

# **STUDIES ON THE PATHOLOGY OF LIVER LESIONS IN CHICKEN**

*By*

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This is to certify that the thesis entitled “**STUDIES ON THE PATHOLOGY OF LIVER LESIONS IN CHICKEN**” submitted in partial fulfillment of the requirements for the degree of “**MASTER OF VETERINARY SCIENCE**” (in the major subject of Pathology) of the Acharya N.G. Ranga Agricultural University, Hyderabad, is a record of the bonified research carried out by **Ms. D.M.PARIMALA** 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 the author of the thesis.

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### **ABSTRACT**

Liver performs a variety of functions and plays important role in the metabolic processes. The liver lesions were reported in chicken only in relation to certain specific poultry diseases and no detailed systematic studies were done. Hence, the present study was under taken to find out the incidence of various pathological conditions affecting the liver in chicken along with their possible etiological basis.

In the present study 1485 birds comprising of 342 broilers and 1143 layers were subjected to post mortem examination that were procured from commercial

poultry farms situated in and around Tirupati. Gross and histopathological studies were made and the lesions found were reported. Bacterial isolations were also attempted from suitable liver lesions to know the etiology.

Histopathological lesions were observed in 797 birds out of 1485 examined with an incidence of 53.67%. Gross lesions could be observed in 746 birds (50.23%). Various pathological conditions such as circulatory (3.57%), degenerative (19.79%) and inflammatory changes (22.36%) and miscellaneous conditions (7.92%) were recorded.

Circulatory disturbances included congestion, which constituted about 4.68% in broilers and 1.57% in layers and haemorrhages observed to an extent of 2.34% in broilers and 0.96% in layers.

Out of 1485 birds examined, degenerative changes were observed in 19.79% of birds. The incidence of cloudy swelling was 2.05% in broilers and 1.31% in layers. Hydropic degeneration was found in 3.22% and 1.62% of broilers and layers respectively. Fatty change was observed in 4.10% of broilers and in 2.36% of layers. Necrosis was categorized into focal, centrilobular, periportal and diffuse necrosis. Of these focal necrosis was recorded in 4.10% of broilers and in 5.42% of layers amounting to 5.12% out of total birds. It was the most frequently observed type of necrosis. Centrilobular necrosis was 2.63% in broilers and 4.72% in layers. Periportal necrosis was noticed in 3.22% and 4.29% of broilers and layers respectively. Diffuse necrosis was found in 0.18% of layers.

An incidence of 22.36% of inflammatory changes was encountered out of 1485 birds examined. Of these, acute hepatitis was noticed in 4.10% and 5.69% of broilers and layers respectively and sub-acute hepatitis was found in 2.92% of broilers and 5.25% of layers. Chronic hepatitis was recorded in 3.80% and 4.90% of broilers and layers respectively. Perihepatitis was seen in 3.51% and 4.63% of broilers and layers respectively and cholangiohepatitis had an incidence of 2.63% in broilers and 3.50% in layers with an overall percentage of 3.30.

Miscellaneous conditions of 7.92% were recorded in the present study out of 1485 birds. Incidence of aflatoxicosis was 2.92% and 3.06% in broilers and layers respectively. Gout was observed in 4.68% of broilers and 3.23% of layers. Fatty liver haemorrhagic syndrome was seen in about 0.58% of broilers, while ascites was found in 2.34% and 0.53% of broilers and layers respectively. Hepatic granuloma was recorded in 0.29% of broilers and 0.09% of layers.

Marek's disease was recorded in of 0.18% of layers. Various microorganisms that were isolated on bacteriological examination from the liver lesions included *Clostridium perfringens*, *E.coli*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Salmonella gallinarum*. Of these, *Clostridium perfringens* was mainly responsible for cholangiohepatitis and *E.coli* for perihepatitis. Few suspected feed samples were collected and analyzed for aflatoxin content. Aflatoxin B<sub>1</sub> was detected that ranged from 0.01 to 0.5ppm.

From the present study, it was evident that considerable number of liver lesions were observed in chicken and a systematic study of liver lesions is helpful in making diagnosis of various poultry diseases.

## CHAPTER – I

### 1. INTRODUCTION

The liver performs a diverse array of functions in birds as in mammals and plays a pivotal role in digestive functions, metabolism of proteins, fats and carbohydrates and detoxification of metabolites. So, the disorders of liver often have far reaching consequences. In general, the liver is frequently affected by various infectious agents, toxic substances and systemic disease processes and the resultant pathological changes are expressed as hepatosis, necrosis, fatty liver, hepatitis, cholangitis etc.

Of these, hepatitis may be caused by viral and bacterial infections, acute mycotoxicosis, toxic agents or obstruction of the biliary system in the liver (Drago and Don 1996). The bacterial agents include *Campylobacter jejuni*, *Salmonella pullorum*, *S.gallinarum*, *Pasteurella* sp., *Clostridium perfringens* and *Escherichia coli*. Avian adenoviruses cause Inclusion body hepatitis. Chicken anemia agent will increase the likelihood of adenoviruses causing liver inflammation and mortality in birds. Likewise, various mycotoxins, especially aflatoxin, too damage the liver leading to increased mortality and decreased production and weight gains. Meat inspection data on liver lesions serves as an indicator of necrotic enteritis, caused by *Clostridium perfringens* in broiler

flocks (Lovland and Kaldhusdal, 1999). In some instances, the liver lesions become responsible for carcass condemnation (Randall *et.al.*1983).

Poultry farming in India has witnessed a tremendous growth during the last three decades and is contributing a lot to GDP. Of late, poultry industry is being confronted by several threats from various quarters. Newer diseases are emerging because of improper bio-security measures and compromised managerial practices and these conditions are also helping the existing pathogens to modify themselves and produce new form of diseases. In this situation, proper disease diagnosis plays a key role to curtail the losses caused by various diseases, by enabling the adaptation of proper control and preventive measures.

Even though, highly sophisticated and costly equipment and modern techniques are available, the value of postmortem diagnosis and histopathological confirmation is unequivocal in disease diagnosis and it is considered as most important tool in early diagnosis of diseases of poultry. The frequent pathological findings that one come across in various diseases of poultry comprise of the involvement of liver. In many instances such lesions and the pattern of changes in this organ are of great value in diagnosis of poultry diseases. Also, perusal of the available literature revealed that the liver lesions

were reported only in relation to certain specific poultry diseases and not many detailed systematic studies were conducted. Hence, the present study on liver lesions occurring in chicken was felt necessary. As poultry rearing in Rayalaseema region of Andhra Pradesh, especially in and around Tirupati has grown up as an established industry, the demands on the services of the pathologist have considerably increased.

Hence, keeping this scenario in view the present study was designed:

1. To study the incidence of various pathological conditions affecting the liver in chicken.
2. To define their etiological basis wherever possible.

## CHAPTER –II

### 2. REVIEW OF LITERATURE

Perusal of the available literature revealed that liver lesions were reported only in relation to certain specific poultry diseases and scanty information was available on spontaneous liver lesions. (Rahamathulla,1972 and Hutchison *et.al.*1990). The common etiological agents or conditions affecting the liver primarily are bacteria viz. *Escherichia coli*, *Salmonella pullorum*, *Salmonella gallinarum*, *Pasteurella multocida* and *Clostridium perfringens*; viruses viz. adenoviruses and tumor inducing viruses; mycotoxins like aflatoxin and metabolic disorders like gout and fatty liver syndrome etc.

The incidence and etiopathology of liver lesions in different diseases of chicken are reviewed here under.

#### 2.1 INCIDENCE:

Clayton and Godfrey (1952) while summarizing the causes of mortality in poultry from 14 years records in Oklahoma found 4% mortality due to liver

disorders. Tudor (1963) examined 2,679 White Leghorn birds and found 24 cases of liver disorders of which 19 cases were of necrosis and 5 were hepatitis.

Sivadas *et.al.* (1970) in their extensive survey for a period of 8 years, recorded mortality of 3.6% due to necrosis of liver in 3-6 months age group poultry and 0.3% due to hepatitis and 0.2% due to cirrhosis in over 6 months age group poultry.

Rahamathulla (1972) reported an incidence of 7.6% liver lesions in poultry excluding Avian Leucosis Complex (ALC) and an incidence of 39.87% including ALC.

Gough and Weber (1978) recognized a massive liver haemorrhage in different flocks of Ontario broiler chickens from 1972-'76. Six flocks that were affected in 1976 showed daily mortality ranging from 0.5-3%. Whereas the liver haemorrhagic syndrome was diagnosed in 1.6% of 2810 adult fowl over a 2 year period by Parthasarathy *et. al.* (1979) in Tamil Nadu.

Epidemiological and pathological studies in out breaks of *Salmonella bareilly* infection in chicken and quails were reported by Kapoor *et.al.*(1980). There was 92.5% mortality with in 17 days in a batch of 148 baby chicks that

were showing liver lesions. Maximum mortality was observed on the 6<sup>th</sup> day, which declined gradually, later on.

Mutalib and Riddell (1982) observed cecal and hepatic granulomas of unknown etiology in chickens. In small flocks of 3-7 month old chicken, 179 outbreaks were diagnosed in Saskatchewan between 1968-'81 and the lesions were generally found at slaughter in the autumn.

Bhattacharyya *et.al.* (1984) investigated broiler chick mortality due to pullorum disease and brooder pneumonia in West Bengal. In chicks with *Salmonella pullorum* infection mortality started on day one and lasted until the 3<sup>rd</sup> week of life, killing 43.52-54.07% of chicks by affecting the liver.

Ascites syndrome was noticed by Coello *et.al.* (1985) as a major cause of mortality for broiler flocks in Mexico with losses averaging 15%.

Bapat *et.al.* (1985) observed mortality in chicks due to *Pseudomonas aeruginosa*. Out of 7840 day old chicks, 2093 chicks died within 4 days. Livers were cultured and *Pseudomonas* was isolated in pure culture.

Moorthy *et.al.* (1985) reported hepatopathology in experimental aflatoxicosis of chickens. Acute toxicosis with 100% mortality was noticed in the chicks fed 6.25 ppm aflatoxin.

Naturally occurring Inclusion body hepatitis (IBH) in Victorian chickens was identified and diagnosed in about 25% of the broiler farms in Victoria with estimated mortality of 20% in some flocks by Reece (1986).

Randall *et.al.* (1986) studied liver abnormality in broilers. Carcass condemnations occurred during December 1985 and early January 1986 because of liver disease in birds of 5 farms. The largest number of affected carcasses and livers was 3.7% and 12%, respectively out of 16,000 broilers.

Lonkar and Prasad (1988) reported 16 cases (38.1%) of fatty liver syndrome in chicken out of 42 cases. Nayak *et.al.* (1988) recorded an outbreak of gout in broiler chickens in West Bengal. Out of 300 broilers, 181 died over a 29-day period. Postmortem examination showed white chalky masses in the liver and other organs.

Asim *et.al.* (1990) studied occurrence of aflatoxins and their associated pathological changes in poultry liver. A total of 110 morbid poultry livers were analysed for aflatoxins and the associated histopathological changes. Aflatoxins

B and G were detected in 82 samples and their levels varied from 12.3-493.0 ppb. Fifty six percent of samples contained <200 ppb aflatoxin, whereas 7.22% of the samples contained high concentration (>300ppb) of aflatoxin.

Incidence of Marek's disease (MD) in Namakkal, Tamil Nadu was reported by Panneerselvam *et.al.* (1990). MD was recorded in 496 (5.78%) of 8582 layers brought to the Poultry Research and Development Center, Namakkal during 1985-1989. The most commonly affected organs were liver (82%) and spleen (74%). Mortality was higher in birds of 9-20 weeks old than in older birds.

Kharole *et.al.* (1991) reported Inclusion body hepatitis ( IBH) in broiler chicks. Six out breaks of IBH were observed in 3-6 weeks old broiler chicks in organized poultry farms around Karnal and Kurukshetra districts. The mortality in these outbreaks varied from 10-60%.

Incidence of *Pseudomonas aeruginosa* in layers of 32 weeks of age was recorded by Kaul *et.al.* (1992). The main symptoms noted were drop in egg production from 80% to 20% and reduced feed consumption by 20%, with an over all mortality of 3%.

Purnima *et.al.* (1993) made pathological studies on natural outbreaks of acute (visceral) Marek's disease in chickens. Deaths were noticed between 11<sup>th</sup> to 45<sup>th</sup> week of age in 6 flocks. Liver (70.5-81.25%) was the most frequently affected organ in all the flocks.

Outbreak of gout in poultry in East Godavari District, Andhra Pradesh was reported by Rao *et.al.* (1993). The mortality rate recorded from day 5 to 3<sup>rd</sup> week of age was 17.9% (358) in the flock of 2000 broilers. Postmortem examination showed chalky white deposits on serosa of the liver and other organs.

Madan Joshi and Bhagwat (1995) studied incidence of fatty liver syndrome in poultry. Mortality data of 10 years (1979-1989) from AICRP on Poultry for Meat and of 6 years (1983-'89) from the Poultry Research Station, Akola, was analysed to find out the incidence of fatty liver syndrome in layers and broilers. The incidence was significantly higher in broilers (8.83%) than layer strain (1.27%), which was probably due to genetic predisposition of broilers.

Fatty Liver Haemorrhagic Syndrome was observed by Reddy *et.al.* (1995) in 294 (3.01%) broiler chickens. Post mortem examinations carried out on 9755

birds from 1985-1993, revealed higher incidence at 7-9 weeks of age (51.1%) and during summer months (65.7%).

Asrani *et.al.* (1997) noticed Hydropericardium – hepatopathy syndrome in Asian poultry. Twenty five broiler flocks, varying in size from 500-4000 birds, suffering from hydropericardum-hepatopathy syndrome were thoroughly examined. The syndrome was evidenced by a sudden high mortality, mostly in 2-6 week old broilers. Rare out breaks were also recorded in broiler breeder flocks (32 weeks old) and commercial layers (17 weeks old) with approximately 5-8% mortality.

Nakamura *et.al.* (1999) studied comparative pathology of liver lesions of broiler chickens that died of ascites, heart failure and others in 120 dead birds where mortality rates were 55.8, 33.3 and 10.8%, respectively.

Jindal *et.al.* (1999) reported *Salmonella gallinarum* and *Salmonella enteritidis* infection in poultry in some parts of Haryana. Mortality due to fowl typhoid was 4.82% during 1994-'95 and had gone up to 12.12% during the year 1996-'97. These infections accounted for 5% of total poultry disease out breaks investigated during that period.

Uma *et.al.* (1999) studied pathology of gout in poultry. A total of 8175 birds were received for necropsy in the department during the year 1992-'93 from various poultry farms. Of these 241 (2.94%) cases revealed lesions of gout in liver, heart, lungs, proventriculus and kidneys.

Lovland and Kaldhusdal (1999) noticed liver lesions at slaughter as an indicator of necrotic enteritis in broiler flock. During the first half of 1998, 33 out of 45 sampled carcasses that were condemned owing to liver lesions showed histopathological changes consistent with earlier described *Clostridium perfringens* associated hepatitis. Further, cholangiohepatitis in broiler chickens in Japan was studied by Sasaki *et.al.*(2000a). Forty five broiler carcasses with the liver lesions were examined bacteriologically and *Clostridium perfringens* was identified and was suggested that it may be important in the pathogenesis of cholangiohepatitis in broiler chickens.

Etiopathological investigation of *Salmonella gallinarum* infection in broilers was studied by Hafeeji *et.al.* (2000). Four hundred and eight birds received for autopsy examination were made use for gross and histological observations. The average mortality due to fowl typhoid was 8.29%.

## **2. 2 ETIOPATHOLOGY OF LIVER LESIONS:**

### **2.2.1 Bacterial diseases:**

Kapoor *et.al.* (1980) conducted pathological studies in outbreaks of *Salmonella bareilly* infection in chickens and quails. Necropsy revealed necrotic foci on the surface of liver. Histologically, focal necrosis, microgranulomae in the parenchyma and perivascular lymphocytic infiltrations in the portal areas were seen in the liver.

Mutalib and Riddell (1982) described cecal and hepatic granulomas of unknown etiology in chickens. Grossly liver was studded with numerous nodules of two types; one smooth, well demarcated and spherical, and the other irregular with rough surface and consisted of a collection of smaller nodules. Histologically smooth nodules consisted of a central core of necrosis, separated from the normal tissue by a ring of inflammatory cells consisting of giant cells, macrophages and heterophils and advanced cases, by a thick fibrous capsule. Whereas the rough nodules had many small areas of necrotic debris surrounded by a similar reaction.

Pathology of spontaneous colibacillosis in a broiler flock was studied by Nakamura *et.al.* (1985). Histopathologically, fibrinous thrombi in sinusoids of the liver with necrosis of hepatic cells and fibrinopurulent inflammation with granulomatous changes in the serosa of liver were observed.

Ling *et.al.* (1987) made observations on the pathology of colibacillosis in chickens. Petechial haemorrhages in the internal parenchymatous organs and a fibrinous serositis were seen grossly. Histology revealed acute – chronic fibrinous inflammation and granulomatoid changes in the serous coverings of the liver capsule.

Shapiro *et.al.* (1988) observed unusual necrotizing cholangiohepatitis in broiler chickens. Grossly livers were of normal size or had slightly rounded borders with distension of gall bladder. Discrete green foci or pinpoint white foci distributed randomly on the liver were also noticed. Microscopically, hepatic parenchyma associated with bile ducts had large, often coalescing, foci of acute and subacute liquefactive necrosis. The bile duct associated with necrotic foci had hydropic changes, necrosis and sloughing of epithelial cells.

Hutchison and Riddell *et.al.* (1990) studied hepatic lesions of broiler chickens in two syndromes. One with hepatosis had enlarged, pale and firm livers. *Clostridium perfringens* type A microorganisms were isolated from this and the liver with hepatosis, was graded as 1-3, basing on mild, moderate to severe loss of hepatocytes with multifocal replacement mainly by proliferation of bile ducts and fibrosis. There were also multifocal heterophilic and lymphocytic infiltrates through out the parenchyma. The other one with ascites

revealed cobblestone –appearing liver with thick capsule, sinusoidal congestion with mild periportal and sinusoidal fibrosis.

Kaul *et.al.* (1992) studied incidence of *Pseudomonas aeruginosa* in layers. Grossly liver revealed congestion and haemorrhages. Livers were collected aseptically and the organisms were isolated and identified as *Pseudomonas aeruginosa* based on colony morphology and biochemical reactions. Microscopically, congestion haemorrhages, degenerative and necrotic changes with heterophil infiltration were prominent.

The birds showed the presence of cheesy yellow pseudomembrane covering the viscera accompanied by perihepatitis and pericarditis in *Escherichia coli* infection (Mukherjee and Khanapurkar 1994). Histopathological examination revealed hepatocytic degeneration and congestion in the liver. Pourbakhsh *et.al.* (1997) observed moderate to severe lesions of perihepatitis and pericarditis in experimentally inoculated *Escherichia coli* infection in chickens. Microscopically, inflammatory cell infiltration, serous to fibrinous exudate and cellular debris on serosal surfaces were present in the liver.

Kokosharov *et.al.* (1997) reported clinical, bacteriological and pathological studies of experimental fowl typhoid. Liver was enlarged, friable

and hyperaemic with distended gall bladder grossly and revealed congestion, diffuse vacuolar degeneration and reactive necrosis histologically.

Pathology of experimentally induced fowl cholera in chickens was studied by Khan *et.al.* (1997). Grossly generalized congestion and haemorrhages, and microscopically focal coagulation necrosis in the liver along with mononuclear cell infiltration were commonly observed.

Singh *et.al.* (1998) studied experimental pathology of *Salmonella gallinarum* cytotoxin in adult White Leghorn birds. Grossly, swollen, greenish brown liver with grayish white foci were evident. Microscopic examination showed congestion, degeneration and fatty changes. At places of hepatic cell necrosis, infiltration of large number of mononuclear cells and few heterophils were observed. Large subcapsular haemorrhagic areas were also recorded.

Sasaki *et.al.* (1998) reported out break of cholangiohepatitis due to *Clostridium perfringens* in broiler chicken. Grossly, liver showed enlargement and discoloration with apparent acinar pattern. The gall bladder was enlarged and extrahepatic bile ducts contained yellow inspissated material. Histopathologically, bile ductules were extensively proliferated with fibrosis forming interlobular bridges between triads. The portal bile ducts were destroyed

and obstructed by multiple granulomas due to bacterial infection and the bile outflow. Infiltration of lymphocytes and plasma cells with germinal centers was also observed around granulomas. Similar findings were again reported by Sasaki *et.al.* (2000b) in cholangiohepatitis of chicken induced by bile duct ligations and inoculation of *Clostridium perfringens*.

Aetiopathological investigation of *Salmonella gallinarum* infection in broilers was reported by Hafeeji *et.al.* (2000). Liver showed marked enlargement, congestion, white grey necrotic foci and necrotic patches that were distributed uniformly on their surfaces. Histopathologically, mild to moderate congestion and haemorrhages, focal to diffuse areas of coagulative necrosis and mononuclear cell infiltration in parenchyma were noticed. Occasionally, septic emboli were observed in hepatic parenchyma indicating invasiveness of the organisms.

Prasanna *et.al.* (2001) studied experimental fowl typhoid and pullorum disease infections in chicken. Histologically, liver in both the diseases showed degeneration, early vacuolar changes in the hepatocytes, hypertrophy of kupffer cells, focal infiltration of mononuclear cells, dilatation of hepatic sinusoids and necrosis.

## **2.2.2 Viral diseases:**

### **2.2.2.1 Diseases caused by Adeno viruses (Group IV):**

Inclusion body hepatitis in chickens was reported by Howell *et.al.* (1970). Grossly, they observed that the livers were swollen, mottled and had a reticular pattern of fine linear and stellate haemorrhages beneath the capsule. Histologically, dissociation of hepatic cords and marked vacuolation of the hepatic cells were apparent. Focal areas of degeneration and necrosis and focal collection of round cell leucocytes were noted in some areas. Large Cowdry type A intranuclear inclusion bodies that were surrounded by a clear halo were also noticed in degenerating liver cells by them. Further, Pettit and Carlson (1972) and Macpherson (1974) also reported Inclusion body hepatitis in broilers.

Balachandran *et.al.* (1993) recorded an outbreak of Inclusion body hepatitis in a layer flock and observed congestion, a few petechiae with yellowish discoloration and circumscribed small greyish white foci on the surface and in parenchyma of the liver. Histologically, liver revealed discrete foci of necrosis with disrupted hepatic cords surrounding these foci. The blood vessels were engorged and few hepatocytes revealed intranuclear eosinophilic spherical inclusion bodies.

Hydropericardium-hepatopathy syndrome in Asian Poultry was reported by Asrani *et.al.* (1997). Grossly, hydropericardium with accumulation of clear straw- colored, watery or jelly-like fluid in the pericardial sac was seen. Liver

was enlarged, congested, friable and pale, containing petechial and ecchymotic haemorrhages. Microscopically, the liver showed fatty change and focal areas of lymphocytic infiltration. The conspicuous intranuclear basophilic inclusion bodies were seen in hepatocytes surrounded by clear halo. Many hepatocytes contained large dense hyperchromatic nuclei.

Ravikumar *et.al.* (1998) studied pathology of hydropericardium syndrome in natural outbreaks. Grossly, typical hydropericardium with 5-15ml of clear, straw colored fluid in pericardial sac along with enlarged, pale to tan yellow colour liver having rounded borders were observed. Microscopic examination of liver revealed congestion, haemorrhage, fatty change, coagulative necrosis, infiltration of inflammatory cells and intranuclear basophilic inclusion bodies in the hepatocytes. Inflammatory cells comprised of heterophils, lymphocytes and macrophages either in the portal areas or in the parenchyma.

Pathological studies on hydropericardium syndrome in broilers (Thakur and Grewal 1999) revealed grossly, distended pericardium containing 5-8ml of fluid and the enlarged and pale liver that had haemorrhagic foci. Microscopically, congestion, haemorrhages, degeneration and necrosis were noticed in liver. Intranuclear basophilic inclusions in hepatic cells were first seen followed by eosinophilic inclusions rarely.

Grossly, accumulation of clear or amber-colored watery or jelly-like fluid in the pericardial sac and discolored, swollen, reticulated and friable liver with focal necrosis were observed in Hydropericardium hepatitis syndrome of broiler poultry (Ganesh and Raghavan 2000). Microscopically, small multifocal areas of coagulative necrosis, mononuclear cell infiltration and the presence of intranuclear basophilic inclusions in the hepatocytes were observed.

#### **2.2.2.2 Neoplastic viral diseases:**

Babjee (1970) studied acute Marek's disease (MD). The gross lesions in the visceral organs were either of nodular form or of smooth and greyish-white in appearance. Massive infiltrations with pleomorphic lymphocytes and with almost complete loss of normal structure were seen histologically in the affected organs including liver.

Mayahi and Sharma (1991) noticed acute Marek's disease in local nondescript chickens in Iran. Grossly the liver was enlarged and greyish white nodules of varying sizes (pinhead to pea) were studded in its parenchyma. Histopathological examination revealed infiltration of pleomorphic lymphoid cells.

Pathological and serological studies on natural outbreaks of acute (visceral) Marek's disease in chickens were conducted by Purnima *et.al.* (1993). Greyish white nodular tumour formation was observed grossly in various organs including liver. Microscopically the cellular composition of lymphoid tumour consisted of pleomorphic lymphocytes including small and medium lymphocytes, blast cells and MD Cells.

Mujeeb Ather (1993) studied histopathological and histochemical changes in Marek's disease and lymphoid leucosis. In Marek's disease, liver showed diffuse infiltration of pleomorphic lymphoid cells in the sinusoids and in the interlobular spaces, with localized accumulation of neoplastic cells especially around the blood vessels. Hepatic cells also revealed degenerative and necrotic changes. Few cells comparatively larger than lymphoid cells with intensely basophilic stained cytoplasm with vacuolation and indistinct nucleus (MD cell) were noticed. Whereas in lymphoid leucosis proliferative lesion was occupied by uniform distribution of lymphoblasts throughout the organ. The tumour cells had moderately basophilic cytoplasm and the nucleus was round and displaced. Accumulation of lymphoblasts was surrounded by endothelial cells of the sinusoids.

Grossly visible tumours almost invariably involving liver and other organs were reported in lymphoid leucosis by Calnek *et.al.* (1997). Tumours were soft, smooth and glistening and either nodular, miliary or diffuse with cut surface appearing slightly greyish to creamy white. Histopathologically nodules in the liver were surrounded by a band of fibroblast like cells that have been shown to be remnants of sinusoidal endothelial cells and the tumours consisted of aggregates of large lymphoid cells at same primitive developmental stage.

Narang *et.al.* (2003) reported occurrence of Marek's disease in vaccinated layer flocks in Haryana. Grossly, small to large sized greyish white tumours were seen on liver, spleen, heart, kidneys, lungs and ovaries along with thickening of sciatic nerves. Histopathologically, infiltration of pleomorphic lymphoid cells such as lymphoblasts, small and large lymphocytes, plasma cells and occasionally MD cells was the characteristic finding.

### **2.2.3 Mycotoxicosis:**

Aflatoxins are the toxic metabolites elaborated by *Aspergillus* sp., of fungi and liver is the target organ.

Livers were pale yellow with subcapsular reticulation in broiler chicks fed with 22.5 ppm aflatoxin (Gabriel, 1971). Histologically, fatty degeneration,

nodular hyperplasia, occlusion of hepatic sinusoids and enlarged nuclei were noticed.

Schroeder *et.al.* (1972) described pale and soft livers in broiler chicks that received crude aflatoxin (3.2mg/Kg). Histologically, hepatic cell degeneration, bile duct hyperplasia and focal lymphoid nodules were observed.

Moorthy *et.al.* (1985) observed pale yellow livers with haemorrhages, hematomas and nodules in White Leghorn chicks fed with 3.12 to 6.25 ppm aflatoxin in the diet. Histopathology revealed lipidosis, sinusoidal congestion, paravascular haemorrhages, bile duct hyperplasia, phlebitis and veno-occlusive lesions.

Pale, yellow, friable livers with rounded borders that had degenerative and necrotic changes histologically were reported in broilers in an aflatoxin B1 (1ppm) outbreak by Rao *et.al.* (1985).

Venkatram (1986) observed pale and firm livers in layer chicks that were fed with 1ppm of aflatoxin and sacrificed at 20 weeks. Microscopically, varying degrees of periportal fibrosis, occlusion of portal vessels by microthrombi, proliferation of kupffer cells and dilated bile ducts with hyperplastic epithelium were noticed.

Discolored and enlarged livers were observed in broiler chicken fed with 1 and 3ppm aflatoxin in the diet for 28 days by Balachandran and Ramakrishnan (1987). Histological examination revealed hepatocytomegaly, bile duct hyperplasia and focal necrosis.

Asim *et.al.* (1990) described occurrence of aflatoxins in poultry liver and associated pathological changes. Histopathological changes included fatty change, cellular dissociation, necrosis, cellular infiltration, fibrosis and bile duct hyperplasia.

Prevalence and pathology of mycotoxicosis in poultry was recorded by Harmeet Singh *et.al.* (1994). Liver was pale yellow, mottled, moderate to severely enlarged with subcapsular haemorrhages. Microscopically, congestion, haemorrhages, degenerative changes in hepatocytes varying from granular degeneration to complete necrosis, mononuclear and heterophilic cell infiltration, bile duct proliferation and individualization of hepatocytes were observed.

Pathomorphological studies on experimental aflatoxicosis in commercial broilers were reported by Bakshi *et.al.* (1995). Enlargement of liver with petechial haemaorrhages and degenerative and necrotic changes histologically were observed at 1.5 and 3.0mg/kg concentration of aflatoxin.

Lalrintluanga and Baruah (1997) noticed aflatoxicosis in broiler chickens. Liver showed enlargement, congestion and pale appearance with extreme distension of gall bladder with bile. The hepatic surface appeared glistening and fatty with small white nodular foci. Histologically, degenerated/ necrosed areas were replaced by newly formed hepatocytes. The biliary epithelium showed active proliferation with hyperplastic changes in hepatocytes in portal region. Mononuclear cell infiltration in the interlobular connective tissue was also present.

Outbreaks of aflatoxicosis in broilers were reported by Al-sadi *et.al.* (2000). Liver showed swelling and yellowish discoloration. Microscopically, severe fatty degeneration, cytoplasmic vacuolization of hepatocytes and proliferation of bile duct epithelium were detected in periportal zone. In some cases, nodular hyperplasia of liver parenchyma was also seen.

Pathomorphological studies on naturally occurring mycotoxicosis indicated visceral gout in chicken (Ashok-Gupta *et.al.* 2002). Liver showed congestion and enlargement. Pale, chalky white deposits were seen on liver and other organs. Microscopic examination of liver revealed congestion, vacuolar degenerative changes in the hepatocytes, biliary cell hyperplasia and focal mononuclear cell aggregations.

## **2.2.4 Metabolic Disorders:**

### **2.2.4.1 Gout:**

Nayak *et.al.* (1988) and Rao *et.al.* (1993) reported outbreak of gout in poultry. Grossly, they noted white chalky masses on the liver. Histopathologically, they observed multiple necrotic foci throughout the parenchyma. There were massive depositions of crystals of urates in the sinusoids and central veins in the form of fine meshes of network and irregular sponges along with marked infiltration of heterophils and mononuclear cells at different zones of liver. The hepatic parenchyma surrounding necrotic zones revealed degeneration of hepatocytes and congestion of the sinusoids. The outer capsule of liver showed marked thickening and proliferation of fibrous tissue and infiltration of heterophils and lymphocytes.

Uma *et.al.* (1999) reported pathology of gout in poultry. Deposition of white urate crystals on liver was observed grossly. Microscopically varying degrees of congestion and /or haemorrhages and deposition of urate crystals were noticed. The urate deposition was most conspicuous on the surface of the liver as compared with the parenchyma. The parenchymal deposition was either focal or multifocal accompanied by necrosis along with inflammatory changes.

#### **2.2.4.2 Fatty Liver Syndrome/ Fatty Liver Haemorrhagic Syndrome:**

Fatty liver haemorrhagic syndrome (FLHS) in poultry in Tamil Nadu was reported by Parthasarathy *et.al.* (1979). Grossly massive blood clots covering the liver and in the abdominal cavity were seen in most of the cases. The liver revealed rupture of the capsule and was pale yellowish in colour and extremely friable in consistency. In others there were haematomas of varying sizes in the parenchyma and/or ecchymoses over the capsule. Histopathologically, varying degrees of fatty changes, focal parenchymal and/or subcapsular haemorrhages, disruption and disappearance of reticulum fibres along with the sinusoids were noticeable features. The reticulum fibres around the blood vessels were just discernible. Frozen sections stained by Oil Red O revealed abundant red staining intracellular fat globules.

Lonkar and Prasad (1988) reported fatty liver syndrome in chickens. Grossly liver was enlarged and friable with rounded edges and with mild to moderate congestion. Histopathologically the architecture of the liver was seen well preserved in mild cases. Only some of the hepatocytes contained fat vacuoles in different cords pushing aside the nucleus. In moderately affected cases, though the architecture of liver was retained, some of the cords were disrupted and most of the hepatocytes were involved. Hepatocytes in pericentral

areas were comparatively more affected. In severely affected cases the normal architecture of liver was moderately to severely disturbed. The cords were distorted and disrupted. In few, the whole microsection was replaced by monotonous sheet of vacuoles.

Madan Joshi and Bhagwat (1995) recorded incidence of fatty liver syndrome in poultry. Grossly liver was enlarged, greasy to touch, pale, fragile with rounded edges and showed capillary haemorrhages and hematoma. Histopathological examination revealed mild to severe fatty changes. Parenchymatous cells showed large fatty vacuoles causing complete distortion of the architecture of hepatic cells and hepatic lobules. Hepatic lobules also revealed haemorrhage.

Grossly small pale liver, covered with a layer of clotted blood throughout parietal surface that was attached with fibrin was reported in FLHS in broiler chicken by Reddy *et.al.* (1995). Also the liver was friable, oily to touch and pale yellow in colour. The hepatic cells were loaded with fat histologically and the cords were distorted with the formation of dilated sinusoids. Hepatic cells contained multiple or fairly big fat globule pushing the nucleus to periphery. Infiltration of mononuclear cells in the portal tract was also noticed.

### **2.2.5 Miscellaneous conditions:**

Cavernous haemangioma were recorded in seven cases by Mahander (1984). Nodular growths of 3-7mm filled with blood were noticed on the liver. Histopathologically, large irregular discrete areas lined by flat endothelial cells and filled with blood were noticed in the hepatic parenchyma. Mild to moderate fibrosis around the cavernous areas was discernible.

Tottori *et.al.* (1995) noticed broiler ascites in summer season. Grossly enlarged right ventricle, pericarditis, swelling, thickening or cyst formation of the liver capsule were seen. Microscopically, focal heterophilic infiltration in liver was present, while, Sakumi *et.al.* (1996) noticed liver capsule thickening characterized by mesothelial cell proliferation with vascularization in broiler ascites syndrome. Grossly, inflammation of liver capsule, right ventricular dilation, pericarditis and peritonitis were present. Histopathology showed liver capsule thickening and focal hepatitis.

One and half year old layer chicken showed numerous solid grey tumor masses interspersed with small pea size cysts filled with red green fluid on the entire visceral surface of the liver in cholangiocarcinoma of chicken (Mayahi and Sharma, 1996). On the parietal surface, liver was seen as a thin rim of tissue, compressed by the tumor. Microscopically, irregular ducts of various sizes

occasionally forming cysts were observed with abundant fibrous stroma. The lining epithelium was cuboidal or flattened. In some ducts, pedunculated growth originating from ductal epithelium was observed. Tumor cells were large with vesicular nuclei. Diffuse infiltration of tumor cells into the rest of the liver tissue was also present.

Habib-ur-Rehman *et.al.* (1999) studied clinical, gross and histopathological observations in spontaneous cases of ascites syndrome in broiler chickens reared at low altitude. Gross lesions included accumulation of straw yellow colored fluid in the abdominal cavity, hydropericardium, right ventricular dilation and indurations and fibrosis of the liver. Histopathology showed congestion and necrosis with proliferation of connective tissue in sinusoidal space along with infiltration of heterophils and mononuclear cells.

Nakamura *et.al.* (1999) recorded comparative pathology of heart and liver lesions of broiler chickens that died of ascites, heart failure and others. Grossly, ascites birds showed abdominal distension with clear, yellow fluid having clots of fibrin in the hepatic peritoneal cavities. The liver was swollen, congested and firm with an irregular surface. Cut surface of livers disclosed a tessellated pattern mixed with congestive and pale areas. Fibrin adhered to the surface of the liver. Microscopically, hepatocytic degeneration/necrosis and/or hepatic capsule fibrosis were seen.

## **CHAPTER –III**

### **3. MATERIALS AND METHODS**

The materials for the study consisted of livers collected from 1143 WLH birds and 342 broilers that were subjected to postmortem examination. These birds were procured from the commercial poultry farms located in and around Tirupati. The duration of study lasted from November-2002 to August-2003.

Different representative portions of livers showing apparent gross lesions as well as those without apparent lesions were collected for histopathological examination. The tissue pieces were preserved in 10% formal saline and absolute alcohol and processed by employing routine paraffin embedding method. Sections of five micron thickness were cut and stained by Haematoxylin and Eosin (H&E) method (Luna, 1968). Special stains such as Van-Gieson stain, De-Galanth's stain (De-Galantha, 1935) and Best's Carmine were used wherever necessary. Frozen sections were also made and stained by using Oil Red O for detecting fat.

For isolation and identification of microorganisms, materials were collected from liver lesions in suitable cases by using sterile swabs. These materials were immediately transferred into suitable bacteriological media. The

bacterial isolates were identified basing on colony morphology and Gram's staining and further confirmed by biochemical tests as described in the Bergey's manual (1974). Feed samples were collected from suspected cases of aflatoxicosis and the toxin content was estimated as described in Tropical Products Institute (TPI), London (1972).

## CHAPTER –IV

### 4. RESULTS

Present investigation on liver lesions was carried out in 1485 birds (WLH) including 342 broilers and 1143 layers that were subjected to postmortem examination during the period from November-2002 to August-2003. These birds were procured from commercial poultry forms located in and around Tirupati. Of these, gross lesions were noticed in 746 birds (50.23%). Microscopical examination revealed an incidence of 53.67% (797 cases) of lesions. The details of various histopathological lesions observed were presented in Table I and Figure 1 and 2.

On bacteriological examination various bacteria viz. *Clostridium perfringens*, *Escherichia coli*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Salmonella gallinarum* were isolated from the liver lesions as presented in Table II.

The various pathological changes encountered in liver were described hereunder.

## **4. LESIONS OF LIVER:**

### **4.1 CIRCULATORY DISTURBANCES:**

Circulatory disturbances were observed in 3.57% of total birds examined.

#### **4.1.1 Congestion (Passive hyperaemia):**

The incidence of congestion encountered was 4.68% in broilers and 1.57% in layers. The liver was enlarged, firm, dark red and the blood oozed out freely from bulged out cut surface (Fig.3). Microscopically, the central vein, sinusoids and portal vein were distended with blood (Fig.4).

#### **4.1.2 Haemorrhages:**

This change was found in 2.34% and 0.96% of broilers and layers respectively. On bacteriological examination, *Pasteurella multocida* were isolated from some cases. The livers were swollen and either dark or slightly pale in colour with petechial haemorrhages (Fig. 5). Microscopically, focal haemorrhages were found in between the hepatocytes and sinusoids (Fig. 6).

### **4.2 DEGENERATIVE CHANGES:**

An incidence of 19.79% of degenerative changes was recorded in 1485 birds.

#### **4.2.1 Cloudy swelling:**

The condition was recorded in 2.05% of broilers and 1.31% of layers. Grossly, the livers were slightly pale, enlarged with rounded edges (Fig. 7). The cut surface bulged out slightly on section. Microscopically, the cells were swollen with indistinct membrane and hazy architecture. The cytoplasm was more granular than normal and sinusoids were also obliterated (Fig. 8).

#### **4.2.2 Hydropic degeneration:**

This condition was present in 3.22% of broilers and 1.62% of layers. Gross appearance of the livers was pale and enlarged with rounded borders. Microscopic examination revealed the presence of small, indistinct vacuoles in the cytoplasm with hazy nucleus (Fig.9). These sections were found to be negative for Oil Red O and Best's Carmine stains.

#### **4.2.3 Fatty change:**

This change was encountered in 4.10% of broilers and 2.36% of layers. Grossly, liver appeared pale with rounded borders (Fig. 10). Presence of small, clear vacuoles of varying size in the cytoplasm was observed microscopically (Fig. 11). Oil Red O staining of section revealed the presence of fat in the cells (Fig. 12).

#### **4.2.4 Necrosis:**

Based on location of lesion, necrosis of the liver was categorized as focal, centrilobular, periportal and diffuse necrosis. On bacteriological examination *Salmonella gallinarum*, *Clostridium pefringens* and *Pasteurella multocida* organisms were isolated from some livers.

##### **4.2.4.1 Focal necrosis:**

The incidence of focal necrosis observed in broilers and layers respectively was 4.10% and 5.42%, amounting to 5.12% in total birds examined. In focal necrosis, the livers appeared grossly as enlarged and friable with greyish or yellowish white circumscribed foci of 0.5-1mm diameter scattered on the surface (Fig.13). Microscopically, small homogenous eosinophilic necrotic areas corresponding to greyish or yellowish foci observed grossly were noticed. Surrounding the areas of necrosis, cellular infiltration consisting of mononuclear cells mixed with few heterophils were also seen (Fig.14).

##### **4.2.4.2 Centrilobular necrosis:**

This type of necrosis was seen in 2.63% of broilers and 4.72% of layers and was about 4.24% of total livers examined. Grossly greyish -white necrotic

areas, varying in size from 1-2mm was found on the surface of the liver. These areas extended into the depth of the parenchyma. Microscopically, the cells surrounding the central vein revealed necrotic changes (Fig.15). Surrounding the necrotic areas, infiltration of mononuclear cells were evident.

#### **4.2.4.3 Periportal necrosis:**

This lesion was encountered in 3.22% of broilers and in 4.29% of layers accounting to 4.04% of birds. Grossly the livers were slightly enlarged, pale and friable. Microscopically, the hepatocytes surrounding the portal triads revealed necrotic changes along with mononuclear cell infiltration (Fig.16).

#### **4.2.4.4 Diffuse necrosis:**

This lesion was observed in 0.18% of layers. The incidence of this lesion was 0.13% out of 1485 birds. The livers showed wide spread patchy areas of necrosis with areas of haemorrhage grossly (Fig.17). Microscopically, necrotic changes of hepatic cells were seen diffusely in several contiguous lobules (Fig.18). At some places homogenous eosinophilic structures were evident in the necrotic areas. Infiltration of mononuclear cells was also seen.

### **4.3 INFLAMMATORY CHANGES:**

The inflammatory changes in livers were noticed in 22.36% of birds. These were categorized into acute, sub-acute and chronic hepatitis, perihepatitis and cholangiohepatitis. *Salmonella gallinarum*, *Clostridium perfringens* and *Pasteurella multocida* organisms were isolated from some livers on bacteriological examination.

#### **4.3.1 Acute hepatitis:**

It was having an overall incidence of 5.32%. Among broilers, it was seen in 4.10% of lesions and in layers 5.69% of lesions. Grossly the livers were enlarged and friable in consistency with rounded borders. Some livers had petechial haemorrhages on the surface. Microscopically, congestion of blood vessels and sinusoids was more conspicuous with focal haemorrhages in the parenchyma. In some areas, the hepatic cells revealed cloudy swelling and necrotic changes. Infiltration of heterophils and mononuclears was present in portal triads. Prominent perivascular lymphoid aggregations were also noticed (Fig.19). Hypertrophy of kupffer cells was also evident. The epithelial cells of the bile ducts revealed degenerative change.

#### **4.3.2 Sub-acute hepatitis:**

This change was seen in 2.92% of broilers and in 5.25% of layers amounting to a total incidence of 4.71% in birds examined. Grossly, the liver was friable, swollen and congested. Microscopically, the hepatic cells revealed degenerative changes like cloudy swelling and hydropic degeneration. Some of the hepatic cells showed necrotic changes followed by infiltration of mononuclear cells and few heterophils. Lymphoid aggregations were noticed around perivascular areas and in some cases in parenchyma also (Fig.20). Mild fibroblastic proliferation was found around portal triads. The blood vessels were engorged and the walls were thickened along with degenerative changes in the epithelium of bile ducts. In some cases mild bile duct proliferation was also evident (Fig.21).

#### **4.3.3 Chronic hepatitis:**

Out of 1485 birds examined, chronic hepatitis was observed in 4.65%. In broilers, it was seen in 3.80% of lesions and in layers, the incidence was 4.90%. Grossly, the liver was shrunken, hard and light tan in colour. Microscopically, chronic hepatitis was characterized by fibrosis of portal triads and necrosis of hepatocytes. Some hepatic cells were atrophic. There was proliferation of new bile ducts in the portal areas. In some large bile ducts, bile stasis was observed

along with necrotic changes in the lining epithelium. Vascular fibrosis was more conspicuous (Fig.22and23). Infiltration of inflammatory cells such as lymphocytes, macrophages and plasma cells with proliferation of connective tissue extending into the parenchyma were also seen in the portal areas. The sinusoids were dilated leading to loss of architecture of liver. Van-Gieson's staining showed presence of fibrous tissue around the portal triad (Fig.24).

#### **4.3.4 Perihepatitis:**

Perihepatitis was observed in 3.51% of broilers and in 4.63% of layers with a total of 4.38% out of 1485 birds examined. Grossly, the liver was slightly enlarged and had cheesy yellow pseudomembrane covering the surface (Fig. 25). From these cases, *Escherichia coli* organisms were isolated. In few cases, the liver was greenish in colour with slight yellowish white deposition on the surface (Fig.26). On bacteriological examination *Pseudomonas aeruginosa* organisms were isolated. Microscopically homogenous eosinophilic material was seen on the capsule of the liver (Fig.27). There was infiltration of lymphocytes and few heterophils with proliferation of fibrous tissue in the capsule (Fig.28). In the parenchyma, the blood vessels revealed congestion and perivascular lymphoid infiltration. At some places the hepatic cells revealed degenerative and necrotic changes.

#### **4.3.5 Cholangiohepatitis:**

This lesion was observed in 2.63% of broilers and in 3.50% of layers with a total of 3.30%. *Clostridium perfringens* organisms were isolated from these cases. The liver was slightly enlarged and hard with grayish-white areas grossly (Fig.29). The gall bladder wall was thickened and contained inspissated bile. In these birds, necrotic enteritis was also found. Microscopically, the intrahepatic bile ducts were dilated with degenerative changes of the epithelium and surrounded by connective tissue and inflammatory cells consisting of mononuclear cells (Fig.30 and 31). In some ducts, hyperplasia of epithelial cells was prominent. The lumen of the bile ducts contained bile casts. Further, there was proliferation of new bile ductules in the portal area. The hepatic parenchyma near these bile ducts also showed focal areas of necrosis with infiltration of heterophils, lymphocytes and plasma cells. Hypertrophy of kupffer cells was also found.

#### **4.4 MISCELLANEOUS CONDITIONS:**

The incidence of miscellaneous conditions observed in the present study was 7.92%. Of these, aflatoxicosis was found in 3.03% of total cases. Gout was recorded in 3.56% of birds examined. The incidence of ascites and hepatic

granuloma was 0.94% and 0.13%, respectively. Each, FLHS and Marek's disease were noticed in 0.13% of total birds.

#### **4.4.1 Aflatoxicosis:**

This condition was noticed in 2.92% of broilers and in 3.06% of layers. Grossly, the liver was pale, yellowish, friable and slightly enlarged with rounded borders in most cases (Fig.32). On microscopic examination, congestion of sinusoids and degenerative changes of hepatic cells along with fatty change were found (Fig.33). In some other livers, in addition, proliferation of bile ducts and infiltration of lymphocytes in portal areas and lymphoid aggregates in parenchyma were observed (Fig.33 and 34). The walls of the blood vessels were thickened due to fibrosis. Suspected feed samples were collected and analyzed for aflatoxin content. The aflatoxin B<sub>1</sub> was found ranging from 0.01-0.5 ppm in these samples.

#### **4.4.2 Gout:**

This condition was noticed in 4.68% of broilers and in 3.23% of layers. The liver was enlarged or of normal size, firm and mottled with chalky white masses on serosa grossly (Fig.35). Microscopic examination revealed multiple massive depositions of urate crystals in the parenchyma of liver in the form of

fine meshes of network and surrounded by necrotic hepatic cells with infiltration of heterophils and mononuclear cells (Fig.36). The urate crystals were also deposited below the capsule. The hepatic cells in other areas showed degenerative changes and congestion of sinusoids. De-Galantha's staining method showed black radiating urate crystals in the parenchyma and on the surface of the liver (Fig.37).

#### **4.4.3 Fatty liver haemorrhagic syndrome:**

This condition was encountered in 0.58% of broilers. Massive blood clot covering the liver was observed grossly (Fig.38). The liver was ruptured, pale, enlarged and friable in consistency. On microscopic examination the hepatic parenchyma showed varying degrees of fatty changes (Fig.39) and subcapsular and parenchymal haemorrhages. There was dilatation of sinusoids and mononuclear cells infiltrated into the portal tracts.

#### **4.4.4 Ascites:**

This condition was noticed in 2.34% of broilers and in 0.53% of layers. Grossly, abdominal distension, hydropericardium and accumulation of fluid between the liver capsule and the peritoneal membrane were noticed (Fig.40 and 41). Microscopic examination revealed congestion, distension of hepatic

sinusoids, necrosis of hepatic cells and infiltration of heterophils and mononuclear cells (Fig.42 and 43). The capsule of liver was thickened.

#### **4.4.5 Hepatic granuloma:**

This condition was seen in 0.29% of broilers and in 0.09% of layers. Grossly, the liver showed congestion and studded with numerous spherical nodules with rough surface that were few mm to 2 cm in diameter (Fig.44). Microscopically granulomatous nodules consisted of central core of necrosis surrounded by a ring of inflammatory cells consisting of epithelioid cells, macrophages, lymphocytes and few fibroblasts were found. Some cells having multiple nuclei resembling giant cells were also found (Fig.45&46). Surrounding these nodules, smaller newly formed nodules consisting of epithelioid cells mainly were also evident (Fig.47).

#### **4.4.6 Marek's disease:**

The incidence of Marek's disease was 0.18% of lesions of layers. The liver was enlarged with greyish white nodules grossly (Fig.48). Other visceral organs also showed nodular growths. Histopathological examination of liver revealed nodular infiltration of pleomorphic lymphoid cells including small and medium lymphoblasts and plasma cells with almost complete loss of normal structure of liver (Fig.49 and 50).



**Table-I Details of various histopathological lesions in broilers and layers**

S.No.	Type of lesion	Broiler(342)	Layer(1143)	Total(1485)
		No. (%)	No. (%)	No. (%)
<b>I.</b>	<b>Circulatory disturbances</b>	<b>24 (7.02)</b>	<b>29 (2.53)</b>	<b>53 (3.57)</b>
	1.Congestion	16(4.68)	18(1.57)	34 (2.29)
	2.Petechial haemorrhages	08(2.34)	11(0.96)	19 (1.28)
<b>II.</b>	<b>Degenerative Changes</b>	<b>66 (19.32)</b>	<b>228(19.88)</b>	<b>294(19.79)</b>
	1.Cloudy swelling	07(2.05)	15(1.31)	22(1.48)
	2.Hydropic degeneration	11(3.22)	19(1.62)	30(2.02)
	3.Fatty Change	14(4.10)	27(2.36)	41(2.76)
	4.Necrosis			
	i. Focal necrosis	14(4.10)	62(5.42)	76(5.12)
	ii. Centrilobular necrosis	09(2.63)	54(4.72)	63(4.24)
iii. Periportal necrosis	11(3.22)	49(4.29)	60(4.04)	
iv. Diffuse necrosis	-----	02(0.18)	02(0.13)	
<b>III.</b>	<b>Inflammatory changes</b>	<b>58 (16.96)</b>	<b>274 (23.97)</b>	<b>332 (22.36)</b>
	1. Acute hepatitis	14(4.10)	65(5.69)	79(5.32)
	2. Sub-acute hepatitis	10(2.92)	60(5.25)	70(4.71)
	3. Chronic hepatitis	13(3.80)	56(4.90)	69(4.65)
	4. Perihepatitis	12(3.51)	53(4.63)	65(4.38)
	5. Cholangiohepatitis	09(2.63)	40(3.50)	49(3.30)
<b>V.</b>	<b>Miscellaneous conditions</b>	<b>37 (10.81)</b>	<b>81 (7.09)</b>	<b>118(7.92)</b>
	1. Aflatoxicosis	10(2.92)	35(3.06)	45(3.03)
	2. Gout	16(4.68)	37(3.23)	53(3.56)
	3. FLHS	02(0.58)	-----	02(0.13)
	4. Ascites	08(2.34)	06(0.53)	14(0.94)
	5. Hepatic granuloma	01(0.29)	01(0.09)	02(0.13)
	6. Marek's disease	-----	02(0.18)	02(0.13)
	<b>Total:</b>			<b>797(53.67)</b>

**Table-II**

**Details of various bacteria isolated from liver lesions in broilers and layers**

S.No.	Bacteria Isolated	Broilers	Layers
1.	<i>Clostridium perfringens</i>	6	15
2.	<i>Escherichia coli</i>	10	24
3.	<i>Pasteurella multocida</i>	-	14
4.	<i>Pseudomonas aeruginosa</i>	-	7
5.	<i>Salmonella gallinarum</i>	15	28

## CHAPTER -V

### 5. DISCUSSION

Liver performs a variety of functions in birds as in mammals and plays very important role in metabolic processes. Liver lesions have been described only in relation to certain specific poultry diseases by several authors but detailed systematic studies on spontaneous liver lesions were undertaken by very few workers (Rahamathulla 1972, Randall 1986 and Hutchison and Riddell *et.al.* 1990). In view of scanty literature, the present study was undertaken to study the incidence of various pathological conditions affecting the liver in chicken. This study indicated that sizable number of livers were affected in chicken. Apart from detecting various gross and microscopic changes, bacterial isolates such as *Escherichia coli*, *Salmonella gallinarum*, *Pasteurella multocida*, *Pseudomonas aeruginosa* and *Clostridium perfringens* were isolated from affected livers.

The present study was carried out in 1485 chicken (WLH) comprising of 342 broilers and 1143 layers that were subjected to post mortem examination. Gross lesions were observed in 746 (50.23 %) birds. In the available literature, gross lesions of livers were reported in relation to specific poultry diseases only (Parthasarathy *et.al* 1979, Moorthy *et.al.*1985, Shapiro *et.al.*1988, Purnima *et.al.* 1993, Mukherjee and Khanapurkar 1994, Tottori *et.al.*1995, Khan *et.al.* 1997

and Hafeeji *et.al.* 2000) and no detailed reports on spontaneous liver lesions were available to compare the incidence of various gross lesions occurred in liver in the present study. Microscopic lesions were noticed in a total of 797 birds (53.67%). Whereas, Rahamathulla (1972) reported an incidence of 39.87 % of lesions microscopically in liver including Avian Leucosis Complex (ALC) and 7.62% lesions excluding ALC, indicating the wide prevalence of ALC during that period. The variation in the incidence of liver lesions might be due to changes in the disease pattern and prevalence of various infectious and toxic diseases in the present scenario. In addition, other factors like emergence of newer diseases or new form of existing diseases, environment, strength of the flocks, industrialized poultry production methods, compromised managerial practices, indiscriminate use of antibiotics leading to drug resistance etc. might have also contributed to the increased incidence of liver lesions.

Bacterial isolations attempted in the present study from suitable cases of liver lesions revealed the presence of various micro-organisms like *Escherichia coli*, *Salmonella gallinarum*, *Pasteurella multocida*, *Pseudomonas aeruginosa* and *Clostridium perfringens*. No detailed systematic reports were available in the literature to compare the overall types of bacteria that could be isolated from spontaneous liver lesions. However in specific disease outbreaks attempts were made to isolate the organisms from the liver as a part of disease investigation

and confirmation (Bhattacharyya *et.al.* 1984, Hutchison and Riddell 1990, Kaul *et.al.*1992, Mukhopadhyaya and Mishra 1992 and Hafeeji *et.al.*2000).

Of the various pathological conditions observed in the liver, circulatory disturbances constituted about 3.57%. This incidence was higher when compared with the reports of Rahamathulla (1972) who recorded an incidence of 1.4%. These findings indicated changes in the mortality pattern and occurrence of infections in the current prevailing conditions. Septicemic organisms like *Pasteurella multocida* were isolated from the livers showing haemorrhages. These circulatory disturbances might have occurred due to septicemic infections, bacterial toxins causing vascular damage, unknown toxic factors and infections.

Out of 1485 birds examined, the incidence of degenerative changes was 19.79%. Cloudy swelling was seen in 2.05% of broilers and 1.31% of layers. Hydropic degeneration was 3.22% in broilers and 1.62% in layers and fatty change was found to an extent of 4.10% in broilers and 2.36% in layers. Rahamathulla (1972) noticed degenerative changes like cloudy swelling and fatty degeneration in about 0.94% of liver lesions in chicken. These degenerative changes might be due to infections, toxins, hypoxia, stress and mild irritants. Circulatory disturbances like passive hyperemia and haemorrhage may also contribute to degenerative changes of liver.

Necrosis has been categorized into focal, centrilobular, periportal and diffuse necrosis based on location of the lesion. *Salmonella gallinarum*, *Clostridium perfringens* and *Pasteurella multocida* microorganisms were isolated on bacteriological examination. Of these focal necrosis was most frequent, constituting of 5.12% in total. Shapiro *et.al.* (1988), Kokosharov *et.al.*(1997), Khan *et.al.* (1997), Singh *et.al.*(1998) and Hafeeji *et.al.* (2000) reported focal necrosis in various bacterial diseases. The diseases that run in a septicemic form were capable of causing focal necrosis (Rahamathulla 1972). Centrilobular necrosis with changes around the central veins was encountered in 4.24% of livers examined. This lesion might be due to bacterial toxins conveyed to the liver by blood from the intestines and also due to hypoxic conditions as also opined by Rahamathulla (1972) and Jones *et.al.* (1997). Periportal necrosis was found in 4.04% of total birds and this might be due to toxins conveyed to portal veins. Whereas, diffuse necrosis was seen in 0.13% of birds indicating the severe manifestation of necrosis.

In the present study, the incidence of inflammatory changes noticed in liver was in 16.96% in broilers and 23.97% in layers, amounting to highest percentage of microscopic lesions (22.36%) observed in the liver out of 1485 birds. On bacteriological isolation, *Salmonella gallinarum*, *Pasteurella*

*multocida* and *Clostridium perfringens* organisms were found. The inflammatory changes of liver were of non suppurative type and classified as acute, sub acute, chronic and perihepatitis basing on vascular, cellular and exudative changes as described by Tudor (1954) and Rahamathulla (1972). In addition, cholangiohepatitis was also recorded in the present study.

Acute hepatitis was observed in 5.32% of cases and characterized by predominant vascular changes like congestion of sinusoids and blood vessels and focal hemorrhages in the parenchyma. Rahamathulla (1972) noted 0.625% of acute hepatitis that was lower than the present findings. The incidence of subacute hepatitis was 2.92% in broilers and 5.25% in layers amounting to a total of 4.71% in total birds. In subacute hepatitis the cellular response was conspicuous with mild fibrous tissue proliferation, whereas the chronic hepatitis was characterized by prominent portal fibrosis, bile duct proliferation, necrosis and atrophy of hepatocytes and infiltration of lymphocytes and observed in 4.65% of cases. In poultry, unlike in mammals the chronic hepatitis was not similar to cirrhosis due to less connective tissue in the liver of poultry with only a few connective tissue septa and thin Glisson's capsule (Bradley, 1950) and there may not be much scar tissue formation and damaged hepatic cells may be replaced by infiltration of lymphocytes.

The incidence of perihepatitis was 3.51% in broilers and 4.63% in layers with a total of 4.38% out of 1485 birds examined. *Escherichia coli* and *Pseudomonas aeruginosa* organisms were isolated from these lesions. The liver had cheesy yellow pseudo membrane covering the surface grossly and presence of eosinophilic homogenous material on the capsule microscopically with infiltration of lymphocytes and few heterophils and proliferation of fibrous tissue. These changes were similar to those described by Rahamathulla (1972), Nakamura *et.al.* (1985), Ling *et.al.* (1987), Mukherjee and Khanapurkar (1994) and Pourbakhsh *et.al.* (1997). Vegad and Katiyar (2001) reported perihepatitis in *Pseudomonas* infection of poultry.

The incidence of cholangiohepatitis was 3.30% in total birds examined and *Clostridium perfringens* was isolated from these cases. Focal areas of necrosis and distended gall bladder with inspissated bile were observed grossly. Microscopically, dilated intrahepatic bile ducts with hypertrophy of bile duct epithelium and infiltration of lymphocytes, focal areas of necrosis in parenchyma near bile ducts with infiltration of lymphocytes, plasma cells and hypertrophy of kupffer cells were noticed. These changes were in accordance with the reports of Shapiro *et.al.* (1988), Hutchison and Riddell (1990) and Sasaki *et.al.*(1998) who reported *Clostridium perferingens* infection in broilers. The histopathological changes observed in these cases suggested that the toxin produced by

*Clostridium perferingens* has caused bile duct proliferation and damaged the epithelium and had allowed the toxin and bile to leak through the walls of ducts into periportal hepatic parenchyma, probably causing necrosis of hepatocytes as opined by Shapiro et.al. (1988) and Hutchison and Riddell (1990). Lovland and Kaldhusdal (1999) stated that the liver lesions seen at slaughter could be taken as an indicator of necrotic enteritis caused by *Clostridium perferingens* and it was further suggested that the occurrence of necrotic enteritis caused by *Clostridium perferingens* in broiler flocks may be monitored by using meat inspection data on liver lesions. Whereas Sasaki et.al. (2000a) suggested that *Clostridium perferingens* may be important in the pathogenesis of cholangiohepatitis in broiler chicken. In the present study, cholangiohepatitis associated with necrotic enteritis was also noticed in layers aged about one year. Calnek et.al.(1997) mentioned the outbreaks of necrotic enteritis in 3-6 months old layers.

In the present study, among the miscellaneous conditions the incidence of aflatoxicosis was 3.03 %. Grossly, the livers were pale and yellowish. In most of the cases microscopic examination revealed degenerative changes of hepatic cells including fatty change. Proliferation of the bile ducts, vascular fibrosis and infiltration of lymphocytes in portal areas and parenchyma were also observed depending on aflatoxin content in some livers. Rao et.al. (1985) reported degenerative and necrotic changes in an aflatoxin B<sub>1</sub> (1ppm) outbreak of

broilers. Rajan and Mohiyuddeen (1973), Edds and Simpson (1976) and Moorthy *et.al.*(1985) also reported lesions in cases of experimental aflatoxicosis of chicken. In these experiments the severity of the lesions varied according to the dose of the aflatoxin given and duration of feeding, indicating dose dependent toxicity of aflatoxin. In the present study, the aflatoxin content was detected as ranging from 0.01 to 0.5 ppm in different feed samples collected from the farms of above birds and duration of the feeding could not be ascertained.

The incidence of gout was 4.68% in broilers and 3.23% in layers accounting to a total of 3.56 % in the birds examined. Grossly, the liver was covered with white chalky masses. Microscopically, multiple massive depositions of urate crystals in the parenchyma and below the capsule were observed. These findings were in agreement with Nayak *et.al* (1988), Rao *et.al.*(1993) and Uma *et.al.* (1999). Diet containing high protein and calcium, Vit.A deficiency, water deprivation, renal injuries and some viral infections might have lead to visceral gout.

In the present study, the incidence of FLHS was 0.58% in broilers and it was characterized by pale, enlarged and ruptured liver with massive blood clot. Microscopically, varying degrees of fatty change, sub capsular haemorrhages

and dilatation of sinusoids were noted. These lesions were in accordance with the findings of Parthasarathy *et.al.* (1979), Mohapatra (1992) and Reddy *et.al* (1995). FLHS might be due to diet containing high protein and energy content and deficiency of lipotropic factors causing excessive accumulation of fat in the hepatocytes. This increased fat may disrupt the architecture of the liver and result in weakening of the reticular framework and blood vessels in the liver. Lysis of the reticulin frame work of the liver leads to rupture and the probability of haemorrhages (Parthasarathy *et.al.* 1979 and Calnek *et.al.* 1997 ).

The incidence of ascites was 2.34% in broilers and 0.53% in layers amounting to 0.94% in total birds. Grossly, distention of the abdomen, hydropericardium and accumulation of fluid between the liver capsule and the peritoneal membrane were found. Microscopically, congestion, distention of hepatic sinusoids, necrotic changes with infiltration of heterophils and mononuclear cells and thickening of liver capsule were noticed. These findings were in confirmity with Tottori *et.al.*(1995), Habib-ur-Rehman *et.al.*(1999) and Nakamura *et.al* (1999) who reported ascites in broilers. This might be due to pulmonary hypertension, right ventricular failure, high growth rate, deficiency of protein, high salt intake and liver damage caused by mouldy toxins.

The incidence of hepatic granuloma in the present study was 0.13% in the total examined birds. Grossly, the liver showed congestion and it was studded with numerous spherical nodules with rough surface. Microscopic examination revealed granulomatous nodule consisting of central core of necrosis surrounded by epithelioid cells, macrophages, lymphocytes and few fibroblasts. This was in agreement with Mutalib and Riddell (1982) who described cecal and hepatic granulomas of unknown etiology in chicken and stated that the common localization of these lesions in the livers suggest that the etiological agent may enter the intestinal tract and then pass via the portal circulation to the liver. The cause of granulomas could not be ascertained in the present study. Whereas, a mucoid form of *E. coli* was incriminated as the etiological agent by Prasad *et.al.*1967.

Marek's disease was encountered in 0.18% of lesions in layers aged about one year. Grossly, the liver was enlarged with greyish white nodules. Microscopical examination revealed nodular infiltration of pleomorphic lymphoid cells and plasma cells with almost complete loss of normal structure. Similar lesions were described by Babjee (1970), Purnima *et.al* (1993) and Narang *et.al.*(2003). They further stated that various conditions like breed susceptibility, variation in antibody levels, break down of immunity and exposure by a virulent MD virus could be the possible causes of MD.

## **CHAPTER – VI**

### **6. SUMMARY**

The systematic study of liver lesions occurring in chicken was undertaken to know the incidence of various pathological conditions affecting the liver along with their possible etiological basis. One thousand four hundred and eighty five birds comprising of 342 broilers and 1143 layers were subjected to post mortem examination. Of these, 797 birds revealed liver lesions microscopically accounting for an incidence of 53.67%. Gross lesions could be noticed in 746 birds (50.23%).

The various histopathological conditions of liver have been described under broad headings such as circulatory, degenerative and inflammatory changes and miscellaneous conditions. The incidences of circulatory, degenerative and inflammatory changes and miscellaneous conditions out of 1485 birds were 3.57, 19.79, 22.36 and 7.92% respectively.

Of the circulatory disturbances (3.57%) congestion constituted about 4.68% in broilers and 1.57% in layers. Haemorrhages were found in 2.34% of broilers and 0.96% of layers.

Among the degenerative changes, cloudy swelling was seen in 1.48% of total birds examined. Hydropic degeneration was found in 2.02% of birds. The incidence of fatty change was 2.76%. Necrosis was categorized into focal, centrilobular, periportal and diffuses necrosis. Of these focal necrosis was most frequent amounting to 5.12%. The incidence of centrilobular necrosis was 4.24%, periportal necrosis was 4.04% and diffuse necrosis was 0.13% of total birds.

Out of 1485 birds, inflammatory changes were encountered in 22.36 % cases and that was the highest of all incidences among all the liver lesions. This included 5.32% of acute hepatitis, 4.71% of sub-acute hepatitis, 4.65% of chronic hepatitis, 4.38% of perihepatitis and 3.30% of cholangiohepatitis.

Miscellaneous conditions included aflalotoxicosis, accounting to 3.03% of cases and gout accounting to 3.56% of the total 1485 birds. Fatty liver haemorrhagic syndrome was recorded in 0.58% of broilers. Ascites was observed in 0.94% of birds. Hepatic granuloma was noticed in 0.13% of cases. Marek's disease was noticed in 0.18% of layers.

On bacteriological examination *Clostridium perfringens*, *E.coli*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, and *Salmonella gallinarum* were isolated from the livers showing lesions. Few suspected feed samples

were collected from different farms and analysed for aflatoxin content. Aflatoxin B<sub>1</sub> was found ranging from 0.01 to 0.5ppm. The incidence of gross and microscopic lesions observed in liver in the present study indicated that sizable number of livers were affected in both broilers and layers. The etiological basis of these lesions was various bacteria, metabolic disturbances and mycotoxins. Further it can be concluded that though the liver lesions are not pathognomic of each and every disease, a study of gross and microscopic lesions of liver is often helpful in making diagnosis of various disease conditions of chicken. Also, it is suggested that, the liver lesions can be taken as an indicator for carcass condemnation at slaughter houses to avoid food borne illnesses.

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