

# COMPARATIVE PATHOLOGICAL STUDIES IN MAREK'S DISEASE AND LYMPHOID LEUCOSIS

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BY  
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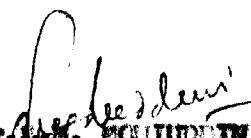
**1986**

**CERTIFICATE**

Sri M.A. Majeed Ather has satisfactorily prosecuted the course of research and that the thesis entitled "COMPARATIVE PATHOLOGICAL STUDIES IN MAREK'S DISEASE AND LYMPHOID LEUCOSIS" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

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# CERTIFICATE

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This is to certify that the thesis entitled "COMPARATIVE PATHOLOGICAL STUDIES IN MAREK'S DISEASE AND ADRENAL LEUCOSIS" submitted in partial fulfillment of Veterinary Science of the Andhra Pradesh Agricultural University, Hyderabad is a record of the bonafide research work carried out by Sri M.A. Majeed Ather under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. Published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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## ABSTRACT

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This study was carried out to determine incidence of Marek's disease and Lymphoid leucosis in relation to age, sex, strain, distribution of gross lesions and histopathologic, histochemical changes, absolute basophil count and enzyme(LDH) estimation. The purpose of this study was to make a comparative study of the changes in two different diseases for proper understanding and early diagnosis. Also to study the behaviour of Absolute basophil count(ABC) and LDH activity in both these conditions.

This study was based on post-mortem examination carried out in 3,220 birds. A total of 522 birds were affected with MD(16.2%), and 789 birds were found to be

affected with LL(84.3%). In MD the growers were found to be more affected than chicks and adults. The frequency of incidence being 98.5%, 13.2% and 27.9% in the growers, adults and chicks respectively. In LL the adults were found to be more affected than chicks and growers. The frequency of incidence being 62.6%, 31.6% and 3.8% in adults, growers and chicks respectively.

Females were more susceptible than males in both MD and LL. The percentage of mortality in males and females due to MD was 10.3% and 89.6% respectively, while in LL 19.3% and 84.7% of males and females were affected respectively.

In the present study visceral type of lesions were found to be more than neural and mixed in MD. The frequency of incidence being 40.2%, 17.2% and 21.0% due to visceral, neural and mixed types respectively.

In LL diffuse type of lesions were found to be more than nodular and mixed types in all the age group of chickens. The frequency of incidence being 48.1%, 8.9% and 12.7% due to diffuse, nodular and mixed types respectively.

In MD the involvement of different organs was different in different age group of birds. In birds 0-8 weeks of age, the incidence of organ involvement being proventriculus(42.4%), liver(39.7%), kidneys(37.0%) and spleen(35.6%) and in 9-20 weeks of age group proventriculus(71.6%), liver(61.9%), spleen(54.7%) and kidneys(53.4%). The frequency of incidence of organ involvement in the age group of 21 weeks and above was peripheral nerves(97.0%), liver(69.5%), kidneys(63.7%) and proventriculus(65.2%).

Liver and spleen were the major organs affected in LL in different age group of birds. The frequency of involvement of the liver and spleen in the age group of 0-8 weeks was 76.0% and 69.0% respectively; while in 9-20 weeks it was 56.2% and 53.0% respectively, and in 21 weeks and above it was 76.0% and 75.3% respectively.

The tumors of MD were composed of anisonorphic and pleomorphic cells, including various-sized lymphoid cells, reticular cells and plasma cells commonly, granulocytes and fibrocytic cells rarely where as the tumors of LL consisted of uniform large lymphoid cells.

Histochemical studies by using methyl-green pyronin-Y staining technique showed that the intensity of pyroninophilia was poor in MD where as it was quite uniform and bright in LL.

The results of Absolute basophil count showed variation in MD and LL. In MD it varied from  $750 \pm 150$  to  $1900 \pm 525$  ABC/mm<sup>3</sup> of blood where as in LL it was  $775 \pm 90$  to  $2025 \pm 200$  ABC/mm<sup>3</sup> of blood. In normal healthy birds it varied from  $475 \pm 75$  to  $725 \pm 225$  ABC/mm<sup>3</sup> of blood.

The levels of serum LDH activity found to be greater in MD than in LL. In MD it ranged from 410 to 1610 LDH units per ml. of serum where as in LL it varied from 450 to 1000 LDH units per ml. of serum. In 20, apparently healthy birds, the LDH activity varied from 260-600 units per ml. of serum.

# INTRODUCTION

## **CHAPTER - I**

### **INTRODUCTION**

Research advances during the past few years have vastly increased our knowledge and understanding of the etiology, pathogenesis and epizootiology of avian neoplastic diseases. The neoplastic disease can now be classified in terms of the causative viruses. Three etiologically distinct neoplastic diseases of avian groups are now recognized.

1. The leucosis / sarcoma group
2. Marek's disease and
3. Reticuloendotheliosis

The Leucosis/sarcoma group includes Lymphoid leucosis(LL), myeloblastosis, myelocytomatosis, erythroblastosis and such solid tumors as fibrosarcomas, endotheliomas, hepatomas, nephroblastomas and osteopetrosis. These tumors are caused by a number of closely related RNA viruses. The most commonly occurring neoplastic disease of this group is Lymphoid leucosis.

Marek's disease, the second neoplastic disease is extremely prevalent throughout the world. It is now known to be caused by a Herpes virus of the B group.

Of the virus induced tumors in chickens, Marek's disease and Lymphoid leucosis are the two major problems. Lymphoid leucosis is a serious threat to the poultry industry since the disease is transmitted vertically and appears only when the hen is about to start laying. Thus control by culling is a difficult task and no vaccine is available to control the disease by prophylaxis.

Marek's disease is characterised by depression, squatting position, debility, drooping of wings and paresis or paralysis and shows an epizootic occurrence, while LL is seen in adult chickens mostly, without exhibiting any collective outbreak.

Marek's disease and Lymphoid leucosis can be differentiated from one another with great difficulty since similar lymphoid tumors may occur in both diseases in the same visceral or one during the same age period. It is not easy to discriminate particularly LL from MD of acute type by gross examination alone. Hence the objective of the present investigation is to make a comprehensive study of the changes in two different diseases so that early diagnosis can be made with ease.

Marek's disease affects younger birds of 6-18 weeks, while LL affects mature birds. The classical form of MD does not pose any problem for diagnosis since the nerve lesions are most conspicuous and such lesions are not found in LL.

Marek's disease is highly contagious and spreads rapidly in a susceptible flock. The diagnosis of the field cases is mainly based upon symptoms and post mortem lesions. In cases where mortality is high without showing any gross lesions, diagnosis is completely based upon histopathological changes of the tissues.

Marek's disease in field flock is recognized in two forms (a) Acute and (b) Classical. This division is based on age of the affected birds, magnitude of mortality and presence of gross lymphoid tumors. The acute form of MD is characterized by sudden and heavy mortality in young stock. Mortality due to MD varies from a few to 70-80%. Usually the classical form affects a small percent of the flock (upto 10%), and the acute form between 20 and 30%. Sporadic cases may also be seen, particularly in flocks of adult chickens. These may have many of the characteristics of lymphoid leucosis and therefore present problem of differential diagnosis.



Investigations on field outbreaks and mortality caused by MD indicated that classical form of the disease is widely prevalent in certain states of India causing a mortality of upto 32%.

Outbreak of acute MD causes heavy losses due to mortality where as in the classical form mortality is low, but continuous because it affects comparatively older birds. More serious is the form, in which the disease does not manifest its characteristic paralysis and death but remain in a state of lower weight gain and production.

Although lymphoid tumors may occur in almost any organ or tissue in both Marek's disease and Lymphoid leucosis, gonad involment is often a feature of Marek's disease and the involment of liver and spleen are frequent in Lymphoid leucosis.

Ordinarily, Lymphoid leucosis does not occur before 16 weeks of age, and most of the mortality occurs between 34 and 40 weeks. Marek's disease on the other hand, may occur as early as 6 weeks of age and the peak of mortality varies from 10-20 weeks. Occasionally losses continue after this age and may reach a peak even after 20 weeks.

Inspite of all the progress made, little had been done to eliminate MD and LL. The main reason was the lack of a sensitive and rapid diagnostic techniques. There appears to be no work taken up in India to simplify and standardise the techniques for field applications for the diagnosis of MD and LL and at present the differential diagnosis of Marek's disease and Lymphoid leucosis is done mainly on post mortem materials.

The main objective of undertaking this investigation is to compare and evaluate a few diagnostic techniques for the diagnosis of Marek's disease and Lymphoid leucosis basing on epidemiological investigation, gross, histopathological, histochemical, Absolute basophil count and serum LPH estimation. The main purpose is to identify a technique to detect MD and LL in early stages of infection.

Histochemical studies on Marek's disease and Lymphoid leucosis together have received very little attention, the present study was carried out to understand the histochemical alterations and their possible significance in cases of MD and LL.

Absolute basophil count was carried out in clinically suspected birds of MD and LL.

Serum lactic dehydrogenase activity has been reported to increase in neoplastic growths. There are few reports on the behaviour of LDH activity in Marek's disease and Lymphoid leucosis. Hence estimation of LDH activity have been given more attention during this study to determine the behaviour of LDH activity in both the diseases.

## REVIEW OF LITERATURE

## CHAPTER - II

### REVIEW OF LITERATURE

#### 2.1 Incidence of Marek's disease and lymphoid leucosis:

Amundson and Biely (1932) recorded the incidence of paralysis and lymphomatous tumors in a flock of 342 pullets of six different breeds, which indicated the presence of both conditions in 14 out of 32 paralysed pullets, while an additional 22 had tumors but were not paralysed.

Biely et al. (1932) reported that the typical lesions of Marek's disease was found in approximately 25% of the inoculated chicks, while in control chicks kept under laboratory conditions the incidence was about 7%.

Benton and Cover (1957) reported an increased incidence of visceral lymphomatosis in the U. S.A. in broiler and replacement birds of eight to ten weeks of age.

Viraraghavan and Blair (1965) studied the incidence of LL in relation to various factors such as breed, age at death, sex and seasonal occurrence in 132 cases of LL out of 93 chickens of 5 months and above age giving an autopsy incidence of 13.9%.

He also reported gross thickening of proventriculus with ulcerations and hemorrhages in LL.

Feldman and Olson (1965) quoted reports in which the incidence of tumors, except neurolymphangitis and osteopetrosis, varied from 3-19%.

Hensley (1966) recorded the tumor incidence in 3,43,600 broiler chickens in 14 flocks on post mortem examination and the incidence of LL was 0.001%.

Losses due to Marek's disease in affected flocks were estimated to range from a few birds to 25 or 30% and occasionally as high as 60% (Biggs, 1968).

5-10% incidence of LL in two inbred line flocks was reported by Sandelink and Estola (1970).

Witter et al. (1970) showed that the infection of MD can often be detected very soon after the placement of chicks in contaminated poultry house. Infection rapidly increased and by 8 weeks 82% had microscopic lesions. He also indicated that the time of exposure to MD was as early as 7-14 days of age.

Twenty-four of 25 White Leghorn pullets from 4 different field outbreaks of transient paralysis were found to have Marek's disease and evidence of Lymphoid leucosis was noted in 14 of 18 chickens suffered with transient paralysis (Cho et al., 1970).

Rajya and Mohanty (1971) recorded the classical form of MD to be widely prevalent in India producing cumulative mortality upto 38% in certain farms. They also reported two outbreaks of acute form of MD with mortality ranging from 26% to above 50%.

Mohiuddin (1972) recorded 277 cases of Lymphoid leucosis and 274 cases of MD out of 1043 autopsied birds and differential diagnosis of MD and LL was based on the age of incidence, pattern of lesions, nerve involvement along with visceral lesions.

Purchose et al. (1972) reported a mortality of 10% due to Lymphoid leucosis in two farms and the vaccination did not have any influence on the occurrence of this disease.

Ramodaran and Thanikachalam (1974) recorded 867 cases of ALC out of 33,133 birds autopsied.

Henderson et al. (1974) reported an increase incidence of solid tumors in 9 week old chickens vaccinated against Marek's disease.

During the year 1970-71, 160(3.4%) cases out of 1,734 and during 1972-74, 709(7.0%) cases out of 9,985 studied was due to Marek's disease (Grewal and Singh, 1976).

Out of 83 cases of MD in Haraknath chicks, 72 suffered with classical form and 11 with that of acute form. The incidence of both the forms was highest 90.9% and 63.9% respectively in the chicks of 9-10 weeks of age, and mortality 9.09% and 13.9% respectively in chicks of 21 weeks and above age (Verna et al., 1978).

Mortality or morbidity due to Marek's disease has been estimated to range from a few birds to 30% and occasionally even upto 60% in certain flocks (Sarunh and Kwatra, 1979).

Zolanski et al. (1980) conducted epidemiological studies, and recorded Leucosis in 5-25% birds up four years in a survey conducted on the breeding and commercial poultry industry.



Rao and Choudary (1981) in a survey during 1972 to 1979 recorded 1393 neoplasms, of which 826 were identified as Lymphoid leucosis, 551 as Marek's disease and in the remaining as fibroma-5, Leiomyoma-4, haemangioma-2 and one each of granuloma cell tumor, hepatoma, adenocarcinoma, fibrosarcoma and chondroma.

826 cases of Lymphoid leucosis and 516 cases of Marek's disease out of 1261 tumorous conditions were reported by Beyer *et al.* (1981).

Singh *et al.* (1981) reported MD with a mortality rate of 41% in 407 unvaccinated fowls from 21 farms and 12.8% in 149 unvaccinated birds from 13 farms.

Over a ten year period (1971-80) the incidence of neoplasms recorded was 10.62% from the post mortem examination of 6760 birds. In the ducks also 8.98% of neoplasms recorded among 267 ducks autopsied during that period. The commonest tumor recorded in fowls was LL, 90% (Sriraman *et al.*, 1981).

Purvulev (1981) recorded 10-17% of mortality due to MD in a flock of about 10,000 birds during 1970 to 1979.

Haleemka and Jurašda (1982) recorded MD in 3.9% of infected chicks.

The percentage mortality due to MD among birds of 9-20 weeks and 21-40 weeks of age group was found to be 8.66% and 28.19% respectively (Panda *et al.*, 1983).

Balani (1983) reported an incidence of 5.6% and 6.3% Lymphoid leucosis and Marek's disease respectively.

Analysis of 479 flocks of broilers at the age of slaughter proved that infection rate of MD reached 85%, skin form of MD was present in 304 flocks (63.0%) and visceral involvement in 101 flocks, 21.0% (Jurašda and Kavela, 1983).

Among 2589 captive and wildbirds examined during 1973 and 1983, leucosis was found in 26 birds belonging to 13 different species and 5 orders (Loupal, 1984).

Durstein *et al.* (1984) reported outbreak of Lymphoid leucosis among layer flock of chicken on 60 farms in Israel, with a mortality upto 20%.

Nicholas (1984) reported Marek's disease outbreak in 1500 layers. A weekly mortality rate of 0.2% at 25 weeks of age, rose to 0.4% at 60 weeks of age.

The lesions were reported to be considerably increased as a result of dual infection with CAA and Virulent strain of MDV. The mortality was 75 - 92% until 16 days after infection (Bulow *et al.*, 1984).

Hinajosa (1984) reported MD in chickens over 35 days of age and the incidence was 1.2%.

Rathore *et al.* (1985) carried out survey on Poultry mortality based on post mortem examination. Mortality due to Marek's disease was recorded in 5748 (1.4%) and Lymphoid leucosis in 4866 (1.8%).

Palaniswami *et al.* (1985) reported MD incidence in a broiler parent stock with a mortality of 15.66% between 12 and 24 weeks of age.

## 2.2 Species susceptibility due to Marek's disease and Lymphoid leucosis

Bamberger (1964) reported that the losses due to Lymphoid leucosis were influenced by genetic resistance, age at the time of infection, contact and composition of diet.

Calnek (1968) carried out a survey in commercial breeding flocks for genetic resistance and the incidence of LL was 4.4%.

The Marek's disease resistant strain had only 12.9% mortality where as in the highly susceptible 90.7% mortality was recorded (Cole, 1968).

The genetic constitution of the host has been shown to influence susceptibility to MD with both classical and acute strains of virus (Biggs et al., 1968).

Recent studies indicated some evidence that the susceptibility of the birds to MD is inversely proportional to age (Biggs, 1970, Vielitz and Landgraf, 1970).

Witter et al. (1971) studied Marek's disease in a naturally infected flock of white Leghorn chickens during 76 week period.

Seneviratna (1972) reported that the susceptibility varied considerably to the different strains or breeds of chickens to the different isolates of MD virus.

Spencer et al. (1972) demonstrated that vaccinated chicks of genetically resistant strains usually had a lower incidence of MD than those of genetically susceptible strains.

Grewal et al. (1977) observed that the incidence of MD in high yielding strains/breeds varied from 65.5% to 100%, however, in desi birds 2 out of 16 birds yielded positive results.

Mohiuddin (1978) made efforts to study the incidence of Lymphoid leucosis in relation to age, sex, involvement of different organs and carcass weight in different strains of white Leghorn over three generations.

The percentage of Lymphoid leucosis virus shedders in 3 strains of pullets selected for upto 27 generations for high egg production and a complex of related commercially important traits was 1.4-3.7% when compared to 2 unselected control strains in which it varied from 18.7-21.4% (Gavara et al., 1980).

Mans et al. (1980) reported mortality due to Lymphoid leucosis was 54.3%, 9.5%, 6.9% and 7.5% in different groups of white Leghorn chicks.

Viraemic tolerant hens classified as congenitally infected had consistently shed virus into albumen of their eggs and also progeny and 18% of these birds died due to tumors. In contrast, contact infected hens had a transient Viraemia, most of them developed antibody to ALC and only 10% shed virus into albumen and progeny (Fadly and Okazaki, 1982).

Lee et al. (1982) recorded MD lesion in 44(43.5%) out of 101 bantam chickens, an Indonesian dwarf fowl of different age groups. All the cases presented visceral involvement, with nerve lesions only in 6 birds (13.6%).

Klucinski et al. (1983) carried out haematological studies in 336 sussex layers aged 35 weeks. The mortality rate reported due to Lymphoid leucosis was 12.3%.

Ree and Burne (1984) reported MD in 64% in a study on 15,000 died chicks of different strains and hatches. The peak mortality was between 18 and 26 weeks of age. The gross lesion mostly affected liver (75%) and spleen(71%).

Ginting (1984) reported an isolated outbreak of Marek's disease recording mortality 25% of a flock of Redu fowls within 3 days and the lesions were present in liver, spleen and ovary.

Of 152 fowls of exotic commercial strains (ten groups) and of 108 local fowls (ten groups), 16.4% and 8.3% respectively died due to Marek's disease (Adene, 1984).

Moreno and Gonzalez (1985) investigated the incidence of Marek's disease in white Leghorn pullets upto 7 months of age and the incidence ranged from 0-38% and averaged to 14%.

Losses of 45000 birds in four lines and their four  $F_1$  hybrids were analysed over 8-13 breeding years for Marek's disease and Lymphoid leucosis. The analysis revealed that the population differed in their susceptibility to both Marek's disease and Lymphoid leucosis (Jahn and Rohland, 1985).

### 2.3 Gross, histopathological and histochemical changes in Marek's disease and Lymphoid leucosis:

Hakagawa (1965) conducted histopathological studies on 81 cases of fowl paralysis, nerve lesions classified as neoplastic proliferation were recorded in 55 cases and non-neoplastic cellular infiltration in 54 cases.

Fayne and Biggs (1967) classified the microscopic lesions of Marek's disease in three forms as type A, B and C.

Neurobiotic lesions have been described in turcs (Purchase and Biggs, 1967; Jakowski et al., 1969; Purchase, 1970; Jakowski et al., 1970).

Wight (1968) described histopathological findings in outbreaks of transient paralysis(MD) affecting 8-12 weeks old fowls.

Goodchild (1969) studied in detailed the microscopic changes in 302 birds suffered with neural Marek's disease.

Prakash and Rajya (1970) observed spastic paralysis of legs and wings and also some of the visceral organs were affected in fowls infected with diseases of peripheral nerves and central nervous system.

Histopathological changes were studied in 66 MD cases collected during severe outbreaks and 17 IL cases collected from sporadic outbreaks and in ten cases of IL (Okada, 1970).

Evans et al. (1971) compared the lesions found in certain lymphoid tissues of chicken with MD. Bursa weights increased significantly than spleen weight in infected chickens.



Differential diagnosis of paralysis in domestic fowl by histological and histochemical examination was discussed by Polival and Rajya (1971).

Fujimoto *et al.* (1971) investigated 161 cases of Marek's disease histopathologically.

Yamamoto *et al.* (1971) distinguished MD and LL based on gross lesion and histopathologic comparison of natural cases of MD with experimental cases of LL was also carried out.

Kakiheta (1973) studied pathological changes in bursa of fabricius in 12 MD affected birds from field cases and observed neoplastic proliferation of the lymphoreticular cells in the interstitial tissue of the BF. Also noticed atrophy and disappearance of the follicles due to cyst formation.

Sabu *et al.* (1976) reported discrete nodular to diffuse granular type of lesions in liver of Lymphoid leucosis. Methyl green and pyronin-Y stained sections revealed few blast cells with distinct pyroninophilia while majority of the cells differentiated poorly. They also reported gross thickening of proventriculus in 10%.

**Mayan et al. (1977)** studied the incidence and histopathology in 52 birds necropsied, revealed Marek's disease lesions in 15(29.2%) cases.

**Krishna et al. (1977)** studied the gross and histopathologic changes in bursa of fabricius in 47 field cases of Marek's disease.

**Fedotov (1978)** considered that the primary tumor affected bone marrow in MB where as in lymphocarcinoma it is bursa of fabricius.

Lymphoid tumors in Marek's disease may occur in one or more of a variety of organs, the gonad (especially the ovary) was most often affected, but lymphomatous lesions can also be found in the lung, heart, mesentery, kidneys, liver, spleen, adrenal, pancreas, proventriculus, intestine, iris, skeletal muscle and skin (Galnek and Bitter, 1978).

**Neumann and Bitter (1979)** carried out differential diagnosis of Marek's disease and Lymphoid leucosis based on tumor associated criteria.

**Robos et al. (1982)** studied detailed pathological changes in the eyes of 30 birds affected with Marek's disease from three different flocks, in which 5-30% incidence of blindness was noticed at 2-12 months of age.

Rhemaiah (1982) recorded proventriculitis associated with Marek's disease in 55 birds(1.90%). Among them 38 were growers, 13 layers and remaining were chicks. Apart from proventriculitis the lesions of MD were also noticed in Ovary, liver and spleen. LI was noticed in 113 growers and 234 pullets and the incidence was 12.0%. He also recorded MD lesions in liver in 22 chicks, 121 growers and 36 adults and the incidence of enteritis associated with MD was 0.34%.

Shikov et al. (1982) carried out histological studies of a large number of chickens from 13 production units which revealed leucotic and sarcomatous lesions in varying proportions.

Elperigin et al. (1983) diagnosed Marek's disease by gross and microscopic examination of affected tissues and concluded as the cause of high mortality noticed in the commercial flocks of white leghorn layers.

The characteristic histological changes like infiltration of kidneys and proventriculus by lymphoid and reticular cells was found in 39% of classical form and similar changes were also found in 72% of acute form (Lapinskaite et al., 1984).

Chen *et al.* (1985) reported an outbreak of Lymphoid leucosis in the state hatchery unit, Dimapur (Nagaland) in the year 1976 and 1977 and the histopathology and epidemiology of the disease has been discussed.

Gunder *et al.* (1983) recorded thickening of the wall of proventriculus, enlargement of the liver, spleen, kidneys and swollen comb in a flock of 30 week old layers affected with MD.

#### 2.4 LDH activity in Marek's disease and Lymphoid leucosis

The measurement of LDH activity is helpful in the diagnosis and prognosis of myocardial infarction, acute hepatitis, leukemia, metastatic carcinoma, meningitis, intravenous hemolytic episodes and other diseases (Philip *et al.*, 1988).

Kallbank *et al.* (1964) reported plasma LDH levels of more than 500 units in chicks suffering with clinical leucosis.

A drop in LDH levels in serum and rise in LDH levels in liver at 12 weeks were observed in chicks injected with leucotic liver extract (DiDomizio *et al.*, 1968).

Chicks inoculated with whole blood of Marek's disease virus developed 5-10 fold increase in serum LDH levels by 120 hours post inoculation(Jones et al., 1969).

Prakash and Rajya (1970) estimated serum LDH levels in birds affected with Marek's disease. The LDH activity was greater in birds having symptoms of paralysis without any histopathologic lesions (700-1000 LDH units)in comparison to healthy birds in which LDH levels varied from 200-300 units per ml. of serum.

LDH values in MD virus inoculated chickens remains at a higher level in comparison to uninoculated chicks. Increase in LDH levels were observed from 4-5 days post inoculation(Mohanty et al., 1974).

## MATERIALS AND METHODS

## **CHAPTER - III**

### **MATERIALS AND METHODS**

The materials for this study were collected from the dead birds brought for post-mortem examination at the Department of Pathology, College of Veterinary Science, Rajendranagar, Hyderabad, All India Co-ordinated Research Project on Poultry for Eggs, Rajendranagar, Hyderabad, Poultry disease investigation wing, Veterinary Biological Research Institute, Shantinagar, Hyderabad and from different private poultry farms in and around twin cities. The practical approach to study this problem was based upon collecting material from 500 cases of Marek's disease and 500 cases of lymphoid leucosis. It was also proposed to investigate Absolute basophil count and LDH activity in both the disease condition by analysing blood and serum respectively from 30 positive cases in each disease. Serum and blood was also analysed from 20 healthy birds which serves as control. The following procedure was adopted for studying the cases.

#### **3.1 Epidemiological investigation:**

1. Mortality rate
2. Age incidence
3. Sex incidence
4. Age incidence in relation to sex
5. Strain susceptibility.

### **3.2 Clinical observation:    Symptoms.**

### **3.3 Pathological studies:**

#### **1. Gross pathology**

- a) Distribution of lesions
- b) Nature of lesions.

### **3.4 Histopathology(Employing Haematoxylin and Eosin stain):**

Small pieces of tissues were collected for histopathological examination from different organs viz., liver, spleen, kidneys, lungs, proventriculus, heart, bursa of fabricius, peripheral nerves and gonads. They were fixed in 10% buffered formal saline, processed and cut at 5  $\mu$  thickness and stained with Haematoxylin and Eosin for routine HP examination.

### **3.5 Histochemistry**

Impression smears were collected from liver and spleen and stained with Methyl green and pyronin-Y stain to detect the presence of pyroninephilic material (Siccardi and Burmester, 1970).

### **Reagents required:**

1. Pyronin-Y	0.15 gm.
2. Methyl green	1.00 gm.
3. Distilled water	100.00 ml.



**Procedure:**

Place the dyes in the flask and hot distilled water. Agitate at intervals, incubate for two days at room temperature and then filter the solution.

Tumors from freshly killed birds are blotted free from blood and gently streaked across previously numbered glass slides until a uniform thin smear is obtained. The slide is then immediately immersed in the fixative of choice (95% Ethyl alcohol for 1-2 minutes) and stained with the following procedure.

1. After fixation in 95% Ethyl alcohol for 1-2 minutes the slide is dipped in 70% alcohol then in distilled water and blot dry.
2. Then the slide is immersed in Methyl green and pyronin-Y stain for 7-10 minutes.
3. Rinse in distilled water by dipping slowly 3 times and gently blot dry.
4. Dehydrate by dipping 3-4 times in each of 3 changes of dioxane and finally clear in xylene but usually 2 changes of dioxane is sufficient.
5. Then mount the slide with coverslip by using D.P.X.

All the sections from liver, spleen, kidneys, lungs, proventriculus, heart, bursa of fabricius, peripheral nerves and ovary were also stained with Methyl green and pyronin-Y stain to demonstrate pyroninophilic material (Culling, 1974).

**Reagents required:**

1. 5% aqueous pyrenin-Y	17.5 ml.
2. 2% aqueous Methyl green(washed)	10.0 ml.
3. Distilled water	250.0 ml.

**Procedure:**

The above reagents were diluted with an equal quantity of acetate buffer pH 4.8 before use. The following steps were adopted for staining the tissue sections.

1. Take paraffin sections to water.
2. Rinse in distilled water and blot dry.
3. Pour prepared staining solution for 15-60 minutes(30 minutes gave good results).
4. Rinse in distilled water and blot on non-fluffy filter paper.
5. Flood the slide with acetone for a second or two and flood again with acetone.
6. Flood the slide with acetone - xylol(equal parts).
7. Then clear in pure xylene and leave the slide until clear.
8. Then mount in D.P.X.

**3.6 Hematology:**

Toluidine-blue stain was employed to determine the absolute basophil count as described by Chand and Syre (1978).

**Reagents required:**

- |  |        |
|--|--------|
| 1. 0.9% acetylpyridinium chloride                  | 25 ml. |
| 2. 0.8% toluidine - blue in 3.0% aluminium sulfate | 20 ml. |
| 3. Distilled water                                 | 25 ml. |

**Procedure:**

1. 0.2 ml. of blood collected from a wing vein, gently mixed with 0.08 ml. of 0.1% EDTA in physiological salt solution.
2. To this was added 1 ml. of staining solution.
3. By using pasteur pipette Neubauer hemocytometer chambers were filled with the blood-stain mixture and left in a wet chamber for 5 minutes to allow the cells to settle.
4. Basophils were counted in both the chambers by a method similar to that for total leukocyte count.
5. Total no. of basophils was divided by 2 and multiplied by 25 to obtain absolute basophil count per  $\text{mm}^3$ .

**3.7 Enzyme Estimation (Determination of serum Lactic Dehydrogenase activity):**

Serum collected from 50 birds clinically suffering from Marek's disease and 50 birds from Lymphoid leucosis from the All India Coordinated Research Project on Poultry for Eggs, Rajendranagar for the estimation of LDH activity. These birds were sacrificed, tissues were collected for histopathological and histochemical studies. Serum was also collected from 20 clinically healthy birds which served as control for LDH activity.

### Principle:

Lactic dehydrogenase(LDH) is an enzyme concerned with the reduction, in the presence of reduced diphosphonucleotide(DPNH), of alpha-keto and alpha, gamma-diketo acids. LDH activity of serum may be measured by the reduction in the presence of DPNH, of pyruvic acid to lactic acid, following this reduction the remaining pyruvic acid reacts with dinitrophenyl hydrazine to form a pyruvate-dinitrophenyl hydrazone, when the pyruvate-dinitrophenyl hydrazone is treated with an alkali, a coloured compound forms, the intensity of which reflects the quantity of pyruvate remaining. This inversely reflects the level of LDH activity, the greater the LDH activity, the less will be the amount of pyruvate remaining in the solution.

### Standard curve for LDH estimation:

The standard curve was prepared by setting up solutions containing decreasing amounts of pyruvic acid which reflect increasing LDH activities(Table 1). This was carried out by the pyruvic acid-buffered substrate solution which contains 200 ug. of pyruvic acid per ml.(Phillip *et al.*, 1958).

Table 1. PYRUVIC ACID CONCENTRATIONS AND EQUIVALENT  
LMU UNITS

Tube No.	Pyruvic acid buffered substrate (mL.)	Pyruvic acid added (mL)	Pyruvic acid added water (mL)	Equivalent LMU units
1	1.0	200	0.1	0
2	0.8	160	0.2	250
3	0.6	120	0.5	750
4	0.4	80	0.7	1250
5	0.2	40	0.9	1610
6	0.1	20	1.0	2000

To each of these tubes 1 ml. of the dinitrophenyl hydrazine solution was added, mixed and stand for 20 minutes, then 10 ml. of 0.4N Sodium hydroxide was added, mixed by inversion and after 5-10 minutes percent transmittance read at 550 mμ. on a photoelectric colorimeter with 100 percent transmittance set with distilled water, the percent transmittance was converted into equivalent LDH units by multiplication with  $10^3$  and plotted against LDH activity which was represented by the decreasing amounts of pyruvic acid in the substrate (Fig.1).

#### Reagents required:

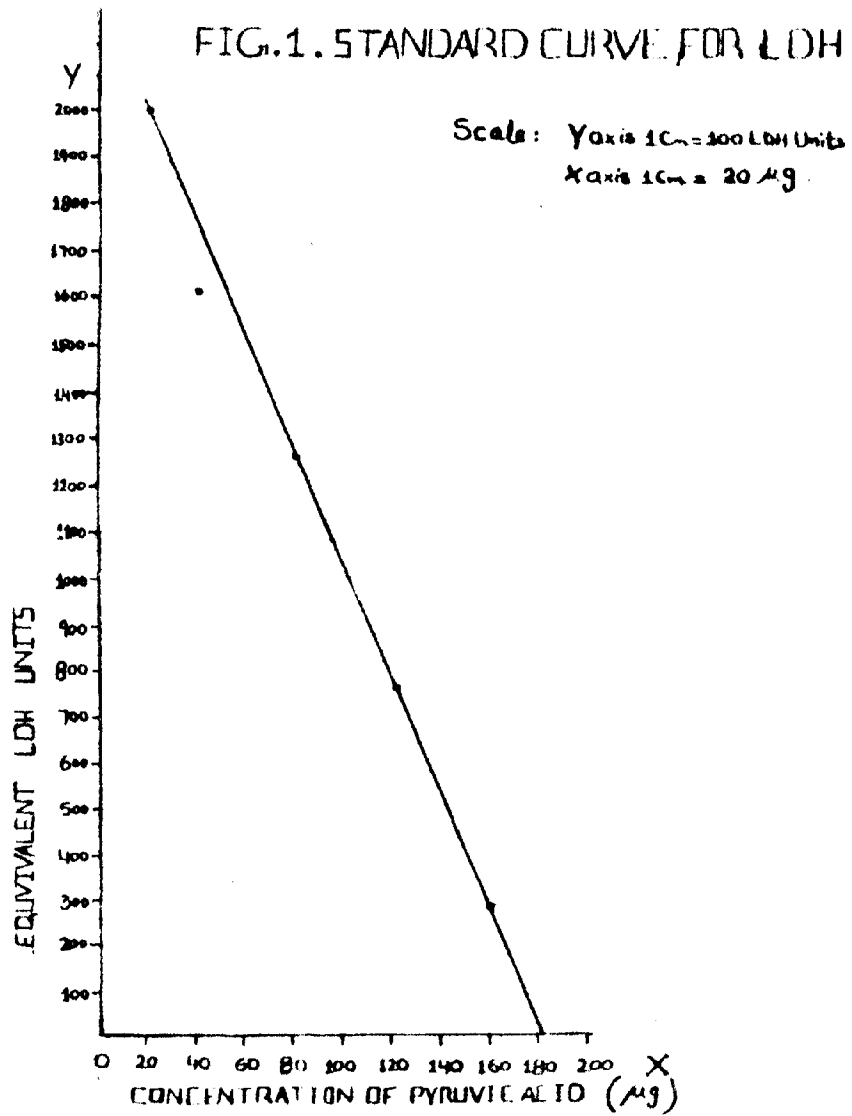
1. Pyruvic acid-buffered substrate (pH 7.8-8.0).
2. Dihydro-diphosphopyridine nucleotide (DPNH) solution.
3. Dinitro phenyl hydrazine solution.
4. 0.4N Sodium hydroxide.

#### Procedure:

1. Dilute serum 1:6 with distilled water (1 part serum and 5 parts water).
2. Place 0.1 ml. of this diluted serum in a larger test tube (150 by 13 mm) and add 0.1 ml. of the DPNH solution.
3. Place in a separate tube enough pyruvic acid-buffered substrate to allow the pipeting of 1 ml. for each determination to be performed.

4. Place all tubes in a 37°C hot water bath till the temperature of the solution equalise at 37°C (10-15 minutes).
5. Leaving the tubes in the 37°C water bath, transfer 1 ml. of the pyruvic acid-buffered substrate to the tubes containing the diluted serum and DPNH, the substrate should be added to each separate tube in order and at a regular interval such as 30 seconds or 1 minute so that step No.6 may be performed at exactly 30 minutes on each tube.
6. Exactly 30 minutes after adding the substrate, add 1 ml. of the dinitrophenyl hydrazine solution, remove the test tubes from the water bath, mix by swirling and let stand for 20 minutes.
7. Add 10 ml. of 0.4N Sodium hydroxide and mix by inversion, let stand for 5-10 minutes and read percent transmittance at 550 mμ. on a photoelectric colorimeter with 100 percent transmittance set with distilled water. The percent transmittance was then converted to units of lactic dehydrogenase activity through the curve determined by the standardisation procedure.

FIG.1. STANDARD CURVE FOR LDH





## RESULTS

## **CHAPTER - IV**

### **RESULTS**

#### **4.1 Sources of material for the study:**

The material for this study was collected from 3,220 birds at the time of post-mortem examination. Of these birds 910, belong to the All India Coordinated Research Project on Poultry for Eggs, Rajendranagar, Hyderabad; 241 birds brought for post-mortem examination at the Department of Pathology, College of Veterinary Science, Rajendranagar, Hyderabad; 975 birds brought for post-mortem examination at Veterinary Biological Research Institute, Shantinagar, Hyderabad and 1,094 dead birds belong to different private poultry farms in and around Hyderabad and Secunderabad (Table 2).

### **HANSEN'S DISEASE**

#### **4.2 Epidemiological Investigation:**

##### **4.2.1 Clinical observations:**

Depression, anorexia, loss of weight, poor feed consumption, pale and shrivelled combs, dehydration and decreased egg production were observed.

**Table 2. THE DIFFERENT SOURCES OF MATERIAL COLLECTED**

Sl.No.	Place of collection	No. of birds examined
1.	All India Co-ordinated Research Project on Poultry for Eggs, Rajendranagar, Hyderabad.	910
2.	Department of Pathology, College of Veterinary Science, Rajendranagar, Hyderabad.	241
3.	Veterinary Biological Research Institute, Shantinagar, Hyderabad.	975
4.	Private Poultry farms of Hyderabad and Secunderabad.	1094
Total:		3220

Considerable number of birds showed paresis of legs and wings, mostly unilateral involvement was more common. Respiratory signs were also recorded in few birds. Partial blindness of one or both the eyes was also observed. In birds of younger age group, vent was pasted with dark greenish coloured droppings. In general, almost all the birds revealed emaciated condition, retarded growth and loss of weight.

#### 4.2.2 Mortality:

Out of a total of 5,220 birds autopsied, 522 were diagnosed as cases of Marek's diseases with mortality rate of 10.2%(Table 3).

#### 4.2.3 Age incidence:

The mortality in the age group of 9-20 weeks were more (58.8%) than in age group 0-8 weeks(28%) and 21 and above weeks(15.2%). The number of birds affected and percentage of mortality in different age groups are summarised in Table 4.

#### 4.2.4 Sex incidence:

Females were more susceptible than males, the incidence was 89.6% and 10.3% in females and males respectively(Table 5).

**Table 3. PARTICULARS OF INVESTIGATION**

-----	
<b>Sl.No.</b>	<b>Particulars</b>
-----	
	<b>Birds</b>
-----	
<b>1.</b>	<b>Total number of birds examined</b>
	<b>3220</b>
<b>2.</b>	<b>Number of birds affected with Marek's disease</b>
	<b>522</b>
<b>3.</b>	<b>Number of birds affected with Lymphoid leucosis</b>
	<b>789</b>
<b>4.</b>	<b>Percent of the birds with Marek's disease</b>
	<b>16.2</b>
<b>5.</b>	<b>Percent of the birds with Lymphoid leucosis</b>
	<b>24.5</b>
-----	

**Table 4. AGE INCIDENCE OF MAREK'S DISEASE**

Age (weeks)	No. of birds affected	Percentage of mortality
0 - 8	146	23.0
9 - 20	307	58.8
21 & above	69	13.2
	<u>522</u>	

**Table 5. SEX INCIDENCE OF MAREK'S DISEASE**

-----		
Sl.No.	Particulars	
-----		
1.	Total deaths due to Marek's disease.	522
2.	Number of females affected (including 7 broilers)	468
3.	Number of males affected	54
4.	Percentage of mortality in females	89.6
5.	Percentage of mortality in males	10.3
-----		

#### 4.2.5 Age incidence in relation to age

In both the sexes, growers were more susceptible than the lower age groups (Table 6).

#### 4.2.6 Strain susceptibility

This information was collected from the records at All India Coordinated Research Project on Poultry for Eggs, Rajendranagar. Among the 322 cases of Marek's disease, 202 belong to different strains of white leghorn. IWA, IWD and IWE strains were more susceptible than IWF and IWK (Table 7).

#### 4.2.7 Gross changes in different organs

Out of 322 suspected cases of MD, 410 were diagnosed based on gross lesions. In the age group of 0-8 weeks and 9-20 weeks visceral type of involvement was more or less predominant, while in birds of 21 weeks and above age group neural involvement was higher than visceral type. The mixed type of lesions were more or less same in all age groups (Table 8 and 9).

The organ involvement in the order of frequency in 0-8 weeks of age group recorded were, proventriculus, liver, kidneys and spleen followed by other organs and in 9-20 weeks of age group were,



proventriculus, liver, spleen and kidneys, while 21 and above weeks includes peripheral nerves, liver, kidneys and proventriculus followed by other organs (Table 10).

### Liver:

It was represented by coarse granular appearance, in few birds, nodular tumor like growths were found within and extended from the parenchyma of the organ. These nodules were grayish coloured, firm and smooth on cutting. In few cases there was no enlargement of the organ (Fig.2).

### Spleen:

Multiple foci of lymphoid tumors were seen, slight enlargement was noticed in few cases and few cases revealed dark colouration with prominent blood vessels and congestion.

### Kidneys:

Most of the cases showed greater enlargement of both the kidneys with multiple greyish nodules. Few cases had enlargement of the organ without any nodular growth, and in few cases the organ was much enlarged and lost its normal structure.

**Table 6. AGE INCIDENCE OF MARER'S DISEASE IN RELATION TO SEX.**

Age (weeks)	No. of females died	Percentage of morta- lity	No. of males died	Percentage of morta- lity
0 - 8	140	29.9	6	11.1
9 - 20	280	59.8	27	50.0
21 & above	48	10.2	21	38.8

**Table 7. STRAIN SUSCEPTIBILITY DUE TO HAREK'S DISEASE**

Strain	No. of birds affected	Percentage of mortality
IWA	102	90.5
IND	48	23.7
IWE	41	20.3
IWF	5	2.4
IVK	6	2.9

**Table 8. TYPES OF INVOLVEMENT IN MAREK'S DISEASE**

Sl. No.	Particulars	No. of birds affected	Percentage of mortality
1. Type of Marek's disease			
(a)	Neural type	90	17.2
(b)	Visceral type	210	40.2
(c)	Mixed type	110	21.0

**Table 9. TYPES OF INVOLVEMENT (AGE WISE) IN HAREK'S DISEASE**

Age (weeks)	No. of birds affec- ted	Macroscopically positive birds	Types of Harek's disease						Total percent- age
			Visce- ral type	Per- cen- tage	Non- ral type	Per- cen- tage	Mixed type	Per- cen- tage	
0 - 8	146	77	45	58.4	15	19.5	17	22.0	100.0
9 - 20	307	285	190	62.6	55	19.3	80	28.0	100.0
21 & above	69	48	15	31.2	20	41.6	13	27.0	100.0

**Table 10. AGE WISE INCIDENCE OF MICROSCOPIC INVOLVEMENT IN VARIOUS ORGANS  
OF MARSH'S DISEASE**

Particulars	0-8 weeks		9-20 weeks		21 weeks & above	
	(146 cases)		(307 cases)		(69 cases)	
	No. of positive cases	Per- centage	No. of positive cases	Per- centage	No. of positive cases	Per- centage
Liver	58	39.7	190	61.9	26	37.7
Spleen	52	35.6	168	54.7	21	30.4
Kidney	54	37.0	164	53.4	24	34.8
Ovary	39	26.7	81	26.4	20	29.0
Bursa of Fabricius	4	2.7	11	3.5	1	1.4
Proventriculus	62	42.4	220	71.7	24	34.8
Peripheral nerves	17	11.6	96	31.2	28	40.6
Lung	43	29.4	109	35.5	18	26.1
Heart	37	25.3	102	33.2	19	27.5



**Fig. 2** Mouse liver (left side) showing H. lesions. The organ is diffusely enlarged with thickened borders. The capsule is tense.

Rat liver (right side) showing H. lesions. The lesions consist of different sizes of the greyish or brownish nodules protruding out the surface or not. They are raised above the surface. The liver is also enlarged.

**Ovary:**

Grayish-white lymphoid tumors were noticed. In few cases the organ was regressed and had translucent appearance, and in others the ovaries appeared cauliflower like.

**Bursa of Fabricius:**

Atrophy and diffuse enlargement was observed.

**Proventriculus:**

All the cases presented diffuse thickening of the organ. Few birds also revealed nodular thickening of the wall.

**Peripheral nerves:**

Sciatic nerves showed more involvement than brachial nerves. The changes included loss of striations, grey and yellow discolouration and thickening. The nerves were also pale and opaque in appearance. Unilateral involvement was greater than bilateral involvement.

**Lungs:**

Slightly enlarged, congested, grayish-white medullary lesions with consolidation of the organ was observed. In few cases half of the organ presented creamy appearance on both the sides.



**Heart:**

Single and multiple tumorous growths was noticed in myocardium. In few birds hydropericardium was also noticed.

**4.2.8 Histopathological changes:**

The incidence of microscopic changes of MD in various organs is presented in Table 11.

**Liver:**

Diffuse infiltration of pleomorphic lymphoid cells were seen in the sinusoids and in the interlobular spaces. Localised accumulation of neoplastic cells were seen especially around the blood vessels. In few cases reticular cells were also noticed in greater proportion. Hepatic cells revealed degenerative and necrotic changes. Few cells comparatively larger than lymphoid cells with intensely basophilic stained cytoplasm with vacuolation and indistinct nucleus (Marek's disease cell) were noticed. Fine network of collagenous fibrils were also seen in the focal cell aggregations. Mitotic changes and cell degeneration frequent. In majority of the cases, the infiltration of lymphoid cells were so diffuse that the normal architecture of the liver was lost (Fig.3).

**Table 11, MICROSCOPIC INVOLVEMENT OF VARIOUS ORGANS IN MD IN DIFFERENT AGE GROUPS**

Particulars	Age(0-8 weeks)		Age(9-20 weeks)		Age (21 weeks & above)	
	No. of positive cases	Percentage	No. of positive cases	Percentage	No. of positive cases	Percentage
Liver	144	98.6	235	92.8	48	69.5
Spleen	122	83.5	265	86.3	41	99.4
Kidney	130	89.0	272	88.6	44	63.7
Ovary	65	44.5	111	36.1	26	37.7
Bursa of fabricius	22	15.0	28	9.1	4	5.8
Proventriculus	146	100.0	301	98.0	45	65.2
Peripheralnerves	35	24.0	132	43.0	67	97.0
Lung	82	56.1	185	60.2	27	39.1
Heart	76	52.0	161	52.4	24	34.8



Fig. 3 Liver — MD: — Lymph in filtration of  
pleomorphic lymphoid cells are seen  
in the entire hepatic tissue.  
H & E x 100.

In most of the cases diffuse infiltration of pleomorphic lymphoid cells was seen. Mitotic changes and plasma cells were frequent. Nodular cells, few histiocytes and few cells were also observed.

## **Grav:**

parenchyma was completely replaced (Fig. 4). Neoplastic cells was so extensive that the entire cells were also observed. In few, proliferation of the tubules showed degenerative changes, few in tissue and between the tubules. Epithelial cells cells were seen in the interstices of parenchymatous focal and diffuse proliferation of lymphoid

## **Liver:**

In almost all the organs proliferation of reticulum cells and lymphoid cells were predominant. In most of the cases central part of the organ was replaced completely by lymphoid cells composed of small, medium and larger size. Plasma cells and mitotic figures were frequent. Few cases showed the presence of tumor cells spreading to the fibrous capsule and to the subserosa. Few MD cells were also observed.

## **Spleen:**



Fig. 4 Kidney — MD: Diffuse infiltration  
of pleomorphic cells in the interstices  
of parenchymatous tissue. H & E (O).

### Area of follicles:

The proliferation of pleomorphic lymphoid cells was more marked in the interstitial spaces of the follicles. Cystic alteration and disappearance of follicles with most of the lymphoid follicles were sharply demarcated and necrobiosis was frequently found in the center of the follicles.

### Peritriculosa:

Focal and diffuse proliferation of tumor cells were observed in the muscularis mucosae, lobules of the glands and subserosa. In few, degeneration and necrosis of lobules of the glands were seen. Tumor cells were composed of mostly small, medium and larger type of lymphoid cells(Fig.5).

### Peripheral nerves:

Nerve changes showed varied distribution of cells between individuals. The distribution of proliferative cells were comparatively sparse, and perivascular cell accumulation was prominent in the interspaces of nerve fibres. Perivascular area showed focal aggregation of pleomorphic lymphoid cells, diffuse proliferation of tumor cells were also observed.

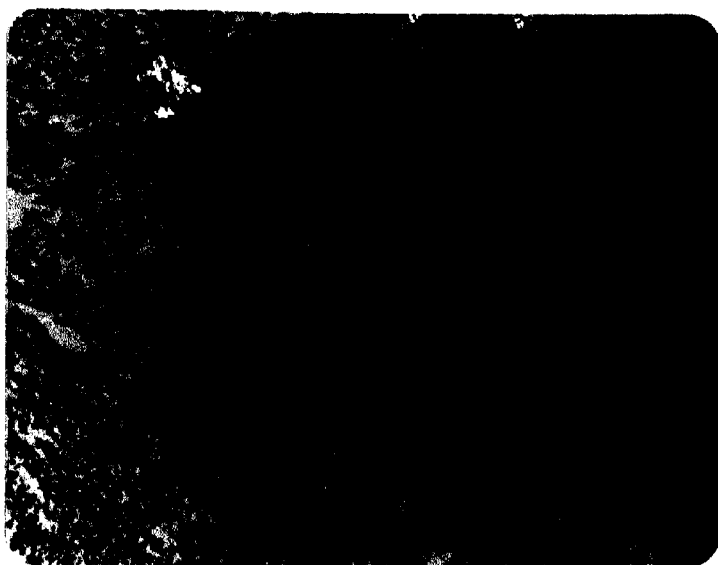


Fig. 3 Placentoculus — MD; proliferation  
 of placental cells in the muscularis  
 externa and in between lobules of the  
 placenta.  $\times 40$ .

Damage of the neurites was often seen in nerves with heavy infiltration of lymphoid cells. In few, uniform distribution of relatively small size lymphoid cells was seen. Few reticular cells having intensely stained basophilic cytoplasm was noticed. In few others fragmentation of axons with oedema was observed(Fig.6).

#### **Lungs:**

Parenchyma was replaced completely by pleomorphic tumor cells. Infiltration of tumor cells was observed in and around the alveoli and bronchioles.

#### **Heart:**

Focal and diffuse aggregation of tumor cells was seen in the intermuscular fibres of connective tissues of the subepicardial area and around the coronary artery. The muscle fibres also showed hyaline changes surrounded by pleomorphic tumor cells.

#### **4.2.9 Histochemistry:**

Impression smears from the fresh affected organs revealed pleomorphic appearance of tumor cells with dull intensity of pyroninephilia, nucleus was dark bluish in colour and in majority of the cases appearance of the nucleus was not clear.



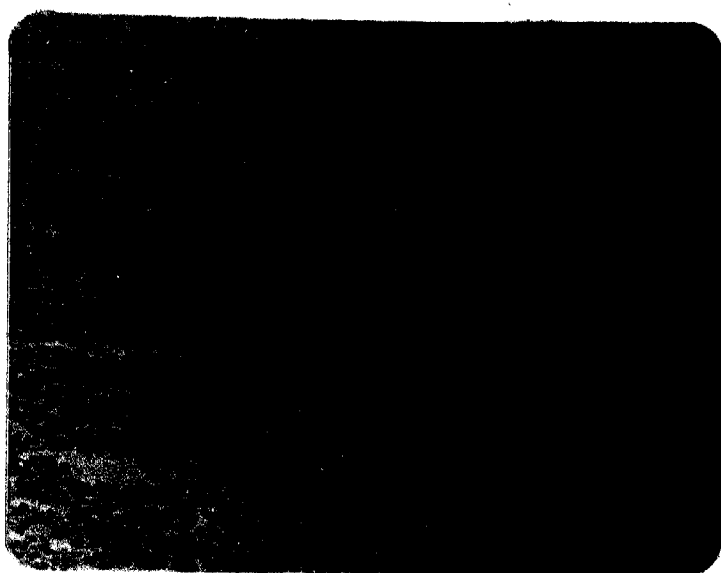


Fig. 6 Sciatic nerve — MD. Accumulation of pleomorphic cells in the interstices of nerve fibres.  $\times 100$ .

Sections from different organs stained with Methyl green and pyronin-Y revealed the presence of poor pyronineophilic appearance of lymphoid cells. Distribution of pleomorphic lymphoid cells was not uniform, the general appearance of the tissues were not clear. Some of the immature cells revealed dark red stained cytoplasm, in most of the cases nucleus showed dark bluish green appearance with vacuolation in the cytoplasm(Fig.7).

## **LYMPHOID LEUCOSIS**

### **4.3 Epidemiological Investigation:**

#### **4.3.1 Clinical observations:**

Distended abdomen, loss of breast muscle with a prominent keel bone, duck walking posture, enlarged liver, pale, shrivelled and synotic combs were the constant symptoms observed in most of the cases. In few birds ascitis was also noticed. In adult birds decrease in egg production, loss of appetite and general weakness were noticed.

#### **4.3.2 Mortality**

Out of a total of 3,220 birds autopsied, 789 were diagnosed suffering with Lymphoid leucosis. The mortality worked out to be 24.5%(Table 3).

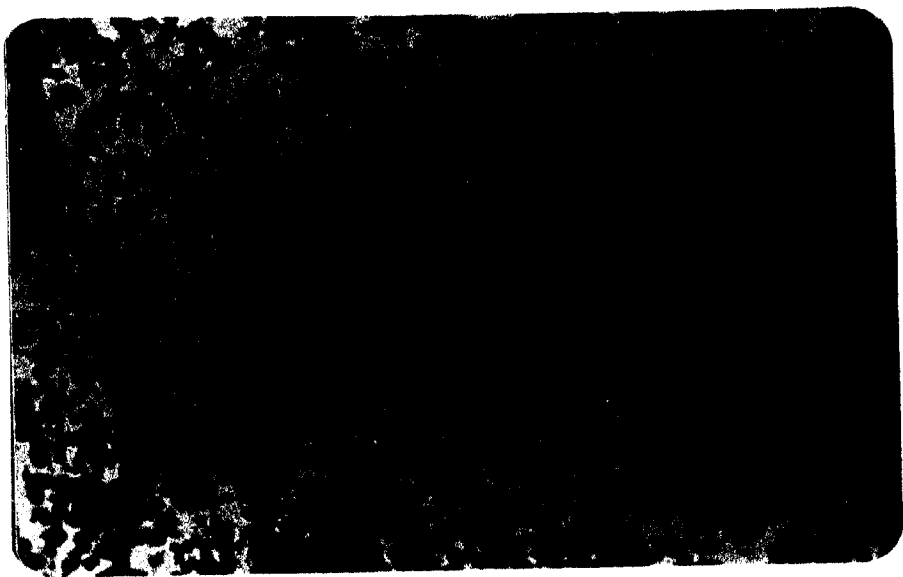


Fig. 7 Heart — MD: Pleomorphic cells accumulated in the intermuscular fibers of the heart. The cytoplasm stained poorly with pyroninophilic stain and the nucleus is deeply stain, cytoplasm also showed few vacuoles. H&E x 100.

#### 4.3.3 Age incidence:

The incidence of LL in birds of 21 and above weeks was more followed by 9-20 weeks and 0-8 weeks. The number of birds affected and percentage of mortality in different age groups is summarised in Table 12.

#### 4.3.4 Sex incidence:

Females were more susceptible than males, the incidence was in females 84.7% and in males 15.3% (Table 13).

#### 4.3.5 Age incidence in relation to sex:

In both the sexes, adults were more susceptible than the lower age groups and among adults the incidence was greater in females than males (Table 14).

#### 4.3.6 Strain susceptibility:

This information was collected from the records at All India Coordinated Research Project on Poultry for Eggs, Rajendranagar. Among the 789 cases of lymphoid leucosis, 132 belong to different strains of white leghorn. The incidence was more in IWA and IF as against IWD, IWE and IWK (Table 15).

#### 4.3.7 Gross changes in different organs:

Out of 789 suspected cases of LL 530 were diagnosed on gross changes. On post mortem examination considerable number of birds showed clear peritoneal fluid measuring 40 ml. to 70 ml. The different types of lesions in LL and different organ involvement in different age groups were presented in Tables 16 and 17. In all the age groups studied, diffuse type of lesions were more frequent followed by mixed and nodular type. Liver, spleen, kidneys and ovary were the major organs affected in all the age groups (Table 18).

#### Liver

In almost all the cases studied, liver was the major organ affected. The liver was enlarged several times and extended up to the vent region. It was dark red in color with fine whitish necrotic lesions distributed throughout, the margins were thin and the organ was friable. Gall bladder was full. Spherical, greyish yellow and glistening nodules were present throughout the surface of the organ. These nodules varied from 0.4mm to 6 mm in diameter. Cut surfaces of the nodules revealed greyish white appearance. The weights of the liver and spleen were recorded in gm. percent in 100 cases and presented in Table 19. The maximum weight of the affected liver was 20.0 gm percent in 24 cases (Fig.2).

**Table 12. AGE INCIDENCE OF LL**

Age (weeks)	No. of birds affected	Percentage of mortality
0 - 8	46	2.8
9 - 20	249	31.6
21 & above	494	62.6
Total		789

**Table 13. SEX INCIDENCE OF LL**

-----	
<b>Sl.No.</b>	<b>Particulars</b>
-----	
<b>1.</b>	<b>Total deaths due to LL 789</b>
<b>2.</b>	<b>Number of males affected 121</b>
<b>3.</b>	<b>Number of females affected 668</b>
<b>4.</b>	<b>Percentage of mortality in males 15.3</b>
<b>5.</b>	<b>Percentage of mortality in females 84.7</b>
-----	

**Table 14. AGE INCIDENCE IN RELATION TO SEX OF LL**

Age (weeks)	No. of males died	Percent- age of mortality	No. of females died	Percentage of morta- lity.
0 - 8	0	0	46	6.9
9 - 20	33	28.9	214	32.0
21 & above	86	71.1	408	61.1



**Table 15. STAIN SUSCEPTIBILITY DUE TO LL**

Strain	No. of birds affected	Percentage of mortality
IMA	36	42.4
IMD	19	14.4
IME	16	12.1
IMF	32	24.2
IMK	9	6.8

**Table 16. TYPE OF INVOLVEMENT IN L.L.**

Sl. No.	Particulars	No. of birds affected	Percentage of mortality
1.	Type of L.L.		
	(a) Diffuse type	380	48.1
	(b) Modular type	70	8.9
	(c) Mixed type	100	12.7

Table 17. TYPE OF INVOLVEMENT(AGE WISE) IN LEUKOID LEUCOSIS

Age (weeks)	No. of birds affected	Macroscopically positive birds	Types of LL						Total percentage.
			Diffuse type	Pericor- type	Nodular type	Pericor- type	Mixed type	Pericor- type	
0 - 8	46	35	20	57.1	5	14.3	10	28.5	100.0
9 -20	249	140	85	60.7	25	17.8	30	21.4	100.0
21 & above	494	375	275	73.3	40	10.6	60	16.0	100.0

Table 18. AGE AND INCIDENCE OF MACROSCOPIC INVOLVEMENT IN VARIOUS ORGANS OF LYMPHOID LEUKOSIS

Particulate	0-9 weeks			9-20 weeks			21 weeks & above		
	No. of Percent- positive tags (46 cases)			No. of Percent- positive tags (249 cases)			No. of Percent- positive tags (494 cases)		
Liver	33	76.0	140	36.2	373	76.0			
Spleen	32	69.5	132	53.0	373	75.3			
Kidney	21	45.6	119	47.8	199	40.2			
Ovary	12	26.0	49	19.6	132	26.1			
Heart	0	0	2	0.8	16	3.2			
Lung	1	2.1	7	2.8	13	2.6			
Proventriculus	2	4.3	9	3.6	9	1.8			
Periphero-nerves	0	0	0	0	0	0			
Course of fibrils	16	34.8	93	37.3	89	18.0			

**Table 19. THE WEIGHTS OF LIVER AND SPLEEN IN LL  
(gm percent)**

Liver	Spleen
14.1(32)	3.0(36)
13.6(22)	2.4(21)
20.0(24)	2.7(19)
13.5(14)	2.2(18)
14.6( 8)	1.5( 6)

Number in parenthesis indicates the number of  
birds included in calculations.

**Spleen:**

In all the cases, the organ was very much swollen, soft, friable and dark coloured. In nodular form, the nodules were seen above the surface of the spleen which varied in size. In the diffuse form, the organ showed whitish necrotic foci in the substance. The weights of the organ were recorded in gm. percent in 100 cases and presented in Table 19, maximum weight recorded was 3.0 gm percent in 36 cases.

**Kidney:**

Gross changes included by slight enlargement of the organ with few to many small tumorous growths. Unilateral involvement of the organ was more frequent. The organ was generally friable in all the cases.

**Ovary:**

Almost all the cases showed the presence of tumorous growth. The growth varied from few nodules of small size to nodules of larger size.

**Heart:**

The lesions were rare in the heart, the affected organs showed similar lesions as described

in other organs. Hydropericardium and hypertrophy was also noticed.

#### Lungs:

Lung lesions were rare. In few cases where the lungs were involved the cut surfaces revealed the presence of small grayish tumorous masses, hence haemorrhages and oedema.

#### Proventriculus:

Lesions were rarely recorded. Where ever recorded the lesions were localised and thickened nodules on the wall.

#### Bursa of Fabricius:

In most of the cases, the organ was enlarged. Greyish white tumorous growth were noticed. Few cases showed slight enlargement of the organ with localized 1 to 2 focal lymphoid nodules.

#### 4.3.8 Histopathological changes:

In all the age groups, highest incidence of microscopic changes were recorded in liver, spleen, kidney, Bursa of fabricius and ovary. In birds of the age group 21 weeks and above involvement of ovary

was slightly greater than the bursa of fabricius. The incidence of microscopic changes of LL in various organs is presented in Table 20.

### Liver:

The proliferative lesion was occupied by uniform distribution of lymphoblasts throughout the organ. In most of the cases accumulation of lymphoblasts was so diffuse that the normal architecture of the liver was lost completely. The tumor cells had moderately basophilic cytoplasm and the nucleus was round and displaced. Hepatic cells showed degenerative changes and associated with necrosis. Accumulation of lymphoblasts was surrounded by endothelial cells of sinusoids(Fig.8).

### Spleen:

Extensive tumorous proliferation with large lymphofollicular structures were observed. Discrete foci of lymphoblasts around the capillary sheath, arteries and also appeared as localized lymphoid follicles. Perivascular tissue showed the presence of large size lymphoid cells with much basophilic cytoplasm and a vesicular nucleus and in few cases



**Table 20. MICROSCOPIC INVOLVEMENT OF VARIOUS ORGANS IN LL IN DIFFERENT AGE GROUPS**

Particulars	0-8 weeks		9-20 weeks		21 weeks & above	
	(46 cases)		(249 cases)		(494 cases)	
	No. of positive cases	Percentage	No. of positive cases	Percentage	No. of positive cases	Percentage
Liver	46	100.0	244	98.0	493	99.8
Spleen	40	86.9	239	96.0	491	99.4
Kidney	29	63.0	190	76.0	240	48.6
Ovary	16	34.8	102	41.0	163	33.4
Heart	3	6.5	4	1.6	31	6.3
Lung	7	15.2	18	7.2	25	5.0
Proventriculus	9	19.5	21	8.4	16	3.2
Peripheral nerves	2	4.3	3	1.2	4	0.8
Bursa of fabricius	19	41.3	113	46.1	111	22.4



Fig. 8 Liver — LI. Most type of large cells are distributed in the sinusoids of the liver. At many places liver parenchyma is replaced by neoplastic cells.  $\times 90$ .

nucleoli was prominent. In majority of the cases diffuse infiltration of lymphoid cells in the parenchyma was seen. Infiltration of few heterophils was also observed in few cases.

#### Kidneys:

Focal accumulation of larger size lymphoid cells was observed in between the tubules, displacing and compressing them. Epithelial cells of the glomeruli showed degenerative changes with accumulation of few histiocytes and heterophils(Fig.9).

#### Ovary:

Localized mild lymphoid cells infiltration was seen. In few cases focal distribution of monomorphic large lymphoid cells were also seen.

#### Heart:

Focal accumulation of lymphoid cells in intermuscular fibres and around the coronary artery. Heart muscle fibres showed degenerative and necrotic changes. Accumulation of few lymphoblasts were found in the connective tissue around the subepicardial area.

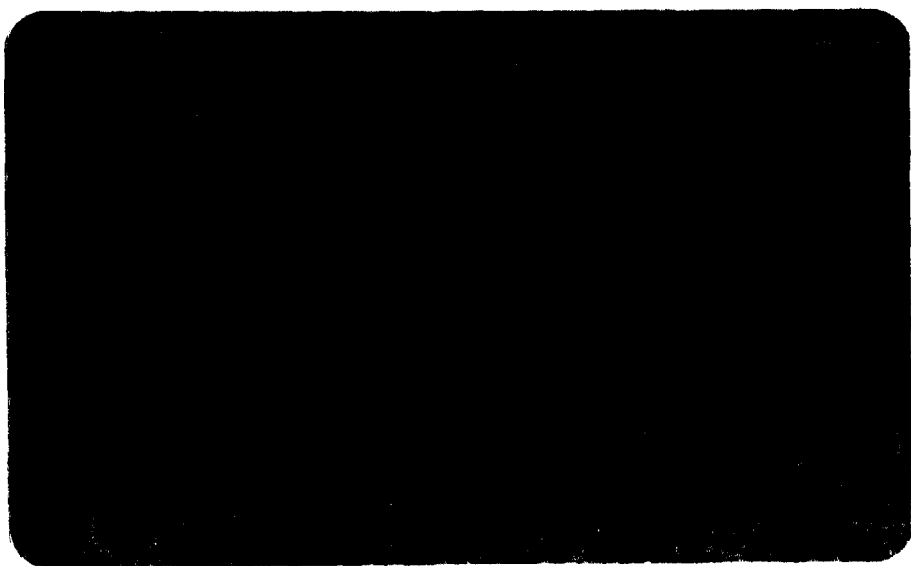


Fig. 9 Kidney — 100x. Section showing the  
large, clear, hyaline, and the small, dark  
colored, cells. x 60.

**Lungs:**

Lymph-follicle-like foci of tumor cells were seen in bronchial mucosa. Lung parenchyma showed degenerative changes and presence of oedema in few cases. Infiltration of histocytes was also noticed in few cases.

**Proventriculus:**

Focal aggregation of tumor cells was observed mostly in the mucosapropria. Lymphoblasts were distributed uniformly, cytoplasm stained basophilic and the nucleus was vesicular. Subserosa layer also showed lymphoblastic infiltration.

**Bursa of Fabricius:**

Lymphoblast proliferation was found within the follicles. The broken interstitial connective tissue was replaced by the lymphoblasts.

**4.3.9. Histochemistry:**

Impression smears from the fresh affected organs revealed infiltration of uniform larger size lymphoid cells having great affinity for pyronine-philic stain.

Sections from various organs stained with Methyl green and pyrenin-Y stain showed clear and uniform distribution of lymphoblasts. Tumor cell cytoplasm stained diffuse red in all the section where as the nucleus stained purple (Fig.10).

#### 4.4 Absolute basophil count in normal healthy birds:

In thirty normal healthy birds the absolute basophil counts were determined, they varied from  $475 \pm 75$  to  $650 \pm 175$  per  $\text{mm}^3$  of blood with a mean of  $594.2 \pm 140.8$  per  $\text{mm}^3$  of blood (Table 21).

##### 4.4.1 Absolute basophil count in Marek's disease:

In the age group of 0-8 weeks there were 24 cases which showed count of  $1025 \pm 300$  to  $1400 \pm 450$  per  $\text{mm}^3$  of blood. In the age group of 9-20 weeks there were 20 cases which showed count of  $750 \pm 150$  to  $1075 \pm 275$  per  $\text{mm}^3$  of blood. 6 cases in the age group of 21 weeks and above showed the value of  $1025 \pm 275$  to  $1500 \pm 525$  per  $\text{mm}^3$  of blood. The mean absolute basophil count per  $\text{mm}^3$  of blood in affected birds of different age groups was  $1127 \pm 300$  (Table 22). It is evident from these studies that the absolute basophil counts were more in Marek's disease than in healthy birds.



Fig. 10. Heart — III. Most type of type of cells are diffusely infiltrated into the heart, replacing the muscle. The cytoplasm is stained bright red with pyronine and the nucleus is deeply stained with methyl green.  $\text{H}\times 100$ .

Table 21. ABSOLUTE BUNOCHIL COUNT IN NORMAL HEALTHY  
BIRDS OF DIFFERENT AGE GROUPS

St. No.	Age (weeks)	No. of birds examined	Absolute base- ball count / mm <sup>3</sup> of blood
1	0 - 8	7	550 ± 150
2	9 - 20	6	650 ± 175
3	21 & above	3	475 ± 75
4	Total No. of birds and mean count	30	594.2 ± 140.8



**Table 22. ABSOLUTE BASOPHIL COUNT IN DIFFERENT AGE  
GROUP OF BIRDS AFFECTED WITH MARX'S DISEASE**

Sl. No.	Age(weeks)	No. of birds examined	Absolute basophil count/mm <sup>3</sup> of blood
1	0 - 8	10 6 3 1	1230 ± 230 1375 ± 325 1025 ± 300 1400 ± 450
2	9 - 20	7 9 4	1025 ± 200 750 ± 150 1075 ± 275
3	21 & above	4 2	1500 ± 325 1025 ± 275
4	Total no. of birds and mean count.	30	1127 ± 300

#### 4.5 Serum lactic dehydrogenase activity

Fifty affected birds were selected based on the clinical symptoms from the All India Coordinated Research Project on Poultry for Eggs, Rajendranagar for estimation of serum LDH activity. There were 24 birds in 0-8 weeks of age group, 20 birds in 9-20 weeks of age group and 6 birds of 21 weeks and above. All the birds were sacrificed and serum was collected for LDH estimation. Gross and histopathological changes were studied in all the organs to confirm the diagnosis. Absolute basophil counts were also determined.

##### 4.5.1 Serum LDH activity in apparently healthy birds

Serum lactic dehydrogenase was estimated in 20 apparently healthy birds. The levels of LDH varied from  $260-600 \pm 841.5$  units per ml. of serum (Table 23).

##### 4.5.2 Serum LDH activity in Marek's disease

###### Age 0-8 weeks

In this age group birds with visceral type of involvement showed levels of LDH activity varied from 620-1350 units per ml. of serum with a mean of 985. Birds with neural type of involvement showed LDH levels of 620-1000 units per ml. of serum with a mean of 796.6.

**Table 23. SERUM LACTIC DEHYDROGENASE ACTIVITY IN APPARENTLY HEALTHY BIRDS**

Sl. No.	No. of birds examined.	Percent transmission (%)	OD values	Equivalent LDH units per ml. of serum ( $\times 10^3$ )	Concentration of pyruvic acid (ug/ml)
1	2	55.0	0.260	260	162
2	1	35.5	0.490	490	146
3	3	29.5	0.590	590	140
4	3	48.0	0.380	380	196
5	1	90.0	0.300	300	198
6	3	27.5	0.560	560	136
7	1	25.6	0.600	600	134
8	1	20.0	0.990	990	138
9	1	39.0	0.410	410	130
10	1	38.0	0.420	420	148
11	1	33.0	0.480	480	144
12	1	51.5	0.290	290	198
13	1	26.5	0.980	980	134

Number of birds examined was 20 with mean LDH levels of 441.5 units per ml. of serum.

Birds with mixed type of lesions represented LDH activity of 360-1300 units per ml. of serum with a mean of 890. Birds which did not reveal any characteristic gross changes but showed positive histopathologic changes had LDH levels of 420-700 units per ml. of serum with a mean of 558 (Table 24).

#### Age 9-20 weeks:

In Birds with visceral type of involvement represented LDH activity varied from 540-1460 units per ml. of serum with a mean of 994.5 LDH units per ml. of serum. Three birds with neural type showed LDH levels of 540, 560 and 690 units per ml. of serum with a mean of 596.6 LDH units per ml. of serum. One bird with mixed type of involvement revealed LDH activity of 800 units per ml. of serum. Birds which did not reveal any specific gross lesions but were found positive by histopathologic studies showed LDH activity varied from 300-940 units per ml. of serum with a mean of 558 LDH units per ml. of serum (Table 23).

#### Age 21 weeks and above:

Two birds with visceral type showed LDH levels of 1260 and 1610 units per ml. of serum respectively.

Table 24. URIC ACID LEVEL IN THE AGG GROUT OF 0-8 WEEKS AFFICTED WITH MARX'S DISEASE

Sl. No.	Particulars	No. of birds	Percent transmittance (ST)	OD value	Equivalent L.H. units per ml. of serum (OD x 80)	Concentration of uric acid (mg/ml)
1.	Birds with visceral type	8	10.0	1.000	1000	100
		1	17.0	0.770	770	120
		1	14.5	0.840	840	114
		1	24.0	0.620	620	132
		1	5.0	1.300	1300	76
		1	4.5	1.350	1350	72
	Total	8		Mean	908	
2.	Birds with neural type	2	24.0	0.620	620	132
		2	10.0	1.000	1000	100
		1	20.0	0.700	700	124
		1	14.5	0.840	840	114
	Total	6		Mean	796.6	
3.	Birds with mixed type	1	17.0	0.770	770	120
		1	27.5	0.560	560	136
		1	24.0	0.620	620	132
		1	10.0	1.000	1000	100
		1	5.0	1.300	1300	76
	Total	5		Mean	830	
4.	Birds without any specific gross lesions but positive histopathologic changes	1	38.0	0.420	420	148
		1	31.0	0.490	490	142
		1	27.5	0.560	560	136
		1	24.0	0.620	620	132
		1	20.0	0.700	700	124
	Total			Mean	528	

**Table 25. SERUM LDH LEVEL IN THE AGE GROUP OF 9-20 WEEKS AFFECTED WITH MARX'S DISEASE**

Sl. No.	Particulars	No. of birds	Percent transmittance(%)	OD Values	Equivalent LDH units per ml. of serum( $OD \times 10^3$ )	Concentration of pyruvic acid( $\mu g/ml$ )
1.	Birds with visceral type	1	11.5	0.940	940	106
		2	10.0	1.000	1000	100
		2	5.5	1.260	1260	80
		2	20.5	0.690	690	126
		1	29.0	0.540	540	138
		1	15.5	0.800	800	118
		1	3.5	1.460	1460	64
		1	5.0	1.300	1300	76
	Total	11		Mean	994.5	
2.	Birds with neural type	1	29.0	0.540	540	138
		1	20.5	0.690	690	126
		1	27.5	0.560	560	136
	Total	3		Mean	596.6	
3.	Birds with mixed type	1	15.5	0.800	800	118
4.	Birds without any specific gross lesions but positive histopathologic changes	2	50.0	0.300	300	198
		1	39.0	0.410	410	190
		1	11.5	0.940	940	106
		1	14.5	0.840	840	114
	Total	5		Mean	598	

940 LDH units per ml. of serum was recorded in one bird with mixed type of involvement. 3 birds which did not reveal any specific gross lesions but showed histopathologic changes showed LDH levels of 690, 340 and 410 units per ml. of serum with a mean of 546.6 LDH units per ml. of serum. The total mean LDH activity was 829.4 units per ml. of serum in 30 studied cases (Table 26).

#### 4.6 Absolute basophil count in L.L.

The count varied from  $775 \pm 50$  to  $2025 \pm 200$  per  $\text{mm}^3$  of blood, 13 cases showed highest value of  $2025 \pm 200$  per  $\text{mm}^3$  of blood. The counts were higher in cases which showed greater involvement of liver and spleen, the mean absolute basophil count was  $1460 \pm 186.5$  per  $\text{mm}^3$  of blood (Table 27).

#### 4.7 Serum Lactic Dehydrogenase Activity:

Fifty clinically suspected birds of above 21 weeks of age were selected for LDH estimation. The birds selected were based on clinical symptoms viz., the presence of enlarged abdomen, pale comb, loss of breast muscle, enlarged liver, duck walking posture and weakness. Few cases showed shrivelled and cyanotic combs with decreased in egg production.

**Table 26. Serum LH Level in the Age Group of 21 Weeks & Above Affected with PAREX'S DISEASE**

Sl. No.	Particulars	No. of birds	Percent transmittance(%)	OD values	Equivalent LH units per ml. of serum( $\times 10^3$ )	Concentration of pyruvic acid (mg/ml)
1.	Birds with visceral type	1	5.3	1.260	1260	80
		1	2.3	1.610	1610	92
	Total	2		Mean	1435	
2.	Birds with mixed type	1	11.5	0.940	940	106
3.	Birds without any specific gross lesion but positive histopathologic changes	1	20.5	0.690	690	136
		1	29.0	0.540	540	138
		1	35.0	0.410	410	139
	Total	3		Mean	546.6	
4.	Grand total	50		Total Mean	829.4	



**Table 27. ABSOLUTE BASOPHIL COUNT OF LL**

<b>Sl. No.</b>	<b>No. of birds examined</b>	<b>Absolute basophil Count per mm<sup>3</sup> of blood</b>
1.	13	2025 $\pm$ 200
2.	9	1375 $\pm$ 125
3.	14	1525 $\pm$ 225
4.	7	1075 $\pm$ 300
5.	7	775 $\pm$ 50
<hr/>		
<b>Total No. of birds and mean count</b>	50	1460 $\pm$ 106.5

All the birds were sacrificed and studied for gross and microscopic changes. LDH activity was estimated in all these birds. Blood was also utilised for determining Absolute basophil counts.

#### 4.7.1 Serum LDH activity in LL:

Lactic dehydrogenase activity varied from 450-1000 units per ml. of serum with a total mean of 659 LDH units per ml. of serum. Birds with diffuse type of involvement showed LDH levels of 540-1000 units per ml. of serum with a mean of 655.9 units per ml. of serum. Birds with nodular type of lesions represented LDH activity of 540-690 units per ml. of serum with a mean of 600 units per ml. of serum. Birds with mixed type of involvement showed LDH levels of 690-1000 units per ml. of serum with a mean of 910 units per ml. of serum.

Birds which did not reveal any gross lesions but were positive with histopathologic studies showed LDH levels of 450-620 units per ml. of serum with a mean of 541.1 units per ml. of serum. The highest activity of LDH was recorded in birds with mixed type of lesions. The LDH activity of LL with different types of involvement have been presented in Table 28.

**Table 23. CONCENTRATIONS OF LI-LI IN FEATHER TIPS OF DUCKWEED**

No.	Particulate	No. of birds	Percent transference (ST)	Or volume	Equivalent LiH units, per ml. of serum ( $10^{-3}$ )	Concentration of pyruvic acid (mg/ml)
<b>1. Birds with diffuse type</b>						
		6	24.0	0.620	620	132
		4	17.0	0.770	770	120
		7	27.5	0.850	850	135
		2	10.0	1.000	1000	100
		6	20.5	0.690	690	125
		4	29.0	0.840	840	130
		Total		Mean	622.9	
<b>2. Birds with nodular type</b>						
		2	20.5	0.690	690	125
		3	27.5	0.850	850	135
		1	29.0	0.840	840	130
		Total		Mean	693	
<b>3. Birds with mixed type</b>						
		4	10.0	1.000	1000	100
		1	20.5	0.690	690	125
		1	17.0	0.770	770	120
		Total		Mean	910	
<b>4. Birds with any specific gross lesions but positive histopathologic changes</b>						
		2	24.0	0.620	620	132
		3	33.0	0.440	440	144
		1	29.0	0.840	840	130
		2	25.0	0.600	600	125
		1	35.5	0.450	450	145
		Total		Mean	541.1	
<b>5. Grand Total</b>						
		20		Total Mean	699	

## DISCUSSION AND CONCLUSIONS

## CHAPTER - V

### DISCUSSION AND CONCLUSIONS

The intensive poultry rearing under crowded conditions using highly inbred and productive strains of chicken resulted in chickens having a higher incidence of viral tumors.

The present study was undertaken on 3,220 birds to study the incidence of Marek's disease and Lymphoid leucosis.

The differential diagnosis of MD and LL were also carried out by Asmundson and Bielý (1932), Okada (1970), Cho *et al.* (1970), Yamamoto *et al.* (1971), Evans *et al.* (1971), Paliwal and Rajya (1971), Mohiuddin (1972), Mohan and McCune (1976), Neumann and Witter (1979), Rao and Choudary (1981), Beyer *et al.* (1981), Bhoomaiah (1982), Balani (1983), Rathore *et al.* (1985) and Jahn and Bohland (1985).

Out of 3,220 birds necropsied, 522(16.2%) birds revealed lesions of MD and 700(24.5%) birds revealed lesions of LL. Similar frequency of the incidence of MD was reported by Bielý *et al.* (1932), Nakagawa (1965), Feldman and Olson (1965), Cole (1968), Biggs (1968), Cho *et al.* (1970), Rajya and Mohanty (1971), Mohiuddin (1972), Grewal *et al.* (1977), Bhuyan *et al.* (1977), Verma *et al.* (1978), Baruah and Khatra (1979), Rao and

Choudary (1981), Singh *et al.* (1981), Purvulev (1981), Dobos *et al.* (1982), Sah *et al.* (1982), Panda *et al.* (1983), JuraJda and Kavela (1983), Bulow *et al.* (1984), Rao and Sarma (1984), Ginting (1984), Adene (1984), Lapinskaite *et al.* (1984), Moreno and Gonzale (1985) and Palaniswami *et al.* (1985). However different workers reported a lesser incidence of MD, 3.4%(Grewal and Singh, 1976), 3.5%(Halounka and JuraJda, 1982), 1.9% (Boomaiah, 1982), 6.3%(Balani, 1983), 0.4-0.8%(Nicholas, 1984), 4.2%(Hinojosa, 1984) and 1.4%(Rathore *et al.*, 1985). Similar frequency of the incidence of LL was reported by Viraraghavan and Nair (1965), Mohiuddin (1972), Purchase *et al.* (1972), Demodaran and Thanikachalam (1974), Mass *et al.* (1980), Rao and Choudary (1981), Beyer *et al.* (1981), Sriaman *et al.* (1981), Bhoomaiah, (1982), Klucinski *et al.* (1983), Leupal (1984), Burstein *et al.* (1984) and Ghosh *et al.* (1985). However, Hensley (1966), Calnek (1968), Sandelink and Estola (1970), Balani (1983) and Rathore *et al.* (1985) reported lesser incidence of LL being 0.001%, 4.4%, 5.10%, 3.6% and 1.8% respectively.

The lesions in MD were found more in chicks (28.0%) and growers (58.8%) than adult birds (13.2%). Similar observations were also recorded by Asmundson and Biely (1932), Wight (1968), Biggs (1970),

Violitz and Landraf (1970), Paliwal and Rajya (1971), Mohiuddin (1972), Verma *et al.* (1978), Bhoomaiah (1982) and Palaniswami *et al.* (1983). However, Panda *et al.* (1983) reported high incidence of mortality due to MD in adult birds (28.13%) than growers (8.66%).

7 broilers among 468 females of 8 weeks old were also affected with MD showed an incidence of 1.3%. Similar incidence of MD in broilers was also reported by Benton and Cover (1957), Hamaley (1966) and Jurejda and Kavelc (1983).

In Lymphoid leucosis the lesions were found more in adults (62.6%) as against growers (31.6%) and chicks (5.8%). The above data coincides with the findings of Bamberger (1964), Viraraghavan and Nair (1965), Mohiuddin (1972), Mohiuddin (1978) and Bhoomaiah (1982).

In both MD and LL females were more commonly affected than males. The frequency of incidence in MD, in females (89.6%) and in males (10.3%). In LL 84.7% and 15.3% of females and males respectively were affected. The above observation was similar with the findings of Burmester and Nelson (1945), Wallbank and Stubbs (1964), Viraraghavan and Nair (1965), Mohiuddin (1972), Biggs (1973) and Mohiuddin (1978).

Among the different strains of white Leghorn affected with MD, IMA and ID strains showed greater mortality over other strains, the mortality rate being 30.3% and 23.7% respectively, while in LL, IMA and IWF strains were found to be more susceptible than others with a mortality rate of 42.4% and 24.2% respectively. This observation coincides with the findings of Mohiuddin (1978).

The frequency of incidence of organ involvement in Marek's disease in the age group of 0-8 weeks was proventriculus (84.2%), Liver (67.8%), kidneys (62.3%) followed by spleen (60.9%); in 9-20 weeks of birds the frequency of incidence being proventriculus (69.0%), liver (63.8%), spleen (61.5%) and kidneys (53.0%) and in 21 and above weeks age of birds the highest frequency of organ involvement was liver (62.3%), kidneys (56.5%) and proventriculus (56.5%). Similar results of organ involvement due to MD were also recorded by Biely *et al.* (1932), Benton and Cover (1957), Prakash and Rajya (1970), Okada (1970), Yamamoto *et al.* (1971), Nayak and Pradhan (1971), Calnek and Witter (1972), Mohiuddin (1972), Bhuyan *et al.* (1977), Calnek and Witter (1978), Neumann and Witter (1979), Bhoomiah (1982), Sah *et al.* (1982), Ekperigin *et al.* (1983), Rao and Narma (1983) and Glunder *et al.* (1985).



In Lymphoid Leucosis the higher frequency of organ involvement was observed in liver and spleen in all age group of birds. The frequency of incidence in 0-8 weeks of birds was liver (91.3%) and spleen (82.6%); in 9-20 weeks of birds liver (95.6%) and spleen (88.7%) and in 21 weeks and above age group liver (95.7%) and spleen (93.3%). These observations were similar to that recorded by Viraraghavan and Nair (1965), Calnek (1968), Mohiuddin (1978), Bhoomaiah (1982) and Shikov *et al.* (1982).

The visceral type of involvement in ML was found to be more than neural and mixed types, the incidence being 40.2%, 17.2% and 21.0% respectively. This observation coincides with the findings of Benton and Cover (1957), Yamamoto *et al.* (1971), Evans *et al.* (1971), Mohiuddin (1972), Babu *et al.* (1976), Calnek and Witter (1978) and Glunder *et al.* (1985).

The diffuse type of LL was more than nodular and mixed types, and the incidence being 48.1%, 8.9% and 12.7% respectively. Similar findings were recorded by Viraraghavan and Nair (1965), Calnek (1968), Yamamoto *et al.* (1971), Damodaran and Thanikachalam (1974) and Mohiuddin (1978).

The weights of liver and spleen were recorded in LL. The highest weights of liver and spleen were 20.0 gms and 3.0 gms, respectively. These weights report was supported by the findings of Viraraghavan and Neir (1965) and Mohiuddin (1978).

The gross pattern of involvement in different organs of MD varied from areas of diffuse infiltration to discrete nodular tumors. In the present investigation, proventriculus showed diffuse thickening in majority of the cases, Bursa of fabricius was atrophied in almost all the cases, slight enlargement of the liver and spleen were also observed along with nodular tumors. Sciatic nerves revealed loss of striation, yellow discoloration and thickening and unilateral involvement was greater than bilateral involvement. Bilateral kidney involvement was more evident Ovaries showed white lymphoid tumors and in few it revealed cauliflower like appearance. Heart showed single and multiple tumorous foci, while lungs showed slight enlargement with creamy appearance. The gross changes were in conformity with the observations of Walker and Gratton (1968), Jakowski *et al.* (1969), Purchase (1970), Pitter *et al.* (1970), Fujimoto *et al.*

(1971), Babu *et al.* (1975), Calnek and Witter (1978), Baruah and Kautra (1979), Beyer *et al.* (1981), Sah *et al.* (1982), Experigin *et al.* (1983), Rao and Sarma (1984) and Ginting (1984).

The histopathologic features of MD lesion constitute anisomorphic and pleomorphic cells which were regarded as differentiated lymphocytic cell, reticular cell, plasma cell and rarely granulocytic and fibrocytic cell. The MD lesion composed of mainly lymphoid cells and reticular cells of various sizes. Diffuse infiltration of pleomorphic lymphoid cells were observed in the sinusoids and interlobular spaces of the liver, in majority of the cases. The infiltration of lymphoid cells were so diffuse that the normal architecture of the liver was lost. Mitotic figures were frequent in all the organs. In most of the cases central part of the spleen was replaced completely by pleomorphic lymphoid cells. Interstices of parenchymatus tissue of kidney showed focal and diffuse proliferation of lymphoid cells. Bursa of fabricius showed proliferation of lymphoid cells in the interstitial spaces. In nerves, perivascular cell accumulation was prominent in the interspaces of nerve fibres. Few cells comparatively

larger than lymphoid cells with intensely basophilic stained cytoplasm with vacuolation and indistinct nucleus (Marek's disease cell) were observed in liver, spleen, ovaries and nerves.

These histopathologic observations were similar to that recorded by Nakagawa (1965), Payne and Biggs (1967), Purchase and Biggs (1967), Walker and Cretten (1968), Wight (1968), GoodChild (1969), Onda (1970), Witter *et al.* (1970), Paliwal and Rajya (1971), Fujimoto *et al.* (1971), Kakinata *et al.* (1973), Mohan and McCune (1976), Bhuyan *et al.* (1977), Krishna *et al.* (1977), Ekperigin *et al.* (1983) and Lapinskaite *et al.* (1984).

The gross changes in LL was seen mostly in liver and spleen with diffuse enlargement of both the organs. In the nodular form, the nodules appeared as spherical, grayish yellow and glistening. Bursa of fabricius showed enlargement in most of the cases. The gross changes in other organs were rare. These gross changes of LL were in conformity with the observations of Viraraghavan and Nair (1965), Sah and Sarma (1967), Chawla (1968), Calnek (1968), Nayak and Pradhan (1971), Evans *et al.* (1971) and Beyer *et al.* (1981).

The Histopathologic features of LL constitute monomorphic and undifferentiated tumor cells in various organs. In the liver, the proliferative lesion was occupied by uniform distribution of lymphoblasts throughout the organ with degenerative changes of hepatic cells. Expansive tumorous proliferation with large lymphofollicular structures were observed in the spleen, in majority of the cases with diffuse infiltration by lymphoblasts. Focal accumulation of lymphoblasts were seen in between the tubules of the kidney thereby compressing them. Bursa of Fabricius showed proliferation of lymphoblasts within the follicles, while the other organs showed similar distribution of lymphoid cells. These histopathologic changes were in conformity with the observations of Gross et al. (1959), Okada (1970), Yamamoto et al. (1971), Paliwal and Bajya (1971), Mohan and McCune (1976) and Shikov et al. (1982).

The tissue sections and impression smears from various organs of MD and LL stained with Methyl green and pyronin-Y staining technique showed that in MD, the tissue sections and impression smears revealed the presence of poor pyroninophilia with infiltration of anisonorphic and pleomorphic lymphoid cells and the

cytoplasm showed vacuolation and nucleus stained dark bluish coloured, while in LL, the tissue sections and impression smears revealed the presence of bright pyreninophilia with infiltration of uniform lymphoblasts, the cytoplasm of which showed diffuse red colour with purple coloured nucleus. These observations was supported by the findings of Cooper *et al.* (1968), Siccardi and Burmester (1970), Prakash and Rajya (1970), Mehan and McCune (1978), Babu *et al.* (1976), Calnek and Witter (1978) and Chaudhan *et al.* (1985).

Absolute basophil count was compared in MD and LL for the first time. In MD it ranged from  $750 \pm 150$  to  $1500 \pm 525$  ABC/mm<sup>3</sup> of blood. In LL, Absolute basophil count activity was  $775 \pm 50$  to  $2025 \pm 200$  ABC/mm<sup>3</sup> of blood. Healthy birds showed a range of  $475 \pm 75$  to  $725 \pm 225$  ABC/mm<sup>3</sup> blood, the Absolute basophil count was greater in LL when compared as against MD.

The serum LDH values in apparently healthy birds were 260-600 units per ml. of serum. The serum LDH values in MD infected birds varied from 500-1610 units per ml. of serum. The highest values of LDH was recorded in birds with greater involvement of visceral organs. In the age group of 0-8 weeks with visceral

type of lesions, the LDH values varied from 620-1390 units per ml. of serum; in neural type values varied from 620-1000 units per ml. of serum; in mixed type it was 560-1300 units per ml. of serum, while in birds which did not reveal any gross lesions but were found positive with histopathologic findings, the LDH levels varied from 420-700 units per ml. of serum.

In the age group of 9-20 weeks with visceral type of lesions, the LDH values varied from 540-1460 units per ml. of serum; in neural type values varied from 540-690 units per ml. of serum; in mixed type it was 800 units per ml. of serum; while in birds which were found positive on histopathologic investigation, the LDH values varied from 300-940 units per ml. of serum.

In birds of 21 weeks and above age group with visceral type of lesions, the LDH values were 1260 and 1610 units pr ml. of serum, there was no neural involvement in this age group, while one bird with mixed type of lesion showed LDH levels of 940 units per ml. of serum. Birds which were found positive on histopathologic investigation showed LDH values of

410-690 units per ml. of serum. These LDH values of confirms to the observations of Jones et al. (1969), Prakash and Rajya (1970) and Mohanty et al. (1974).

The serum LDH activity in LL varied from 450-1000 units per ml. of serum with highest activity recorded in birds affected with mixed type of involvement. In birds with diffuse type of lesions, the LDH activity varied from 540-1000 units per ml. of serum and in nodular type LDH values varied from 540-690 units per ml. of serum, while in mixed type it was 690-1000 units per ml. of serum. Wallbank et al. (1964) recorded an increased plasma LDH levels from chicks with clinical leucosis, while DiDonizio et al. (1968) reported a drop in serum LDH activity and an increased liver LDH activity.



## SUMMARY

## CHAPTER - VI

### SUMMARY

Of the virus induced tumors in chickens, Marek's disease and Lymphoid leucosis are the two major problems. Therefore this study was carried out to determine incidence of Marek's disease and Lymphoid leucosis in relation to age, sex, strain, distribution of gross lesions and histopathologic, histochemical changes, absolute basophil count and enzyme(LDH) estimation. The purpose of this study is to make a comparative study of the changes in both the diseases for proper understanding and early diagnosis and as well as to study the behaviour of LDH activity and Absolute basophilic count.

It was proposed to collect and study 500 cases each for Marek's disease and Lymphoid leucosis from the post-mortem examination was carried out in 3,220 birds so as to study the incidence of both diseases. Out of 3,220 birds autopsied, 522 birds were found to be affected with MD(16.2%), and 789 birds were affected with LL(24.5%). In MD growers were found to be more affected than chicks and adults. The frequency of incidence being 58.5%, 13.2% and 27.9% in growers, adults and chicks respectively. In LL adults were

found to be more affected than chicks and growers. The frequency of incidence being 62.6%, 31.6% and 5.8% in adults, growers and chicks respectively.

Females were more susceptible than males in both MD and LL. The percentage of mortality in males and females due to MD was 10.3% and 89.6% respectively, while in LL 15.3% and 84.7% of males and females were affected respectively.

In the present study in MD, visceral type of lesions were found to be more than neural and mixed. The frequency of incidence being 40.2%, 17.2% and 21.0% due to visceral, neural and mixed types respectively.

In LL diffuse type of lesions were found to be more than nodular and mixed types in all the age groups of chickens. The frequency of incidence being 48.1%, 8.9% and 12.7% due to diffuse, nodular and mixed types respectively.

In MD the involvement of different organs was different in different age group of birds. In birds 0-8 weeks of age, the incidence of organ involvement being proventriculus(42.4%), liver (39.7%), kidneys(37.0%)

and spleen (33.6%) and in 9-20 weeks of age group proventriculus(71.6%), liver (61.9%), spleen (54.7%) and kidneys(53.4%), while in the age group of 21 weeks and above it was peripheral nerves(97.0%), liver (69.5%), kidneys(63.7%) and proventriculus(63.2%).

Birds of different age groups affected due to LL showed that liver and spleen were the major organs affected. The frequency of involvement of the liver and spleen in the age group of 0-8 weeks was 76.0% and 69.0% respectively, while in 9-20 weeks it was 36.2% and 53.0% respectively, and in 21 weeks and above it was 76.0% and 75.3% respectively.

Histopathologic studies showed that the tumors of MD were composed of anisonorphic and pleomorphic cells, including various - sized lymphoid cells, reticular cells and plasma cells commonly and granulocytes and fibrocytic cells rarely, whereas the tumors of LL consisted of uniform large blasts cells.

Histochemical studies by using Methyl - green pyronin-Y staining technique showed that the intensity of pyriminophilia was poor in MD where as in LL it was quite uniform and bright.

The birds used for estimating LDH activity were also utilised to study the behaviour of basophils by doing Absolute basophil count. In MD, absolute basophil count varied from  $750 \pm 150$  to  $1500 \pm 525 \text{ ABC/mm}^3$  of blood, whereas in LL it was  $775 \pm 50$  to  $2025 \pm 200 \text{ ABC/mm}^3$  of blood. Absolute basophil count in 30 normal healthy birds varied from  $475 \pm 75$  to  $725 \pm 225 \text{ ABC/mm}^3$  blood.

The serum LDH activity was studied in 30 affected cases each of Marek's disease and Lymphoid leucosis and in 20 apparently healthy birds. In healthy birds, the LDH activity varied from 260-600 units per ml. of serum. In MD it has a range of 410-1610 LDH units per ml. of serum where as in LL it varied from 450-1000 units per ml. of serum. There was a definite increase in LDH activity in MD as against LL and control.

There were only few studies where in LDH activity was determined in MD and LL. The behaviour of basophil count have not been studied in MD and LL. Attempt was made in the present investigation to study the LDH activity and Absolute basophil counts in both MD and LL.

Hence, the estimation of LDH activity and the determination of Absolute basophil count were found to be much useful techniques to be employed in field laboratories for early detection of both MD and LL. Besides this, staining of impression smears with Methyl green and pyronin-Y stain was found to be a good test for differentiating between MD and LL.

## LITERATURE CITED

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- Adams, D.F., 1984. Serological survey of Marek's disease in exotic and local chickens in Nigeria. *Vet.Bull.* 54:4499.
- Asmundson, V.S., J. Biely, 1932. Inheritance of resistance to fowl paralysis (*Neurolymphomatosis gallinarum*) I. Differences in susceptibility. *Can.J.Res.* 6:371.
- Babu, K.S.J., S.J. Seshadri and S.Mahmuddin, 1976. Some observation on the epidemiology and pathology of lymphoid leucosis and Marek's disease. *Mysore J.Agr.Sci.* 10:133-144.
- Balani, D.K., 1983. Incidence of various poultry diseases in Bahthak and Senepet district (Haryana). *Poultry Adviser* 16:21-33.
- Bamberger, K., 1964. Survey on the experimental and literary basis of the control of avian leucosis complex in Hungary. *Acta. Vet.Hung.* 14:253-263 (*Vet.Bull.* 39:679).
- Baruah, G.K., and M.S. Kwatra, 1979. Studies on Marek's disease in Assam I. Prevalence. *Indian Vety.J.* 56:983-987.
- Benton, W.J., and W.L. Cover, 1957. Incidence of visceral lymphomatosis in broilers. *Avian Dis.* 1:320.
- Beyer, J., R. Heol, K. Vogel, O. Werner and C. Plonio, 1981. Reliability of flock diagnosis of Marek's disease and lymphoid leucosis of fowls, on the basis of pathological findings. *Archiv fur experimentelle Veterinarmedizin* 34:827-846. (*Vet.Bull.* 51:5133).
- Bhoomaiah, S., 1982. Studies on pathology of digestive system in poultry. Thesis submitted to Andhra Pradesh Agricultural University.
- Bhuyan, P.R., and D.C. Nayak, 1977. Incidence and Histopathology of Marek's disease in Poultry in Orissa. *Indian J.Poult.Sci.* 12:33-35.
- Biggs, P.M., 1968. Marek's disease. *Curr.Top.Microbiol. Immunol.* 43:93-123 (*Vet.Bull.* 39:2642).



- \_\_\_\_\_. 1970. In Gordon, R.F. and Freeman, B.M(Eds). Poultry disease and world economy British Poultry Science Ltd., 33, Hantgarny Street, Edinburgh, pp.195(Vet.Bull.42:4708).
- \_\_\_\_\_. 1973. In A.S. Kaplan(ed)., The Herpesviruses, pp.957-94. Academic press, New York.
- \_\_\_\_\_. R.J. Thorpe and L.M. Payne. 1968. Studies on genetic resistance to Marek's disease in the domestic chicken. Br.Poult.Sci.9:57-52.
- Biely, J., E. Palmer and V.O. Asmundson. 1932. Inheritance of resistance to fowl paralysis(*Neurolymphomatosis gallinarum*) II. On a significant difference in the incidence of fowl paralysis in two groups of chicks. Can.J.Res.6:574.
- Bulow, V., B. Fuchs, E. Vialitz and H. Landgraf. 1984. Early mortality syndrome in chicks after double infection with Marek's disease virus and chicken anaemia agent. Vet.Bull.54:2517.
- Burnester, B.R., and M.M. Nelson. 1945. The effect of castration and sex hormones upon the incidence of lymphomatosis in chickens. Poultry Sci.34: 509-515.
- Burstein, H., H. Giload, U. Dondheim and H. Kotler. 1984. Viral aetiology of haemangioendothelioma outbreaks among layer hens. Avian Pathol.13: 715-726(Vet.Bull.55:366).
- Calnek, B.M., 1968. Lymphoid leucosis virus. A survey of commercial breeding flocks for genetic resistance and incidence of embryo infection. Avian Dis.12:404-411.
- \_\_\_\_\_. 1968. Lesions in young chickens induced by lymphoid leucosis virus. Avian Dis.12:111-129.
- \_\_\_\_\_. and R.C. Jitter. 1978. Marek's disease. In Loftstad, H., and Calnek, B.M(eds). Diseases of Poultry, 7th ed.pp.400. Iowa State Univ. Press, Ames.
- Campbell, J.G., 1964. A proposed classification of the leucosis complex and fowl paralysis. Br.Vt.J.147:316-323.

- Chand, N., and P. Byre, 1978. Rapid method for Basophil count in domestic fowl. *Avian Dis.* 22:639-643.
- Chauhan, H.V. ., A.L. Panisup and H. White, 1983. Some histochemical studies on the nervous system and skeletal muscles of poultry affected with Marek's disease. *Indian Vet.J.* 62:282-288.
- Chawla, R. ., 1968. Studies on the Pathology of the affections of gastrointestinal tract including liver and pancreas in domestic fowl. Thesis submitted to Agra University.
- Cho, B.A., S.O. Kenzy and S.H. Mathey, 1970. Histologic and microbiologic studies of chickens with transient paralysis. *Avian Dis.* 14:587-590.
- Cole, L.R., 1968. Studies on genetic resistance to Marek's disease. *Avian Dis.* 12:9-28.
- Cooper, N. ., L.H. Payne, J.H. Dent, B.R. Burnester and L.A. Cook, 1968. In Hofstad, M.G. and Calnek, D.W. (eds). *Diseases of Poultry*, 7th ed. pp.438. Iowa State Univ. Press, Ames.
- Culling, C.F.A., 1974. *Handbook of Histopathological and histochemical Techniques*. 3rd ed. pp.254-255. Butterworths & Co. Ltd., London.
- Emofaran, A., and E. Thanilinchalam, 1974. A survey of poultry necropoles. *Cheiron* 3:148-160.
- Elionizio, S., M. Minoccheri and L. Totara, 1968. Behaviour of Lactic dehydrogenase(LDH) and of its iso-enzymes in fowls that are healthy carriers of avian leucosis and in organs with leukotic lesions. *Vet.Bull.* 38:4675.
- Obos-kovacs, S., A. Erdevari and J. Rajer, 1982. Entophthalmitis(Uveitis) in Poultry flocks repeatedly immunized against Marek's disease. *Vet. Bull* 9:656.
- Ex origin, J. ., A.L. Pelly, L.F. Lee, X. Liu and H.H. McCaper, 1983. Comb lesions and mortality pattern in white leghorn layers affected by Marek's disease. *Avian Pathol.* 27:503-512.

- Evans, D.L., J.S. Beasley and L.T. Patterson, 1971. Correlation of immunological competence with lesions in selected lymphoid tissues from chickens with Marek's disease. *Avian Dis.* 15:680-687.
- Feldman, W.H., and C. Olson, 1969. In Hofstad, M.S. and Calnek, B.W. (eds). *Diseases of Poultry*, 7th ed., pp.389. Iowa State Univ. Press, Ames.
- Fujimoto, Y., H. Nakagawa, K. Okada, M. Okada and K. Matsukawa, 1971. Pathological studies of Marek's disease I. The Histopathology on field cases in Japan. *Jap.J.Vet.Med.* 19:7-26.
- Fadly, A.M., and B. Okazaki, 1982. Studies of avian leukosis virus infection in chickens from a commercial breeder flock. *Poult.Sci.* 61:1055-1060.
- Fedotov, A.V., 1978. Pathological and Immunological diagnosis of Marek's disease and Lymphoid leucosis in fowls. *Veterinariya, Moscow, U.S.S.R.* 6:62-56 (*Vet.Bull.* 48:7519).
- Gavorn, J.P., J.L. Spencer, R.C. Cowe and D.L. Harris, 1980. Lymphoid leucosis virus infection. Effects on production and mortality and consequences in selection for high egg production. *Poult.Sci.* 59:2165-2178.
- Ghosh, S., N.C. Khatra, G.P.R. Sharma, D.C. Verma, D. Anubrata and S. Barua, 1985. Studies on the occurrence of lymphoid leucosis in Nagaland. *Indian J. Anim. Health.* 24:17-19.
- Ginting, B., 1984. Marek's disease in Kedu chickens (case report). *Vet.Bull.* 34:2737.
- Glander, G., U. Hounam, U. Hoffels-Rodmann and M. Friederichs, 1985. Comb lesions as manifestations of Marek's disease in chickens. *Vet.Bull.* 55:7969.
- Goodchild, W.H., 1969. Some observations on Marek's disease. *Vet.Rec.* 84:87-89.

Grewal, G.S., and D. Singh. 1976. Incidence of Marek's disease virus infection in domestic fowl of Punjab (India). Avian Dis. 20:191-194.

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\_\_\_\_\_ and H.P. Singh. 1977. Epidemiology of Marek's disease: incidence of viral specific antigen in feather follicle epithelium of domestic fowl of Punjab. Indian J. Poul. Sci. 12:1-5.

Gross, M.A., B.R. Burmester and W.G. Walter. 1959. Submicroscopic morphology of avian neoplasms. J. Nat. Cancer Inst. 22:33-101. (Vet. Bull. 29: 3345).

Halouzka, R., and V. Jurajda. 1982. Marek's disease: influence of the type of infective inoculum on the development of pathological changes in the skin of fowls. Vet. Bull. 52:7902.

Hensley, L.A., 1966. The incidence of tumors in young chicken. J. Path. Bact. 92:91-96.

Henderson, B.F., M.B. Gardner, I.P. Cherman and E.Y. Johnson. 1974. Investigation of an increase of solid tumors in chickens vaccinated against Marek's disease. Avian Dis. 18:58.

Pinojosa, J.E., 1984. Prevalence of Marek's disease in Matanzas province of Cuba during recent years. Vet. Bull. 54:2735.

Jahn, G., and K. Bohland. 1985. Marek's disease and Lymphoid leucosis in purebred lines of white leghorn and their crosses. Vet. Bull. 55:2294.

Jakowski, R.M., T.H. Fredrickson, R.E. Luginbuhl and J. Helmbold. 1960. Early changes in bursa of fabricius from Marek's disease. Avian Dis. 13:215-222.

---

\_\_\_\_\_ and T. S. C. Osiak. 1970. Hematopoietic destruction in Marek's disease. Avian Dis. 14:374-385.

Jones, R.D., A.J. Kenyon, G.P. Helmboldt and M. Devolan. 1969. Lymphoproliferative diseases of fowl - high LPA levels associated with lymphoblastic leukemia. Avian Dis. 13:579-584.

Jurajda, V., and S. Raveis. 1983. Occurrence of the skin form of Marek's disease and its relation to the age of slaughtered broilers. Poultry Abst. 9:2452.

- Kakihata, K., 1973. Pathological observations of the Bursa of Fabricius in Marek's disease, *Jap.J.Vet. Res.* 21:99-100.
- Klucinski, W., J. Kopeć-Golezak, J. Szesepanska, W. Borzeczka, E. Jitarska, P. Szeleszenek, M. Pfeiffer and G. Jamialkowska, 1983. Use of lymphocyte "Nucleolar test" in the diagnosis of avian leukosis in hens. *Poultry Abst.* 9:1676.
- Krishna, S.P., S.J. Seshadri, I. Mohiuddin and A.V. Bui, 1977. Marek's disease, bursal lesions in spontaneous cases of India. *Vet.J.* 94:1033-1034.
- Lapinskaite, R.A., A.G. Bogushyavichyute and Z.K. Matakevich, 1984. Pathology of Marek's disease (in the Lithuanian SSR). *Vet.Bull.* 94:3579.
- Loupal, G., 1984. Leukosis among Zoo and freeliving birds. *Avian Pathol.* 13:703-714.
- Mass, H.J.L., M.H. Rispens, J.L. Groenendael and J. Vloten, 1980. Age related resistance to avian leukosis virus 1. Influence of age at exposure on mortality and congenital transmission. *Avian Pathol.* 9:227-243.
- Mohanty, G.C., B. Rajya, R.M. Sharma and S. Gopala-krishna, 1974. Studies on Marek's disease. Annual Scientific report of the Pathology division, I.V.R.I., Izatnagar (U.P.).
- Mohan, K., and E.L. McCune, 1976. Comparative evaluation of a few diagnostic techniques in Marek's disease and Lymphoid leucosis. *Indian Vet.J.* 53:743-745.
- Mohiuddin, S., 1972. Report presented at uniser Inst. Avian Path., I.V.R.I., Izatnagar (U.P.).
- Mohiuddin, S.M., 1978. A study on the incidence of Lymphoid leukosis in different strains of white leghorn over three generations. *Indian J. Poul.* 13:113-124.
- Moreno, A., and L. Gonzalez, 1985. Influence of continuous cycle and closed systems of rearing commercial replacement pullets on the incidence of Marek's disease. *Vet.Bull.* 55:1031.

- Nakagawa, M., 1965. Pathological studies of fowl paralysis - The relationship between the lesions in the nervous system and those in the visceral organs. *Jap.J.Vet.Res.*13:35-56.
- Nayak, B.C., and H.K. Pradhan, 1971. A case record of proventricular avian leucosis with ulceration. *Indian J.Poult.Sci.*6:6-8.
- Neumann, U., and A.L. Witter, 1979. Differential diagnosis of LL and MD by tumor-associated criteria I. Studies on experimentally infected chickens. II. Studies on field cases. *Avian Dis.*23:417-426.
- Nicholas, T.J., 1984. Marek's disease in sixty week old laying chickens. *Aust.Vet.J.*61:243.
- Okada, M., 1970. Light and Electron microscopic observations on the visceral lesions in Marek's disease. *Jap.J.Vet.Res.*18:97.
- Palaniswami, K.S., E. Thyagrajan, N. Mahalingam, G.A. Pothanulla Khan and V.J. Gajapathy, 1985. Pattern of Marek's disease outbreak in a broiler parent stock. *Poultry Adviser* 18:53-56.
- Palival, G.P., and B. Rajya, 1971. Paralysis in domestic fowl. Histopathological and histochemical studies. *Indian J.Anim.Sci.*41:1144-1150 (*Vet.Bull.*42:6478).
- Panda, B.K., S.C. Arya, H.K. Pradhan and T.C. Johari, 1985. Marek's disease incidence in various strains of white leghorn. *Indian J. cult.Sci.* 18:100-103.
- Payne, L.H., and P.M. Biggs, 1967. Studies on Marek's disease II. Pathogenesis. *Vet.Bull.*38:1053.
- Phillip, G., Cebaude and P. Broblewski, 1958. Colorimetric measurement of lactic dehydrogenase activity of body fluids. *Am.J. Clin.Pathol.*30: 234-236.
- Prakash, D., and B. Rajya, 1970. Avian leucosis complex. II. Pathoanatomy and serum lactic dehydrogenase level of MD in natural infection. *Indian J. Anim.Sci.*40:297-308.

- Purchase, H.C., 1970. Virus specific immune fluorescent and precipitation antigens and cell free virus in the tissues of birds infected with Marek's disease. *Cancer Res.* 30:1898:1908(*Vet. Bull.* 41:13).
- \_\_\_\_\_, and P.M. Biggs. 1957. Characterisation of five isolates of Marek's disease. *Res. Vet. Sc.* 8:440-449(*Vet. Bull.* 38:664).
- \_\_\_\_\_, W. Okazaki and B.R. Burnester. 1972. Longterm field trials with the HVT vaccine against MD. *Avian Dis.* 16:57.
- Purvulov, B., 1981. Economic effectiveness of Marek's disease vaccination in a poultry flock. *Vet. Bull.* 51:276.
- Rajya, B.S., and G.C. Mohanty. 1971. Report on Marek's disease Annual symposium All India Poultry Science Association, Bangalore.
- Rao, B.R., and C. Choudary. 1981. Certain poultry neoplasms encountered in Andhra Pradesh. *Poultry Adviser* 14:37-39(*Vet. Bull.* 52:299).
- Rao, T.C., and P.L.N. Sarma. 1984. Some observations of Marek's disease in an organised flock of white leghorn. *Indian J. Poult. Sci.* 19:74-76.
- Rathore, B.S., . . . Rajendra and S.S. Khara. 1985. Survey on causes of poultry mortality in India based on post mortem examinations conducted at ten diagnostic centres. *Poultry Guide* 10:95-99.
- Roh, R.L., and R.M. Sharma. 1967. Proceedings of Poultry Conference held at I.V.M.I., Izatnagar (U.P.).
- \_\_\_\_\_, G.C. Mohanty and R.C. Verma. 1982. Marek's disease in bantam chickens (*Gallus gallus*). *Indian J. Poult.* 17:57-62(*Vet. Bull.* 52:5688).
- Sandelink, K., and T. Estole. 1970. Studies on the possibility of obtaining leukosis free eggs from commercial poultry flocks, with reference to current laboratory methods. *Acta. Path. Microbiol. scand.* 78B:473-478(*Vet. Bull.* 41:3560).
- Schat, L.A., D.L. Salnok and J. Fabricant. 1981. Influence of the bursa of Fabricius on the pathogenesis of Marek's disease. *Infection & Immunity* 31:190.

- Benevirath, P., 1972. Marek's disease: A new threat to Poultry industry, Poultry Patrika 1:197.**
- Sharma, J.M., D. Burger, and S.G. Keny, 1972. Serological relationship among herpes viruses: Cross-reaction between Marek's disease virus and pseudorabies virus as detected by immune fluorescence, Infection and Immunity 5:406-411 (Vet.Bull.42:3023).**
- Shikov, A.T., S.I. Lutokhin, G.A. Kravnikov, V.S. Tsymbal and L.R. Soloveva, 1982. The leukaemia sarcoma complex of fowls (in the Ukraine) Vet.Bull.5: 3323.**
- Stoccardi, P.J., and S.R. Burnester, 1970. The differential diagnosis of Lymphoid leukaemia and Marek's disease. Tech.Bull.U.S. Dep. Agric.No.1412, PP.23 (Vety.Bull.41:820).**
- Singh, J., P.M. Kaira and M.U. Kharole, 1981. Note on the status of Marek's disease in the Poultry farms of Haryana, Indian J.Anim.Sci.51:1016-1017.**
- Spencer, J.L., A.M. Grunder, A. Robertson and G.W. Speckmann, 1972. Attenuated Marek's disease herpes virus, protection conferred on strains of chickens varying in genetic resistance, Avian Dis.16:94-107**
- Srinanan, P.K., S.R. Ahmed, H.R. J. Naidu and P.R. Rao, 1981. Neoplasia in chicken and ducks, Indian J.Poult.Sci.16:436-437.**
- Verma, R.C., H.V.S. Chauhan, A.C. Arya, G.V. Rao, R.L. Sah and K.L. Inha, 1976. Studies on source aspects of epidemiology and vaccination against Marek's disease in H line breed (Haralmath) in Poultry, Indian J.Poult.Sci.13:102-108.**
- Vielitz, E., and . Landgraf, 1970. Epidemiology and control of Marek's disease, Vet.Bull.41:3027.**
- Viraraghavan, R., and R.P.C. Nair, 1965. Studies on avian leucosis complex (autopsy incidence) Indian Vet.J.42:901-908.**



- Walker, D., and D.A.F. Gratten, 1968. A temporary paralytic syndrome of growing pullets. *Vet. Rec.* 82:43-47.
- Wallbank, A.M., and E.L. Stubbs, 1964. Studies of avian sarcoma and erythroblastosis (Strain 13) III effect of chick sex on virus susceptibility. *J. Natl. Cancer Inst.* 33:807-811 (*Vet. Bull.* 35:2244).
- 
- \_\_\_\_\_, J. Sahl, R. Hubben, C.J. Hollister, E.C. Sterling and E.L. Stubbs, 1964. Use of lactic dehydrogenase for detection of avian leucosis. *Nature* 201:844-846.
- Wight, P.A.L., 1968. The histopathology of transient paralysis of the domestic fowl. *Vet. Rec.* 82: 749-755.
- Witter, R.L., J.I.H. Moulthrop, G.H. Murgoyne and H.C. Cornell, 1970. Studies on the epidemiology of Marek's disease herpesvirus in broiler flocks. *Avian Dis.* 14:253-267.
- 
- \_\_\_\_\_, J.E. Solomon, L.R. Champion and E. Nazarian, 1971. Longterm studies of Marek's disease infection in individual chickens. *Avian Dis.* 15:346-365.
- Yamamoto, H., T. Yoshino, H. Mihara and R. Ishitani, 1971. Histopathologic comparison of Marek's disease with avian lymphoid leucosis. *Nat. Inst. Anim. Hlth. Quart.* 12:29-42.
- Zelenskii, V.P., B.P. Vedvin, L.L. Popova, G.K. Perova, L.I. Zaretskaya, V.A. Oganensyan and L.V. Borisenki, 1980. Epidemiology and prophylaxis of avian leukosis. *Veterinariye, Moscow, U.S.S.R.* 9:34-35. (*Vet. Bull.* 51:1880.)

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