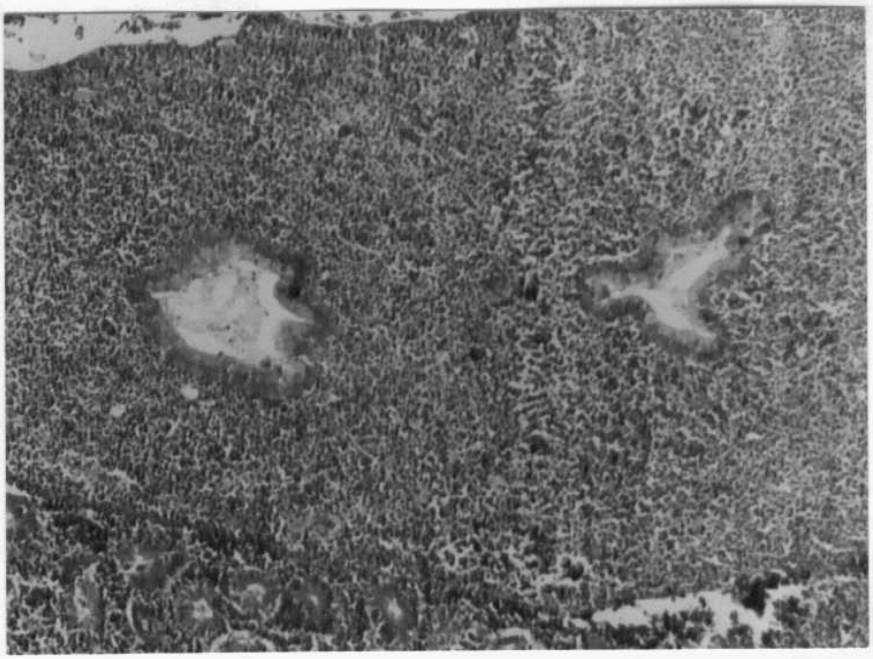

Fig (14) Lymphoid leukosis, kidney showing diffuse infiltration of Lymphoblasts, surrounding the collecting ducts. (H&E x40)

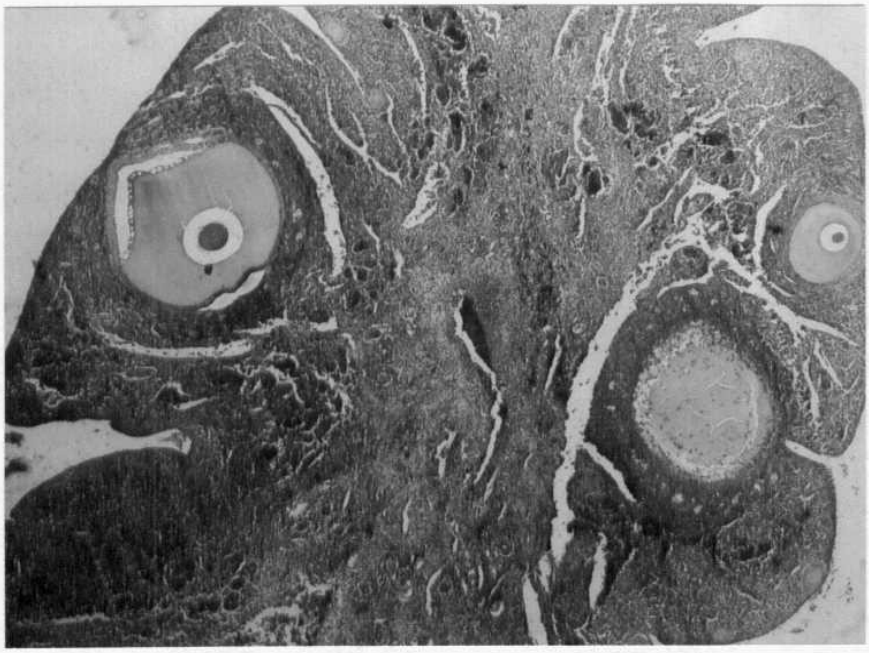

Fig (15) Lymphoid leukosis, ovary showing diffuse infiltration of lymphoblastic cells with atrophied ovarian follicles (H&E x40).

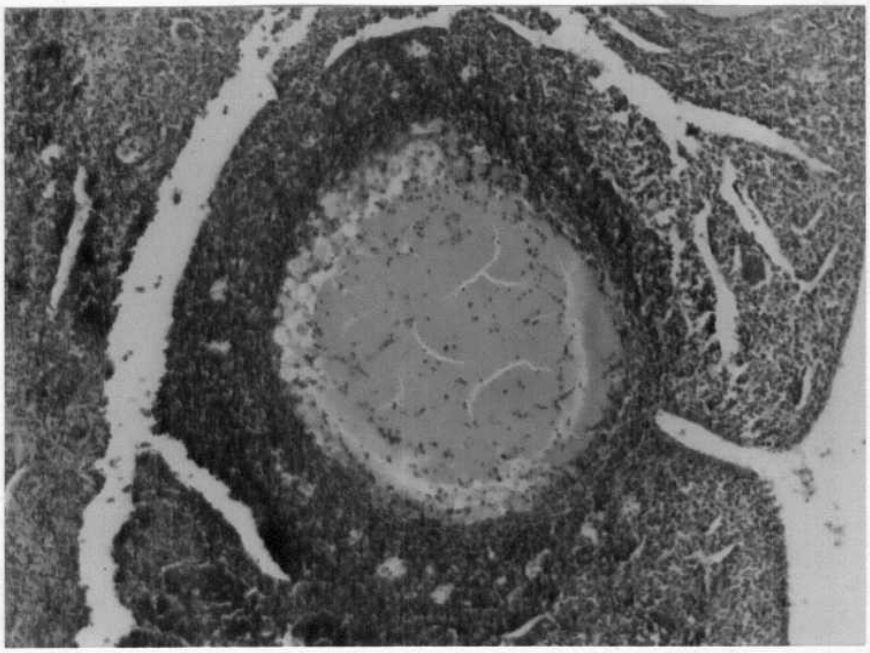

Fig. (16) lymphoid leukosis, ovary showing lymphoblastic cells infiltration surrounding mature graffian follicle (H&E x100).

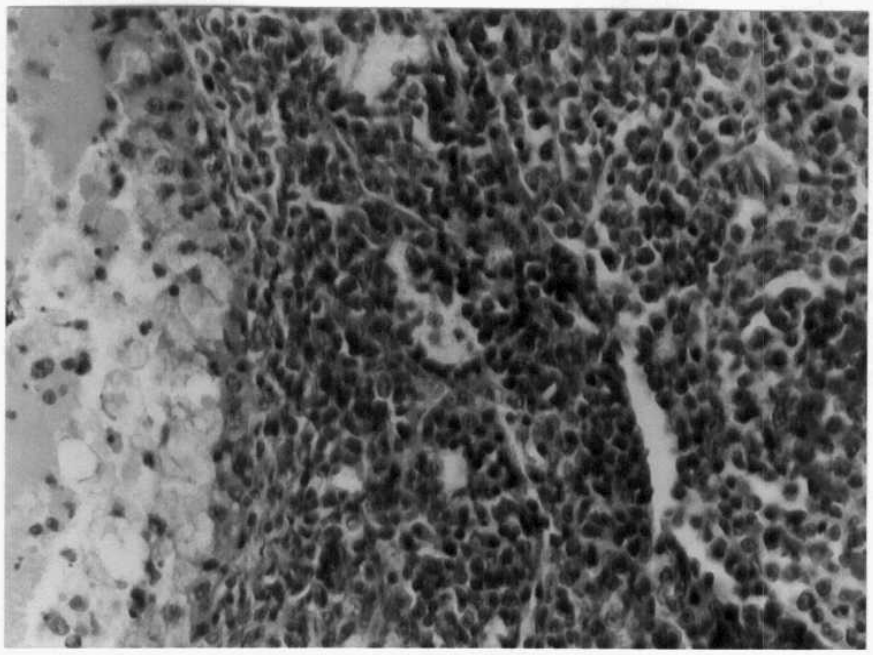

Fig (17) Lymphoid leukosis, ovary showing lymphoblastic cells infiltration .notice the homogenous cell population.(H&E x200)

Fig (18) Lymphoid leukosis, spleen showing enlargement with grayish white nodules .

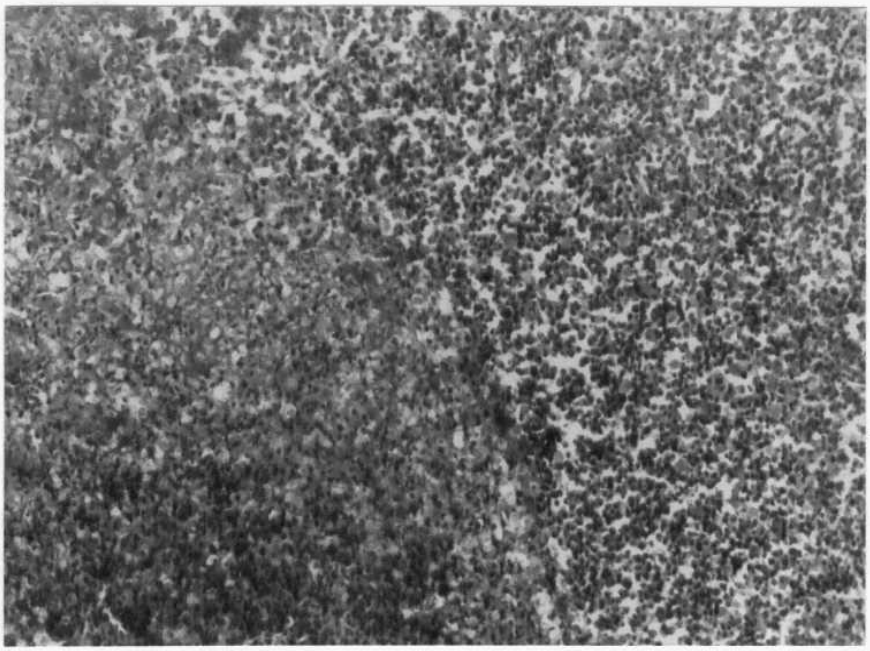

Fig.(19) Lymphoid leukosis, spleen showing lymphoblastic cells infiltration in white pulp.(H&E x100)

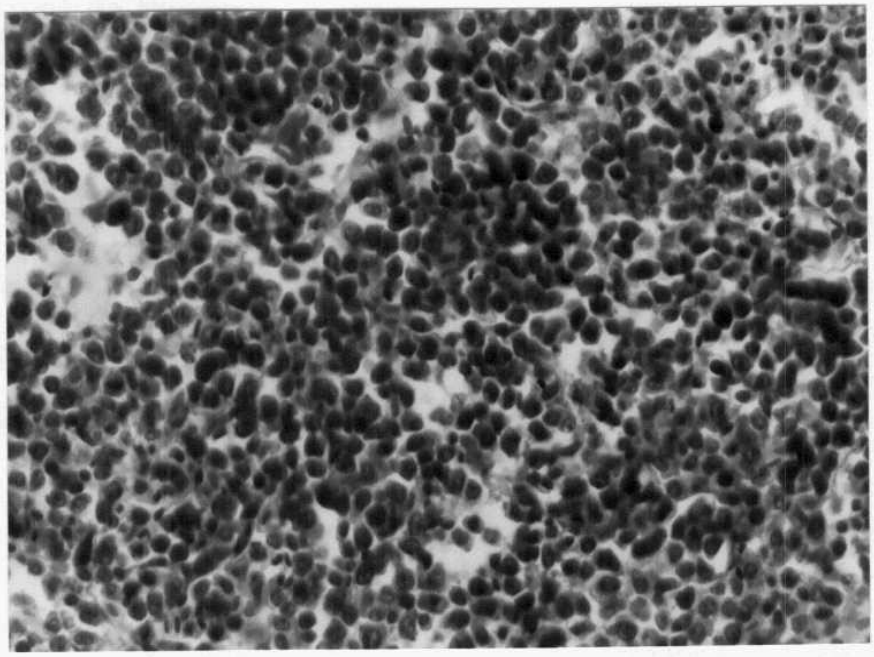

Fig. (20) Lymphoid leukosis, spleen showing lymphoblastic cells with mitotic figures.(H&E x200)

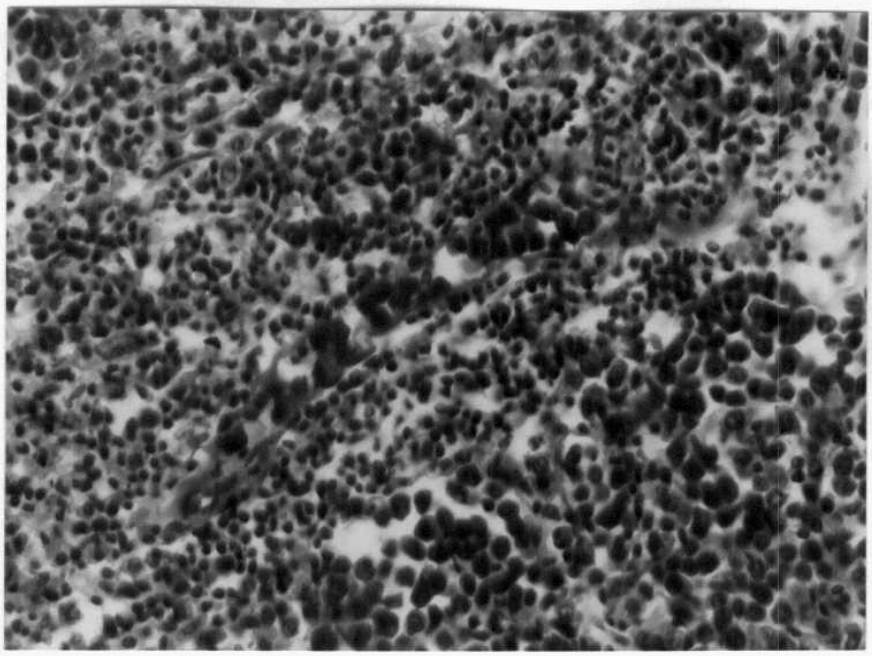

Fig. (21) Lymphoid leukosis, spleen showing lymphoblastic cells inside blood vessles.(H&E x400)

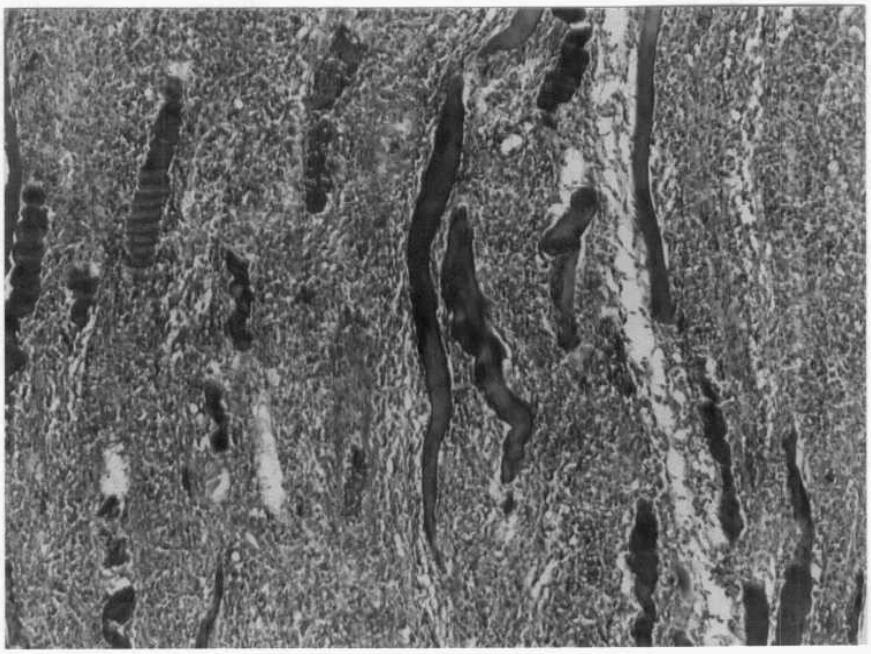

Fig. (22) Lymphoid leukosis, skeletal muscles showing lymphoblastic cells infiltration with Zenker's necrosis of muscle bundles. (H&E x40)

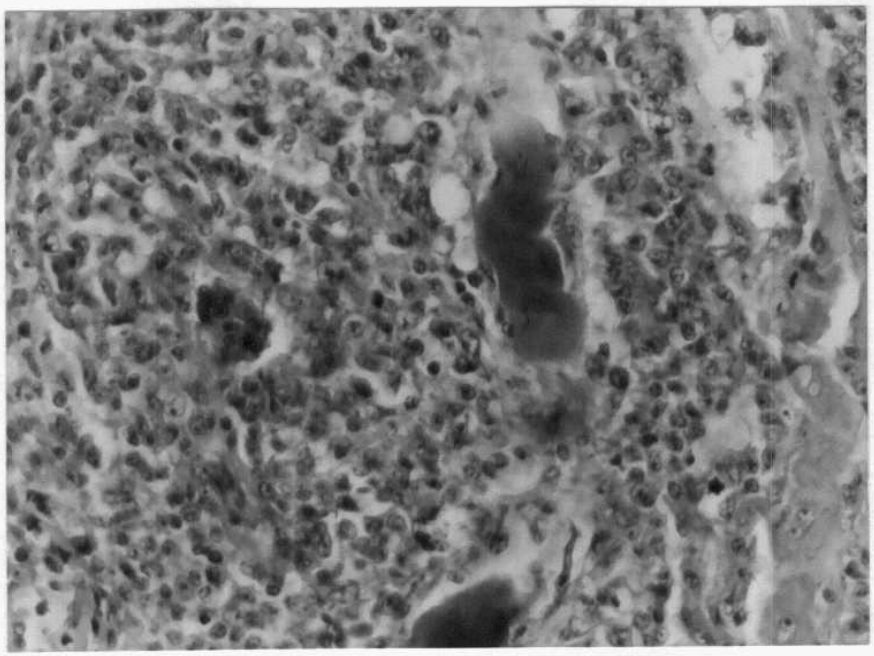

Fig. (23) Lymphoid leukosis, skeletal muscles showing lymphoblastic cell infiltration with mitotic figures.(H&E x200)

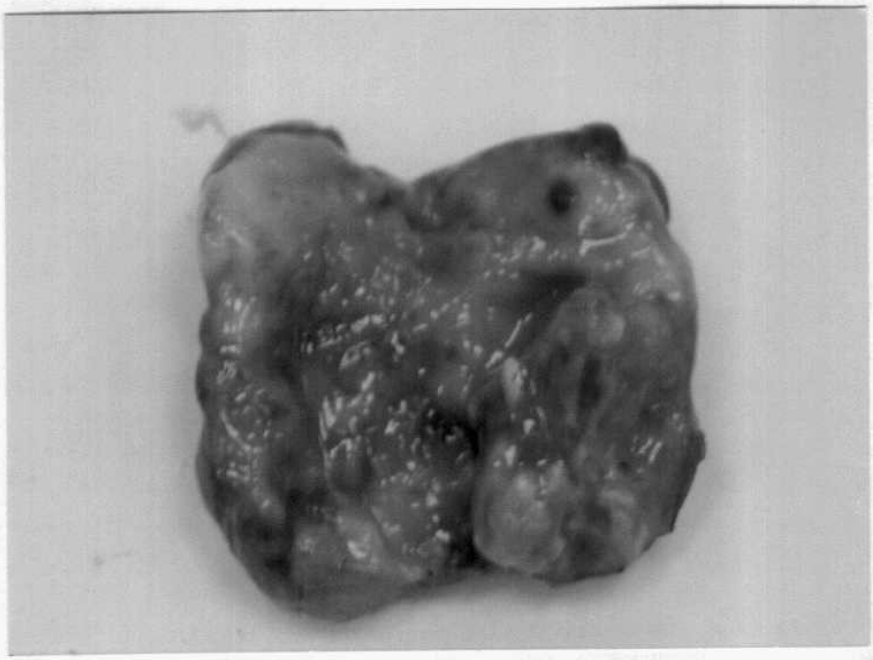

Fig. (24) Lymphoid leukosis, bursa of fabricius showing enlargement and loss of bursal folds.

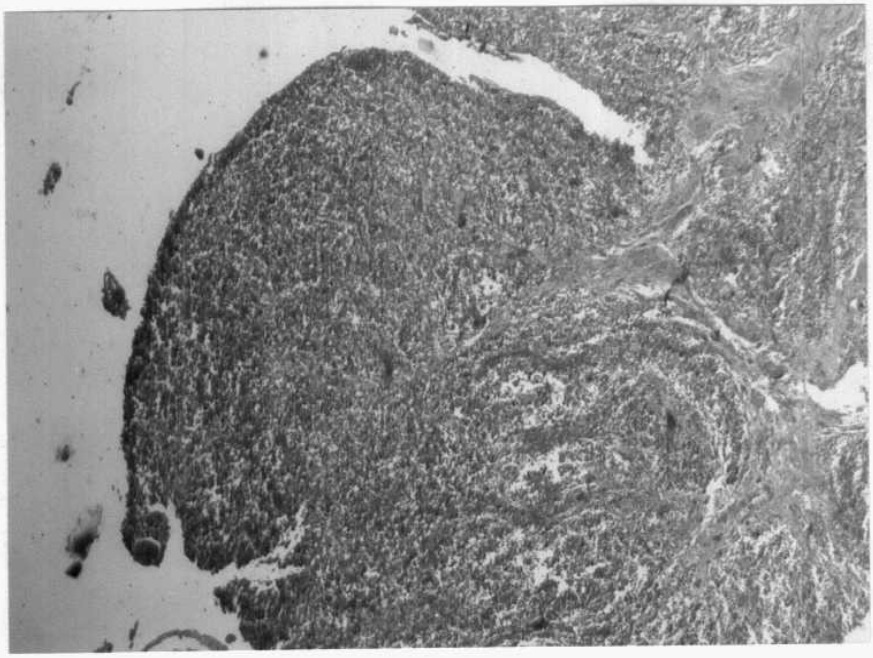

Fig. (25) Lymphoid leukosis, bursa of fabricius showing severe enlargement of bursal lymphoid follicle. (H&E x40)

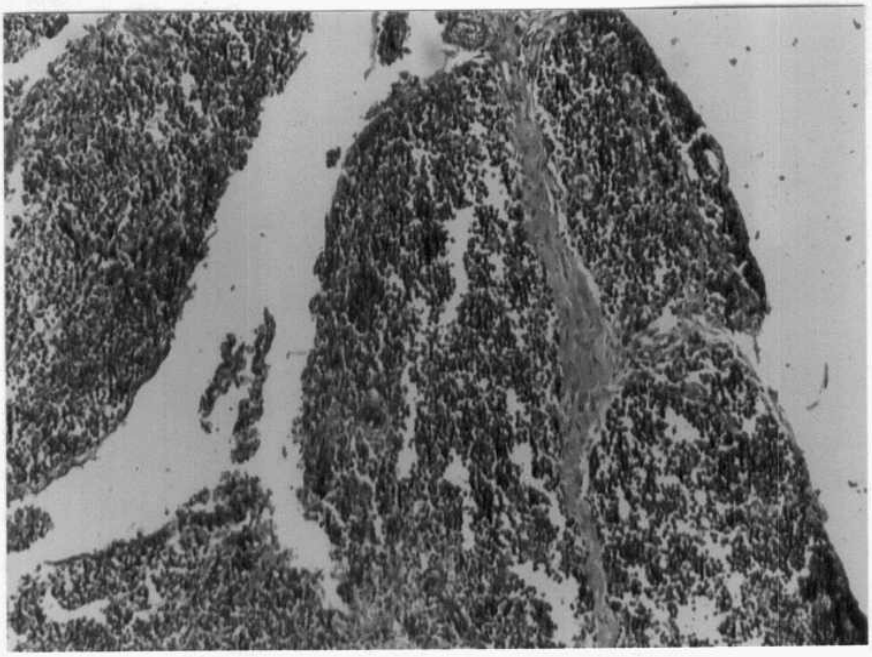

Fig. (26) Lymphoid leukosis, bursa of fabricius showing lymphpblastic proliferation with fibroplasia.(H&E x100)

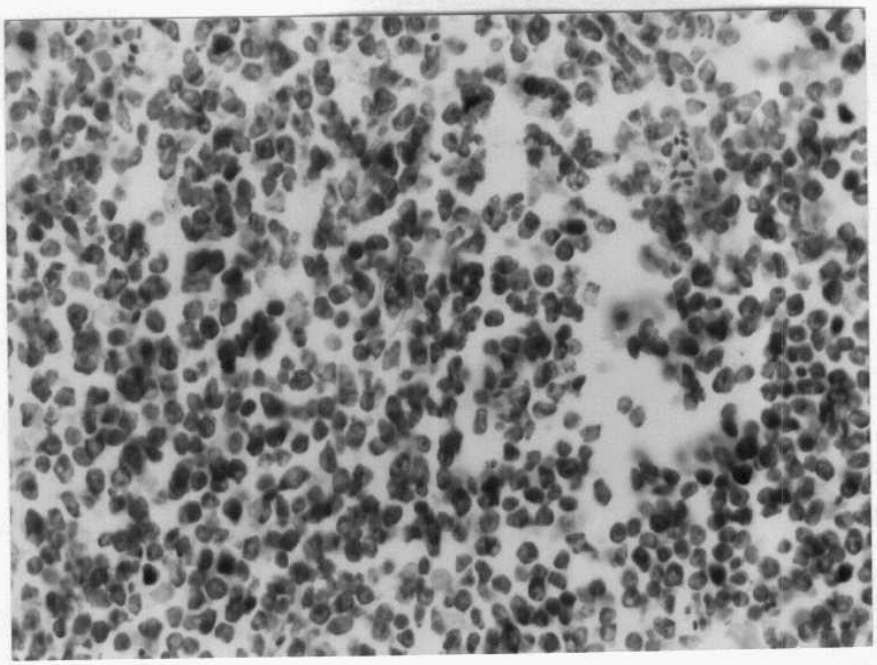

Fig. (27) Lymphoid leukosis, bursa of fabricius showing lymphoblastic cells stained deeply red with Methyl Green Pyronin (x200).

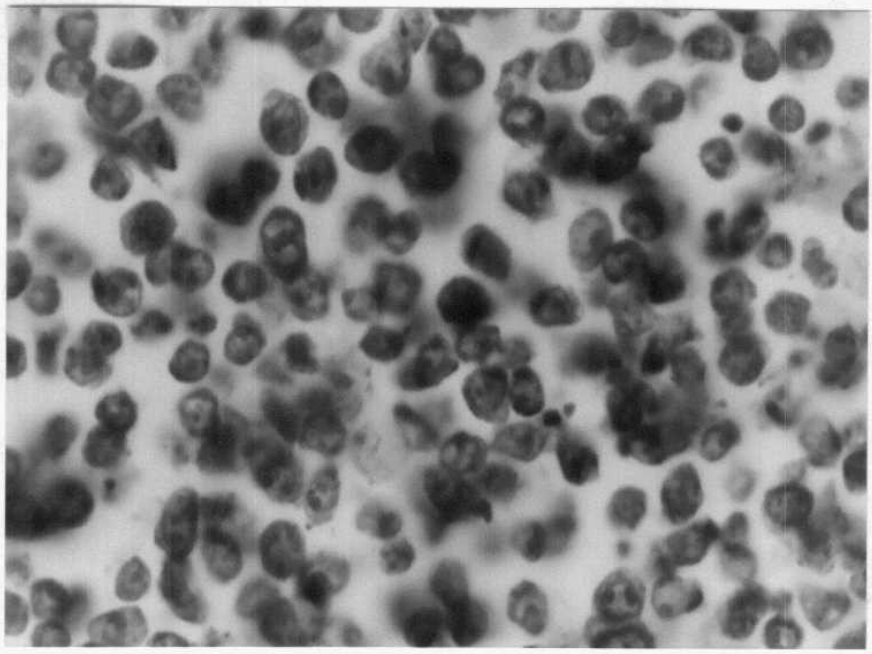

Fig. (28) Lymphoid leukosis, bursa of fabricius showing lymphoblastic cells with high pyroninophilic cytoplasm (Methyl Green Pyronin x1000)

Fig. (29) Lymphoid leukosis, intestine showing grayish white nodules on its surface.

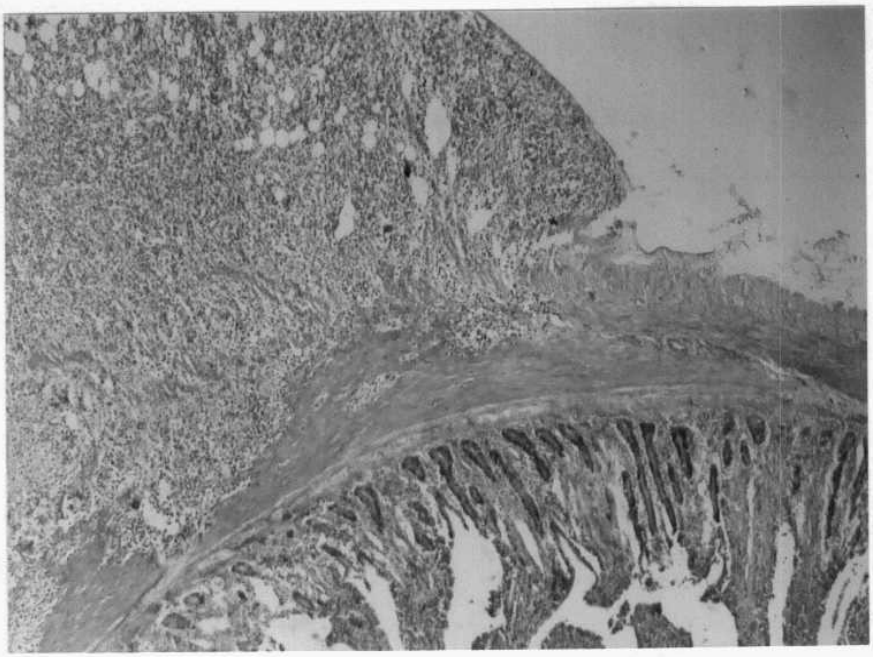

Fig. (30) Lymphoid leukosis, intestine showing lymphoid nodules attached on serosa by fibrous C.T. (H&E x40)

Fig. (31) Subcutaneous tumor on head and neck of chickens.

Fig. (32) Subcutaneous tumor appeared on lower lid.

Fig. (33) Subcutaneous tumor showing great protrusion on eye lid with ulceration of the overlying skin.

Fig. (34) Subcutaneous tumor showing swelling from the upper eyelid.

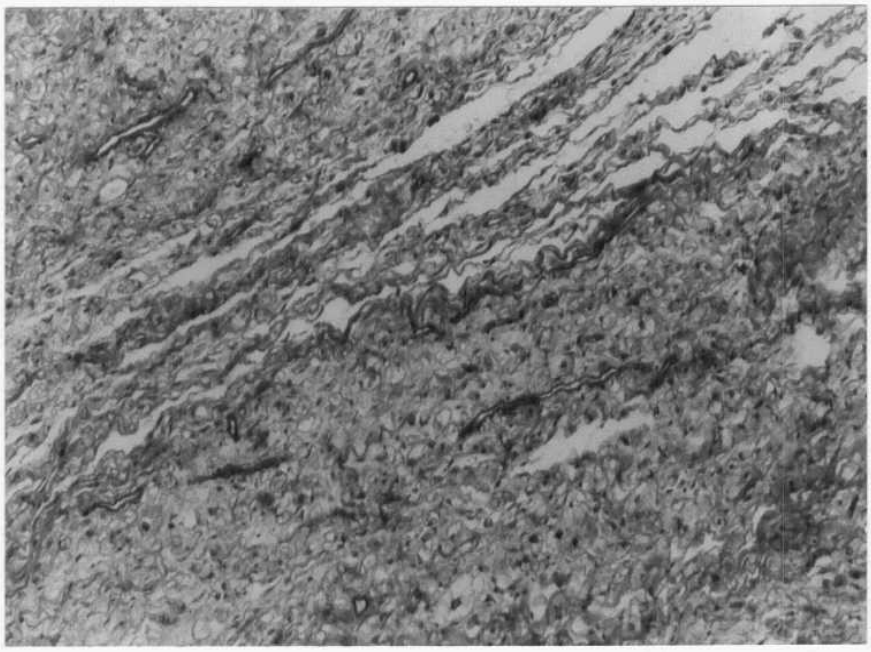

Fig. (35) Subcutaneous tumor showing myxoma with loose fibrous Connective tissue. (H&E x40)

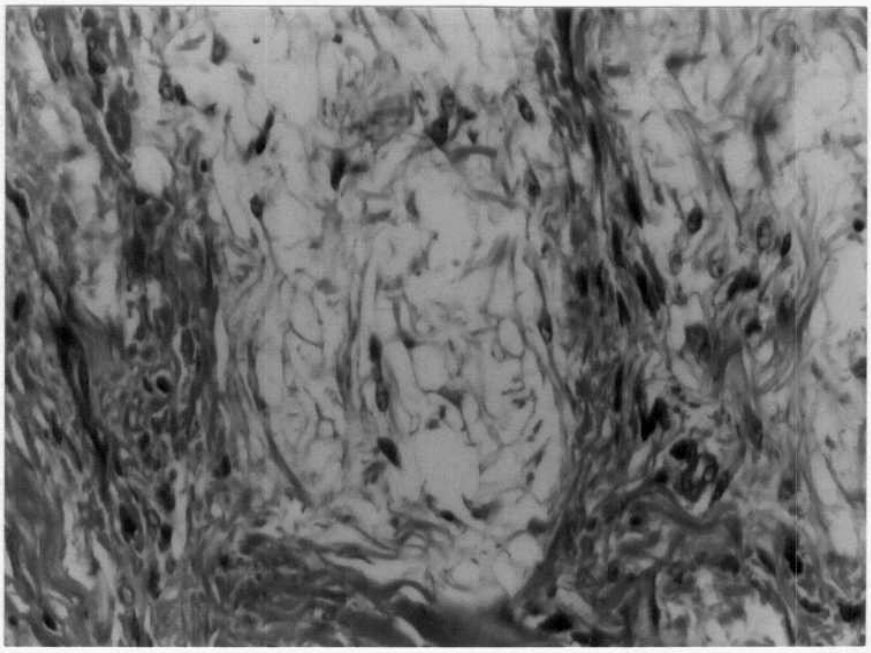

Fig (36) Subcutaneous tumor showing myxoma with stellate shaped cells. (H&E x 400)

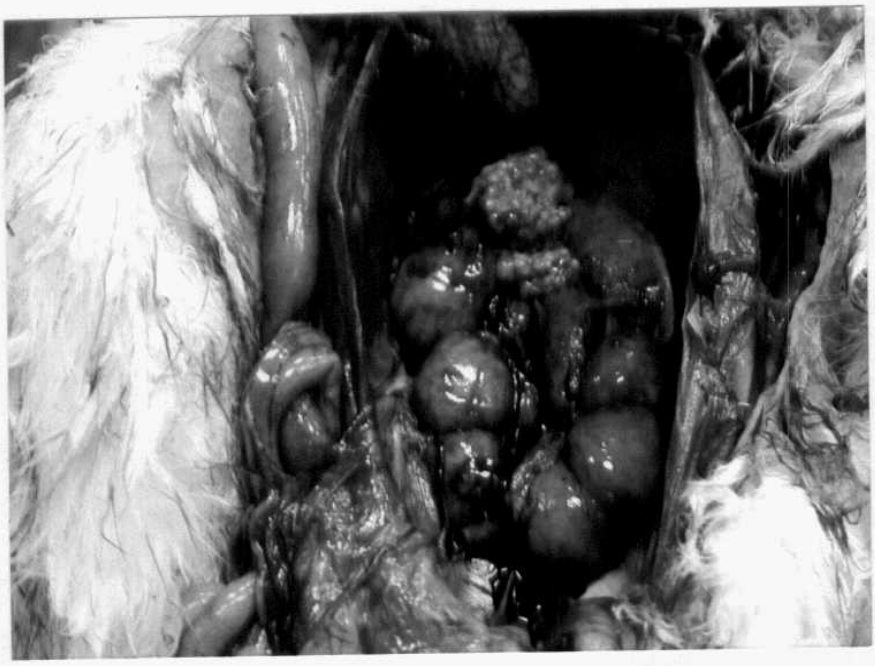

Fig (37) Myeloid leukosis, kidneys showing enlargement with grayish white patches and subcapsular haemorrhagic areas.

A B C

Fig (38) Myeloid leukosis, kidneys showing A, B severely enlargement in comparison with C non-infected bird.

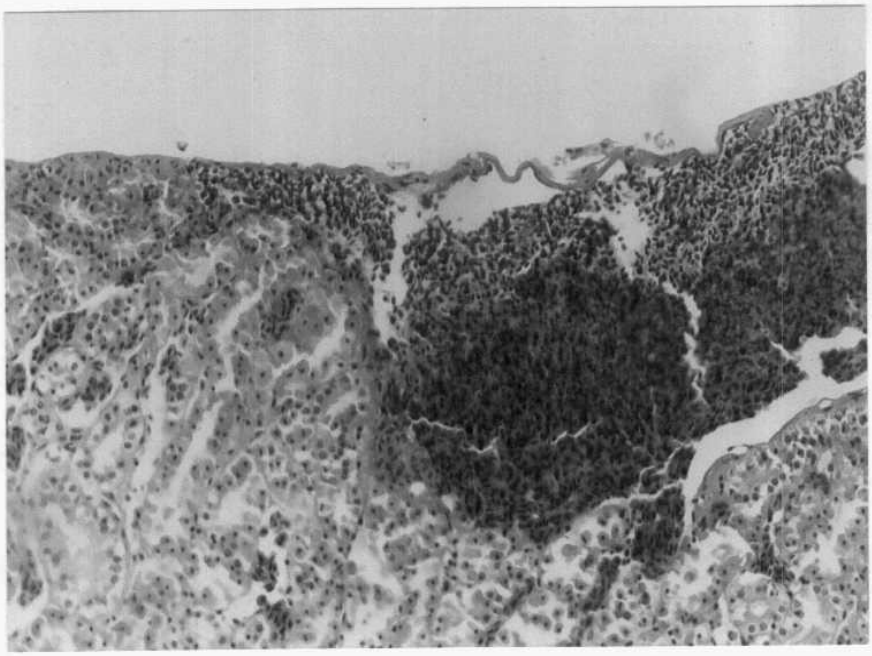

Fig (39) Myeloid leukosis, kidney showing sub capsular haemorrhage and tubular degeneration (H &E x200).

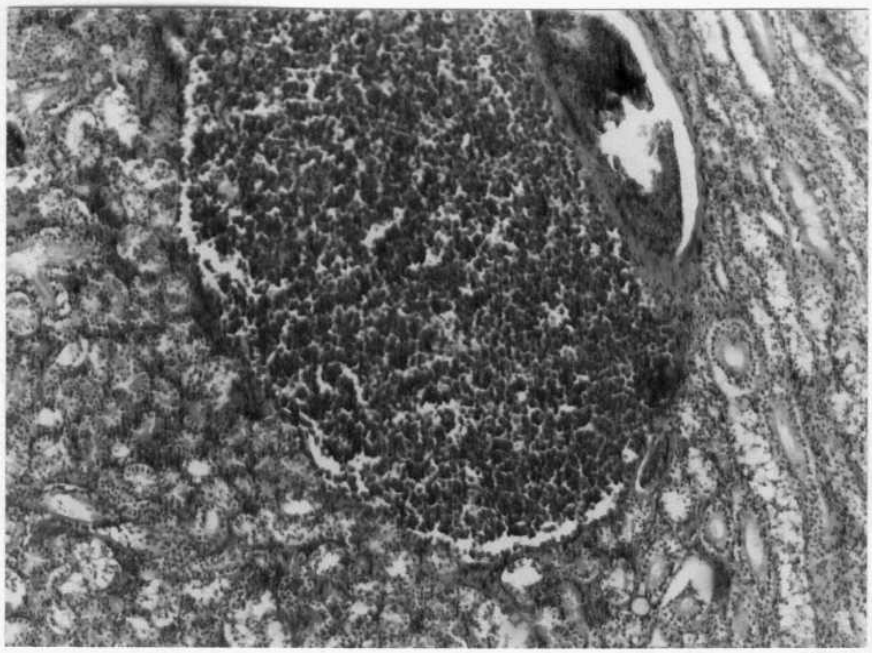

Fig (40) Myeloid leukosis, kidney showing focal aggregation of myelocytes surrounding the dilated blood vessels. (H &E x100)

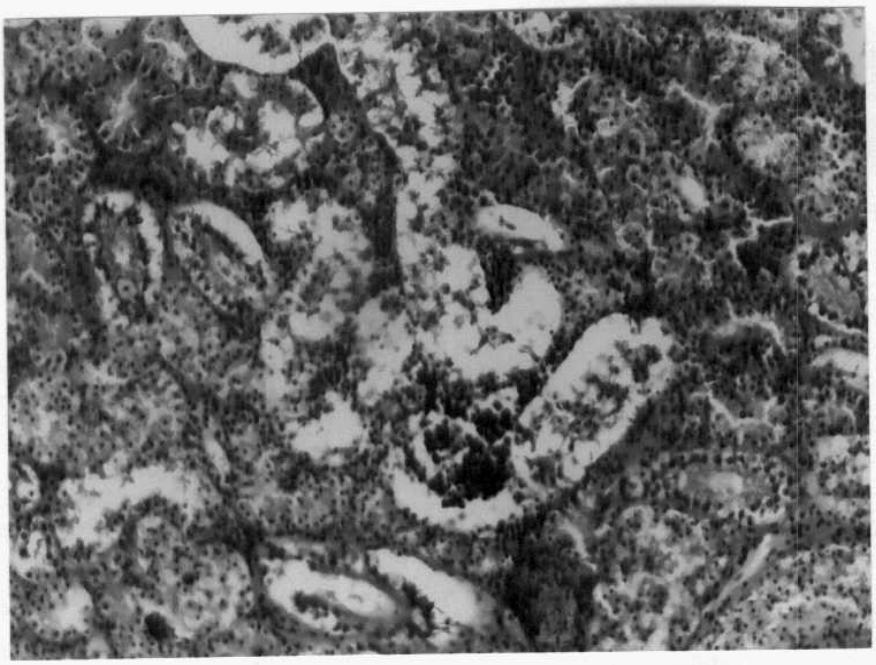

Fig (41) Myeloid leukosis, kidney showing necrobiotic changes of tubular epithelium with severe sloughing (H& E x100).

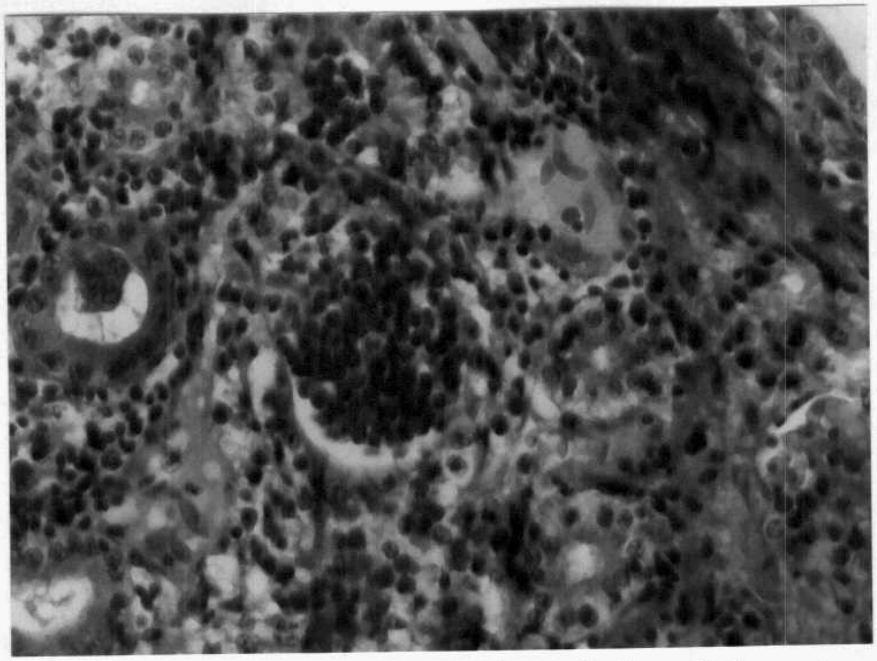

Fig. (42) Myeloid leukosis, kidney showing intratubular cellular cast composed mainly of myelocytes and epithelial cells (H & E x200).

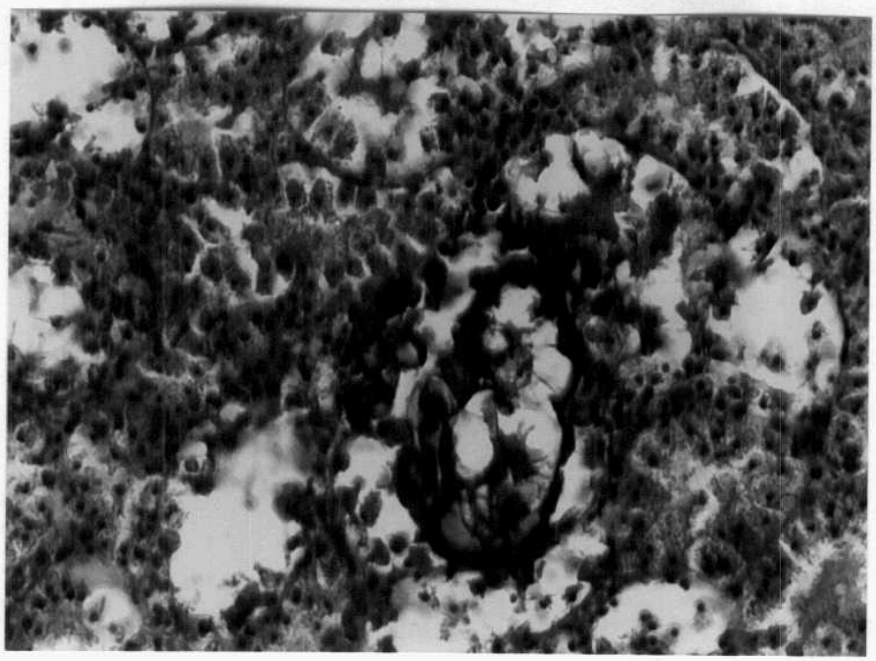

Fig. (43) Myeloid leukosis, kidney showing mineralization of degenerated renal tubules. (H & E x200)

Fig (44) Myeloid leukosis, thoracic cavity showing white to creamy color nodules on the inner surface of sternum.

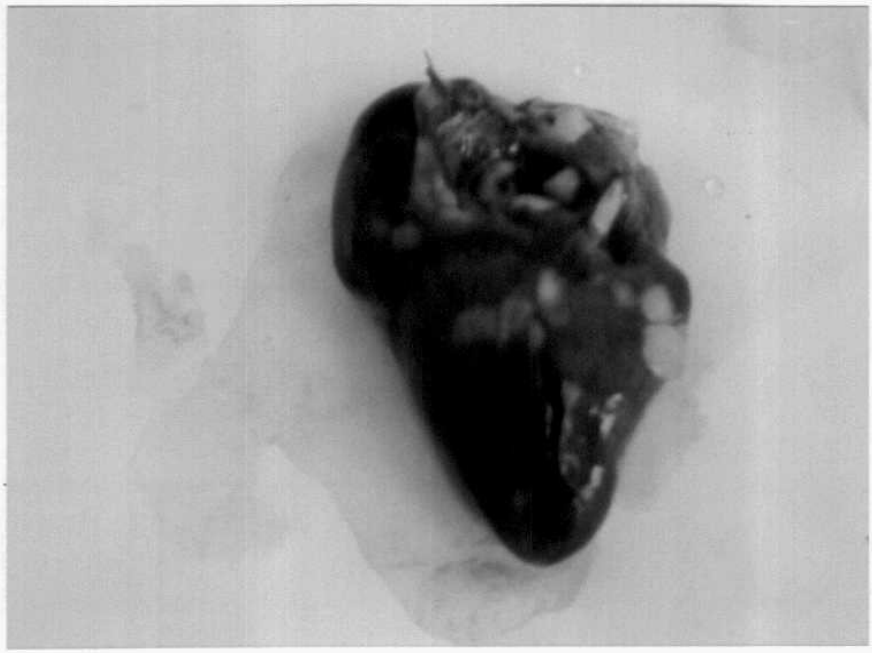

Fig (45) Myeloid leukosis, heart showing multiple yellowish white nodules of varied size and shape.

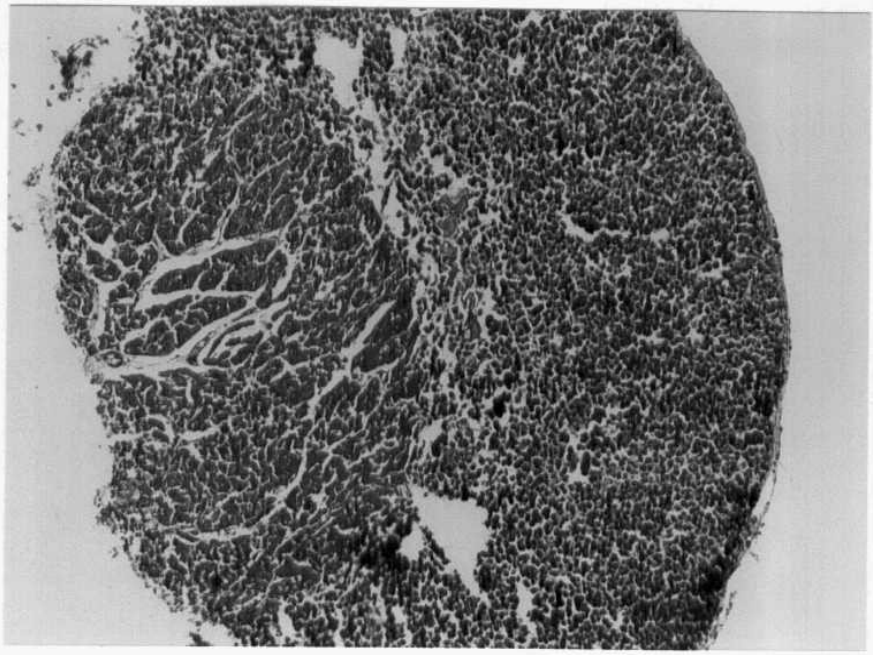

Fig. (46) Myeloid leukosis, heart showing diffuse aggregation of myelocytic cells (H & E x40).

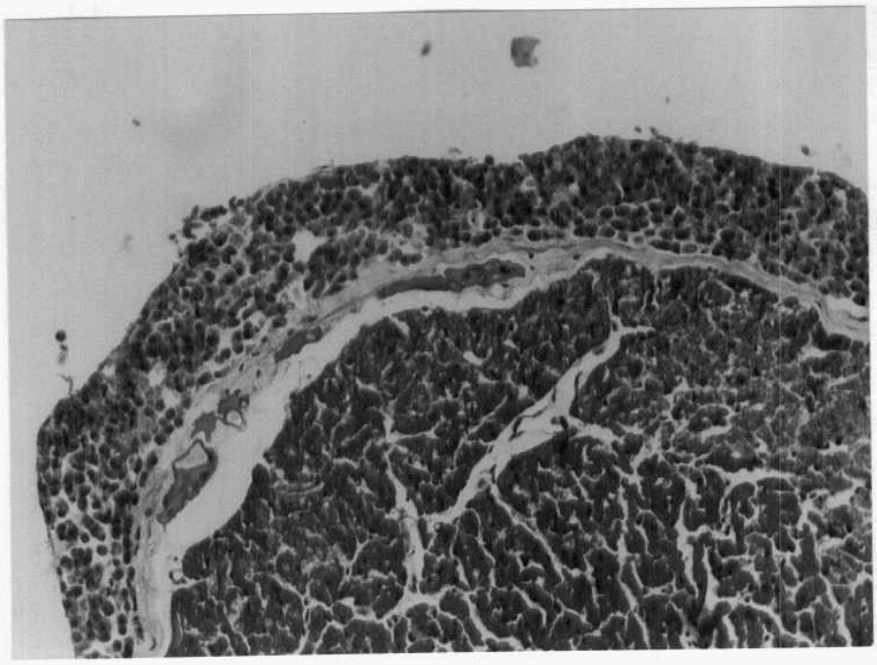

Fig (47) Myeloid leukosis, heart showing epicardium infiltrated with aggregated myelocytes. (H&E x100).

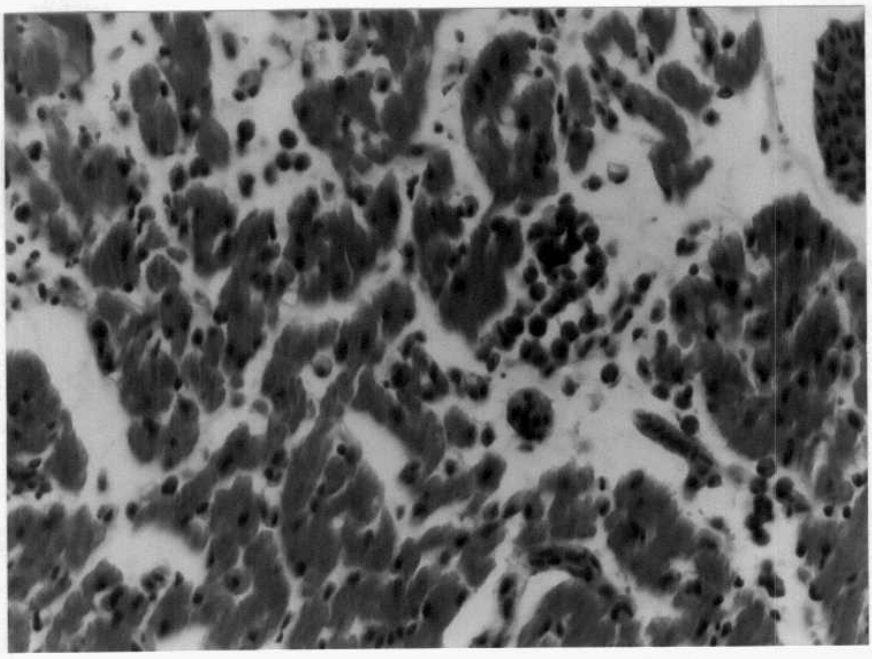

Fig (48) Myeloid leukosis, heart showing myelocytic infiltration between the cardiac muscle fibers (H & E x200).

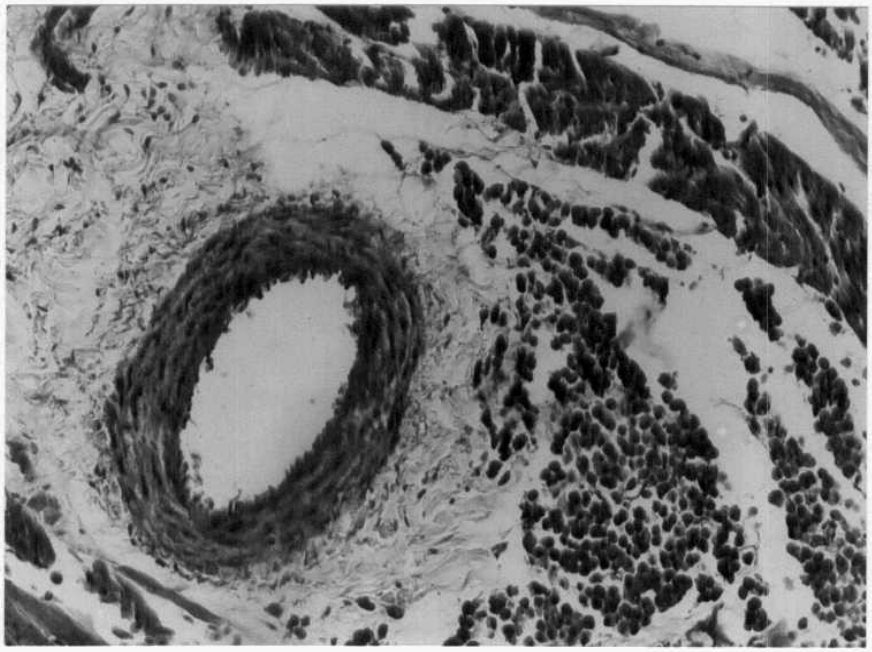

Fig (49) Myeloid leukosis, heart showing perivascular edema with focal aggregation of myelocytes around blood vessels (H & E x100).

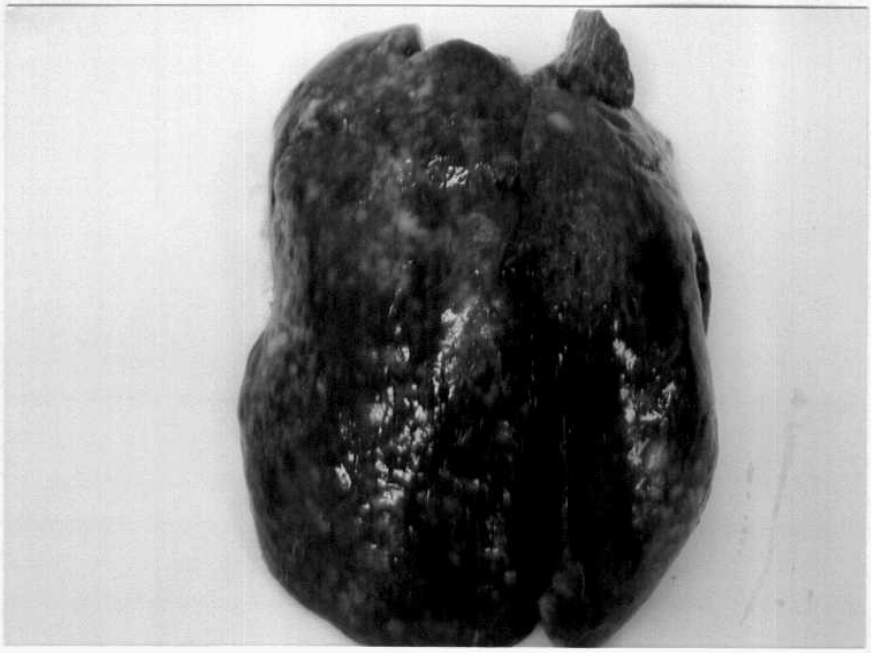

Fig. (50) Myeloid leukosis, liver showing diffuse enlargement with white creamy nodules on its surface.

Fig (51) Myeloid leukosis, liver showing diffuse enlargement of hepatic lobes in comparison with the liver of non-infected bird.

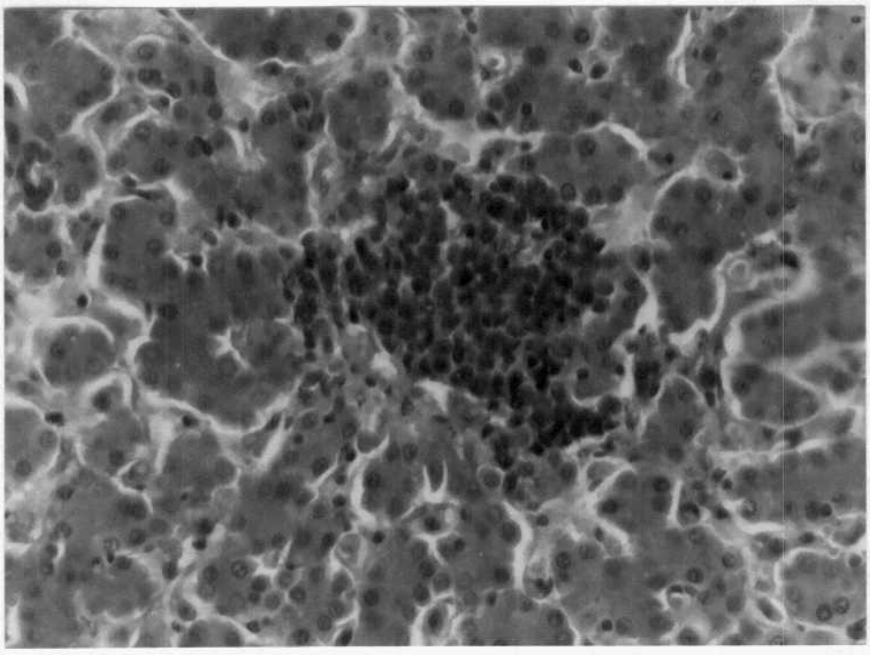

Fig (52) Myeloid leukosis, liver showing small focal aggregation of myelocytic cells, with dilated disse's space (H & E x200).

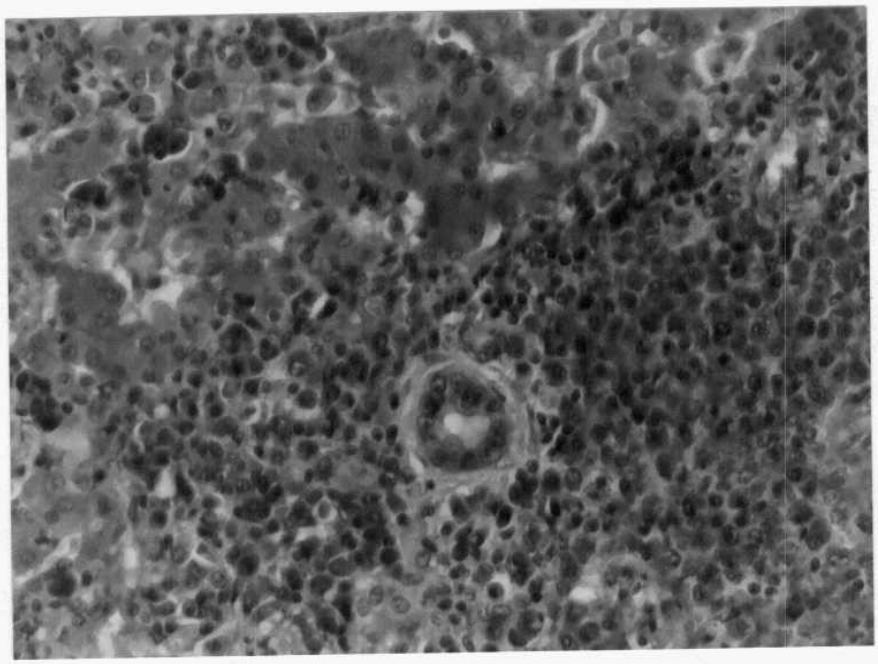

Fig (53) Myeloid leukosis, liver showing aggregation of myelocytes in portal triad (H&E x400).

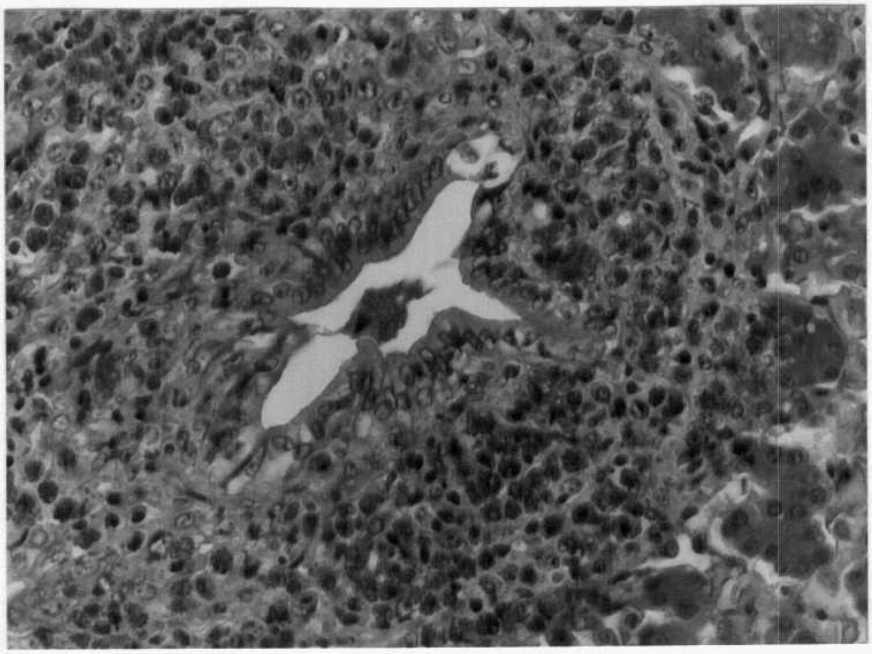

Fig (54) Myeloid leukosis, liver showing hyperplasia of bile duct surrounded by myelocytic cells. (H & E x400).

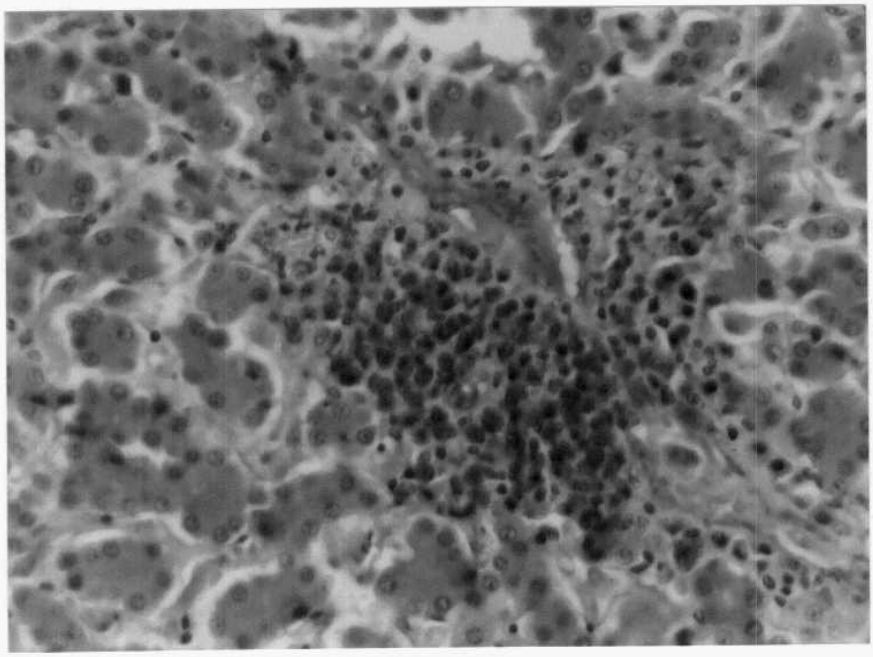

Fig (55) Myeloid leukosis, liver showing perivascular myelocytic cell aggregation. (H&E x400).

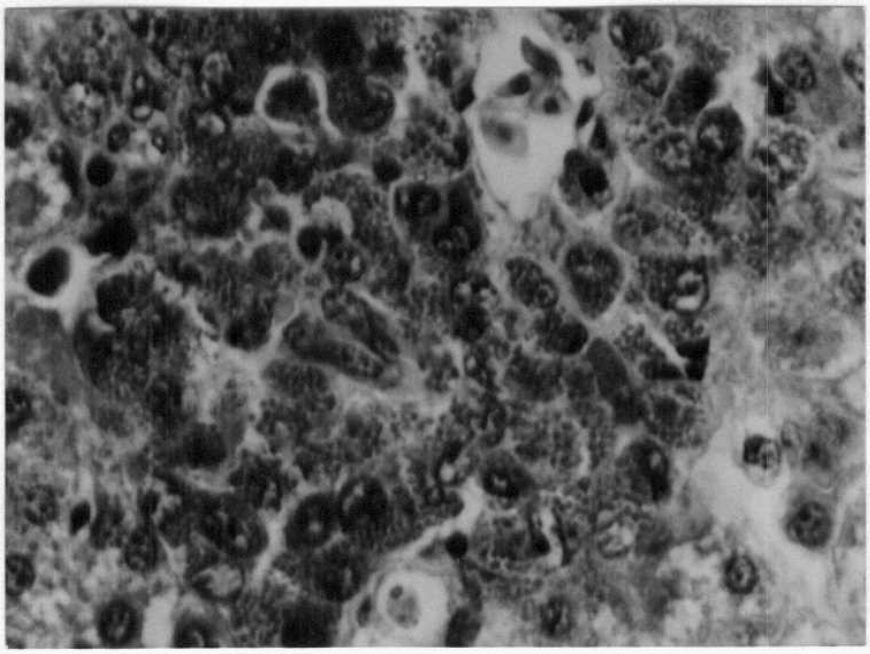

Fig. (56) Myeloid leukosis, liver showing myelocytic cells with eccentric nucleus and cytoplasm packed with conspicuous coarse eosinophilic granules.(H & E x1000).

Fig. (57) Myeloid leukosis in skeletal muscles showing multiple grayish white nodules.

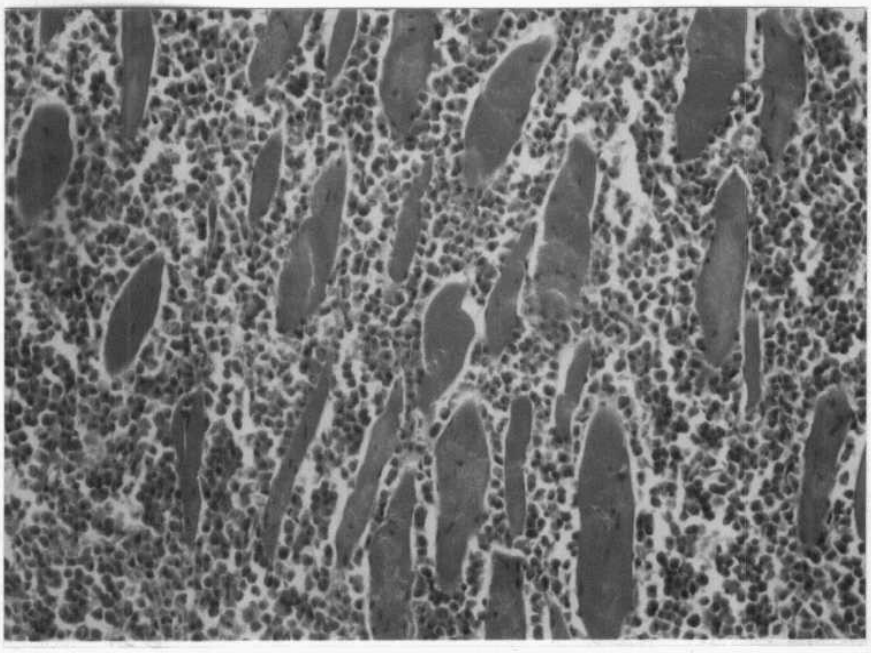

Fig. (58) Myeloid leukosis ,skeletal muscles showing diffuse infiltration of myelocytes with Zenker's necrosis of muscle bundles. (H & E X100).

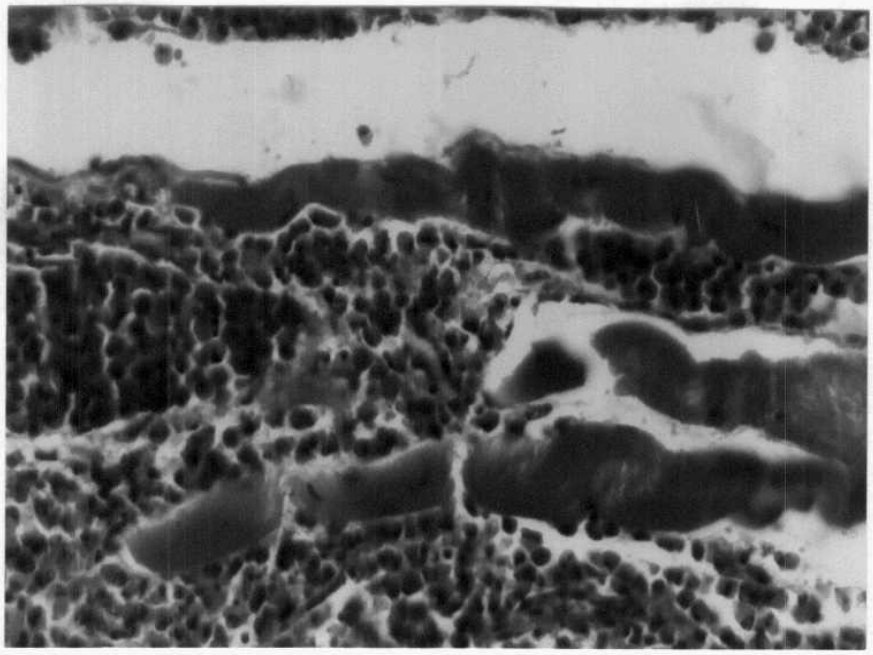

Fig (59) Myeloid leukosis ,skeletal muscle showing diffuse infiltration of myelocytes.(H & E x400)

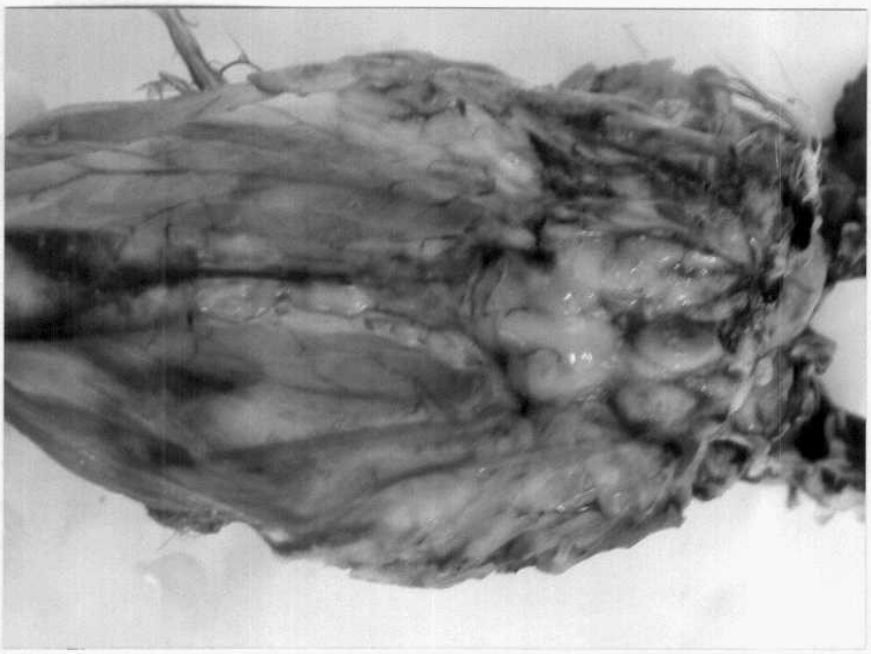

Fig (60) Myeloid leukosis ,sternum showing multiple creamy white nodules scattered on the inner surface of the sternum.

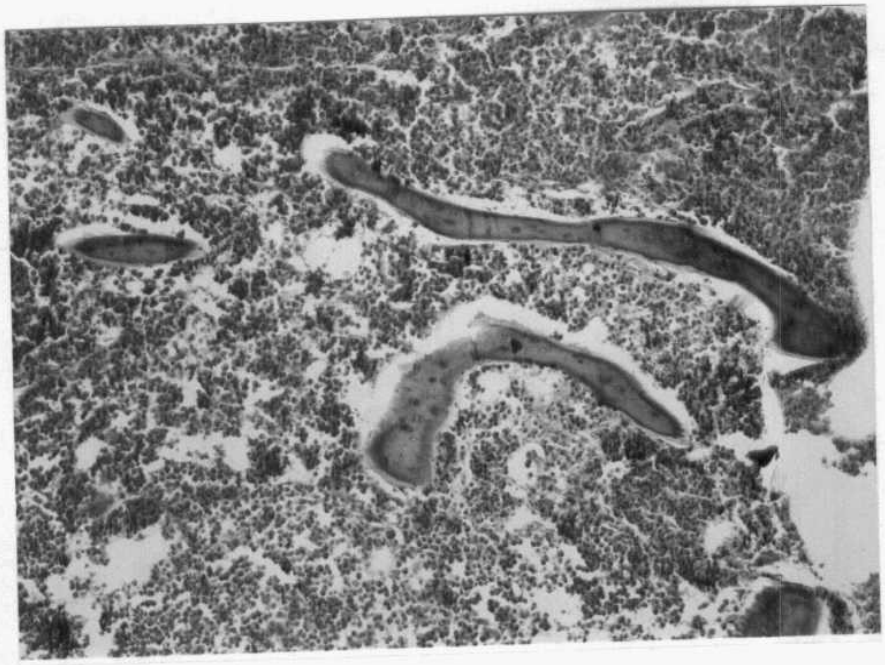

Fig (61) Myeloid leukosis ,sternum showing diffuse infiltration of myelocytes between cartilaginous part of sternum. (H & E X40)

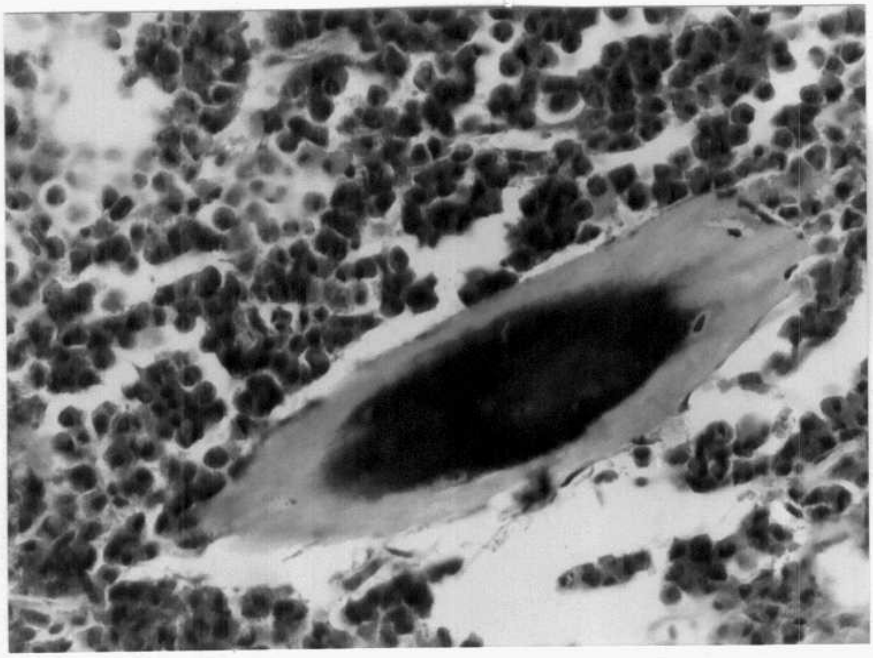

Fig (62) Myeloid leukosis ,sternum showing diffuse infiltration of myelocytes around degenerated cartilage (H & E x400).

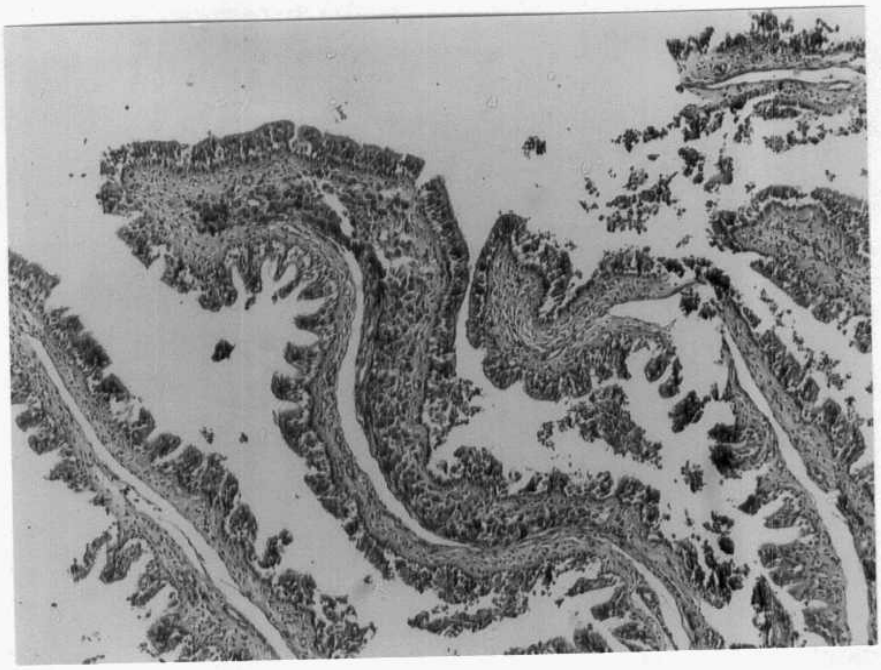

Fig (63) Myeloid leukosis , intestine showing myelocytic infiltration in intestinal villi. (H & E x100)

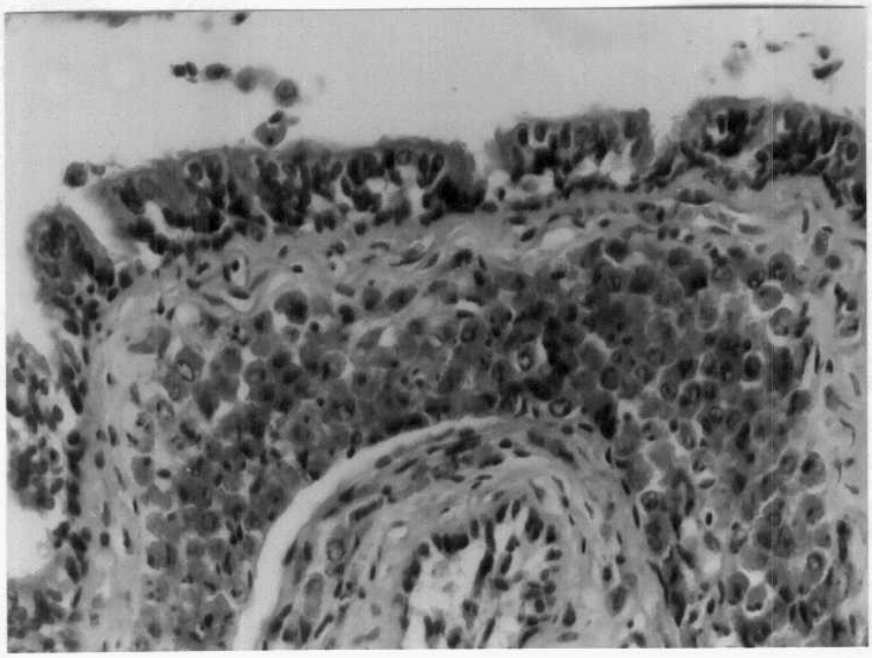

Fig (64) Myeloid leukosis ,intestine showing infiltration of myelocytes in the lamina propria of the intestinal villi. (H & E x200)

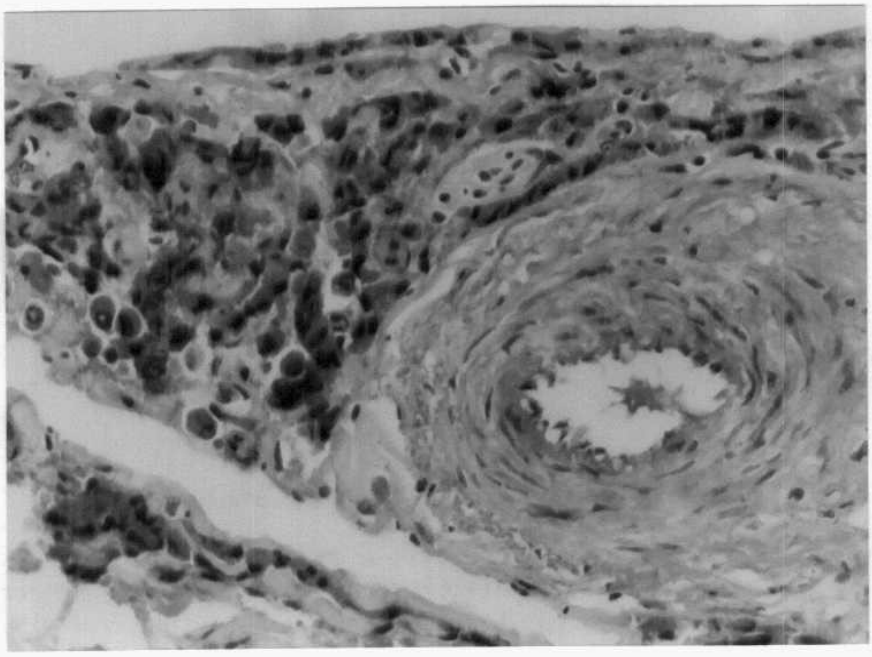

Fig (65) Myeloid leukosis ,intestine showing perivascular aggregation of myelocytes (H & E x200)

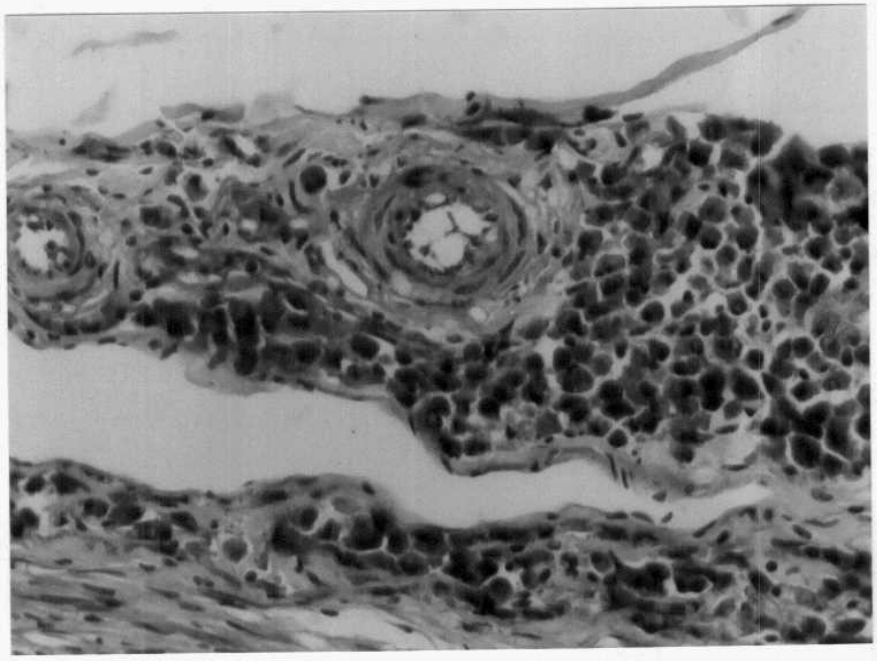

Fig. (66) Myeloid leukosis ,intestine showing central lactae surrounded by mature myelocytic cells (H&E x200)

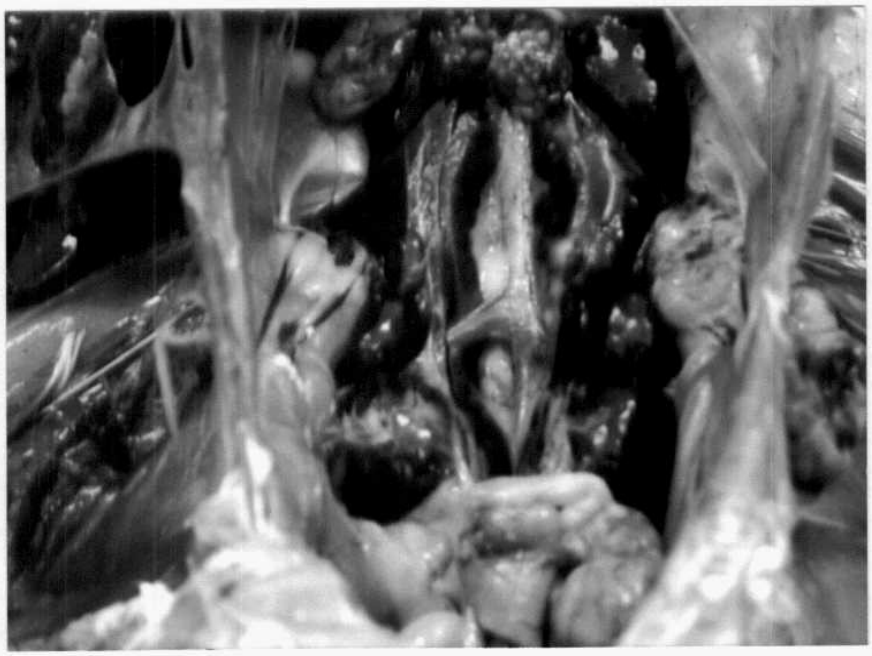

Fig (67) Myeloid leukosis ,ovary showing atrophied ovarian follicles.

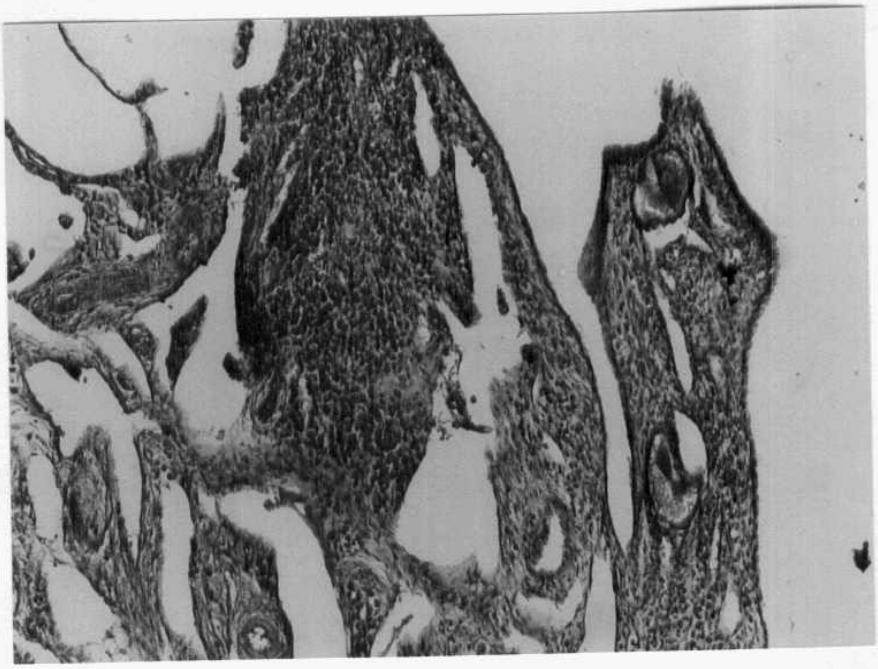

Fig (68) Myeloid leukosis ,ovary showing atrophy of ovarian follicles with focal aggregation of myelocytes (H&E x 100)

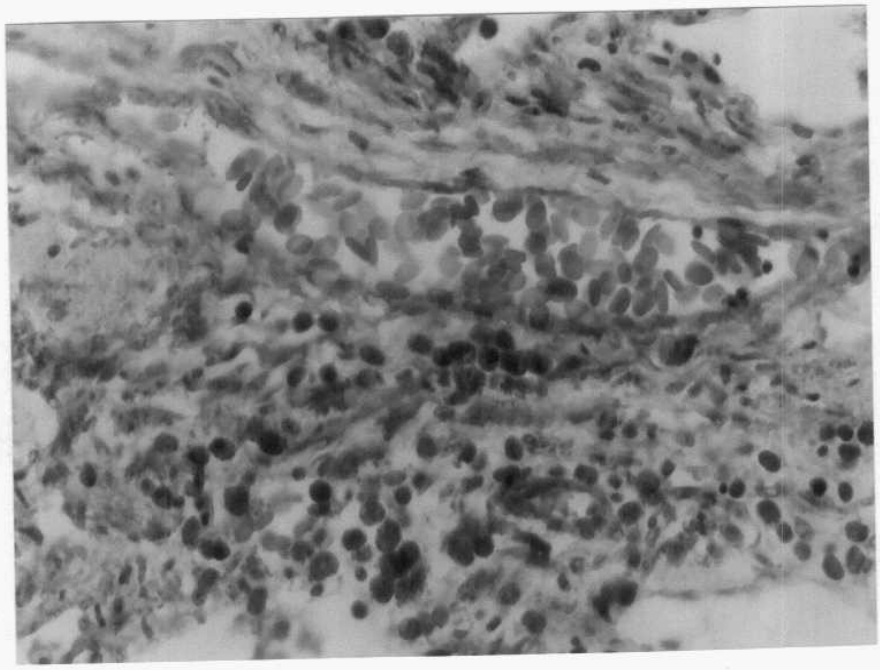

Fig (69) Myeloid leukosis ,ovary showing perivascular myelocytic infiltration (H&E x200).

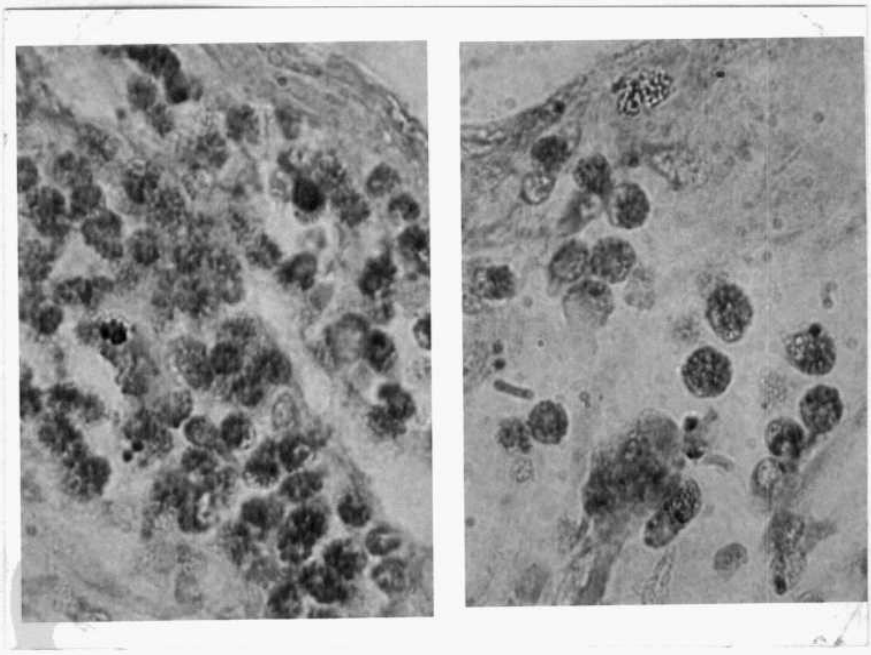

Fig (70) Myeloid leukosis ,ovary showing myelocytes with brilliant red cytoplasmic granules stained with May Grunwald Gimsa. (x400)

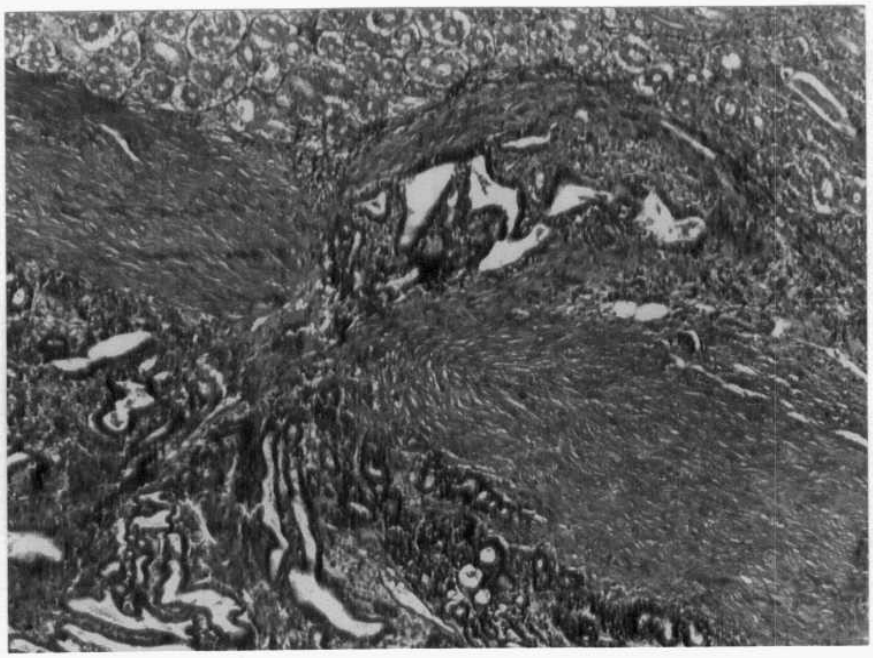

Fig (71) Nephroblastoma showing fibrous C.T. separate the apparently healthy renal tissue from the tumor part (H&E x100)

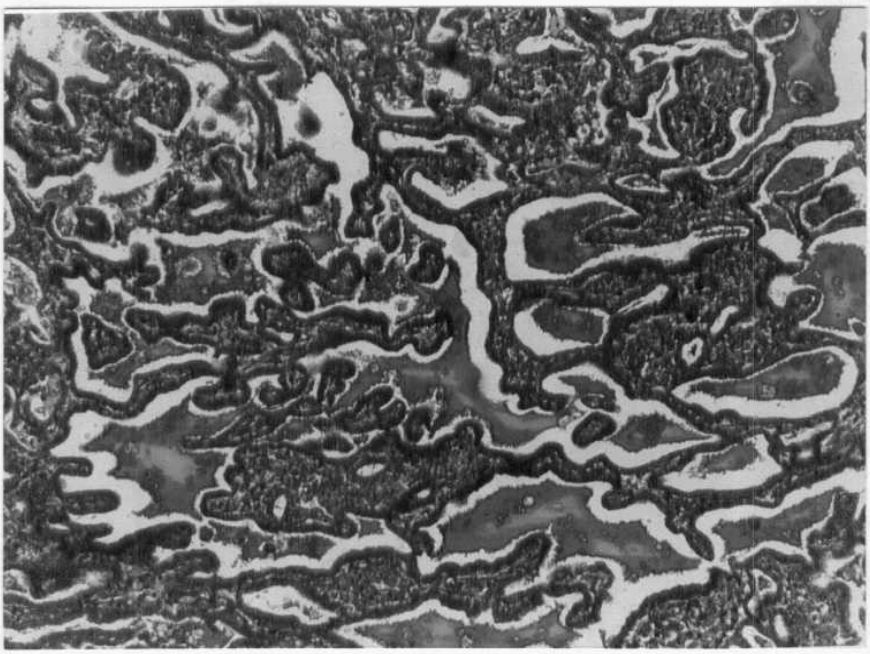

Fig (72) Nephroblastoma showing undifferentiated cystic renal tubule with intraluminal proteinacious cast. (H&E x100)

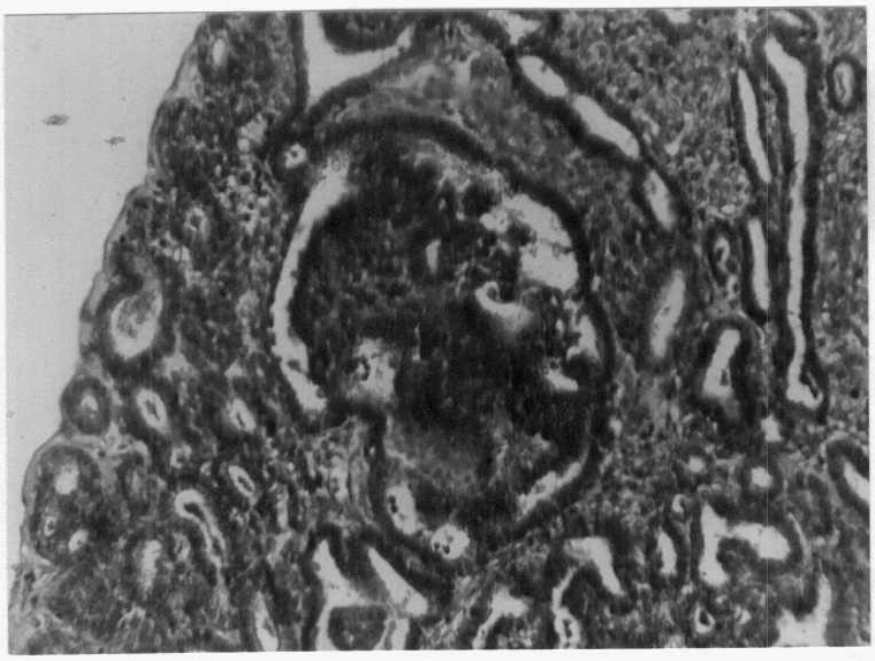

Fig (73) Nephroblastoma showing primitive glomerulus.(H&E x200)

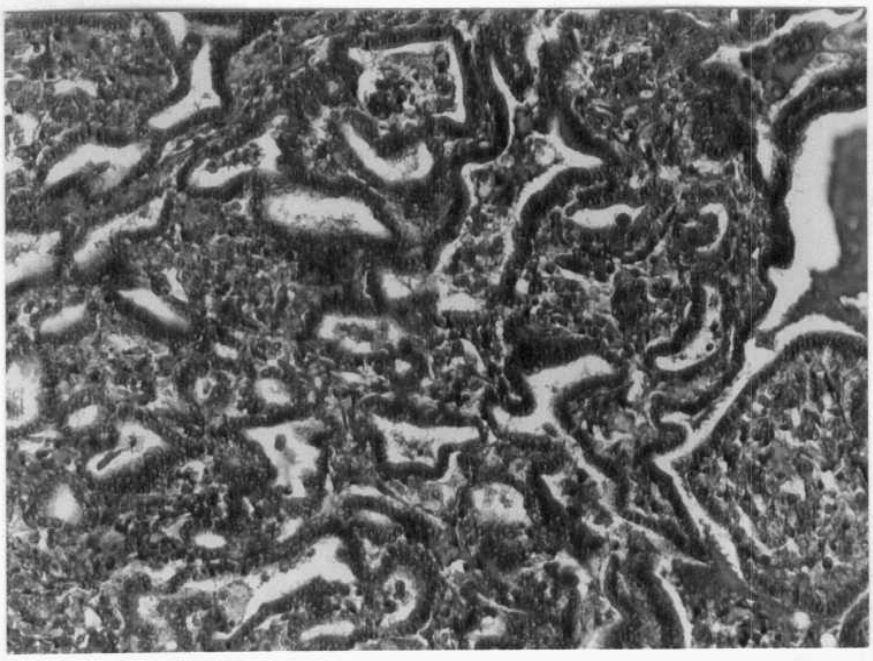

Fig (74) Nephroblastoma showing undifferentiated basophilic tubular epithelial lining (H&E x200)

Discussion

The present work is designated to study the diagnosis of avian leukosis through investigation of the histopathological changes in the affected chicken flocks of both layers and broiler parents with avian leukosis viruses in different provinces and to differentiate between the lymphoid leukosis and the myeloid leukosis using pathological, serological, and molecular biological methods.

Lymphoid Leukosis

Avian leukosis viruses (ALVs) are well known for their ability to induce neoplasia in chickens. Other manifestations met with lymphoid leukosis virus infection were observed during the present study as decrease in egg production reached 30% in broiler parents, increasing mortalities up to 15%, and decrease of hatchability. These findings come in agreement with that reported by **Gavora *et al.* (1980).**

Lymphoid leukosis tumors that occurred in chickens become grossly visible after 4 months of age or older. Lymphoid leukosis affect several organs and tissues but primarily appeared in the liver, spleen and bursa of fabricius however; many other organs and tissues may be affected such as kidneys, gonads, heart and mesentery.

The size of tumor and the type of affected organs are highly variable. Tumors were soft, smooth and glistening and on cut section appeared grayish to creamy white. Tumors may be nodular, miliary and diffuse or a combination of these forms but the most common form was the nodular one. It was usually spherical but may be flattened when located close to the surface of an organ. The miliary form, which was the most obvious one in the liver, consisted of numerous small nodules uniformly distributed throughout the parenchyma. In the diffuse form, the organ was uniformly enlarged, slightly grayish in color, and usually very friable. These findings come in agreement with that stated by **Purchase (1987)** and **Payne & Fadly (1997)**

Microscopically, lymphoid leukosis tumors were focal and multiple in origin, even in the diffuse form it can be considered as coalescing of multifocal aggregations of neoplastic lymphoblastic cells. As tumour cells proliferated, leading to displacement and compressing the cells of the affected organ rather than infiltrate between them. These findings coincide with that attributed by **Payne & Fadly (1997).**

The nodular tumour form of liver appeared as focal aggregations of neoplastic lymphoblastic cells that usually surrounded by band of fibroblast like cells. These findings have shown to be remnant of sinusoidal endothelial cells and it has been previously elucidated by **Gross *et al.* (1959)**

In the diffuse form massive lymphoblastic cells were infiltrating the hepatic lobules and replacing most of the hepatic parenchyma. The hepatocytes appeared as solitary islets inside the massive infiltration of lymphoblastic cells.

Hepatocytes showed degenerative changes characterized by swelling and hypergranularity of cytoplasm. Apoptosis of hepatocytes were observed in the form of apoptotic eosinophilic bodies contained remnants of nuclear material. The neoplastic cell populations were characterized by large lymphoblastic cells in uniform sizes with poorly defined cytoplasmic membrane and basophilic cytoplasm. These findings coincide with that described by **Payne & Fadly (1997).**

Tumors of lymphoid leukosis consisted of large lymphoid cells (lymphoblasts) aggregates at the same primitive developmental stages, these cells have poorly defined cytoplasmic membrane, much basophilic cytoplasm and vesicular nucleus with margination and clumping of chromatin and obvious one or two eosinophilic nucleoli.

The cytoplasm of most tumor cells stained deeply red with methyl green pyronin and that finding attributed by **Cooper *et al.* (1968)**

These tumor cells contained large amount of RNA which stained red due to high pyroninophilic affinity of the cytoplasm of tumor cells, indicating that the cells are immature and rapidly dividing and that justified by **Cooper *et al.* (1974).**

Bursa of affected birds by lymphoid leukosis was greatly enlarged. On incision it showed loss of distinct bursal pelicae (bursal folds) and great thickening of the wall with prominent grayish white nodules which appeared on the inner surface of the bursa of fabricius.

The histopathological picture of the bursa of fabricius revealed great enlargement of bursal lymphoid follicles. There was loss of distinction between cortex and medulla of bursal lymphoid follicles. The affected bursal follicles became engorged with uniformly neoplastic lymphoblast cells. These cells showed high pyroninophilic cytoplasm with methyl green pyronin stain. These lymphoid follicles were surrounded by thickened fibrous connective tissue. The affected follicles showed expansion and displacement of the adjacent normal bursal follicles.

The bursa of fabricius plays the master role in transformation of B-lymphoid cells and act as a source of metastasis of malignant B- lymphoma. The histopathological examination of bursa in the present work has provided the evidence that lymphoid leukosis is malignant tumor-bursal dependant lymphoid system, and it was explained by **Neiman *et al.* (1980), Crittenden & Kung (1984).**

Baba & Humphries (1985) gave the justification of early transformation of malignant lymphoma of bursa. The infection persists longer in bursal lymphocytes than in other

hematopoietic tissues, and cells of the bursa of fabricius are the target cells that neoplastically transformed. The target cells must be resident in the bursa, because surgical bursectomy up to 5 months of age and other treatments that destroy the bursa of Fabricius will eliminate the disease and that was explained by **Purchase and Gilmour (1975), Chievelle *et al.* (1978).** Medullary macrophages appeared to be the principal bursal cells for virus replication and may be important in transmitting infection to the lymphoid cells (**Gilka and Spencer, 1987).** The proliferation of lymphoblasts occurred in one or more lymphoid follicles in the bursa. These altered bursal follicles are termed transformed follicles (**Purchase, 1987),** and the change is regarded as a focal preneoplastic hyperplasia as reported by **Humphries and Baba (1984)**.

The transformed follicle is a consequence of activation of the c-*myc* gene by nearby insertion of ALV. The c-*myc* gene under the control of the enhancers of the viral LTR, results in over-expression of *myc*, causing a maturation arrest and proliferation of bursal stem cells. Arrest of maturation of the transformed B cells results in interference of the bursal follicle and is not neoplastic. Sometimes, many follicles are transformed, but the majority of these appear to regress, and only a few continue to grow giving rise to nodular tumors in the

bursa, which are visible grossly from about 14 weeks of age as reported by **Cooper *et al.* (1974) and Neiman *et al.*(1980).**

From about 12 weeks of age, cells in the clonal bursal tumors metastasize to other organs and tissues and result in the terminal disease. Metastatic tumors in the visceral organs usually have the same DNA fragments as bursal tumors from the same birds, supporting their clonal origin, these findings are greatly parallel with that reported by **Crittenden and Kung (1984).**

Subcutaneous Tumor:

Connective tissue tumours appeared as swelling or protrusion of the integument and were observed in head and neck of chickens. These usually are solitary, firm but not hard and creamy white to dull red in colour. The overlying skin was wounded resulting in ulceration. Histopathologically, the tumour identified as myxoma which composed of loose C.T. areas with abundant mucinous matrix. Stellate and spindle shaped cells were predominant. Long cytoplasmic processes may extended from the cells and became fused with sparse collagen fibers.

These findings come parallel with **Purchase *et al.* (1972)** as they reported that connective tissue tumors caused by ALSVs containing *v-onc* gene and **Payne & Fadly (1997)** who explained that member of ALV causes connective tissue tumors as fibroma, myxoma, myxosarcoma, fibrosarcoma, and histiocytic sarcoma.

Murphy *et al* (1999) estimated the incidence of C.T. tumor and they found that its occurance is not less than 1 in 1000 of cases.

These findings come in agreement with **Masaaki *et al.* (2004)** that extensively investigated and approved an outbreak of subcutaneous tumour associated with subgroup-A ALV in young layer chickens.

Myeloid Leukosis (Myelocytomatosis):

The gross lesions of myeloid tumours are distinctive and can be recognized. Characteristically myeloid tumors affected bone occurred on its surface in association with periosteum and near the cartilages, so any adjacent tissue or organ may be affected. Tumours often developed at the costochondral junction of the ribs and inner sternum. Flat bones of the pelvis and synsacrum were also affected. These findings coincided with **Payne *et al.* (1991) and Payne & Fadly (1997).**

Our results revealed that myeloid tumours were dull, grayish white or creamy white in colour, friable to cheesy and nodular or diffuse in arrangement. These findings come in agreement with **Payne *et al.* (1991)**.

Histopathologically, tumours consisted of aggregated masses of uniformly arranged myelocytes with very little stroma so, the myeloid tumours characterized by high cellular and less stromal connective tissue.

The myelocytic cells characterized by large vesicular eccentrically located nucleus. The cytoplasm was tightly packed with eosinophilic granules which were usually spherical. When these cells stained by May- Grünwald- Gimsa the granules appeared brilliant red or purple. These findings come in agreement with that reported by **Mladenov *et al.* (1967).**

In the liver, myelocytes firstly accumulated near blood vessels and portal triads then invaded the hepatic cords. Also; myelocytes were replacing the hepatocytes leads to degenerative changes and atrophy of the hepatic cells. The principal phenomena of this pattern are the formation of invasive growth in the parenchymatous organs **(Beard, 1980).**

Two types of cells were reported in myeloid leukosis, the myeloid stem cell that differentiated into myelocytic level which can be granulated or arrested at non-granulated stage. These findings coincide with that reported by **Mladenov *et al.* (1967).**

Myeloid leukosis was associated with the infection of a novel subgroup of ALV designated J in meat type chickens in UK as firstly recorded by **Payne *et al.* (1991).**

Also, **Mona,(2000)** reported ALV –J associated with myeloid leukosis in meat type chickens in Egypt. Since the first report, myeloid leukosis has occurred in broiler breeder flocks in many countries causing serious losses. The virus spreads vertically and horizontally and has carried out severe damage to

the poultry industry. In recent years, studies have shown that sequence changes of *env* gene in variable regions caused rapid variation of the antigenicity of ALV-J leading to emerging of variant viruses due to the antigenic variation as elucidated by **Venugopal *et al.* (1998).**

Acutely transforming strains of ALV that induce myelocytomatosis, such as MC29 and CMII, which carry the v-*myc* oncogene was reported by **Enrietto and Hayman (1987).** Slowly transforming strains of subgroup- J ALV that also induce myelocytomatosis, such as HPRS-103 and ADOL-Hc1, do not carry an oncogene, but molecular studies of HPRS-103 that induced myelocytomatosis indicated that c-*myc* was activated **(Chesters *et al.*,2001)**. The acutely transforming strain 966 ALV, derived from myelocytoma and induced by strain HPRS-103 of subgroup- J ALV, has been shown to carry v-*myc* as reported by **Payne *et al.* (1993).**

Studies on HPRS-103 and 966 showed that they have a tropism for the myelomonocytic cell lineage rather than the lymphoid cell lineage, which may related to their ability to cause myelocytomas as reported by **Arshad *et al.* (1997), (1999)**.

The earliest alterations occurred in bone marrow in which there was crowding of intrasinusoidal spaces, principally by myelocytes, and destruction of sinusoidal walls. The spaces may contained 2 types of cells, the myeloid stem cell and the neoplastic myelocyte. The latter appears to arise directly from

the stem cell, and differentiation is arrested both at the nongranulated and granulated myelocyte level. Myelocytes proliferated and soon overgrow the bone marrow. Tumours formed by expansion of marrow growth and may crowd through the bone and periosteum. Extramedullary tumours may also arised by blood-borne metastasis as stated by **Payne and Fadly (1997).**

Nephroblastoma:

Nephroblastoma considered as embryonal highly malignant tumour. This type of tumour was recorded in two examined birds infected with ALV-J. The kidneys of infected birds showed spherical, well demarcated tumour mass replaced part of the parenchyma. On cut section, the tumor appeared soft, friable, grayish white with haemorrhagic spots.

Microscopically, the tumour mass was separated from apparently healthy tissue by thick fibrous connective tissue and leukocytic infiltration. Structures like glomeruli or metanephric precursor of glomeruli were observed. The interstitial tissue showed polymorphic stromal cells which appeared round or stellate in shape. The renal tubules lined by cuboidal or columnar epithelium with hyperchromatic nuclei. The epithelium of the renal tubule was merged with stromal cells.

The finding reveal that nephroblastoma was found in association with ALV-J infection. This comes in accordance with **Payne *et al.* (1993)**

Serological results shown in table 4 revealed that 4 of 10 suspected flocks suffered from decrease in egg production, high mortalities and progressive visceral tumours were positive for detection avian leukosis virus antibodies by different percentage and titer.

Presence of ALV antibodies indicated either current or past infection with ALV. The negative flocks were the rest 6 flocks that were negative for ALV antibodies in serum by ELISA which indicated that these flocks may be free from ALV infection but, may be infected with other subgroup of ALV. It may be due to that, these birds was immune tolerant which do not produce antibodies against ALV as elaborated by **Arafa (2000).** These results supported our histopathological results of lymphoid leukosis.

The results shown in table 5 revealed that 6 flocks were positive for ALV-J antibodies by different percentage and titer. Presence of ALV-J antibodies indicated that, these flocks exposed either to the current or past infection. The rest 4 flocks were negative for ALV-J antibodies. Identification of ALV-J infected flocks can be made by detecting ALV-J specific antibodies in the serum. This can either be done by

neutralization test or by using an antibody ELISA on plate coated ALV-J infected cell lysate or a recombinant baculovirus derived HPRS-103 gp85 envelope glycoprotein (**Venugopal *et al.*, 1997**) and as reviewed by **Venugopal (1999).**

Results shown in Table 6 described the detection of ALV-J DNA in tissues from the affected organs (liver, kidney, and ovary) with tumours. The results of polymerase chain reaction gave the confirmation to the results of histopathology for myeloid leukosis. It gave positive amplifications at 545 bp to the 4 flocks but the 2 flocks which gave negative result supported the results of lymphoid leukosis in histopathological and serological examination. These results coincide and came parallel with the data reported by **Smith *et al.* (1998), and Binrui *et al.* (2004).**

Summary&Conclusions

- This work was planned to study the gross and microscopical lesions of different tumors induced by avian leukosis ALV in particular the new subgroup-J in broiler breeders flocks in order to inspect the diagnostic aspects to differentiate macroscopically and microscopically between the lymphoid leukosis and myeloid leukosis.
- This study was carried out on 245 as total number of chickens of 10 flocks from different provinces. The examined cases were classified into 47 commercial layers and 198 broiler parents.
- Serological test were conducted on serum samples to detect the antibodies of avian leukosis virus ALV that reflect the exposure of virus infection.
- Polymerase chain reaction PCR was carried out on tissue samples to confirm the presence of DNA of ALV subgroup J.
- The histopathological results revealed that 4 flocks showed lymphoid leukosis tumors (2 layers and 2 broiler parents). The affected organs with LL were bursa of fabricius, liver, heart, kidneys, ovaries, spleen, intestine and skeletal muscles. These affected organs showed focal or diffuse form of tumor lesion particularly the liver showed 3 forms of tumors which were milliary, nodular and diffuse aggregations of infiltrated large lymphobalstic cells.

- These cells made displacement and compression to the adjacent tissues leading to degenerative changes and necrosis. In lymphoid leukosis the bursa was considered as the master organ of transformation of the lymphoblastic cells to the neoplastic form and the origin of metastasis to the other visceral organs. The neoplastic cell populations characterized by large lymphoblastic cells, homogenous in size with poorly defined cytoplasmic membrane and basophilic cytoplasm. The nuclei appeared vesicular in which margination and clumping of chromatin with mitotic figures. These cells showed high pyroninophilic cytoplasm with methyl green pyronin stain due to high RNA in the cytoplasm.

- Another type of tumors induced by ALV was subcutaneous myxoma tumor. This myxoma appeared as protrusion of the integument observed on head and neck and consisted mainly of loose embryonal connective tissue with abundant mucinous matrix. The cells were stellate or spindle shaped cells which were predominated.

- The results of ELISA for detection of ALV antibodies in sera of the examined flocks revealed that 4 flocks showed lymphoid leukosis were positive out of 10 flocks (4 /10) by percentage ranged from 60% to 75% of total (68 / 245) 27.7%.

- The other 6 flocks (6 broiler parents) showed myeloid leukosis induced by the novel subgroup-J of ALV. The affected organs with ML were Bones and cartilages, liver, heart, kidneys, ovary,

spleen, intestine and skeletal muscles. These affected organs showed diffuse enlargement of the organs and myeloid tumors appeared as nodules on the inner surface of sternum, ribs and synsacrum. The myeloid cells were observed around the veins and arteries in the visceral organs. Myelocytic cells characterized by eccentrically located nuclei and had marked nucleolus with clear mitotic figure. The cytoplasm was filled with conspicuous spherical eosinophilic granules. These myelocytic cells showed characteristic brilliant red colored cytoplasmic granules when stained with May- Grünwald Gimsa.

- Another type of tumors induced by ALV subgroup-J was observed. Nephroblastoma considered as embryonal highly malignant tumor. The kidney of infected birds showed spherical well demarcated tumor mass, replaced part of the parenchyma. The tumor tissue showed undifferentiated cystic renal tubules with intraluminal proteinacious cast. The interstitial tissue showed polymorphic stromal cells which appeared round or stellate in shape. Metanephric precursors of glomeruli were observed. The renal tubule lined by cuboidal or columnar epithelium with hyperchromatic nuclei.

- The results of ELISA for detection of ALV -J antibodies in sera of the examined flocks revealed that 6 flocks showed myeloid leukosis were positive out of 10 flocks (6 /10) by percentage ranged from 70 % (21/30) up to 88% (22/25) of total (107/245) 43.7%.

- The result of PCR test of 6 examined flocks indicated positive amplification of 545 bp fragment with the extracted DNA of ALV-J for 4 flocks showed myeloid tumor lesion (4 broiler parent) and negative amplification for 2 flocks showed lymphoid tumor lesions (1 broiler parent and 1 layer).
- This study concluded that the gross lesions of lymphoid and myeloid leukosis were considered as the first aid in diagnosis.
- The histopathological examination plays a crucial and decisive role in diagnosis among different types of neoplasms caused by avian leukosis virus due to the different targeted cells affected (lymphoblastic and myelomonocytic lineage) that differentiate between the lymphoid and myeloid leukosis.
- Epithelial tumors as nephroblastoma had observed in our study in association with ALV subgroup –J infection. Also, connective tissue tumors as myxoma had observed in our study caused by ALV infection.
- Application of specific stains gave help in differential diagnosis between the lymphoid and myeloid leukosis.
- Detection of antibodies either of ALV or ALV-J through ELISA test assisted in differentiation between the lymphoid and myeloid leukosis.
- Application of PCR test verified the presence of DNA of ALV-J that induced the tumors of myeloid leukosis.

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Arabic Summary

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قسم الباثولوجيا

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الدرجة: الماجستير فى العلوم الطبية البيطرية
التخصص: باثولوجيا عام وخاص وتشريح مرضى

عنوان الرسالة: دراسات باثولوجية و مناعية لتشخيص الفيروسات المسببة لأورام الدجاج (مرض الليوكوزس فى الطيور).

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رئيس بحوث أمراض الدواجن و وكيل معهد بحوث صحة الحيوان.

المستخلص

تم فحص عدد ٢٤٥ عينة من الدجاج ممثلا ١٠ قطعان من محافظات مختلفة(٤٧من قطعان البياض التجارى و١٩٨ من قطعان أمهات التسمين) لدراسة التغيرات المرضية والهيستوباثولوجية للأورام المختلفة التى يحدثها فيروس الليكوزس في الطيور.تم فحص عدد ٢٤٥ عينة سيرم لاستبيان وجود الأجسام المضادة لفيروس الليكوزس الليمفاوى ومجموعة(J) كل على حدة باستخدام اختبار الاليزا.تم التحقق من وجود الحامض النووي الديوكسى ريبوزى لفيروس الليكوزس المجموعة(J) فى الأنسجة المصابة باستخدام اختبار أنزيم البلمرة المتسلسل. أظهرت النتائج الهيستوباثولوجية اصابة ٤ قطعان بأورام الليكوزس الليمفاوى(المصاحبة بخلايا الليمفوبلاست) و ايجابيةالأربع قطعان فى اختبار الاليزا لاستبيان وجود الأجسام المضادة لفيروس الليكوزس الليمفاوى. أظهرت النتائج الهيستوباثولوجية اصابة ٦ قطعان من ١٠قطعان (٦ قطعان أمهات تسمين) بأورام الليكوزس الميلوسايتومى(المصاحبة بخلايا الميلوسايت) المسببة بفيروس الليكوزس المجموعة (J)و ايجابية (٦) قطعان فى نتائج اختبار الاليزا. كانت نتائج اختبار تفاعل أنزيم البلمرة المتسلسل التى أجريت على ٦ قطعان للتحقق من وجود الحامض النووى الديوكسى ريبوزى لفيروس الليكوزس الميلوسايتومى المجموعة (J) ايجابية فى ٤ قطعان (٤ امهات تسمين) وسلبية فى قطيعين. توصلت الدراسة ان الفحص الهيستوباثولوجى وسيله هامه وفاصله فى تشخيص الامراض السرطانيه المختلفه الناجمه عن الاصابه بفيروس الليكوزس فى الطيور وذلك لاختلاف الخلايا المستهدفة من الفيروس (خلايا اللمفوبلاست و خلايا الميلوسايت) والتى تفرق بين اورام الليكوزس الليمفاوى وأورام الليكوزس الميلوسايتومى . ان استخدام اختبار تفاعل انزيم البلمرة المتسلسل يثبت وجود الحامض النووى الديوكسى ريبوزى لفيروس الليكوزس المجموعة J المسبب لاورام الليكوزس الميلوسايتومى.

الملخص العربى

- تم تخطيط هذا العمل لدراسة التغيرات المرضية والهيستوباثولوجية للأورام المختلفة التى يحدثها فيروس الليكوزس في الطيوروبخاصة المجموعة (J) في قطعان أمهات التسمين وقطعان البياض فى الدجاج وذلك للوصول الى النقاط التشخيصية للتفرقة بين أورام الليكوزس الليمفاوي وأورام الليكوزس المصاحبة بخلايا الميلوسايت.
- تم فحص عدد ٢٤٥ عينة من الدجاج ممثلا ١٠ قطعان من محافظات مختلفة. ينقسم هذا العدد الى ٤٧من قطعان البياض التجارى و١٩٨ من قطعان أمهات التسمين.
- تم فحص عدد ٢٤٥ عينة سيرم لاستبيان وجود الأجسام المضادة لفيروس الليكوزس الليمفاوى ومجموعة(J) كل على حدة باستخدام اختبار الاليزا.
- تم التحقق من وجود الحامض النووي الديوكسى ريبوزى لفيروس الليكوزس المجموعة(J) فى الأنسجة المصابة باستخدام اختبار أنزيم البلمرة المتسلسل.
- أظهرت النتائج الهيستوباثولوجية فى ١٠قطعان (قطيعين بياض و٨ قطعان أمهات تسمين) أصابة ٤ قطعان بأورام الليكوزس الليمفاوى(المصاحبة بخلايا الليمفوبلاست).
- تم فحص الأعضاء التالية : غدة فابريشيا, الكبد, القلب, الكلى, المبيض, الطحال, الأمعاء و العضلات الهيكلية.
- أوضحت النتائج وجود أورام بؤرية ومنتشرة فى الأعضاء المصابة وهى تتكون من تجمعات من ارتشاحات خلايا الليمفوبلاست.
- تؤدى هذه التجمعات الخلوية الى ازاحة وضغط للأنسجة المجاورة ممايؤدى الى تنكرز لهذه الأعضاء.

- تعتبرغدة فابريشيا المصابة بورم الليكوزس الليمفاوى عضوا أساسيا فى تحورخلايا الليمفوبلاست الىخلايا سرطانية كماأنهاتعتبرمصدرا لاصابة الأعضاء الداخلية الأخرى.وتتكون تجمعات هذه الخلايا السرطانية من خلايا ليمفوبلاست كبيرة, متماثلة فى الحجم, ويتميز السيتوبلازم بلونه الأزرق وصغرحجمه مقارنة بالنواة.تتميز أنويتها بتجمع الكروماتين على حوافها وأشكال متعددة من الأنقسامات الميتوزية.تصبغ هذه الخلايابصبغةالميثيل الأخضر والبيرونين وتأخذ لونا أحمر لأن هذه الخلايا تحتوى على نسبة عالية من الحمض النووى الريبوزى فى السيتوبلازم.
- كما أظهرت الدراسة وجود نوعا من الأورام فى الأنسجة الضامة تحت الجلد(الميكزوما) نتيجة الاصابة بفيروس الليكوزس الليمفاوى وذلك لوجود نسب عالية من الأجسام المضادة لهذا الفيروس ويتميز هذ الورم بنتؤات تحت سطح الجلد فى الرأس والرقبة ويتكون من نسيج ضام جنينى والخلايا مغزلية الشكل.
- أظهرت الدراسة أن نتائج اختبار الاليزا لاستبيان وجود الأجسام المضادة لفيروس الليكوزس الليمفاوى ايجابيةلأربع قطعان من ١٠ (٤ /١٠)لعدد (٦٨/ ٢٤٥) بنسبة مئوية ٧ , ٢٧ %.
- أظهرت النتائج الهيستوباثولوجية اصابة ٦ قطعان من ١٠قطعان (٦ قطعان أمهات تسمين) بأورام الليكوزس الميلوسايتومى(المصاحبة بخلايا الميلوسايت) المسببة بفيروس الليكوزس المجموعة (J).
- و كانت الأعضاء المصابةهى العظام ,الغضاريف, الكبد, القلب, الكلى, المبيض, الطحال, الأمعاء والعضلات الهيكلية وتتميز باورام منتشرة فى الاعضاء الداخلية والسطح الداخلى لعظمة القص وضلوع الصدر.
- أظهرت الدراسة ان هذه الاورام تتكون من ارتشاحات خلوية من الميلوسايت ذات الانوية الجانبية ويتميز السيتوبلازم بامتلائه بحبيبات حمراء اللون وتصبغ بصبغة ماي جرون والد جيمزا وتأخذ لون احمرلامع.

- كما أظهرت الدراسة وجود نوعا من الأورام فى الأنسجة الطلائية فى الكلى(نفروبلاستوما) نتيجة الاصابة بفيروس الليكوزس الميلوسايتومى.
- يعتبر اورام النفروبلاستوما من الاورام الخبيثة الجنينية يحتل هذا الورم جزءا من نسيج الكلى ويتميز بوجود القنويات الكلوية متحوصلة وغير متميزة والنسيج البينى متعدد الاشكال الخلوية وتغير في الكبيبات الى الشكل الجنينى.
- أظهرت الدراسةأن نتائج اختبار الاليزا لاستبيان وجود الأجسام المضادة لفيروس الليكوزس الميلوسايتومى المجموعة (J) ايجابية (٦) قطعان من ١٠ (٦ /١٠)لعدد (١٠٧/ ٢٤٥) بنسبة مئوية ٧ , ٤٥ %.
- كانت نتائج اختبار تفاعل أنزيم البلمرة المتسلسل التى أجريت على ٦ قطعان للتحقق من وجود الحامض النووى الديوكسى ريبوزى لفيروس الليكوزس الميلوسايتومى المجموعة (J) ايجابية فى ٤ قطعان (٤ امهات تسمين) وسلبية فى قطيعين.
- من هذه النتائج انتهت الدراسة الى وجود اختلاف فى التغيرات المرضية الظاهرية بين نوعي الاورام محل الدراسة (اورام الليكوزس الليمفاوى وأورام الليكوزس الميلوسايتومى).
- و يعتبر الفحص الهيستوباثولوجى وسيله هامه وفاصله فى تشخيص الامراض السرطانيه المختلفه الناجمه عن الاصابه بفيروس الليكوزس فى الطيور وذلك لاختلاف الخلايا المستهدفة من الفيروس (خلايا اللمفوبلاست و خلايا الميلوسايت) والتى تفرق بين اورام الليكوزس الليمفاوى وأورام الليكوزس الميلوسايتومى.
- أظهرت الدراسة وجود بعض الاورام المصاحبة للاصابة بفيروس اليكوزس المجموعة J مثل اورام النفروبلاستوما وايضا اورام الميكزوما المصاحبة للاصابة بفيروس الليكوزس الليمفاوى.

- كما ان استخدام الصبغات الخاصة تساعد فى تشخيص انواع الاورام المختلفة لفيروس الليكوزس فى الدجاج.
- ان استخدام اختبار الاليزا فى الكشف عن الاجسام المناعية لفيروس الليكوزس الليمفاوى والميلوسايتومى المجموعة J يساعد فى تشخيص المرض.
- ان استخدام اختبار تفاعل انزيم البلمرة المتسلسل يثبت وجود الحامض النووى الديوكسى ريبوزى لفيروس الليكوزس المجموعة J المسبب لاورام الليكوزس الميلوسايتومى.

جامعة القاهرة
كلية الطب البيطرى
قسم الباثولوجى

قرار لجنة الحكم والمناقشة

قررت لجنة الحكم و المناقشة اليوم الخميس الموافق ٢٢/ ٩/ ٢٠٠٥
ترشيح السيد ط.ب. / محمد أحمد محمد علي سليمان للحصول علي درجة الماجستير في العلوم الطبية البيطرية (باثولوجيا عام وخاص و تشريح مرضي).

عنوان الرسالة باللغة العربية:
دراسات باثولوجية و مناعية لتشخيص الفيروسات المسببة لأورام الدجاج (مرض الليوكوزس فى الطيور)

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تحريرا في٢٢ / ٩/ ٢٠٠٥

جامعة القاهرة
كلية الطب البيطرى
قسم الباثولوجى

دراسات باثولوجية و مناعية لتشخيص الفيروسات المسببة لأورام الدجاج
(مرض الليوكوزس فى الطيور)

رسالة مقدمة من طبيب بيطرى
محمد أحمد محمد على سليمان

بكالوريوس العلوم الطبية البيطرية ١٩٩٢
جامعة القاهرة

للحصول على درجة الماجستير فى العلوم الطبية البيطرية
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