HEPATO –RENAL PATHOLOGY WITH SPECIAL REFERENCE TO AFLATOXICOSIS IN CHICKEN (Gallus domesticus)

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**HEPATO-RENAL PATHOLOGY WITH SPECIAL REFERENCE TO AFLATOXICOSIS IN CHICKEN (*Gallus domesticus*)"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that the thesis entitled **"HEPATO-RENAL PATHOLOGY WITH SPECIAL REFERENCE TO AFLATOXICOSIS IN CHICKEN (*Gallus domesticus*)"** is a record of research work done independently by **Dr. Daly C. Davis** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Dr. Daly C. Davis**, a candidate for the degree of Master of Veterinary Science in Veterinary Pathology, agree that the thesis entitled **"HEPATO-RENAL PATHOLOGY WITH SPECIAL REFERENCE TO AFLATOXICOSIS IN CHICKEN (*Gallus domesticus*)"** may be submitted by **Dr. Daly C. Davis** in partial fulfillment of the requirement for the degree.

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Daly C. Davis

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Introduction

1. INTRODUCTION

Chicken, the most popular domesticated poultry accounts for more than 90 percent of the total poultry population of the country. Poultry farming in India was mostly a backyard venture almost upto 1960. During the last two and a half decades the entire scenario of poultry farming in the country has changed. It is now recognized as an organized and scientifically based industry and a potential resource to fight poverty and malnutrition. Thousands of poultry farms, both small and large, have come up all over the country and indigenous desi birds have given way to highly specialized layers and broilers. The importance of poultry sector in solving the problems of unemployment and under-employment is well-conceived by planners and personnel in the developmental programmes. Since poultry farming requires only minimum capital investment and ensures quick returns, it helps to improve the quality of life of rural poor.

The occurrence of disease problems of poultry in India and their relative importance have been related to differences in climate, methods of management and husbandry and levels of development of poultry production. In recent years, frequent outbreaks of poultry diseases have caused significant economic losses resulting in adverse impact on poultry production. Disease outbreaks in commercial poultry can be attributed to high density of birds associated with close proximity of hundreds of farms in major pockets.

The heavy economic losses faced by the poultry industry either by death of birds or by the production losses are due to the feeding of mouldy feeds. Aflatoxins, which are toxic secondary metabolites produced by the fungal species, *Aspergillus flavus* and *Aspergillus parasiticus* are potentially carcinogenic and mutagenic. It was only during 1961, following the outbreak of Turkey X disease resulted in heavy mortality among birds fed contaminated feed that the role of mycotoxins in the etiology of diseases in livestock and poultry assumed considerable importance (Asplin and Carnaghan, 1961).

Aflatoxins have received worldwide attention due to their potentialities as toxic chemical agents entering the food chains thus posing a threat to human and animal health. Contamination by aflatoxin is generally considered to be a problem in tropical and subtropical regions of Asia, Africa and Latin America, where the temperature and humidity conditions are favourable for the growth of *Aspergillus flavus*, mycotoxins have also been found in temperate countries of Europe and North America thus signifying that mycotoxins are indeed a global problem. In Kerala, out of 1261 chicken feed samples analyzed during the period of 1986-1990 about 50.1 % percent of the feed samples were reported to be contaminated with aflatoxin (Rajan *et al*. 1991).

Aflatoxicosis lowers the profitability in livestock and poultry industry due to huge economic losses by decreasing growth rate, feed conversion efficacy, carcass yield, egg production and male and female reproductive performances. Aflatoxins at low level in poultry feed interact with causative agents of diseases like Pasteurellosis, Salmonellosis, coccidiosis, Infectious Bursal Disease, Marek's disease and candidiasis and cause relapse of latent infections Aflatoxins are very stable and they resist degradation by ordinary drying and cooking. Hence the possible contamination of foods of animal origin by residual mycotoxins is a matter of concern with regard to human health (Maryamma *et al*.1992).

Liver is an important organ which controls the health and productivity of birds. Several specific and non specific disease conditions which affect chicken involve the liver. No other organ is involved in as many complex interrelationships with other organs in maintaining the internal homeostasis as the liver. Important functions of liver are secretory, defensive, haemopoietic, metabolic, excretory and detoxification. Liver falls victim to malfunctions because of the complexity of structural organization and functional activities. Hepatocytes are highly specialized cells in the body and this high degree of specialization makes them vulnerable even to apparently mild irritant. However, the effects of minor lesions of the liver are not manifested clinically.

The kidney is a vital organ of the bird with diverse metabolic and excretory functions which include maintaining the chemical composition of body fluids, removal of metabolic waste and toxic products, regulation of blood pressure and blood volume and conservation of fluids and electrolytes. The function of kidneys is affected by a number of specific diseases, disorders and toxicities. The damage to the liver could induce concurrent morphological and functional alterations in the kidney, since the toxic metabolites that tend to accumulate following hepatic damage could bring about pathomorphological alterations in the kidneys as well. Hence considering the significance of these two organs, an in-depth study on the various pathological changes encountered in them during various disease conditions and aflatoxicosis was undertaken with the following objectives:

1. To study the gross and histopathological lesions of liver and kidney of chicken.

2. Classify the gross and histopathological lesions of liver and kidneys.

3. To identify the mycotoxin residues in liver and kidney.

Review of Literature

2. REVIEW OF LITERATURE

2.1 INCIDENCE

Surumay *et al*. (1995) in an epidemiological study of broiler diseases over a period of 20 years in Mississippi observed 6979 cases of bacterial diseases, 3660 cases of diseases of unknown etiology, 2515 cases of parasitic diseases, 1121cases of viral diseases, 771 cases of management errors, 339 cases of nutritional deficiencies and 304 cases of fungal diseases.

Anjaneyulu *et al*. (1998) in his study on the mortality pattern of broilers in Prakasam district examined 427 carcasses . They observed that highest mortality was due to colisepticemia (13.4 percent) followed by coryza (9.6 percent), Gumboro disease (9.1 percent), respiratory mycoplasmosis (8.4 percent), coccidiosis (7.0 percent) and ascites (6.78 percent).

Singh (1998) in his etiopathological studies on chick mortality from selected farms of Ludhiana district observed maximum mortality of 1.3 percent by omphalitis, followed by infectious bursal disease (0.9 percent), coccidiosis (0.6 percent), fowl typhoid (0.5 percent), colibacillosis (0.3 percent), inclusion body hepatitis (0.2 percent), starvation syndrome(0.1 percent), ascites (0.06 percent), nephrosis (0.05 percent) aspergillosis (0.05 percent), rickets (0.03 percent), hepatitis (0.04 percent), gout (0.03 percent), pneumonia (0.01 percent) and tracheitis (0.05 percent).

Farooq *et al.* (2002) in his study on the prevalence of diseases and mortality in layers under subtropical environment observed that coccidiosis was the major problem causing 19.1 percent mortality. Egg prolapse and enteritis resulted in 12.1 percent and 8.4 percent mortality respectively. About seven percent loss was due to disease outbreaks including E. coli, infectious coryza and chronic respiratory disease (CRD). Losses due to infectious bronchitis, omphalitis, yolk sac infection, ascites and feed toxicity in each case were around

six percent while hydropericardium syndrome and cannibalism caused less than five percent loss.

Bacterio-pathological investigation on 1751 dead chicken during one year period from January to December 2002 at the BRAC poultry disease diagnostic centre, Gazipur showed that 39.81 percent cases were with seven types of different bacteriological diseases, of which salmonellosis, colibacillosis and fowl cholera had significantly higher rate of prevalence than staphylococcosis, gangrenous dermatitis, necrotic enteritis and infectious coryza. (Rahman *et al*., 2004).

Goyal (2005) observed the prevalence of hepatic lesions associated with various diseases of chicken and quail was 46.03 and 26.83 percent respectively. The diseases primarily affecting liver were inclusion body hepatitis, hydropericardium syndrome and lymphoproliferative diseases in poultry and Marek's disease in quail.

Mustafa and Ali (2005) in their study on the prevalence of infectious diseases in local and Fayoumi breeds of rural poultry observed high prevalence of disease (57 percent) in Fayoumi than local breed (43 percent). The overall prevalence of various diseases was Newcastle disease (40.33 percent), E.coli (5 percent), infectious bronchitis (12.66 percent), chronic respiratory disease (7 percent), infectious coryza(8.33 percent), salmonellosis (6.33 percent), fowl pox (15.66 percent), hydropericardium (4 percent) and coccidiosis (10.66 percent).

Rahman *et al.* (2007) examined a total of 8169 dead chickens from five different farms in selected areas of Bangladesh. Among bacterial diseases salmonellosis was found in 53.90 percent of chicken followed by omphalitis in 28.42 percent, colibacillosis in 13.36 percent, mycoplasmosis in 2.55 percent, necrotic enteritis in 1.18 percent and infectious coryza in 0.59 percent.

New castle disease, infectious bursal disease, yolk sac infections and coccidiosis were found to cause maximum mortality (over 30 percent) in layers.

Infectious laryngeotracheitis caused mortality with in a range of 0.81-20 percent in layers. Cannibalism was also reported to be a major cause of death in egg type layers. A drop of 10-40 percent in egg production was found with the incidence of infectious coryza, E. coli, Mycoplasmosis, coccidiosis, egg prolapse and aflatoxicosis in their study on prevalent diseases and mortality in egg type layers.(Usman and Diarra, 2008).

2.2 AFLATOXICOSIS

2.2.1 Incidence of Aflatoxin in Poultry Feed

Out of 1261 chicken feed samples analyzed 50.1 percent were found to be contaminated and 22.2 percent had aflatoxin levels above 100 ppb during the period from 1986 to1990 in Kerala (Rajan *et al.,* 1991).

During the period between 1983 and 1993, 4818 samples of agricultural commodities, comprising cereals, oilseed cakes, compound feeds, and other ingredients were examined for aflatoxin contamination. High quantities of aflatoxins were found in groundnut cake, deoiled groundnut cake, maize, and mixed feeds. Highest incidence of aflatoxin contamination was observed in groundnut cake (96.35 percent) and deoiled groundnut cake (96.20 percent); and the highest level of aflatoxin B_1 of 8260 ppb was observed for aflatoxin B_1 in maize in Bangalore (Dhavan and Choudary, 1995).

Dutta *et al*. (2005) in a survey to indicate the magnitude of AFB_1 contamination in various livestock and poultry feeds and feed stuffs in Jammu and Kashmir found that 319 feed samples comprising maize (24), ground nut cake (14), cattle ration (89), poultry ration (75), wheat bran (32), linseed cake (14), gram's husk (26), cotton seed (29) and mustard cake (16) were positive for AFB_1.

2.2.2 Residual Aflatoxin in Tissues

Mariyamma *et al.* (1992) screened and quantified residual aflatoxin in the liver, kidney, gizzard and thigh muscle of eight White Leghorn chicks given aflatoxin at the rate of 100 mg/kg body weight for 30 days. Liver and gizzard samples of three chicks contained aflatoxin.

Yadav *et al.* (1995) analyzed liver and muscle pieces of broiler chicks treated with 0.5 ppm aflatoxin B_1.The results indicated that only a small fraction of aflatoxin B_1 was retained in the liver and muscles. The highest values of aflatoxin in these tissues were found on 45^{th} day and were 4.7 and 2.0 ppb respectively.

Aflatoxin B_1 started to appear in eggs after four weeks of feeding 200 parts per 10^9 aflatoxin B_1 per day in White Leghorn chicken. The concentration of aflatoxin in the eggs appeared to peak at nine weeks from the start of feeding and leveled off from 10 weeks till the end of the experiment. (Azzam and Gabbal, 1998).

The aflatoxin residues in the liver, muscle and eggs of laying ducks, hens, quails and in broiler chicken were examined after feeding with a diet containing three ppm aflatoxin B_1. The tissue levels of aflatoxin B_1 and its metabolites were higher in quail than in other birds. The levels of aflatoxin B_1 and its metabolites were more than 10 fold higher in liver than in muscle in all species. (Bintvihok *et al.,* 2002).

Two groups of 32 hens and broiler chickens were experimentally fed with a feed contaminated with 2.5 and 5 mg of aflatoxin per kg for a period of 32 days. The tissues (liver, muscle, kidney, gizzard and eggs) were analyzed and revealed a wide range of concentrations, the lowest was found in hen muscle (0.05 µg per kg of AFB_1) and the highest in gizzards from the 5 mg per kg group of the hens (9.01 µg per kg of AFB_1) (Fernandaz *et al*., 2006)

Residual aflatoxin was not detected in the liver on the 15th and 30th day of experiment except for the trace residue of 2.35 (ng/g) in 100 ppb treated group on the 30th day. On the 45th day, all toxin treated (20, 40, 60, 80 and 100 ppb) groups recorded residual AFB_1 in liver. (Arulmozhi *et al*., 2007)

Churchil *et al.* (2009) in a study conducted to assess aflatoxin deposition and its clearance from the tissues of broiler chicken fed with aflatoxin B_1 at one ppm level from day-old to 56 days of age. Aflatoxin B1 feeding resulted in its increase in tissue residues to reach a plateau at second week. No residue was detectable in the tissues after 15 days of withdrawal.

A total of fifty random table egg samples were collected from EL-Beida city markets and subjected to mycological examination and detection of aflatoxin residues. Aflatoxin residues were detected only in seven samples and the mean aflatoxin residue was 0.542 ± 0.226 ppb (Salem *et al*., 2009)

2.2.3 Gross and Histopathology

2.2.3.1 Liver

The liver of broilers fed with aflatoxin was pale yellow and enlarged with petechial hemorrhages. Microscopically, lipidosis, lymphocytic infiltration around portal tracts, sinusoidal congestion and dilatation, villous appearance of bile duct epithelium, phlebitis, mild bile duct proliferation, thickened blood vessels, focal areas of necrosis and tubular pattern of regenerating hepatocytes were noticed (Ramadevi *et al*., 1990; Anjaneyulu and Rama Rao., 1993).

In an experimentally induced aflatoxicosis trial at 1.5 and three ppm levels in commercial broilers, the liver exhibited enlargement, yellow discoloration, petechial hemorrhage and fatty change. Histologically, degeneration, focal areas of necrosis, heterophilic and lymphocytic infiltration in parenchyma and around bile duct were observed (Bakshi *et al.,*1995)

Kalra *et al.* (1995) observed enlarged, pale and fragile liver in chicken fed with aflatoxin B_1 at the rate of 0.6 ppm. Varying degrees of congestion and hemorrhage, marked granular and vacuolar degeneration, mononuclear cell infiltration, prominent Kupffer's cells along with hyperplasia and proliferation of bile duct were the lesions observed histologically.

Of the 1113 birds examined, 117 cases of aflatoxicosis was diagnosed. Gross alterations in the liver were hepatomegaly, 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, at places the degenerated or necrosed areas were replaced by newly formed hepatocytes. The biliary epithelium showed proliferation and hyperplastic changes. Tendency to form ductular structures, mononuclear cell infiltration in the interlobular connective tissue were also noticed (Lalrintluanga and Baruah, 1997).

In broilers experimentally fed with aflatoxin at the rate of 2.5 mg per Kg of the diet (Kiran *et al*., 1998) observed pale, enlarged and friable liver. Microscopically, the hepatocytes were swollen and the swelling was so great that individual cells had ruptured in some areas, accompanied by mild fatty changes. In some livers, more basophilic hepatocytes were arranged in an acinar pattern with a central lumen. Other hepatic lesions included bile duct hyperplasia, periportal fibrosis, perivascular mononuclear cell, heterophil and nodular lymphoid cell infiltrations.

Srivani *et al.* (2003) observed that the livers were pale yellow, enlarged and with rounded borders. Microscopic picture revealed congestion of central vein and dilated sinusoidal spaces in broiler chicks fed with aflatoxin B_1 at one ppm level for 14 days

Madheswaran *et al.* (2005) observed enlarged and pale or yellowish discolored liver. Histologically, liver showed vacuolar degeneration of hepatocytes, bile duct hyperplasia, focal necrosis and mononuclear cell

infiltration in Japanese quail treated with aflatoxin at the rate of three ppm level from day of hatch to 35 days of age.

In livers of chicken with chronic exposure of aflatoxin at the rate of 50 and 100 ppb for 42 days (Ortatatli *et al*., 2005) observed slight to moderate hydropic degeneration, small fatty vacuoles in hepatocytes in centrilobular and midzonal areas and mild bile duct proliferation and periportal fibrosis.

In aflatoxin fed birds at the rate of 80 and 100 ppb levels (Arulmozhi *et al.,* 2007) observed that the livers were yellow in color with a congested appearance, subcapsular hemorrhage and focal areas of necrosis. Histologically degeneration of hepatocytes, phlebosclerosis, periportal fibrosis, biliary hyperplasia and Kupffer cell reaction were observed.

In broilers fed with aflatoxin at the rate of 0.2 ppm aflatoxin, liver was found to be enlarged, yellowish with raised nodules. Histologically, hepatocytes were swollen and vacuolated. The area of necrosis was diffusely spread in the liver parenchyma and was infiltrated with heterophils and lymphocytic aggregates. Areas of fibrous tissue proliferation and adenomatous arrangement of hepatocytes were also observed (Sakhare *et al*., 2007).

Grossly the livers from birds fed with aflatoxin at the rate of one ppm for 28 days showed hepatomegaly, pallor or yellowish discoloration, occasional mottling and gall bladder distension. Microscopically, the liver revealed vacuolar degeneration, macro vesicular fatty degeneration and ballooning degeneration of the hepatocytes. The hepatocytes showed fatty changes coalescing to form fatty cysts. The regenerating hepatocytes were arranged in acinar or ductular patterns .The acinar hepatocytes also showed fatty degeneration and fatty cysts in some areas. Congestion, bile duct hyperplasia, focal infiltration of heterophils and mononuclear cells, perivascular infiltration of mononuclear cells and heterophils along with fibrosis were also noticed in the livers (Kumar and Balachandran, 2009).

2.2.3.2 Kidney

In broilers fed with aflatoxin at two ppm in the feed the kidneys were slightly enlarged, pale or congested with few petechial hemorrhages. Microscopically, focal hemorrhages, lymphocytic infiltration, hydropic changes, mild thickening of glomerular basement membrane were observed (Ramadevi *et al*., 1990).

In broiler chicken fed with aflatoxin at the rate of two ppm (Anjaneyulu and Rama Rao, 1993) observed enlarged kidneys with hemorrhages. Histology, revealed parenchymatous degeneration of tubules with casts, swollen glomerular tufts, intertubular hemorrhages and atrophied glomeruli.

Kalra *et al*. (1995) observed individualization and desquamation of tubular epithelium in kidneys of birds fed with aflatoxin B_1 at the rate of 0.6 ppm.

Lalrintluanga and Baruah (1997) observed that the kidneys were slightly enlarged and pale having ecchymotic hemorrhagic foci. Histologically, diffuse interstitial hemorrhage, dilatation of blood vessels and degeneration of tubular epithelium with bizarre nuclei were noted in their study on incidence and pathology of aflatoxicosis in broiler chicken.

In broilers experimentally fed with aflatoxin at the rate of 2.5 mg per kg of the diet (Kiran *et al.*, 1998) observed pale kidneys. Histologically, kidneys of the chickens showed degeneration and necrosis of tubular epithelium. Degenerating tubular epithelium was swollen and had granular cytoplasm and pyknotic nuclei.

Srivani *et al.* (2003) observed only mild congestion and enlargement of kidneys. Histological lesions included mild intertubular hemorrhages and degenerative changes in the tubules and focal areas of lymphoid aggregates in broiler chicks fed with aflatoxin B_1 at one ppm level for 14 days

In Japanese quail treated with aflatoxin at three ppm level from day of hatch to 35 days of age Madheswaran *et al*. (2005) observed enlarged and congested kidneys. Histologically, kidneys showed congestion and tubular epithelial degeneration and necrosis.

Sakhare *et al.* (2007) observed enlarged and pale kidneys in broilers fed with aflatoxin at the rate of 0.2 ppm. Histologically, degenerative changes in tubular epithelium, condensation of nuclear material, necrosis, separation of epithelial cells from basement membrane and areas of hemorrhage were observed.

In broiler chicken fed aflatoxin at the rate of one ppm for 28 days Kumar and Balachandran (2009) observed that the kidneys were enlarged, pale or congested with a few petechiae. Microscopic features were congestion, focal hemorrhages, degeneration of tubular epithelium and occasional thickening of basement membrane.

2.3 CONDITIONS ASSOCIATED WITH HEPATIC LESION

2.3.1 Bacterial Diseases

2.3.1.1 Colibacillosis

On *Escherichia coli* infection in layer chicks previously infected with *Mycoplasma gallisepticum* Bajwa *et al.* (1992) observed perihepatitis with white cloudy covering and enlargement of liver and congestion, hemorrhages and urate deposition was recorded in the kidneys. Histologically liver showed congestion, leucocytic infiltration, degeneration and necrosis of hepatocytes.

In a study on the dynamics of *Escherichia coli* infection in experimentally inoculated chicken, moderate to severe lesions of air sacculitis, perihepatitis, pericarditis and splenic hypertrophy were observed. Histology revealed inflammatory cell infiltration, serous to fibrinous exudates and cellular debris on the serosal surfaces of the liver, spleen and air sacs (Pourbakash *et al*., 1997).

Mitra *et al*. (1999) observed grayish mottled and enlarged friable liver with fibrinous covering on its surface, swollen pale kidneys and atrophied bursa and thymus. Microscopically, the liver lobules were found to be encircled by fibrous connective tissue outside and the kidney lesions consisted of degenerative changes and necrosis of lining tubular epithelial cells, congestion and hemorrhages in interstitial areas in spontaneous aflatoxicosis associated with *Escherichia coli* infection in ducks.

Shah *et al*. (2003) noticed fibrinous perihepatitis, hepatomegaly with pale coloured necrotic foci, fibrinous pericarditis and caseous exudates in the air sacs in their study conducted to observe the prevalence of infection, gross and histopathological changes in various organs of broilers in colibacillosis.

Srinivasan *et al*. (2003) noticed fibrinous layer of varying thickness with moderate to severe infiltration of heterophils and mononuclear cells in the liver of chicken infected with *Escherichia coli*.

Pericarditis, perihepatitis and moderate to severe air sacculitis were observed in chicken infected with colibacillosis. (Vandekerchove *et al*., 2004; Someya *et al*., 2007)

Sylvester *et al.* (2008) in their study on *E.coli* infection using organisms isolated from different species of ailing or dead birds observed perihepatitis with a thick yellow granular layer covering the liver and the kidneys showed moderate to severe degree of hemorrhages and congestion. Histologically, changes in the liver included thickening of the Glisson's capsule, necrotic foci with patchy degenerative changes in hepatocytes, focal infiltration of mononuclear cells, sinusoidal dilatation, congestion and haemorrhages.

2.3.1.2 Salmonellosis

Brito *et al.* (1995) observed miliary white foci on the liver of chicks exposed to *Salmonella typhimurium*. Histologically multiple foci of necrotic

hepatocytes along with heterophil and mononuclear cell infiltration were observed.

In an experimental infection of *Salmonella enteritidis* Dhillon *et al*. (2001) observed severe perihepatitis, moderate to severe peritonitis, severe air sacculitis, pneumonia and omphalitis

Prasanna *et al.* (2001) noticed early vacuolar degenerative changes in the hepatocytes, hypertrophy of Kupffer cells, focal infiltration of mononuclear cells, dilatation of hepatic sinusoids and necrosis of hepatocytes in chicken experimentally infected with *Salmonella gallinarum* and *Salmonella pullorum.*

Madhuri and Sadana (2005) observed typical bronze discoloration of the liver in chicken after seven days of infection with broth culture of *Salmonella gallinarum* by subcutaneous route. Histologically moderate to severe congestion, hemorrhages, isolated foci of necrotic hepatocytes were noticed.

In Salmonella infected layer chicken, Islam *et al.* (2006) observed congested and enlarged liver with focal necrosis. Microscopically, liver showed congestion and focal necrosis with multifocal infiltration of histiocytes in the liver parenchyma.

2.3.1.3 Pasteurellosis

Locke *et al.* (1970) observed hemorrhages on the heart and liver, which varied in size from petechiae to large irregular shaped blotches. Extensive hemmorhagic enteritis involving the entire duodenum, hemorrhages in the lungs, air sac membrane and pericardium were also noticed in ducks submitted to the laboratory from an outbreak of fowl cholera in the Chesapeake Bay.

In an outbreak of fowl cholera among wild ducks Fujihara *et al*. (1986) noted multiple petechiae over the epicardium, serosal surfaces of the liver and the spleen. Light yellow thin fibrin were matted on the surface of tunica serosa in the liver. Microscopically, congestion and multifocal necrotic areas were present in

the liver. Those necrotic areas and sinusoids contained a large number of bacteria. Small areas of necrosis were scattered throughout and bacteria were present within the necrotic foci in the spleen.

Rahman *et al.* (2004) noticed marked congestion, petechial hemorrhage in the heart, lungs, intestinal mucosa, epicardium and endocardium. Liver appeared to be swollen bearing multiple foci of coagulation necrosis in some cases of Pasteurellosis affected birds.

In an investigation on avian *Mannheimia hemolytica* and *Pasteurella multocida* in different poultry farms at Beni-seuf governorate, (Afifi *et al.*, 2007) observed necrotic foci and multiple petechiae on the liver and spleen and congestion and edema of the lungs. Histologically, liver showed multifocal heterophilic infiltrations associated with coagulative necrosis.

In their study to estimate the prevalence of carriers of *Pasteurella multocida* among healthy looking chicken and ducks Mbuthia *et al.* (2008) observed fibrin remnants on the air sacs, fibrosis of the lungs and air sacs, necrotic liver lesions and splenomegaly in chicken and pericarditis, perihepatitis, fibrin strands on air sacs and necrotic spots on spleen and liver in the ducks.

Ducks inoculated with Pasteurella multocida by the subcutaneous route revealed hemorrhages on the epicardium, pinpoint necrotic foci on the liver, congestion and enlargement of kidneys, congestion and hemorrhages in the lungs. Histologically, congestion of blood vessels and sinusoids, multifocal hemorrhages, degeneration, multifocal coagulative necrosis, focal heterophilic infiltration, fatty change and vacuolation of hepatocytes were evident in the liver. In the kidney sections, multifocal hemorrhages in the renal parenchyma, widespread coagulation necrosis of tubulae and glomeruli and moderate heterophilic infiltration were observed. Degeneration, necrosis and desquamation of tubular epithelium were also evident (Pramod, 2009).

2.1.2.4 Staphylococcosis

Kibenge *et al.* (1983) on gross examination of chickens infected with Staphylococci observed white foci 1 to 2 mm in diameter on liver surfaces and livers were enlarged, congested and greenish while the kidneys showed moderate enlargement and streaky deposits of urate throughout their substance. The most common histological lesion was a staphylococcal granuloma which was present in the sections of the heart, lung, kidney and bursa of Fabricius. There were also heterophilic hepatitis, epicarditis, glomerular and interstitial nephritis.

Schmidt *et al.* (2003) observed that gross lesions caused by staphylococci and streptococci included variable hepatic swelling and multifocal to confluent yellow-white foci within the parenchyma. There were abscess formation in some chronic cases. Histologically, multifocal to confluent necrosis and an inflammatory reaction comprising primarily of heterophils and macrophages were observed in birds.

Andreasen (2008) reported that the gross lesions of septicaemic staphylococcal infection in poultry consisted of necrosis and vascular congestion in many internal organs including the liver, spleen, kidneys and lungs. Histologically, staphylococcal lesions consisted of necrosis, heterophils and bacterial colonies composed of large number of gram positive coccoid bacteria.

2.1.2.5 Tuberculosis

In a cross sectional study on Avian Mycobacteriosis in 95 randomly indigenous breeds of chicken from three selected agroclimatic regions, Tadesse *et al.* (2003) observed grayish- yellow to grayish-white pinpoint to irregularly round and few to innumerable nodules measuring upto two cm in diameter raised above the surface of liver, spleen, small intestine, bone marrow and skin. Histologically, granuloma is characterized by caseonecrotic cores that were surrounded by a broad ring of palisading epithelioid cells, macrophages, multinucleate giant cells with a moderate mixture of heterophils, lymphocytes and plasma cells.

In a study on Avian Mycobacteriosis in chicken from a rural area of Argentina, Coles *et al.* (2007) observed numerous yellow, firm, nodular formations between 1mm to 2 cm in diameter in the spleen and liver. Multiple nodules and ulcers were present in the crop, proventriculus and all layers of the small and large intestines. Histologically, granulomatous lesions varied from small cluster of uncapsulated macrophages with foamy cytoplasm to large necrotic centers surrounded with giant cells, epithelioid cells, macrophages and an outer fibrous capsule containing some mononuclear cells.

Fulton and Sanchez (2008) reported that lesions of avian tuberculosis are characterized by pinpoint to several centimeter, irregular grayish yellow or grayish white nodules in the spleen, liver and intestine. Histologically in larger nodules, the central area of granuloma sometimes had coagulative or caseous necrotic core surrounded by multinucleate giant cells. Immediately peripheral to the multinucleate giant cells there was a collection of both epithelioid and histiocytic macrophages. A fibrous capsule consisting of fibrocytes and minute blood vessels were also seen near the outer portion of the peripheral area.

2.3.2 VIRAL DISAESES

2.3.2.1 Inclusion Body Hepatitis

In his epidemiological and experimental studies of inclusion body hepatitis in broiler chicks, Brar, (1993) reported swollen and pale liver with petechial and ecchymotic hemorrhages and necrotic foci. Spleen appeared swollen and mottled in some cases. Histologically, liver revealed congestion, hemorrhage and fatty changes with types of intranuclear inclusion bodies in the hepatocytes. Mononuclear cell infiltration and bile duct hyperplasia were also evident with perihepatitis in a few cases.

In an outbreak of inclusion body hepatitis in a young broiler breeder pullet flock in Ontario (Philippe *et al*., 2005) observed friable, pale yellow white livers bearing hemorrhages and pale and swollen kidneys. Histologically, necrotizing hepatitis with large basophilic intranuclear inclusion bodies in the hepatocytes compatible with inclusion body hepatitis was present. The bursas had histologically evident follicular lymphoid depletion.

2.3.2.2 Marek's disease

In a prevalence study of Marek's disease and Avian Leucosis between 1975-1984, Fatunmbi and Adene, (1986) observed diffuse or focal neoplastic lesions in one or more parenchymatous organs like the liver, spleen and kidney, which were often enlarged and grayish. The focal lesions produced nodules of varying sizes which in some cases, accompanied the diffuse types. Liver and spleen were not only enlarged but also hemorrhagic and congested. Histologically, liver was infiltrated by a mixed population of lymphoid cells and plasma cells.

In a study on incidence of Marek's disease in Tamil Nadu, Paneerselvam *et al.* (1990) observed that gross lesions in internal organs were common in liver and spleen. Multiple nodules were noticed in the liver, spleen, kidney and heart. The ovaries were leathery and had cauliflower like growths. The histopathological examination revealed the presence of pleomorphic lymphoid cell infiltration in the affected organs.

In an outbreak of Marek's disease in chickens in central Ethiopia Lobago and Woldemeskel, (2004) observed nodules varying in size from pinpoint white or grayish to large ones of 25 mm in diameter in the liver. They had smooth surfaces with firm consistencies. Microscopically, there was diffuse infiltration or massive aggregation of pleomorphic mononuclear cells in the liver.

In a study conducted on Marek's disease in local strains reared under confined management regime in central Ethiopia Duguma *et al*. (2005) observed that white or grayish lesions in visceral organs took either nodular, diffuse, or mixed forms of varying degrees in size with deep, superficial, or mixed depth in the parenchyma of organs. The gross lesions were in the visceral organs namely liver, spleen, lung, heart, proventriculus and bursa. Histologically, in the visceral organs diffuse infiltration or aggregates of lymphocytes were noted.

In an outbreak of acute Marek's disease in commercial chicken in Aizwal Rajkhowa (2005) observed diffuse, nodular and miliary forms of tumors in the visceral organs. Diffuse forms of the tumors were seen commonly in liver, spleen, intestine and in the bursa of Fabricius. In the liver diffuse infiltration caused loss of normal architecture and gave the surface a coarse nodular appearance. Microscopically, the lesions were uniformly proliferative and focal in nature. The cellular composition consisted of small to medium lymphocytes, lymphoblasts and activated and primitive reticulum cells.

2.3.2.3 Avian leucosis

Purchase and Burmester (1978) observed tumor masses which were nodular, miliary or diffuse or a combination of these forms particularly in the liver and spleen in chicken with Lymphoid Leucosis. Microscopically, the tumors are focal and multicentric in orgin. As the tumor cells proliferate, they displace and compress the cells of organ rather than infiltrate them. Nodules in the liver are usually surrounded by a band of fibroblast like cells that have been shown to be remnants of sinusoidal endothelial cells.

An investigation on avian leucosis virus infection in a broiler parent farm in Sudan by Latif and Khalafalla (2005) revealed hepatomegaly upto three times of the normal size, splenomegaly, congestion and consolidation of lungs and enlarged, congested and pale kidneys. Histologically, liver showed diffuse infiltration of pleomorphic population of lymphoid cells bearing mitotic

figures.These cells consisted of small and large lymphocytes and some cells with plasmacytoid differentiation. The majority of these cells were large lymphocytes. Liver also revealed packing of the congested dilated sinusoids with lymphoid cells resulting in pressure atrophy of the hepatocytes. In severely affected areas, the hepatic parenchyma was replaced by the accumulation of the infiltrated lymphoid tissue.

2.3.3 Protozoan Diseases

2.3.3.4 Histomoniasis

In a wild turkey with histomoniasis and reticuloendotheliosis (Ley *et al*., 1989) observed large multifocal and irregular pale areas on the liver. The cut surface indicated this lesion throughout. Spleen had a mottled appearance. Microscopic changes in the liver consisted of large multifocal coalescing areas of necrosis. Occasionally spherical organisms 10 to 15 micrometer in diameter consistent with *Histomoniasis meleagridis* were present in the necrotic areas. Viable hepatic parenchyma contained multifocal infiltrations of numerous mononuclear cells interpreted as neoplastic cells resembling lymphoblasts and plasma cells.

In an outbreak of histomoniasis in free range layer hens, Esquenet *et al.* (2003) observed small livers with a pale aspect, fibrinous hemorrhagic peritonitis and enlarged caeca with thickened and hyperemic walls. In one chicken multiple white raised foci were observed in the kidneys. Histologically, histomonads were observed in caecal walls and kidneys but not in the livers, although they contained multiple foci of necrosis surrounded by macrophages.

Kamil *et al.* (2006) observed enlarged livers with brilliant greenish discoloration in broiler breeder chicken with histomoniasis. Numerous round to oval, circumscribed lesions ranging from 0.5 to 1.5 cm in diameter resembling a bull's eye target were visible. These lesions had depressed centers circumscribed by a well defined ringed edges. When cut, each lesion extended deep into the

parenchyma visible as diffuse pale necrosed areas. Microscopically, there were multiple confluent areas of necrosis mostly around the thrombosed blood vessels. The necrotic hepatocytes were sparsely distributed or disappeared completely leaving the stroma and a few cellular debris. An extensive infiltration of lymphocytes, mononuclear cells, and scarce heterophils were noticed. Hepatocytes towards the periphery of the lesions contained punched out ovoid bodies resembling vacuoles in the cytoplasm. The ovoid bodies stained positively and became readily visible with PAS stain.

Cue *et al.* (2009) during the necropsy of 9-11 week old male turkeys in an outbreak of systemic histomoniasis revealed enlargement of livers, most of which had numerous pale white nodules ranging in size from 0.3 to 1.5 cm in diameter. The ceacal walls were severely thickened. The kidneys, pancreas and spleen had pale or yellow foci in a few birds. Microscopically, there were multifocal necrosis and granulomatous inflammation in the liver, kidneys, lungs, proventriculus and spleen.

2.3.4. NUTRITIONAL AND METABOLIC DISEASES

2.3.4.1 Fatty Liver Hemorrhagic Syndrome

Lonkar and Prasad (1988) observed enlarged and fragile liver with rounded edges and mild to moderate congestion in chicken with fatty liver syndrome. Microscopically, in mild cases only some of the hepatocytes contained fat vacuoles in different cords pushing aside the nucleus. In moderately affected cases, 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 was moderately or severely affected. The cords were distorted and disrupted. In a few cases, the whole microsection was replaced by monotonous sheet of vacuoles. Tubular lining epithelial cells of the kidneys revealed mild fatty changes in severely affected cases.

Rosemary *et al.* (1993) in their studies to characterize selected aspects of fatty liver hemorrhagic syndrome induced by overfeeding in laying hens noticed poorly defined hepatic cords, whose liver architecture was distorted by numerous large lipid droplets that frequently displaced hepatocyte nuclei. Telangiectasia and compression of hepatic cords was common among overfed hens. The overfed hens had grossly abnormal reticulin. Overfeeding of hens for more than one week increased the severity of liver hemorrhage.

Fatty liver hemorrhagic syndrome was characterized by very fatty liver accompanied by hemorrhage. There was an increased fat content of the liver, which becomes putty coloured and very friable, kidneys were pale and swollen and the abdomen contained large accumulations of fat, usually yellow. Histologically, the hepatocytes were grossly distended with fat globules, which disorganize internal structure and eventually rupture cell membranes in chicken (Jordan and Pattison,1996).

In some cases of chicken with fatty liver hemorrhagic syndrome the liver was significantly larger having yellowish discoloration and fragile in consistency. Histologically, majority of hepatic cells were swollen and sinusoidal dilatation was noticed. Extensive fatty changes and complete distortion of hepatic parenchyma were prominently seen. Focal or diffuse areas of hemorrhages were also noticed (Chawak *et al*.,1997; Karadas *et al*.,1999)

Turkeys that die of hepatic lipidosis were in good body conditions with abundant visceral fat. The liver was typically enlarged and its surface was mottled due to numerous hemorrhagic foci. Some livers had irregularly shaped discrete or confluent pale areas representing lipid accumulation. Microscopically, in some areas the normal architecture of the liver was distorted by marked vacuolation of the cytoplasm of hepatocytes. In other areas, groups of vacuolated

hepatocytes and proliferating bile ductules were separated by hemorrhagic areas. (Aziz, 2008).

Crespo and Shivaprasad (2008) observed that the liver was greatly enlarged, pale and friable in chicken with fatty liver hemorrhagic syndrome. It sometimes had smaller hematomas within the parenchyma. Microscopic examination of the liver showed hepatocytes distended with fat vacuoles, hemorrhage of various sizes and organizing hematomas and often small irregular masses of uniform eosinophilic material.

2.3.4.2 Gout

Uma *et al.* (1999) observed deposition of white urate crystals on the visceral organs during the necropsy of the birds with gout received from various poultry farms. Histologically, liver revealed congestion, hemorrhage and urate deposition. The urate deposition was most conspicuous on the surface of liver as compared with the parenchyma. The parenchymal deposition was either focal or multifocal accompanied by necrosis along with inflammatory changes. In De Galantha's stained sections urate crystals appeared as black radiating structures.

Ahmed *et al.* (2003) noticed deposition of chalky white precipitate of urates on the surface of heart, lungs, liver, proventriculus, spleen, gizzard and intestines in broiler breeders with urolithiasis. Liver section revealed degenerative changes, congestion and dilated sinusoids and mononuclear cell infiltration.

In a Black Vulture with uric acid nephritis, Singh *et al*. (2003) microscopically observed in the liver, sinusoidal congestion, vacuolation of hepatocytes and disorganization of hepatic cords.

In an outbreak of gout in Kashmir Favorella chicken, Mir *et al*. (2005) observed dry platery patches of white chalky urate deposits on the the liver, spleen, lungs, air sacs, kidneys and joints. Histologically, liver showed focal

hepatitis and hemorrhage associated with urate deposits which was numerous especially in subcapsular region. Moreover, the subcapsular hepatocytes were flattened with elongated nucleus and intense cytoplasmic basophilia. Glisson's capsule revealed proliferation of fibrous connective tissue. In De Galantha's stained liver sections urates stained characteristically black, showing cotton ball appearance with radiating crystals at periphery.

2.3.4.3 Ascites syndrome

In sodium chloride induced ascites syndrome of broiler chicken histologically, severe diffuse granular degeneration of the hepatocytes with multifocal areas of necrosis and hepatocytes showing loss of chord-like arrangement assuming an acinar pattern in many places were observed (Jacob, 1996).

In a case of natural ascites syndrome dark breast muscle, marked abdominal distension, clear yellow fluid with lots of fibrin in the abdominal cavity, hydropericardium, congestion of lungs, liver, kidneys and intestines were observed. Histologically, pronounced dilatation of sinusoids, atrophy and degeneration of hepatocytes, marked thickening of Glisson's capsule, infiltration of inflammatory cells in the perivascular areas and in some cases fatty change of liver were observed. In the kidney sections, many glomeruli appeared congested and scattered foci of lymphocytes were present in the interstitial tissue of broiler chicken (Tafti and Karima, 2000).

Rajkhowa (2004) observed accumulation of clear straw colored fluid in the abdominal cavity. The heart appeared enlarged with mild to severe hydropericardium in chicken with ascites syndrome. The liver in affected birds showed severe congestion, mottling, enlargement, shrunken appearance with a grayish thickened capsule and irregular surface. The fibrin clots were found adherent to the liver surfaces. In a few cases, the left lobe was found to be atrophied and cirrhotic. Microscopically, in the liver there was marked dilatation

and congestion of sinusoids and in the kidneys there were massive congestion and hemorrhages in the intertubular areas. Varying degrees of degenerative changes with desquamation of tubular epithelial cells were evident.

Goyal *et al.* (2005) observed gross abdominal distension in poultry with ascites syndrome. The liver was mottled, fatty, occasionally hemorrhagic and swollen but mostly shrunken with thickened capsule and rounded borders. Mild fibrinous perihepatitis was also noticed in a few cases. Heart showed varying degrees of hydropericardium. Spleen and kidneys were slightly enlarged and congested. Besides these, clear yellowish fluid with or without fibrin clots were noticed in the abdominal cavity. Microscopically, liver showed varying degrees of chronic venous congestion, sinusoidal dilatation, hemorrhage, hepatocellular degeneration and fatty change with atrophy of hepatocytes. Moreover, the other changes were mild to severe fibrinopurulent to active perihepatitis, periportal hepatitis, mild to severe perivascular and periportal fibrosis, and bile duct proliferation. Kidneys exhibited congestion, varying degrees of nephrosis and chronic glomerulitis with narrowing of Bowman's capsule.

2.4 CONDITIONS ASSOCIATED WITH RENAL LESIONS

2.4.1 Viral Diseases

2.4.1.1 Infectious Bursal Disease (IBD)

Ley *et al.* (1984) on histopathologic examination of lymphoid and non-lymphoid tissues from IBD virus infected specific pathogen free chicken observed lymphoid necrosis in the bursa of Fabricius. Kidney sections from infected group showed prominent lymphoid foci, edema, tubular necrosis and intratubular crystalline material. In the liver, multifocal periportal accumulations of lymphoid cells, sinusoidal infiltration of heterophils and mild fatty changes were observed.

With regard to the kidneys of IBD virus infected birds, Abdu *et al.* (1986) observed grossly enlarged kidneys, while the tubules and ureters were distended with accumulated urate crystals. The kidneys varied in color from white or pale grey to brown. Histological features were interstitial hemorrhages, perivascular accumulations of lymphoid cells, edema, tubular necrosis and glomerular necrosis.

Hemalatha *et al*. (2005) observed congestion and enlargement of bursa, extensive skeletal muscle hemorrhages with pallor of liver and kidneys and grayish white foci scattered on the surface of spleen in experimentally induced Infectious Bursal Disease in chicken. Histologically, kidneys revealed congestion of blood vessels with mononuclear cell infiltration in the interstitium and mild tubular epithelial degenerative changes with interstitial hemorrhages. Liver sections showed congestion, focal mononuclear cell infiltration, hypertrophy of Kupffer cells and mild vacuolar degenerative changes.

Dutta *et al.* (2007) noticed enlarged, distended, congested bursa filled with gelatinous fluid. The lesions in kidneys were characterized by unilateral and bilateral enlargement with congestion in natural outbreaks of Infectious Bursal Disease in Vanaraja birds of Meghalaya. Histologically, renal parenchyma showed areas of degeneration, congestion, hyperplasia and hypertrophy of cells of glomeruli, focal interstitial nephritis and mild fatty changes. In some birds, there were areas of nephrosis of the proximal convoluted tubules.

2.4.1.2 Marek's disease

Fujimoto *et al.* (1971) observed gray-white nodular tumors and in some cases ill defined tumorous masses with a diffuse reticular appearance in various organs like liver, spleen, kidney, proventriculus, lungs and adrenals. Histologically in the kidneys, lymphoid cell proliferation varying in extent was found between tubules. In some, proliferation was so extreme that the parenchyma was completely replaced by tumor cells and only small parts of

parenchyma could be seen. Lymphoid cell infiltration was seen in and around the tunica propria of the ureter in almost every case in their histopathological investigation on Marek's disease in chicken.

Lobago and Woldemeskel (2004) noticed that the lesions in the visceral organs including liver, kidney and spleen were either nodular or diffuse or mixed in an outbreak of Marek's disease in chickens in central Ethiopia. The nodules ranged in size from small pinpoint white to grayish to large ones of 25 mm in diameter. They were either localized deep in parenchyma and partly rose above the surface of organs involved. Microscopically, there were diffuse infiltration or massive aggregates of pleomorphic mononuclear cells in the kidneys.

Rajkhowa (2005) during an outbreak of acute Marek's disease among commercial chicken in Aizawal observed diffuse, nodular and miliary forms of tumors in proventriculus, liver, spleen, kidneys, bursa of Fabricius, lungs, intestine and mesentry. Miliary form of the disease involving mainly kidneys was recorded in a layer farm in a bird of 8-10 weeks age. Miliary grayish colored soft tumors measuring 2 to 3 mm in diameter were distributed throughout all the lobes of kidney. Microscopically in the kidneys, lesions were uniformly proliferative and focal in nature. The cellular composition consisted of small to medium lymphocytes, lymphoblasts and activated and primitive reticulum.

In MD virus infected chicken lymphamatous lesions in the gonads, lungs, kidneys, liver, spleen, intestines and skin were observed. Lymphomas were focal, nodular growths, which are white or grey in color and firm in some cases. Histologically, diffuse proliferating small to medium lymphocytes, lymphoblasts, activated and primitive reticulum cells were present in the kidneys. Kidney tubules showed degeneration caused by tumor cell pressure. In some cases, immune complexes were found deposited in the kidney leading to glomerulopathy (Schat and Nair, 2008).

2.4.1.3 Avian leucosis

Nobel (1972) observed that kidneys were pale, swollen with numerous, round, white spots and white areas of irregular size and distribution in an egret with avian leucosis. Microscopically, kidneys had nodular infiltration with lymphoid cells, predominantely lymphoblasts. Urate deposits were also observed in the kidneys.

Nephroblastomas vary greatly in appearance from small, pinkish grey nodules that may be embedded in the kidney parenchyma to large, yellowish grey, lobulated masses that replace most of the kidney tissue in chickens experimentally infected with BA_1 strain A myeloblastosis virus. Tumors are often pedunculated. Histologically, there is usually a neoplastic proliferation of both epithelial and mesenchymal elements. The epithelial structures vary from enlarged tubules with invaginated epithelium and malformed glomeruli and irregular masses of distorted tubules to groups of large, irregular, cuboidal and undifferentiated cells with little tubular organization. There may be islands of keratinizing stratified squamous epithelial structures, cartilage or bone (Purchase and Burmester, 1978).

The nephroblastomas consisted of abnormally differentiated kidney tissue containing elements of sarcoma, carcinoma, chondroma, osteoid and keratinized tissue areas in chicken infected with avian nephroblastoma virus MAV-2(N). These non invasive neoplasms were multifocal in origin and progressed to form massive, thickly encapsulated structures. The tumors comprised of fluid filled cysts, solid tissue, necrotic areas and hemorrhagic (Watts and Smith, 1980).

In a study to determine whether c-fos was involved in avian leucosis virus induced nephroblastomas, Collart *et al*. (1990) observed abnormal growth of epithelial as well as mesenchymal cells. All tumors contained aberrant tubular arrangement of epithelial cells. The mesenchymal cells in the tumors exhibited

various stages of differentiation, with some nephroblastomas containing mesenchymal cells that ranged from primitive mesenchyme to fully differentiated cartilage and bone while others contained only blastema or blastema and fibroblasts.

In the kidney of a Copper pheasant with nephroblastoma Singh and Mohanty (2007) microscopically observed proliferation of varying shaped deeply stained tubuli in fairly cellular connective tissue stroma. The neoplastic tissue had replaced the normal architecture of the kidney and there were attempts to form new not well differentiated glomeruli. There were both large and small cystic tubules arranged irregularly among scattered primitive tubules. There were also areas of degeneration at times with hemorrhages. Some areas of kidney tissue including the neoplastic areas also revealed presence of uric acid granuloma.

Fadly and Nair (2008) observed grossly visible tumor masses on liver, spleen, bursa of Fabricius, kidney, heart, gonad and mesentry in chickens affected with avian Leucosis. Tumor growth were sometimes nodular, miliary, diffuse or a combination of these forms. Histologically, kidney tissue consisted of aggregates of large lymphoid cells varying slightly in size but are all of the same early developmental stage.

2.4.2 NUTRITIONAL AND METABOLIC DISEASES

2.4.2.1 Fatty liver and kidney syndrome

Karadas *et al.* (1999) observed an increase in the size of the liver, kidney and heart in white broiler chickens fed with ration including rendering oil. Microscopically, a diffuse fatty degeneration involving the epithelial cells of proximal convoluted tubules of the kidney and also generalized diffuse glomerular lipidosis were observed.

Fatty liver and kidney syndrome in laying hens occurred as a result of feeding a low protein, high calorie rations to hens that were not laying enough eggs. At postmortem examination, the liver and kidneys were maedly enlarged, pale and fatty with abnormal deposits of fat in the subcutaneous tissue, abdominal cavity and visceral organs (Sathyanarayanan, 2007).

2.4.2.2 **Gout**

Onderka *et al.* (1987) observed visceral urate deposits in chicks following water deprivation. Renal tubular changes consisted of increased spaces between membrane infoldings of the distal convoluted tubular epithelium, increased number of cytoplasmic vacuoles in proximal convoluted tubules and increased production of mucin by the collecting ducts.

In an outbreak of urolithiasis in White Leghorn pullets, several birds showed extensive uric acid deposits throughout the visceral organs, peritoneal membrane and subcutaneous tissues. Many birds had unilateral or bilateral urinary involvement with either severe atrophy or irregular hypertrophy of one or several lobes of the kidneys. The ureters were markedly enlarged with irregular white uroliths. The swollen kidneys had an irregular mottled grey-red appearance and cut firmly. Microscopic lesions in kidneys were severe. Urate granulomas were disseminated through all the kidneys and were associated with necrosis and loss of parenchyma. The remaining renal parenchyma showed compensatory change of tubular dilatation, hypertrophy and hyperplasia of tubular epithelium (Coy *et al*., 1988).

Uma *et al.* (1999) observed deposition of white urate crystals on the visceral organs in birds with gout. Microscopically, urate deposits in the kidneys were seen as dense amorphous structure on the surface and in the parenchyma.

They appeared as spongy balls either in the glomeruli or in the interstitial tissue along with infiltration of inflammatory cells including giant cells. Additional changes included hyaline casts and infiltration of lymphoid cells. Urate crystals appeared as black radiating structures in De Galantha's stained sections.

Ahmed *et al.* (2003) noticed unilateral or bilateral urolithiasis in broiler breeders in their pathological study on urolithiasis. The ureters and its branches were seen dilated with uroliths and clear thick mucus. The lobes of kidneys drained by these were atrophied due to hypertrophy of unaffected lobes. Histopathology of kidneys revealed dilated tubules with degenerative changes and sloughing of the epithelium. Some tubules revealed necrotic debris with hyaline casts in their lumen. The kidneys showed radiating crystals of urates which appeared black with Gomori's methanamine Leishman stain.

In an outbreak of gout in Kashmir Favorella chicken, Mir *et al*. (2005) observed dry platery patches of white chalky urate deposits on the kidneys. Occasionally, the cranial lobes of the both kidneys were enlarged. Ureters of either side were found to be distended with retained semifluid to semisolid chalky white urates, giving cord like appearance to ureters. Histologically, kidney parenchyma was atrophied and revealed degenerative and necrotic changes associated with hemorrhages. Glomerular changes included atrophy, distortion and segmentation. The tubules showed degeneration and desquamation. Uric acid deposits replacing parenchyma was surrounded by inflammatory cells including heterophils, lymphocytes, macrophages and giant cells. In De Galantha's stained kidney sections, the urates stained characteristically black, showing cotton ball appearance with radiating crystals at periphery.

Crespo and Shivaprasad (2008) noticed histologically, in the kidneys dilatation of ureter branches and tubules, tubular degeneration and loss of tubules, presences of celluler casts, urate crystals and varying degrees of fibrosis in chicken affected with gout.

2.5 TOXINS OTHER THAN AFLATOXIN AFFECTING LIVER AND KIDNEY

Shrivastava *et al.* (1988) in a feeding trial conducted in chicks with diet containing raw and treated castor meals observed enlargement, discoloration, congestion and petechial hemorrhages in liver and kidneys. The histopathological changes in the liver ranged from early degenerative changes to necrosis with sinusoidal dilatation and congestion, which in a most severe form had a diffuse distribution, whereas the mild form showed focal or centrilobular necrosis. The kidneys showed granular degeneration and desquamation of tubular lining epithelial cells.

Leenadevi *et al.* (1990) in a toxicity study on superchlorinated water in ducks observed enlargement of thyroids and congestion of liver and kidney.

Pathological changes were observed in organs of chicks which were destroyed after 12 hour of citrinin administration. The liver and kidneys were slightly pale. The kidneys were moderately swollen. Mild focal degenerative changes were seen in the liver. Cytoplasm of some of the periportal hepatocytes showed granular and fatty degeneration. The nuclei of these cells was eccentrically placed. Mononuclear cell infiltration was evident in occasional foci (Maryamma *et al.,* 1990).

Kulkarni *et al.* (1995) in an experimental study on butocarboxim toxicity in chicken observed congestion of kidneys, liver and brain. Histologically, kidneys showed severe congestion and degenerative changes including hydropic degeneration.

In broiler chicken with ochratoxicosis Ramadevi *et al.* (1998) observed pale, enlarged liver and kidneys with petechial hemorrhages on the surface. Microscopically, congestion, degeneration and focal interstitial nephritis were observed in the kidneys while congestion, degenerative changes, focal lymphoid aggregation and total necrosis of hepatocytes were observed in the liver.

Materials and Methods

3. MATERIALS AND METHODS

The present study was conducted at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy to investigate the pathology of liver and kidney in chicken as well as to find whether aflatoxin residues were present in the liver and kidney of chicken during natural cases of aflatoxicosis.

3.1 PATHOLOGY OF LIVER AND KIDNEY

3.1.1 Sample Collection

A total of 200 liver and kidneys having lesions were collected from chicken brought for autopsy to the Centre of Excellence in Pathology during the period of study. The samples collected were subjected to detailed gross and histopathological examination.

3.1.2 Gross Examination

A detailed systematic post mortem examination of the chicken carcasses brought for autopsy was performed. The liver and kidneys were carefully studied for gross lesions like change in size, shape, colour and consistency and presence of cysts, abscesses or tumor. Representative samples were collected for histopathological examination.

3.1.3 Histopathological Examination

The representative parts of liver and kidney showing gross pathological changes were collected and preserved in 10 % neutral buffered formaldehyde solution for histological examination. They were then processed and paraffin embedded as described by Sheehans and Hrapachak (1980). The paraffin embedded tissues were cut at four microns thickness and stained with Haematoxylin and Eosin stain (H&E stain) as described by Bancroft and Cook

(1995). The stained sections were subjected to detailed examination under the light microscope.

To demonstrate uric acid crystals, tissue pieces were collected in absolute alcohol and were directly cleared, embedded in paraffin and the sections were stained by De-Galantha's method (Luna, 1968). Masson's trichrome staining was done to demonstrate fibrous tissue proliferation in the kidney (Luna, 1968).

3.1.4 Microbiological Studies

Bacterial isolation was attempted from the liver and kidney in all the fresh cases and identification of the organisms was done as per Cowan (1974). Blood smears were also prepared from heart blood from cases suspecting for Pasteurellosis.

3.2 ESTIMATION OF AFLATOXIN

3.2.1 Aflatoxin Residues in Tissues

Aflatoxin residues in the liver and kidney of cases suspected for aflatoxicosis were determined by modified Pons method (Pons *et al*., 1966) using Thin Layer Chromatography (TLC). For quantification, the plate was scanned fluorimetrically using Hitachi-3000 model computerized fluorescence spectrometer at a wave length of 365 nm for excitation and 420 nm for emission.

3.2.2 Aflatoxin in the Feed

Feed samples were also randomly collected and detected for the presence of aflatoxin by modified Pons method using TLC and also quantified using fluorescence spectrometer.

Results

4. RESULTS

During the course of this study, 200 samples of lesion bearing livers and kidneys were collected from chicken brought for autopsy to the Centre of Excellence of Pathology.

Feed samples were collected randomly from the poultry farmers, who brought poultry for postmortem examination and analyzed for the presence of aflatoxin. Liver and kidney tissues were also collected from suspected cases of aflatoxicosis for analyzing aflatoxin residues .

The level of aflatoxin in the feed and tissue samples was shown in the Table 1. The percentage incidence of various conditions associated with hepatic lesions is presented in Table 2 and Fig. 1 and renal lesions in Table 3 and Fig.2

The conditions associated with hepatic lesions were classified into bacterial, viral, protozoan, nutritional and metabolic, aflatoxicosis, hepatic congestion and non-specific hepatosis and hepatitis. Among bacterial diseases colibacillosis accounted for 16 percent, pasteurellosis, 5.5 percent and staphylococcosis, 0.5 percent. Marek's is a viral disease which accounted for 21.5 percent. Histomoniasis a protozoan disease accounted for one percent. Nutritional and metabolic diseases consisted of Fatty liver syndrome (20.5 percent), gout (nine percent) and ascites syndrome (6.5 percent). Aflatoxicosis accounted for two percent , hepatic congestion, 11 percent and non-specific hepatosis and hepatitis, 6.5 percent.

The percent incidence of various conditions associated with renal lesions included IBD, 38.5 percent, Marek's disease, 21.5 percent, nephroblastoma, 0.5 percent , gout, nine percent, fatty liver kidney syndrome, 3.5 percent, aflatoxicosis, two percent, vascular disturbances, 17 percent and non specific nephrosis and nephritis, eight percent.

4.1. DETECTION OF AFLATOXIN AND AFLATOXIN RESIDUES

Feed samples were collected from the poultry farms, from which cases suspected for aflatoxicosis were brought for postmortem examination. Out of the 14 feed samples examined for aflatoxin, four feed samples were found to be contaminated at the rate of 0.05, 0.1, 2 and 2.5 ppm levels. Out of the 38 tissue samples examined for aflatoxin residues, two were found to be positive. Feed samples of these two cases had aflatoxin at the level of 2 and 2.5 ppm. The levels of aflatoxin residues in the liver and kidneys were 15 ppb and six ppb respectively in chicken fed with 2.5 ppm level of aflatoxin. In chicken fed with 2 ppm level of aflatoxin had seven ppb aflatoxin residue in the liver and no detectable level of aflatoxin residue in the kidney.

4.2. CONDITIONS ASSOCIATED WITH HEPATIC LESIONS.

4.2.1 Bacterial diseases

4.2.1.1 Colibacillosis

4.2.1.1.1 Incidence

Colibacillosis was the most common bacterial disease observed in chicken, in which 16 percent of the cases were associated with hepatic lesions. These cases were confirmed by the isolation of *Esherichia coli* from the representative samples.

4.2.1.1.2 Gross pathology

Pericarditis, perihepatitis, air sacculitis, enteritis and salpingitis in mature laying hens were observed. The surface of the liver was covered by yellow-white granular layer (Fig.3) in most of the cases. Hepatomegaly and diffuse grayish white patches of necrosis were observed. Kidneys showed slight enlargement and hemorrhages on their surface.

4.2.1.1.2. Histopathology

In the liver, the areas of necrosis were homogenous and eosinophilic with hyperchromatic pyknotic nuclei. In many of the hepatic cells the nucleus had completely disintegrated and disappeared retaining only the outline of the cell. Diffuse infiltration of inflammatory cells, sinusoidal dilatation and congestion were also observed. Extensive fibrin deposition in the subcapsular region along with granulation tissue formation and infiltration of inflammatory cells were evident (Fig.4). Kidney sections showed degeneration and necrosis of the tubular epithelium and hemorrhages in the interstitial spaces.

4.2.1.2. Pasteurellosis

4.2.1.2.1 Incidence

In 5.5 percent cases, hepatic lesions were associated with pasteurellosis. Pasteurellosis was confirmed by the isolation of *Pasteurella multocida* from the samples. On blood smear examination bipolar stained organisms could be detected.

4.2.1.2.2. Gross pathology

On gross examination, petechial epicardial hemorrhage, enlargement of liver, spleen and kidney and congestion of the lungs were observed in most of the cases. The surface of the liver showed multifocal pinpoint grayish white necrotic foci (Fig.5).

4.2.1.2.2. Histopathology

Congestion of blood vessels and sinusoids, multifocal coagulative necrosis (Fig.6), focal heterophilic infiltration and vacuolation of the hepatocytes were evident in the liver. The kidneys showed infiltration of the inflammatory cells in the interstitium and marked tubular degeneration and desquamation of the lining epithelial cells.

4.2.1.3 Staphylococcosis

4.2.1.3.1 Incidence

Staphylococcosis was observed only in one chicken. The percentage of incidence was only 0.5 percent. It was confirmed by bacterial isolation.

4.2.1.3.2 Gross pathology

Liver was found to be slightly enlarged. Hepatomegaly with multifocal yellowish- white foci (Fig.7) were seen distributed on the surface of the liver. Kidneys were slightly congested.

4.2.1.3.3 Histopathology

Periportal coagulative necrosis (Fig.8), focal leucocytic infiltration and congestion of blood vessels were observed in the liver. Tubular degeneration and desquamation of lining epithelial cells, congestion and glomerulitis (Fig.9) were noticed in the kidney.

4.2.2 Viral Diseases

4.2.2.1.Marek's disease

4.2.2.1.1 Incidence

Marek's disease was the most common disease among all the conditions affecting the liver. The percentage of incidence of Marek's disease was 21.5 percent.

4.2.2.1.2 Gross pathology

Out of the 43 cases of Marek's disease, nodular type of lesions in the liver was observed in 29 cases and diffuse enlargement of the liver in 14 cases.

Nodules were grayish white or grayish colored (Fig.10) They were distributed evenly throughout the surface of parenchyma and within the substance of the liver tissue. The nodules were firm to cut and cut surface was smooth to touch. In diffuse type there was moderate to severe enlargement of the liver (Fig.11). In one case a large grayish-white colored nodular tumourous mass (Fig.12) was found attached to the left lobe of the liver. There was moderate to severe congestion of the liver in most of the cases. Neoplastic lesions were also observed in other visceral organs also.

4.2.2.1.3 Histopathology

Histologic lesions consisted of both focal and diffuse proliferation of neoplastic lymphocytes (Fig.13). The cellular composition consisted of diffuse or focal proliferation of small to medium sized lymphocytes and large lymphoblasts. Focal collection of proliferating lymphocytes was mostly seen in the periportal areas. In the case of the diffusely proliferating type, most of the hepatocytes were replaced by pleomorphic neoplastic lymphocytes. The cell types of the tumors were same in all cases, even though the gross pattern of involvement varied. Most of the blood vessels were found to be dilated and congested.

4.2.3 Protozoan Diseases

4.2.3.1 Histomoniasis

4.2.3.1.1 Incidence

The incidence of histomoniasis in chicken was one percent.

4.2.3.1.2 Gross pathology

Enlarged livers with numerous round to oval, circumscribed lesions (Fig.14) ranging from 0.5 to 1.5 cm in diameter were visible. These lesions had a

slight depression at the center and circumscribed by well defined ringed edges. When incised, the lesions were found to be extending deep into the parenchyma.

4.2.3.1.3 Histopathology

In the liver, an extensive infiltration of inflammatory cells was noticed. Hepatocytes mostly surrounding the blood vessels contained histomonads visible as punched out ovoid bodies resembling vacuoles in the cytoplasm (Fig.15). Multiple confluent areas of necrosis were observed. The necrotic hepatocytes were sparsely distributed or disappeared completely leaving the stroma and a few cellular debris. In the kidney, degeneration of tubular epithelium and necrosis were observed.

4.2.4 Nutritional and Metabolic Diseases

4.2.4.1 Fatty Liver Syndrome

4.2.4.1.1 Incidence

Fatty liver syndrome includes Fatty liver hemorrhagic syndrome and Fatty liver kidney syndrome. Fatty liver hemorrhagic syndrome was observed in 34 chicken and fatty liver kidney syndrome in seven cases. The percentage of incidence of fatty liver syndrome was 20.5 percent.

4.2.4.1.2 Gross pathology

Liver showed moderate to severe enlargement with rounded borders. Liver had light yellow or yellowish brown discoloration (Fig. 16). Mild to moderate congestion was observed in most cases. The liver was friable and cut surface bulged out and has oily appearance. Blood clots were present on the surface of livers and on the heart in cases of FLHS. The kidneys were found to be pale and enlarged in FLHS. The abdominal cavity contained large amount of fat.

4.2.4.1.3 Histopathology

In mild cases, only some of the hepatocytes were swollen with fat vacuoles in different cords pushing the nucleus to one side. In severely affected cases, the normal architecture of the liver was disturbed by the distorted and disrupted hepatic cords by the large lipid droplets (Fig.17). Focal or diffuse areas of hemorrhages were also observed in most of the cases in the liver. The tubular epithelium of the kidneys showed mild degenerative changes in cases of FLHS.

4.2.4.2 Gout

4.2.4.2.1 Incidence

The incidence of gout was found to be nine percent.

4.2.4.2.2 Gross pathology

There was deposition of dry platery patches of white chalky urate crystals on the surface of the liver , other visceral organs (Fig.18) and joints in most of the cases. In some cases, the deposition of urate crystals on the surface of the liver was not evident grossly.

4.2.4.2.3 Histopathology

Liver showed focal hepatitis, sinusoidal dilatation and congestion, degeneration and areas of focal hepatic necrosis. The urate deposition (Fig.19) was most conspicuous on the surface of the liver as compared with the parenchyma. Glissons capsule revealed proliferation of fibrous connective tissue. Dissociation of hepatic cords (Fig.20) and fatty vacoulation of few hepatocytes were observed in some cases.

Liver sections stained with De Galantha's, the urates stained characteristically black, showing cotton ball appearance with radiating crystals at the periphery.

4.2.4.3 Ascites Syndrome

4.2.4.3.1 Incidence

Ascites syndrome was encountered in 13 chicken. The incidence was 6.5 percent.

4.2.4.3.2 Gross pathology

Marked abdominal distension with accumulation of clear yellowish fluid with or without fibrin clots (Fig.21) were the gross changes noticed in the abdominal cavity. The liver was swollen and congested. Focal pale areas and thickened capsule were noticed in most cases. Mild fibrinous perihepatitis was observed in a few cases. The heart appeared enlarged with mild to moderate hydropericardium and kidneys were also found to be enlarged.

4.2.4.3.2 Histopathology

The sinusoids, portal and central veins were dilated showing varying degrees of congestion. Focal infiltration of mononuclear cells (Fig.22) and multifocal random areas of hepatic necrosis (Fig.23) were noted. Disorganization of hepatic cords with individualization of hepatocytes was also observed in the liver. In the kidneys, there were glomerular and interstitial nephritis, congestion and hemorrhages in the intertubular areas (Fig.24). Varying degrees of degenerative changes with desquamation of the tubular epithelial cells were evident.

4.2.5 Aflatoxicosis

4.2.5.1 Incidence

Aflatoxicosis was observed in four chicken and the incidence of aflatoxicosis was two percent. Aflatoxicosis was the main cause of death in two chicken which had aflatoxin in the feed at 2 ppm and 2.5 ppm. In one case, aflatoxicosis was combined with IBD, in which aflatoxin in the feed was 0.1

ppm. Aflatoxicosis combined with coccidiosis was observed in another case which had aflatoxin in the feed at 0.05 ppm.

4.2.5.2 Gross pathology

With aflatoxin at 0.1 and 0.05 ppm levels in the feed, liver showed slight enlargement and yellowish discoloration (Fig.25). At 2.5 and 2 ppm levels of aflatoxin in the feed, moderate to severe enlargement, yellowish discoloration, petechial hemorrhages and focal areas of necrosis (Fig.26) were observed in the liver.

4.2.5.3 Histopathology

At 0.05 and 0.1 ppm levels, liver showed marked granular and vacuolar degeneration (Fig.27), periportal heterophilic and lymphocytic infiltration and congestion of blood vessels. The hepatocytes showed marked fatty change with disruption of cord like arrangement, diffuse areas of necrosis, periportal infiltration of heterophils and lymphocytes, mild bile duct proliferation (Fig.28), sinusoidal dilatation and congestion in chicken with 2 and 2.5 ppm levels of aflatoxin in the feed.

4.2.6. Non – Specific Hepatosis and Hepatitis

4.2.6.1 Incidence

Non-specific hepatosis accounted for 6.5 percent of the cases

4.2.5.2 Gross pathology

The areas of degeneration and necrosis appeared as diffuse, pale to yellow streaks extending into the parenchyma. The cut surface revealed a cooked appearance.

4.2.5.3 Histopathology

The cytoplasm of the hepatocytes contained vacuoles in cases of vacuolar degeneration. Vacuolar degeneration was observed in five cases. In cases of necrosis the normal architecture and outline of the hepatocytes were lost. The cytoplasm was homogenous and stained pink. The nuclei were in varying stages of pyknosis, karyorrexis and karyolysis. Necrosis of the hepatocytes were observed in eight cases out of thirteen cases of non-specific hepatosis and hepatitis. Infiltration of the inflammatory cells was noticed along with necrosis in three cases. Necrosis was mostly accompanied by vascular disturbances and degenerative changes.

4.2.6 Hepatic Congestion

4.2.6.1 Incidence

Congestion as the predominant lesion was observed in 11 percent of the cases. Congestion of the liver was seen in most of the diseases along with other lesions. Hemorrhage was also seen along with congestion in most of the cases.

4.2.6.2 Gross pathology

Liver showed mild to moderate enlargement with rounded borders and was dark brown color. On incision a large quantity of blood oozed out from the cut surface.

4.2.6.3 Histopathology

There was dilatation and engorgement of the sinusoids with erythrocytes. The central veins and portal vessels were found to be dilated and filled with erythrocytes in severe cases. Thinning of the hepatic cords were also observed due to the dilatation of the sinusoids.

4.3 CONDITIONS ASSOCIATED WITH RENAL LESIONS

4.3.1 Infectious Bursal Disease

4.3.1.1 Incidence

IBD was the most common disease among all the conditions associated with renal lesion. It accounted for 38.5 percent .

4.3.1.2 Gross pathology

On gross examination, the bursa was found to be enlarged and distended with hemorrhage and yellow exudate. Extensive skeletal muscle hemorrhage, paleness of the liver and splenomegaly were observed. Kidneys were found to be enlarged and congested in most cases. The ureters were distended with accumulated urate crystals (Fig.29). The kidneys varied in color from white or pale grey to brown.

4.3.1.3Histopathology

Kidney sections revealed tubular degeneration and desquamation, congestion of blood vessels and intertubular hemorrhages in focal areas. Scattered and focal infiltration of lymphoid cells predominantly in the cortical surface (Fig.30) was observed. In five cases mild fatty changes of the tubular lining epithelial cells were also noticed. Liver sections revealed periportal mononuclear cell infiltration, congestion and vacuolar degenerative changes.

4.3.2 Marek's Disease

4.3.2.1.Incidence

The percentage of incidence of Marek's disease was 21.5 percent among all the conditions associated with renal lesion.

4.3.2.2. Gross pathology

Grossly small grayish white colored soft tumors (Fig.31) were found to be distributed in some lobes of the kidneys. However in some cases there were only diffuse enlargement without any gross lesion on the surface of the kidneys.

4.3.2.3 Histopathology

In the kidneys, there was infiltration of proliferating populations of small to medium sized lymphocytes and lymphoblasts. Kidney tubules showed degeneration and desquamation (Fig.32).The infiltration of pleomorphic lymphoid cells between the tubules replaced the renal parenchyma. Congestion of blood vessels and intertubular hemorrhages were also observed in most of the cases.

4.3.3 Nephroblastoma

4.3.3.1Incidence

The percentage of incidence of nephroblastoma was found to be 0.5 percent.

4.3.3.2 Gross pathology

A large tumorous growth was found attached to the right kidney. It was grayish white colored (Fig.33) and enclosed a fluid filled cyst. The solid part was firm to cut.

4.3.3.3 Histopathology

The neoplastic tissue had replaced the normal architecture of the kidney. There was neoplastic proliferation of both epithelial and mesenchymal elements. The epithelial structures varied from enlarged tubules with invaginations and malformed glomeruli and irregular masses of distorted tubules to groups of large, irregular, cuboidal, undifferentiated cells with little tubular organization in a

fairly cellular connective tissue stroma (Fig.34). There were also areas of degeneration and hemorrhages.

4.3.4 Gout

4.3.4.1 Incidence

The percentage of incidence of gout was about nine percent among diseases associated with renal lesions.

4.3.4.2 Gross pathology

Kidneys were enlarged and extensive deposition of white urate crystals was observed on the surface of the kidneys in most of the cases. On incision of the kidneys large white chalky urate crystals were present inside the kidneys (Fig.35) in some of the cases. Ureters were found to be distended with retained chalky white urates giving cord like appearance to the ureters.

4.3.4.3 Histopathology

Uric acid deposits were surrounded by inflammatory cells including heterophils, lymphocytes, macrophages and giant cells (Fig.36). In De Galantha's stained kidney sections, the urates stained characteristically black, showing cotton ball appearance with radiating crystal (Fig.37).

The kidney parenchyma was atrophied and revealed degenerative and necrotic changes associated with intertubular hemorrhages. Varying degrees of fibrosis was also evident in the kidney parenchyma (Fig.38) in two cases. Glomerular changes included atrophy and distortion. The tubular epithelium showed degeneration and desquamation. Certain tubules showed dilatation, necrosis of lining epithelium and these tubules were found to be filled with inflammatory cells (Fig.39). Masson's Trichrome staining was done to demonstrate fibrous tissue proliferation where in collagen fibres took a blue colour (Fig.40).

4.3.5 Fatty Liver and Kidney Syndrome

4.3.5.1 Incidence

The percentage of occurrence of fatty liver and kidney syndrome was 3.5 percent among various conditions associated with renal lesion.

4.3.5.2 Gross pathology

Kidneys showed slight enlargement with pale or yellowish discoloration. Kidneys were fragile in consistency. Liver was enlarged and friable with light yellow discoloration (Fig.41). Large amount of fat was observed in the abdominal cavity and around the viscera

4.3.5.3 Histopathology

In the kidneys, tubular epithelium cells showed diffuse fatty degeneration. The lining epithelial cells were found to be distended with fat globules displacing the nucleus to one side (Fig.42). The glomerulus also contained fat vacuoles (Fig.43).

4.3.6 Aflatoxicosis

4.3.6.1 Incidence

The percentage of incidence of aflatoxicosis was two percent.

4.3.6.2 Gross pathology

The kidneys were found to be congested (Fig.44) at 0.05 and 0.1 ppm aflatoxicosis and pale and enlarged kidneys (Fig.45) at 2 and 2.5 ppm aflatoxicosis.

4.3.6.3.Histopathology

Degeneration and desquamation of the lining tubular epithelial cells and congestion of blood vessels were observed at 0.1 ppm aflatoxicosis. Moderate to severe vacuolar degeneration, necrotic changes with desquamation of lining tubular epithelial cells (Fig.46), vascular changes like congestion and intertubular hemorrhages and focal areas of lymphoid aggregates were observed at 2.5 ppm aflatoxicosis.

4.3.7 Vascular Disturbances

4.3.7.1 Incidence

Vascular disturbances accounted for 17 percent of the cases among the conditions associated with renal lesions. In most of the cases hemorrhage was seen along with congestion. Out of the 34 cases of vascular disturbances, congestion was observed along with hemorrhage in 22 cases and congestion alone was noticed in 12 cases.

4.3.7.2 Gross pathology

Kidneys were slightly enlarged and exhibited patchy reddish or pinpoint areas of petechiae, occasionally blood oozed out from the cut surfaces.

4.3.7.3 Histopathology

The blood vessels were found to be dilated and filled with erythrocytes. Erythrocytes were also found to be extravasated into the interstitium and subcapsular areas.

4.3.8 ***Non-Specific Nephrosis and Nephritis***

4.3.8.1 *Incidence*

Non-specific renal lesions like nephrosis and nephritis accounted for eight percent of the cases.

4.3.8.2 Histopathology

In six cases changes were of vacuolar degeneration characterized by the formation of vacuoles in the cytoplasm of the tubular epithelial cells. In some tubules, lining epithelial cells were desquamated into the lumen. In cases with necrosis, cytoplasm of the lining tubular epithelial cells were eosinophilic and the nuclear changes were pyknotic, karryorrhectic and karyolytic. Infiltration of inflammatory cells into the interstitium was observed in two cases along with necrosis.

4.3 MICROBIOLOGICAL STUDIES

A total of 14 bacterial isolations were obtained from representative samples of different cases. The isolates obtained included *Escherichia coli* (9), *Pasteurella sp*.(4) and *Staphylococcus sp*. (1).

Table 1 Aflatoxin residues (ppb) in the tissues of chicken received different levels of dietary aflatoxin (ppm)

Aflatoxin in the feed (ppm)	Aflatoxin residues in the tissues (ppb)	
	Liver	Kidney
0.05	-	-
0.1	-	-
2	7	-
2.5	15	6

Table 2 Incidence of the conditions associated with hepatic lesions

Conditions associated with hepatic lesions	Number of cases	In percentage (%)
Bacterial diseases		
Colibacillosis	32	16
Pasteurellosis	11	5.5
Staphylococcosis	1	0.5
Viral diseases		
Mareks disease	43	21.5
Protozoan diseases		
Histomoniasis	2	1
Nutritional and metabolic diseases		
Fatty liver syndrome	41	20.5
Gout	18	9
Ascites syndrome	13	6.5
Aflatoxicosis	4	2
Hepatic congestion	22	11
Non-specific hepatosis	13	6.5
Total	200	100

Table 3 Incidence of the conditions associated with renal lesions

Conditions associated with renal lesions	Number of cases	In percentage(%)
IBD	**77**	**38.5**
Marek's disease	**43**	**21.5**
Nephroblastoma	**1**	**0.5**
Gout	**18**	**9**
FLKS	**7**	**3.5**
Aflatoxicosis	**4**	**2**
Vascular disturbances	**34**	**17**
Non-specific nephrosis	**16**	**8**
Total	**200**	**100**

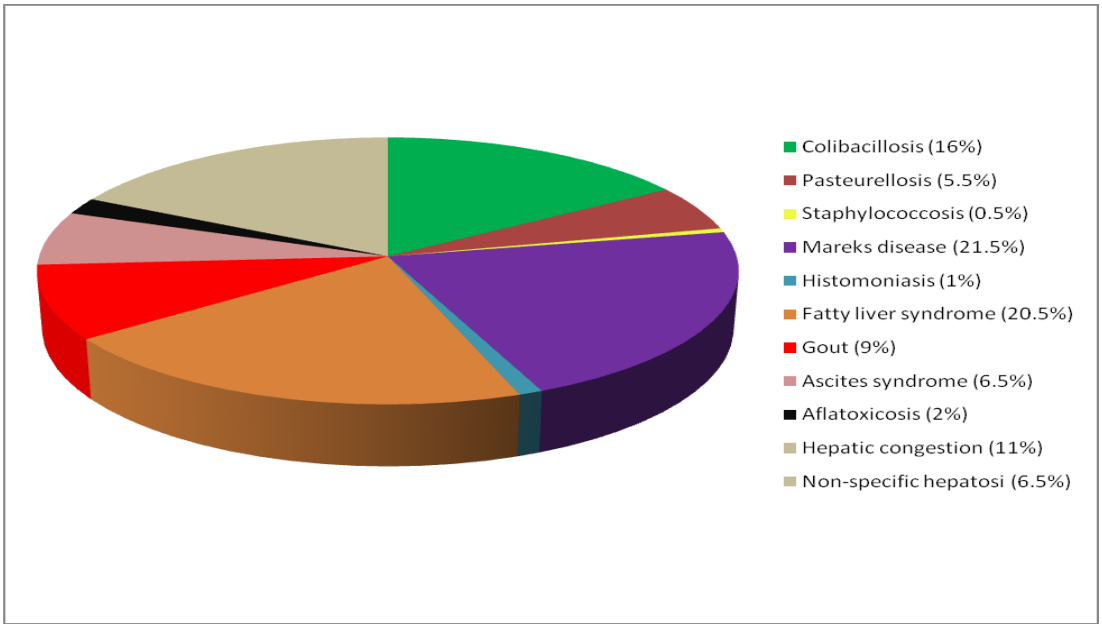

Fig. 1. Percentage of conditions associated with hepatic lesions

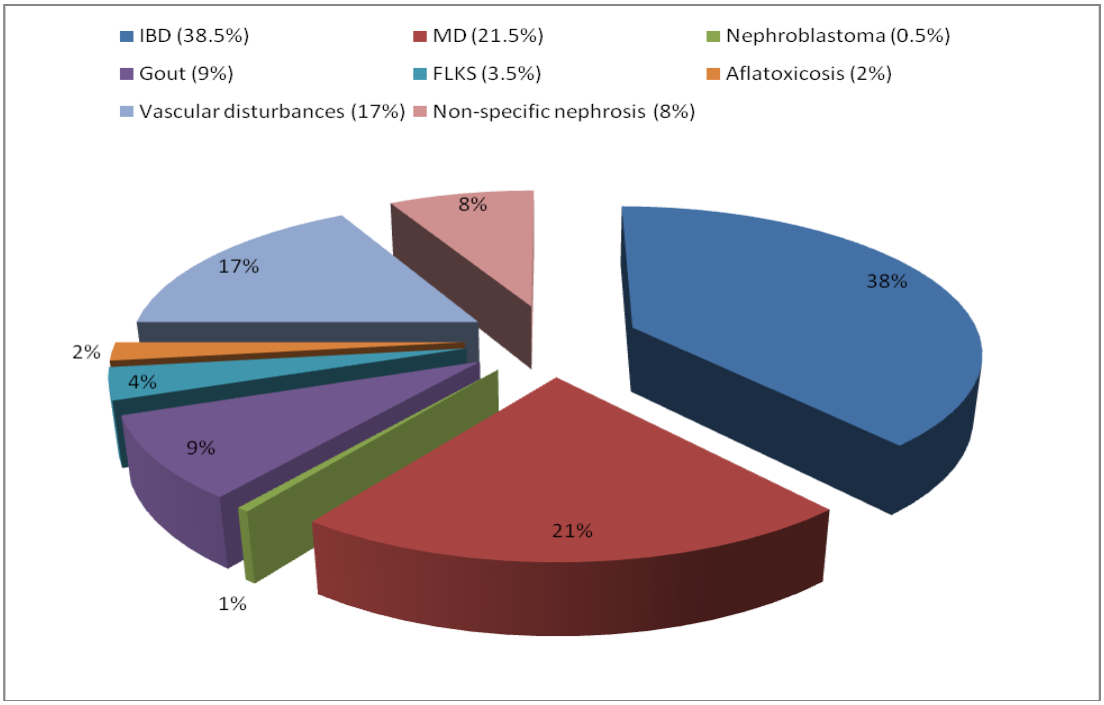

Fig.2 Percentage of conditions associated with renal lesions

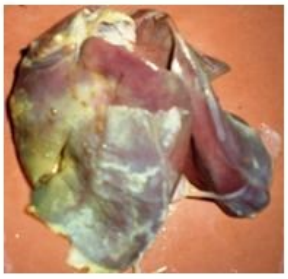

Fig. 3. Colibacillosis: Liver- yellowish- white granular layer on the surface of liver

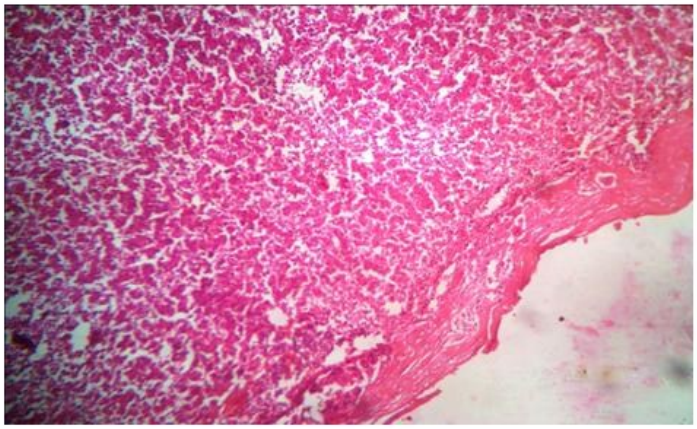

Fig. 4. Colibacillosis: Liver- fibrin deposition on the Glisson's capsule with infiltration of inflammatory cells(A), diffuse infiltration of inflammatory cells in the parenchyma (B) (H & E × 400)

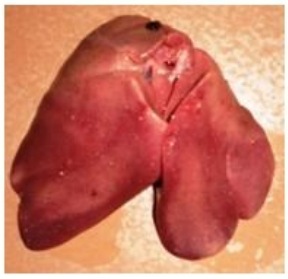

Fig. 5. Pasteurellosis: Liver- multifocal pinpoint grayish- white necrotic foci on the surface

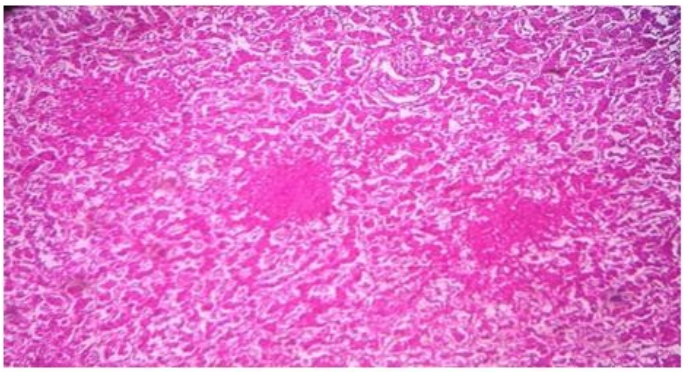

Fig. 6. Pasteurellosis: Liver- multifocal coagulative necrosis(H & E ×400)

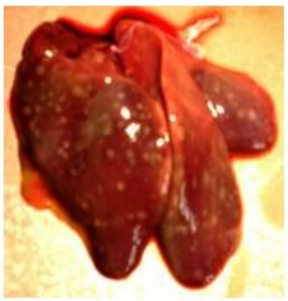

Fig. 7. Staphylococcosis: Liver- multifocal yellowish- white foci on the surface

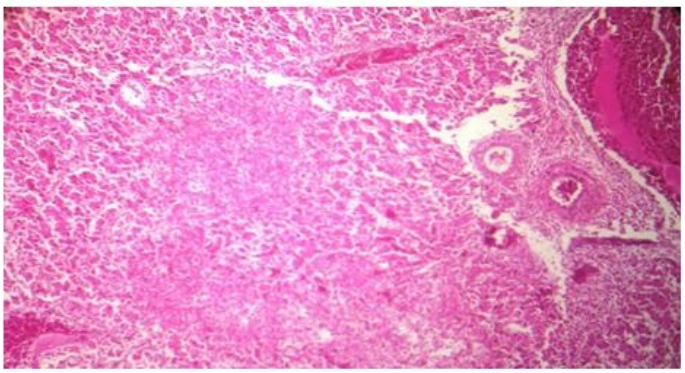

Fig. 8. Staphylococcosis: Liver- periportal coagulative necrosis (A), congestion of blood vessels (B) H&E ×400

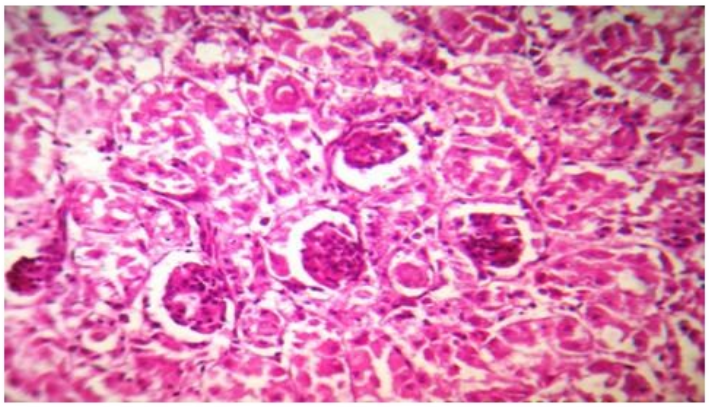

Fig. 9. Staphylococcosis: Kidney- degeneration and denudation of renal tubular epithelial cells(A), glomerulitis (B) (H & E 400)

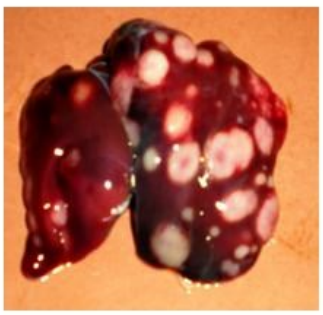

Fig. 10 Mareks disease: Liver- grayish-white nodules on the surface.

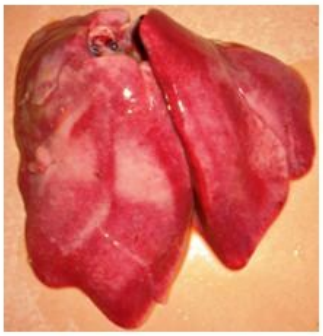

Fig. 11. Marek's disease : Liver- diffuse enlargement

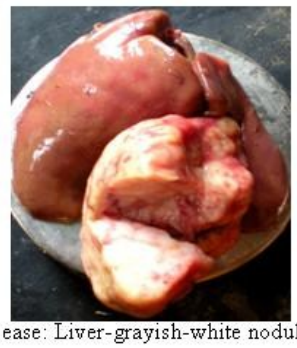

Fig. 12 Mareks disease: Liver-grayish-white nodular tumourous mass on the left lobe.

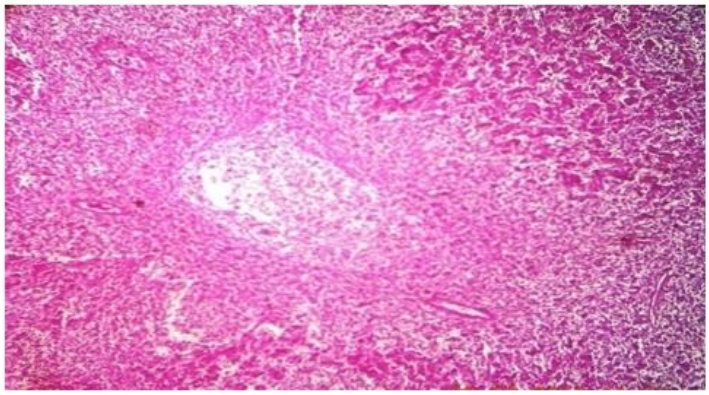

Fig. 13. Mareks disease: Liver- proliferation of neoplastic lymphocytes replacing the hepatocytes(H & E 100)

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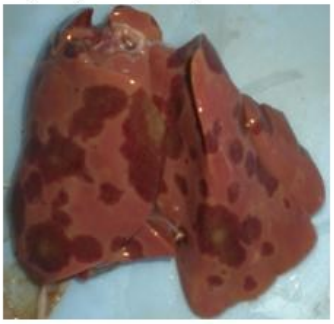

Fig. 14. Histomoniasis: Liver- round to oval circumscribed lesions on the surface

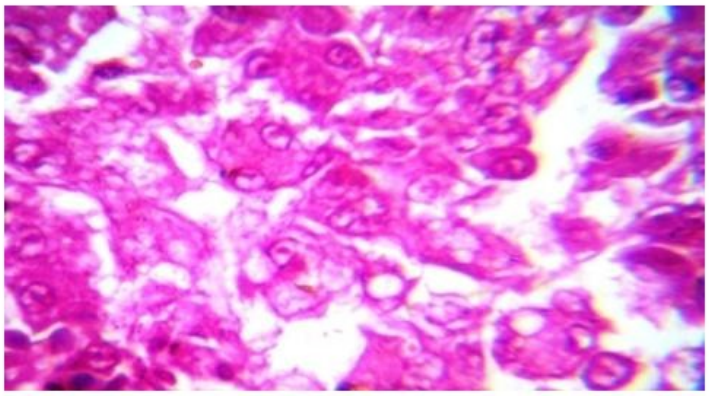

Fig. 15 Histomoniasis: Liver- ovoid bodies representing *Histomonas meleagridis* resembling vacuoles in the cytoplasm of hepatocytes (H & E× 400).

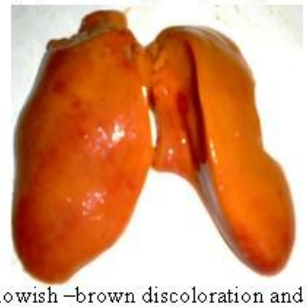

Fig. 16. FLHS: Liver- yellowish –brown discoloration and moderate congestion

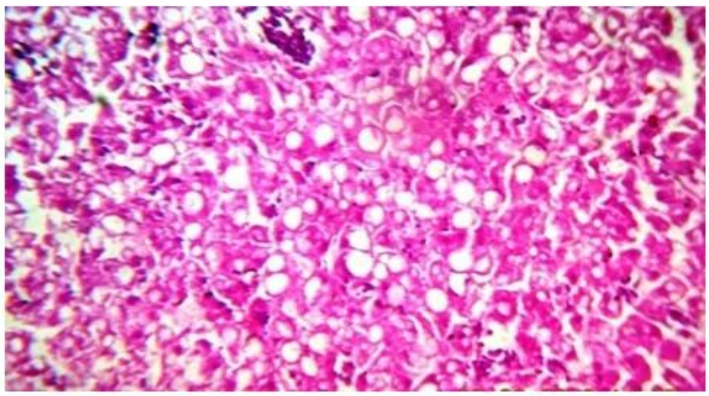

Fig. 17. FLHS: Liver- large fat vacuoles in the cytoplasm of hepatocytes(H&E×400)

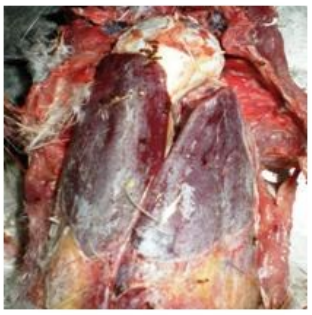

Fig. 18. Gout: deposition of white chalky urate crystals on the surface of the liver (A), heart (B)

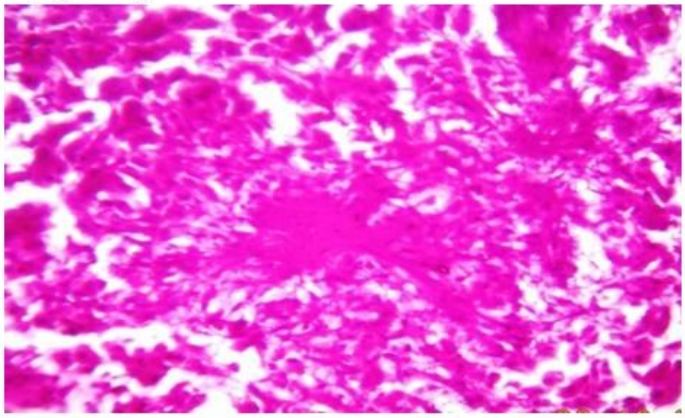

Fig. 19. Gout: Liver- showing urate deposition (H & E 400).

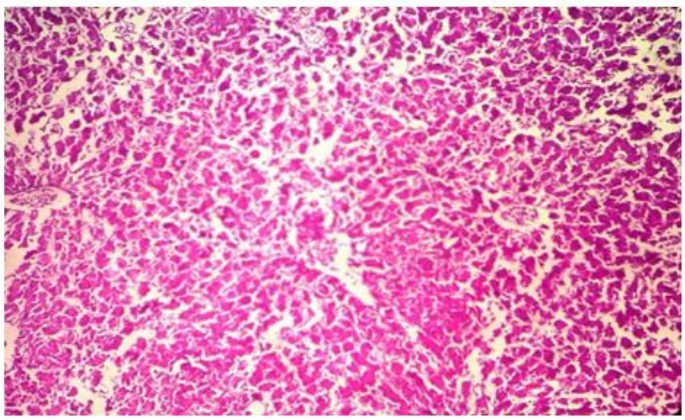

Fig. 20. Gout: Liver- dissociation of hepatic cords (H & E × 400)

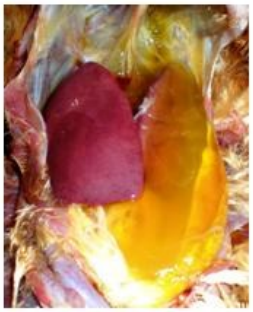

Fig. 21. Ascites syndrome: accumulation of clear yellowish fluid with fibrin clots in the abdominal cavity and pale areas on the surface of the liver

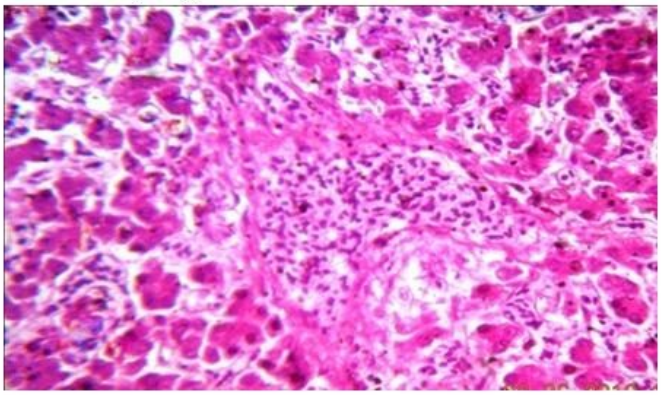

Fig. 22. Ascites syndrome: Liver- sinusoidal dilatation and congestion (A), focal infiltration of mononuclear cells (B) (H & E 400)

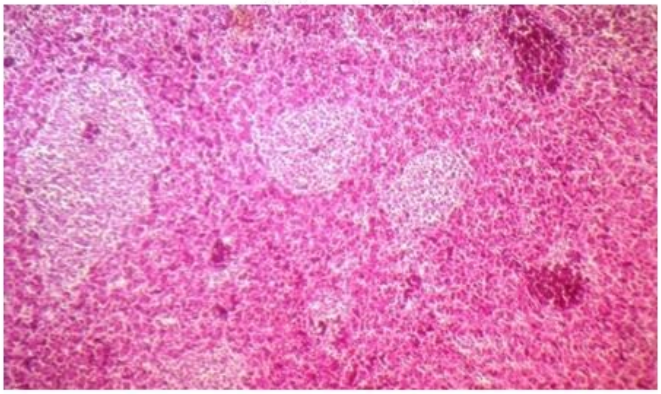

Fig. 23. Ascites syndrome: Liver- multifocal random areas of necrosis(A), congestion (B) (H & E × 400)

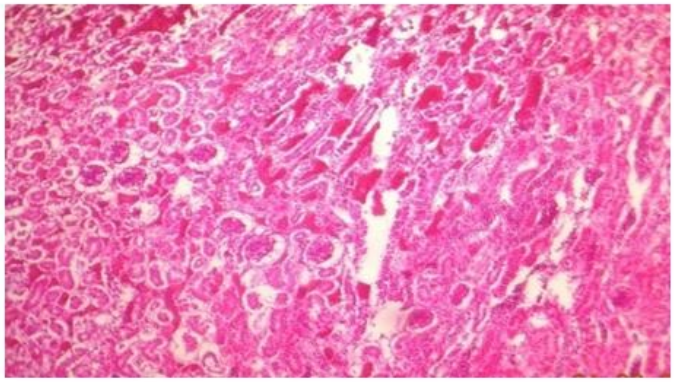

Fig. 24. Ascites syndrome: Kidney- glomerulitis (A), intertubular hemorrhages (B) (H & E 100)

Fig. 25. Aflatoxicosis: Liver- pale- yellowish discoloration and enlargement, 0.1 ppm level of aflatoxin

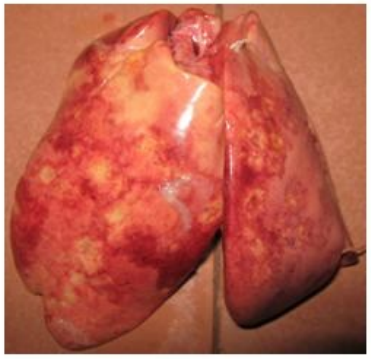

Fig. 26. Aflatoxicosis: Liver- yellowish discoloration and petechial hemorrhages on the surface, 2.5 ppm level of aflatoxin

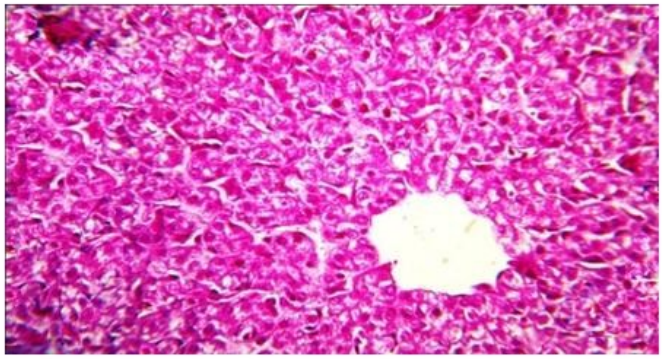

Fig. 27. Aflatoxicosis: Liver- vacuolar degeneration, sinusoidal congestion (A),0.1 ppm level of aflatoxin (H & E 400)

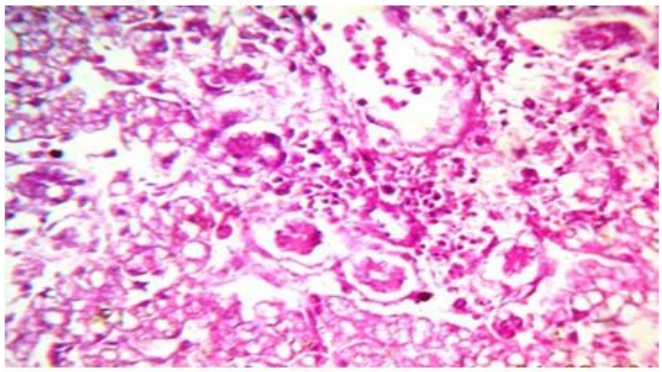

Fig. 28. Aflatoxicosis: Liver- mild bile duct proliferation (A), fatty change (B), periportal infiltration of inflammatory cells (C), 2.5 ppm level of aflatoxin(H & E 400)

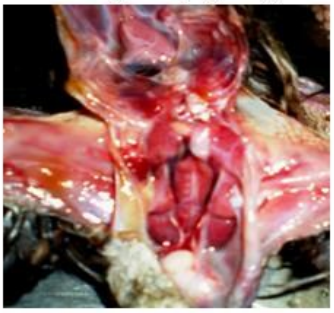

Fig.29. IBD: enlarged and congested kidneys, distended ureters (A), enlarged and oedematous bursa (B)

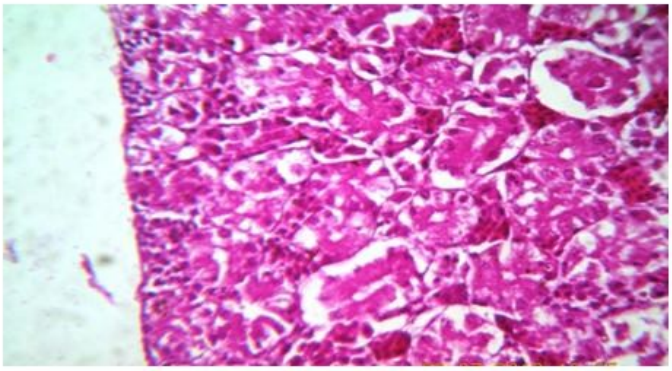

Fig. 30. IBD: Kidney- focal infiltration of lymphoid cells in the cortical surface (A),tubular degeneration and desquamation (B), intertubular hemorrhages (C)(H & E 400)

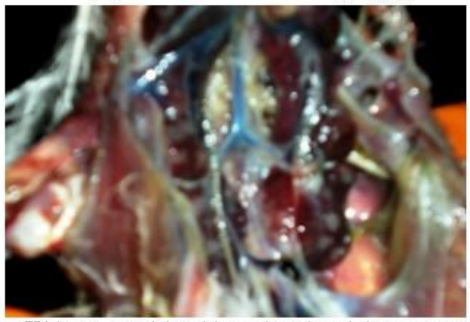

Fig. 31. Mareks disease: Kidney- grayish-white colored nodular tumours on the surface

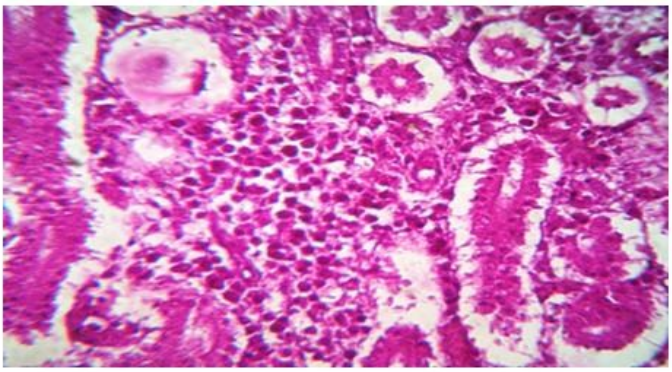

Fig. 32. Mareks disease: Kidney- infiltration of pleomorphic lymphoid cells between the tubules(A),degeneration and desquamation of tubular lining epithelial cells (B) (H & E 400)

Fig. 33. Nephroblastoma: grayish-white colored tumorous mass found on the kidney

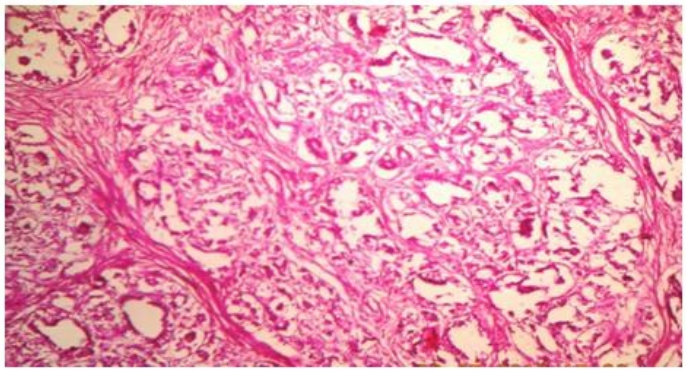

Fig. 34. Nephroblastoma: Kidney- fibrous tissue proliferation (A), irregular masses of distorted tubules (B) (H & E 400)

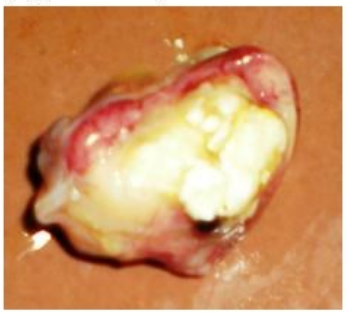

Fig. 35. Gout: Large white chalky urate crystal inside the kidney

×

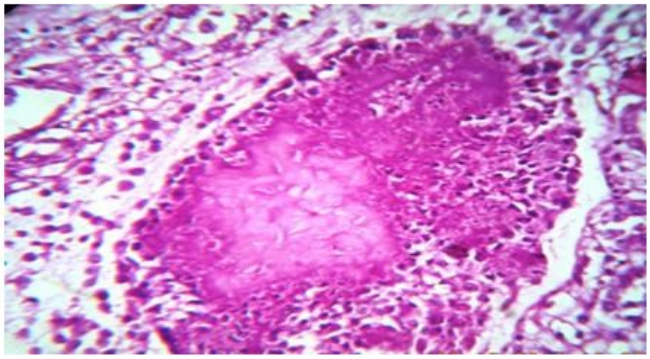

Fig. 36. Gout: Kidney- uric acid deposits surrounded by inflammatory cells(H & E 400)

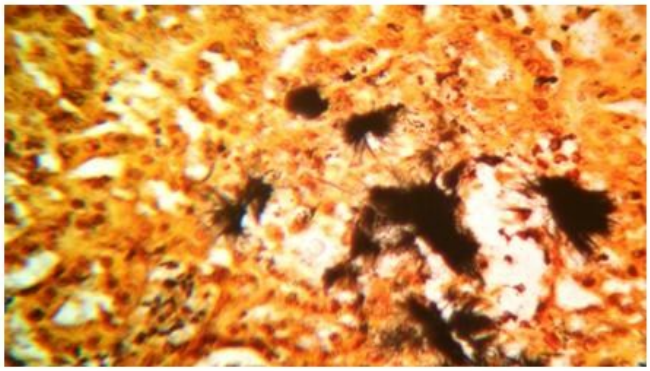

Fig. 37. Gout: Kidney-multifocal areas of characteristic black colored radiating urate crystal deposition (De Galantha's 400)

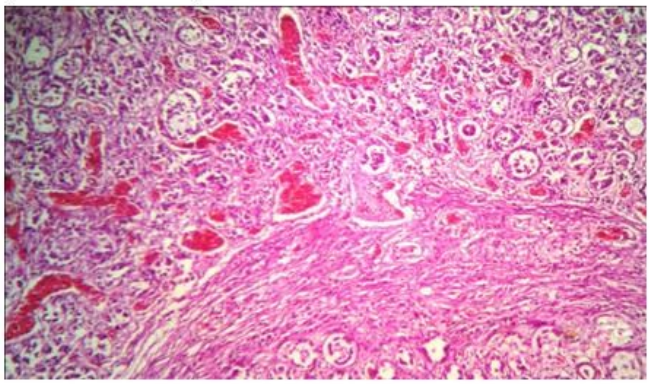

Fig. 38. Gout: kidney-fibrous tissue proliferation(A), intertubular hemorrhages (B), necrosis of lining tubular epithelial cells(C)(H & E 400)

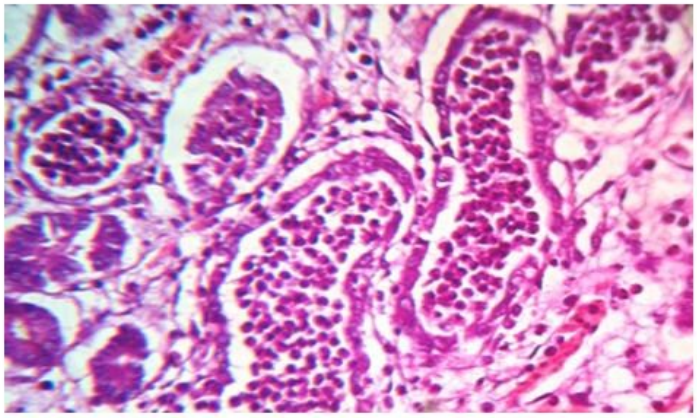

Fig. 39. Gout: Kidney- tubules filled with inflammatory cells(H & E 400)

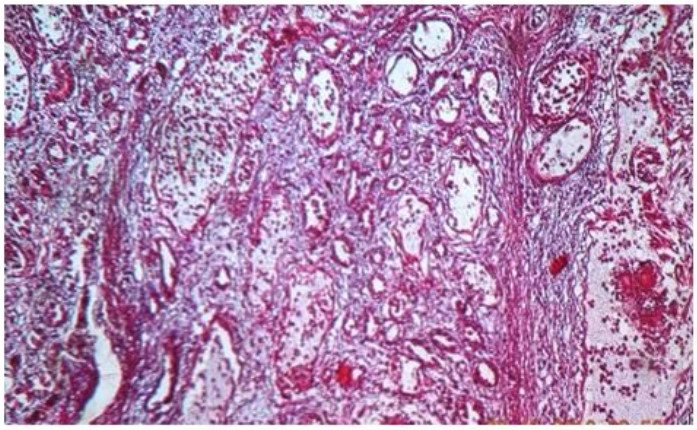

Fig. 40. Gout: kidney-Masson's trichrome staining to demonstrate fibrous tissue proliferation, collagen fibres took blue color(Masson's trichrome stain×400)

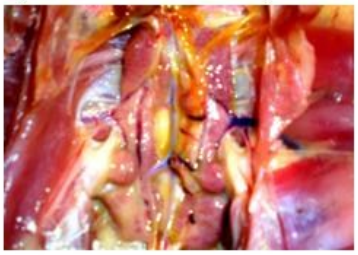

Fig. 41. FLKS: Kidney – slight enlargement and yellowish discoloration

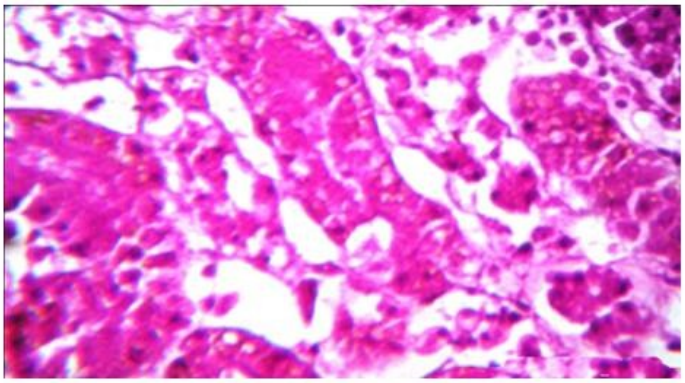

Fig. 42. FLKS:Kidney- fat vacuoles in the tubular lining epithelial cells(H & E 400)

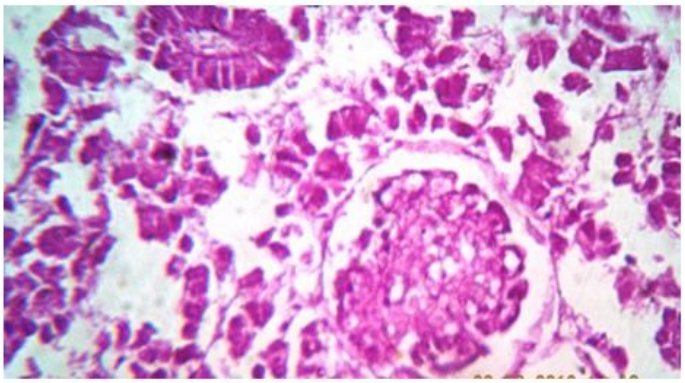

Fig. 43. FLKS:Kidney- fat vacuoles in the glomerulus(H & E 400)

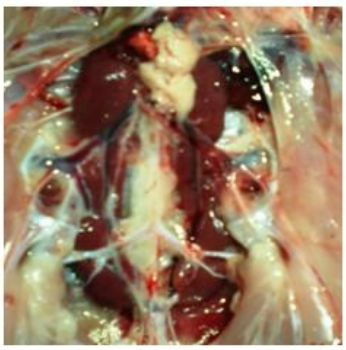

Fig.44. Aflatoxicosis: Kidney- congested and blood clot on the surface(A),0.1 ppm aflatoxin

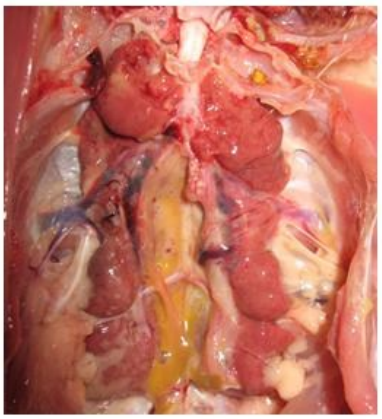

Fig. 45. Aflatoxicosis: Kidney- pale and enlarged, 2.5 ppm aflatoxin

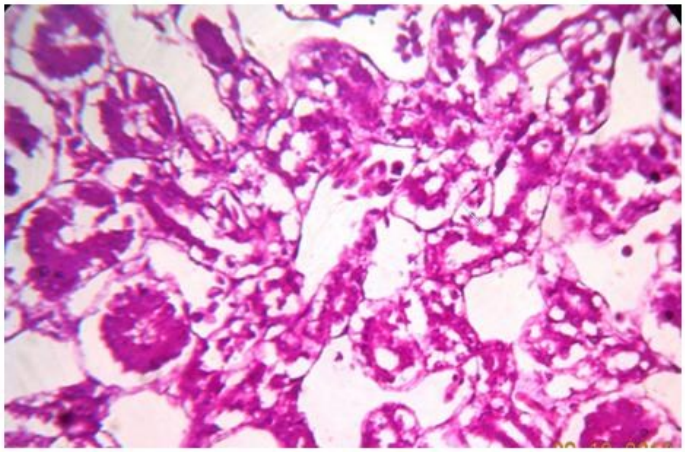

Fig. 46. Aflatoxicosis: Kidney-necrotic changes (A), desquamation of the lining tubular epithelial cells (B)(H & E 400)

Discussion

5 DISCUSSION

The present study was undertaken to classify and elucidate the pathological lesions in the liver and kidney of chicken encountered during various diseases and aflatoxicosis, utilizing systematic autopsy. Further investigations were also conducted to ascertain aflatoxin residues in the liver and kidney in natural cases of aflatoxicosis.

The hepatic lesions encountered were divided and classified under bacterial diseases, viral diseases, protozoan diseases, nutritional and metabolic diseases, aflatoxicois, hepatic congestion and non-specific hepatosis. Among bacterial diseases, colibacillosis accounted for 16 percent, pasteurellosis for 5.5 percent and staphylococcosis for 0.5 percent. The viral disease namely Marek's disease accounted for 21.5 percent while the protozoan disease namely histomoniasis one percent. Nutritional and metabolic diseases comprising fatty liver syndrome accounted for 20.5 percent, gout nine percent and ascites syndrome 6.5 percent. Aflatoxicosis accounted for two percent, hepatic congestion about 11 percent while non-specific hepatosis together with hepatitis accounting for 6.5 percent.

The percentage incidence of various conditions associated with renal lesions included IBD 38.5 percent, Marek's disease 21.5 percent, nephroblastoma 0.5 percent, gout nine percent, Fatty liver and kidney syndrome 3.5 percent, aflatoxicosis two percent, vascular disturbances 17 percent and non specific nephrosis and nephritis 8 percent.

The incidence of aflatoxicosis was found to be two percent. Aflatoxicosis in poultry occurs from the consumption of toxin contaminated feeds. The disease leads to depressed growth, poor feed efficiency, liver and kidney damage, immunosuppresion and increased mortality in chicken. (Kalra *et al*., 1995 and Arulmozhi *et al*., 2007). Aflatoxicosis is a major worldwide threat to poultry industry.

The residual aflatoxin in the meat and eggs is a matter of concern to human health. In the present study residual aflatoxin was detected only in two cases which had aflatoxin in the feed at rates of 2 and 2.5 ppm. The amount of aflatoxin residues was 15 ppb in the liver and 6 ppb in the kidney of chicken with 2.5 ppm level of aflatoxin in the feed while aflatoxin residue of 7 ppb was detected from the liver of chicken with 2 ppm level of aflatoxin in the feed but no detectable residues in the kidney. Maryamma *et al.* (1992) estimated toxin residues ranging from 10-20 µg per kg in the liver and 10-80 µg per kg in the kidney of White Leghorn chicks fed with 100 ppb of aflatoxin for 30 days. Yadav *et al.* (1995) noticed 4.7 ppb of aflatoxin residue in the liver and 2.0 ppb of residues in the kidney of broiler chicks treated with 0.5 ppm aflatoxin B_1 for 45 days. Arulmozhi *et al.* (2007) detected toxin residue ranging from 1.5 to 6.7 ppb in the liver and 1 and 3 ppb in the kidney on 45 days of age by contaminating the feed of broiler chicken with aflatoxin at 20, 40, 60, 80 and 100 ppb levels.

Residual aflatoxin was not detected in the present study in the tissues of chicken with aflatoxin in the feed at 0.05 and 0.1 ppm level. Arulmozhi *et al.* (2007) did not detect residual aflatoxin in the tissues on 15 and 30 days of the experiment except for the trace residual toxins detected in the liver (2 ppb) and muscle (2.4 ppb) on the thirtieth day in broiler chicken fed for 30 days with feed contaminated at 100 ppb level. Churchil *et al.* (2009) noticed no detectable residues in the tissues of broiler chicks after 15 days of withdrawal of feed contaminated with Aflatoxin B_1 at the level of one ppm from day old to 56 days of age. The observations by the above authors suggested that the chicken, from which aflatoxin residue was not detected, but had aflatoxin in the feed at the rate of 0.05 and 0.1 ppm, could be due to short term exposure to the aflatoxin contaminated feed so that no detectable level of aflatoxin could have accumulated in the tissues.

Concurrent occurrence of aflatoxicosis and IBD was observed in one chicken which had aflatoxin level of 0.1ppm in the feed. Aflatoxicosis and Coccidiosis was noticed together in one chicken which had aflatoxin in the fed at

the rate of 0.05 ppm. Aflatoxin at low level in the poultry feed could interact with organisms of diseases like Pasteurellosis, Salmonellosis, Candidiasis, Infectious Bursal disease, Marek's disease and Coccidiosis (Kalra *et al*., 1995, Mitra *et al*., 1999, Mahajan *et al*., 2002 and Gupta and Singh, 2003)

Enlarged and pale yellow liver and congested kidneys were observed in the chicken with 0.05 and 0.1 ppm levels of aflatoxin in the feed. Petechial hemorrhages and focal areas of necrosis along with enlargement and yellow discoloration of the liver and pale and enlarged kidneys were observed in chicken with aflatoxin at 2 and 2.5 ppm levels. The observations of Rama Devi *et al.* (1990), Anjaneyulu and Rama Rao (1993) and Arulmozhi *et al*.(2007) supports these gross findings. The enlarged pale yellow livers noticed in the present study could be due to the accumulation of lipids in the hepatocytes as evidenced histopathologically.

At 0.05 and 0.1 ppm levels microscopic picture of the liver revealed marked granular and vacuolar degeneration, marked fatty change with disruption of cord like arrangement, diffuse areas of necrosis, infiltration of heterophils and lymphocytes, mild bile duct proliferation, sinusoidal dilatation and congestion in chicken maintained on feed with 2 and 2.5 ppm levels of aflatoxin. In the kidneys, extensive degenerative changes with disruption and desquamation of the lining epithelial cells leaving intact the basement membrane, vascular changes like congestion and intertubular hemorrhages and focal areas of lymphoid aggregates were also observed. These histological findings correspond with those of Lalrintluanga and Baruah (1997), Kiran *et al.* (1998), Srivani *et al.* (2003) and Kumar and Balachandran (2009).

The incidence of Colibacillosis was 16 percent which was among the most common bacterial disease confirmed by bacteriological examination. Gross findings included pericarditis, perihepatitis and moderate to severe air sacculitis, enteritis and salpingitis in mature laying hens. The kidneys showed slight enlargement and hemorrhages on its surface. Histologically the liver showed

extensive fibrin deposition in the subcapsular region and degeneration and necrosis of hepatocytes. Kidneys showed degeneration and necrosis of the tubules. These findings were in agreement with the observations of Bajwa *et al.* (1992), Mitra *et al*. (1999), Srinivasan *et al*. (2003) and Sylvester *et al.* (2008).

The incidence of Pasteurellosis was about 5.5 percent. Pasteurellosis was confirmed by gross, histological and bacteriological examination. On gross examination epicardial petechiae, enlargement of the liver ,spleen and kidneys were observed. The surface of the liver showed multifocal pinpoint grayish-white necrotic foci similar to the findings of Locke *et al*. (1970), Rahman *et al*. (2004) and Afifi *et al*. (2007).

Microscopically, congestion of the blood vessels and sinusoids and multifocal coagulative necrosis and heterophilic infiltration were observed in the liver. The kidneys showed marked tubular degeneration and desquamation of the lining epithelial cells and infiltration of inflammatory cells in the interstitium. Similar histological features were noticed by Afifi *et al.* (2007) and Pramod (2009).

Staphylococcosis was observed in 0.5 percent cases and was confirmed by the isolation of the bacteria. On gross examination, the liver was found to be slightly enlarged bearing multifocal yellowish- white foci on the surface. The kidneys were found to be slightly congested. Microscopically, periportal coagulative necrosis and focal infiltration of leucocytes in the liver and degeneration and desquamation of the tubular lining epithelial cells , glomerulitis were observed in the kidneys. The gross and histopathological findings of the liver and kidney were in agreement with observations of Kibenge *et al.* (1983), Schmidt *et al.* (2003) and Andreasen (2008).

The percentage of incidence of Marek's disease was 21.5 percent. Marek's disease was found to be the most common disease condition affecting the liver among the chicken brought for autopsy to the Centre of Excellence in

Pathology. This was because most of the chicken brought for autopsy were from the University Poultry Farm, where the chicken are not vaccinated against Marek's disease and are reared under intensive system.

Grossly nodular, focal and diffuse type of lesions were observed in different organs like liver, spleen, kidney and heart. These nodules were distributed evenly throughout the surface of the parenchyma and within the substance of the liver tissue. Moderate to severe congestion of the liver was also observed. There was only diffuse enlargement of kidneys without any grossly visible lesions in the kidneys in most of the cases. Histologic lesions consisted of diffusely or focally proliferating small to medium sized lymphocytes and large lymphoblasts resulting in the loss of normal architecture of the liver. The kidney tubules showed degeneration and desquamation and infiltration of pleomorphic lymphoid cells between the tubules. The gross and histological findings of liver and kidneys were in accordance with observations of Dugma *et al.* (2005), Rajkhowa (2005) and Schat and Nair (2008).

Nephroblastoma accounts for 0.5 percent cases caused by Myeloblastosis-associated virus (MAV). Grossly a large grayish white tumorous growth was found attached to the left kidney replacing most of the kidney tissue while on incision revealed fluid filled cyst inside. Similar findings were observed by Purchase and Burmester (1978) and Watts and Smith (1980).

Histologically, there was neoplastic proliferation of both epithelial and mesenchymal elements. The epithelial structures varied from enlarged tubules with invaginations and malformed glomeruli in a fairly cellular connective tissue stroma. These histological findings were in agreement with those of Purchase and Burmester (1978), Watts and Smith (1980), Collart *et al.* (1990) and Singh and Mohanty, (2007).

Infectious bursal disease, caused by IBD virus (IBDV), posing a serious problem to the poultry industry causing heavy economic losses. The percentage

of incidence of IBD was 38.5 percent in the present study. IBD was diagnosed from the gross and histopathological findings. Grossly the bursa was enlarged and distended containing hemorrhages and yellowish exudates. Extensive skeletal hemorrhages, paleness of liver splenomegaly were also observed. Kidneys were found to be enlarged and congested and ureters were distended with accumulated urate crystals. Histologically the kidneys revealed tubular degeneration and focal infiltration of lymphoid cells, congestion of blood vessels and intertubular hemorrhages in focal areas. These gross and histological findings agreed with the findings of Abdu *et al*. (1986), Hemalatha *et al*. (2005) and Dutta *et al*. (2007).

Histomoniasis was found to be one percentage as confirmed by the gross and histopathological findings. These two cases were from outside, where the poultry was reared under backyard system. Histomoniasis is a disease common in poultry reared under backyard system because *Histomonas meleagridis* is transmitted horizontally through intermediate hosts such as *Heterakis gallinae* and earthworms.

On gross examination the liver was found to be enlarged with numerous round to oval, circumscribed lesions. Microscopically the hepatocytes mostly surrounding the blood vessel contained histomonads visible as punched out ovoid resembling vacuoles in the cytoplasm. Similar gross and histological findings were observed in the liver by Kamil *et al.* (2006). The gross findings were not in agreement with observations of Ley *et al*. (1989) that the macroscopic liver lesions, as traditionally seen in turkeys, are often absent in chicken. He noticed only focal hepatic necrosis in the liver of chicken.

Fatty Liver Syndrome includes Fatty liver hemorrhagic syndrome (FLHS) and Fatty liver kidney syndrome (FLKS). The percentage of incidence of Fatty liver syndrome was 20.5 percent.

Excessive energy in the diet induces FLHS regardless of the source. Excessive consumption of high energy diets by birds with restricted movement in

cages is considered to result in a positive energy balance and excessive fat deposition. Excess fat may disrupt the architecture of the liver resulting in the weakening of the reticular framework and blood vessels in the liver. A pathogenic relationship between hepatic steatosis and hemorrhage has been suggested by Crespo and Shivaprasad, (2008). FLKS is a biotin deficiency related metabolic disease in chicken, resulting in impaired hepatic gluconeogenesis and increased fat deposition. The problem is caused by low activity of biotin dependent enzyme- pyruvate carboxylase. Birds die from hypoglycaemia and the clinical signs and death are related to hypoglycaemia (Julian, 2005).

Grossly the liver showed moderate to severe enlargement with light yellow or yellowish brown discoloration. Blood clots were present on the surface of the liver and heart (Sathyanarayanan, 2007). Kidneys were found to be pale and enlarged in cases of FLHS In cases of Fatty liver kidney syndrome, the changes observed in the liver was almost similar to that of FLHS except for the absence of any blood clots on the surface of the liver and heart. The kidneys were slightly enlarged with pale or yellowish discoloration and fragile in consistency. Histologically, the hepatocytes were distended with fat vacuoles, the normal architecture of the liver was disturbed by the distorted and disrupted hepatic cords containing large lipid droplets. Focal or diffuse areas of hemorrhages were also observed The tubular epithelium of the kidneys showed mild degenerative changes in cases of FLHS. The lining epithelial cells of the tubules were distended with fat globules displacing the nucleus to one side and the glomerulus also contained fat vacuoles in cases of FLKS. These observations were in accordance with observations of Lonkar and Prasad, (1988), Jordan and Pattison, (1996), Karadas *et al*., (1999) and Sathyanarayanan, (2007).

The incidence of gout was found to be nine percent. Grossly deposition of white chalky urate crystals on the surface of the liver, kidney, other visceral organs and joints were observed. Kidneys were found to be enlarged and large white chalky crystals were present inside the kidneys. Ureters were found to be

distended with urates. Microscopically liver showed urate deposition, focal hepatitis, areas of focal hepatic necrosis, dissociation of hepatic cords and fatty vacuolation of few hepatocytes were observed. In the kidneys, uric acid deposits were found surrounded by inflammatory cells The kidney parenchyma was atrophied and degenerative and necrotic changes associated with intertubular hemorrhages was observed Varying degrees of fibrosis were also evident in the kidney parenchyma. These gross and histological findings agreed with observations of Coy *et al.* (1988), Ahmed *et al*. (2003), Mir *et al.* (2005) and Crespo and Shivaprasad (2008). In liver and kidney sections stained with De Galanthas, the urates stained characteristically black, showing cotton ball appearance with radiating crystals at the periphery which was in agreement with observations of Uma *et al*. (1999) and Mir *et al*. (2005).

There are many causes for gout. Higher levels of dietary proteins cause excess uric acid production and nephropathy while higher dietary levels of calcium and low phosphorus lead to increased retention and decreased excretion of uric acid. (Mir *et al*., 2005). Dehydration due to water deprivation was also a common cause for visceral deposition of urates. (Onderka *et al*., 1987). Infectious agent like Infectious Bronchitis virus, also induces gout (Ahmed *et al*., 2003).

The incidence of Ascites syndrome in the present study was found to be 6.5 percentage. On gross examination marked abdominal distension and accumulation of clear yellowish fluid with or without fibrin clots were noticed in the abdominal cavity. The heart appeared enlarged with mild to moderate hydropericardium. The liver was swollen and congested. Focal pale areas and thickened capsule were also noticed. The kidneys were also slightly enlarged. Histologically, in the liver, the sinusoids, portal and central veins were found to be dilated showing varying degrees of congestion, multifocal random areas of hepatic necrosis and disorganization of hepatic cords. The kidney sections showed congestion and hemorrhages in the intertubular areas and varying degrees of degeneration. These gross and histological findings were in agreement with the observations of Jacob (1996), Rajkhowa (2004) and Goyal *et al.* (2005).

Several factors such as atmospheric hypoxia, housing environment, respiratory diseases, rapid growth rates, high-energy rations, toxins, nutritional aspects and feed additives are known to influence the incidence of ascites in broiler chickens.

Vascular disturbances like hemorrhage and congestion was mostly observed together. Out of the 22 cases of hepatic congestion and 34 cases of renal congestion, congestion of both organs were observed together in 18 cases. Hepatic congestion and renal congestion may occur as a part of generalized congestion, which is a common cause of death of many chicken in farm. The earliest response of any organ to injury is congestion. So there is a possibility of toxic agents in the feed and hypoxia induced damage to the vascular endothelium responsible for the congestion of the liver and kidney. The observations of Leenadevi *et al*. (1990) and Kulkarni *et al*. (1998) supports the possibility of toxic factors as responsible for renal and hepatic congestion.

Vacuolar degeneration of non-specific etiology was observed in five cases in the liver and four cases in the kidney, while hepatic necrosis was seen in 8 and renal necrosis in 12 cases. Bacterial isolates were not obtained from these cases. Vacoular degeneration is a common change observed in a number of diseases ranging from toxic insult, activity of microorganisms, inflammatory reaction or by nutriotional deficiencies and severe metabolic disturbances including hypoxia. In 11 cases, nephrosis was seen along with Hepatosis. Toxins other than aflatoxins were not detected in the feed in the present study. The observations of Maryamma *et al.* (1990) and Ramadevi *et al.* (1998) supports the fact that toxins in the feed results in degeneration of liver and kidney.

Summary

6. SUMMARY

A study was undertaken to elucidate the pathology of liver and kidney in chicken during various disease conditions and to ascertain whether aflatoxin residues were present in the liver and kidney of chicken during natural cases of aflatoxicosis.

Two hundred samples of lesion bearing liver and kidneys collected from the chicken brought for autopsy to the Centre of Excellence in Pathology, Mannuthy were used for the study. Aflatoxin residues in the liver and kidney from cases suspected for aflatoxicosis were determined by modified Pons method using thin layer chromatography (TLC). Samples for bacteriological studies were collected from liver, kidney and heart blood in appropriate cases. The conditions encountered were classified and pathological features were recorded.

The conditions associated with hepatic lesions were divided into bacterial diseases, viral diseases, protozoan diseases, nutrititional and metabolic diseases, aflatoxicosis, hepatic congestion and non-specific hepatosis and hepatitis. Among bacterial diseases colibacillosis accounted for 16 percent, pasteurellosis for 5.5 percent and staphylococcosis for 0.5 percent. Viral disease, namely Marek's disease accounted for 21.5 percent, while the protozoan disease, histomoniasis accounted for one percent. Nutritional and metabolic diseases comprised Fatty liver syndrome (20.5 percent), gout (nine percent) and ascites syndrome (6.5 percen). Aflatoxicosis accounted for two percent, hepatic congestion 11 percent and non-specific hepatosis and hepatitis for 6.5 percent.

The percentage incidence of various conditions associated with renal lesions included Infectious bursal disease (IBD) 38.5 percent, Marek's disease 21.5 percent, nephroblastoma 0.5 percent , gout nine percent, fatty liver kidney syndrome 3.5 percent, aflatoxicosis two percent, vascular disturbances 17 percent and non specific nephrosis and nephritis 8 percent.

Aflatoxin residues from the tissues were detected only in two cases; aflatoxin contamination in the feed in these cases were 2 and 2.5 ppm.

Among bacterial diseases, Colibacillosis was the most common disease and was encountered in 32 cases. *Escherichia coli* could be isolated from nine representative samples from these cases. The most common liver lesion observed grossly was perihepatitis and diffuse grayish-white patches of necrosis and microscopically subcapsular fibrin deposition, necrosis and diffuse infiltration of inflammatory cells. Pasteurellosis was observed in 11 cases and the bacteria, *Pasteurella multocida* was isolated from four representative samples. The most common gross lesion was multifocal coagulative necrosis and focal heterophilic infiltration. Staphylococcosis was confirmed only in one case by bacteriological examination and characterized grossly by multifocal yellowish-white foci in the liver and microscopically by multifocal coagulative necrosis of the hepatocytes.

Marek's disease, one of the most common viral disease affecting liver and kidney was observed in 43 chicken. The most important gross finding was nodular or diffuse type of enlargement of the liver. Histologically, proliferating neoplastic lymphocytes were observed in the liver and kidney. Nephroblastoma, was seen in one chicken. Grossly, a large tumorous growth was found attached to the right kidney. Histological examination revealed neoplastic proliferation of both mesenchymal and epithelial elements. One of the most common viral disease affecting kidney was IBD. The kidneys were pale colored and slightly enlarged. Tubular degeneration and desquamation and infiltration of lymphoid cells were the common histological findings.

The protozoan disease, histomoniasis was observed in two cases. Grossly, the liver was enlarged bearing numerous round to oval circumscribed lesions and microscopically extensive infiltration of inflammatory cells and histomonads were visible as punched out ovoid bodies resembling vacuoles in the cytoplasm of hepatocytes.

Fatty liver syndrome one of the most common nutritional and metabolic disease was observed in 41 chicken. It included fatty liver hemorrhagic syndrome (FLHS) and fatty liver kidney syndrome (FLKS). FLKS was observed in seven cases. In fatty liver syndrome, liver was found to be enlarged with yellow discoloration. Besides this in FLKS, kidneys were also slightly enlarged with yellow discoloration. The hepatocytes, kidney tubular epithelial cells and glomerulus contained fat vacuoles histologically.

Gout, an important metabolic disease was encountered in 18 cases. It was characterized by the deposition of chalky white crystals on the surface of the liver and kidneys. Histologically, urate crystals could be appreciated in the liver and kidney by H and E stain. In De Galanthas stained sections the urate crystals took black color. Ascites syndrome was noticed in 13 chicken. The liver was found to be swollen and congested. The common histologic findings were sinusoidal dilatation and congestion.

Aflatoxicosis was observed in four chicken. The birds maintained on feed containing aflatoxin at levels of 0.05 and 0.1 ppm had pale yellow enlarged livers, while those maintained on the feed containing aflatoxin at 2 and 2.5 ppm levels showed petechial hemorrhages and focal areas of necrosis along with the above changes. The kidneys were congested at 0.1 ppm level of aflatoxin while pale and enlarged kidneys were observed at 2 and 2.5 ppm levels. The prominent histological lesion in chicken were marked vacuolar degeneration and infiltration of inflammatory cells at 0.05 and 0.1 ppm levels and marked fatty change, diffuse areas of necrosis, mild bile duct proliferation and congestion at 2 and 2.5 ppm levels. The kidneys showed extensive degenerative changes.

Vascular disturbance in the form of congestion of non-specific etiology was observed in 22 liver and 34 kidney samples. Hepatic and renal congestion may occur as a part of generalized congestion, due to effects of toxins in the feed, hypoxia induced damage to the vascular endothelium and certain viral agents.

Degenerative changes in the liver and kidney of non-specific etiology was observed in 13 liver and 16 kidney samples. The possible causes suggested were hypoxia, nutritional deficiencies or viral agents.

The result of the present study will help to create an awareness about the common diseases affecting the liver and kidney of chicken.

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HEPATO-RENAL PATHOLOGY WITH SPECIAL REFERENCE TO AFLATOXICOSIS IN CHICKEN (*Gallus domesticus*)

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ABSTRACT

The present study was undertaken to study the pathology of liver and kidney in chicken under various disease conditions and to find whether aflatoxin residues were present in the liver and kidney of chicken during natural cases of aflatoxicosis.

A total of 200 liver and kidney samples having lesions were collected during the period of study after detailed systematic examination of chicken brought for autopsy to the Centre of Excellence in Pathology. Aflatoxin residues in the liver and kidney were determined by Modified Pons method using Thin layer chromatography (TLC) from cases suspected for aflatoxicosis.

The conditions associated with hepatic lesions were divided into bacterial diseases, viral diseases, protozoan diseases, nutrititional and metabolic diseases, aflatoxicosis , hepatic congestion and non-specific hepatosis and hepatitis. Among bacterial diseases, colibacillosis accounted for 16 percent, pasteurellosis 5.5 percent and staphylococcosis 0.5 percent. Viral disease, namely Marek's disease accounted for 21.5 percent. Protozoan disease, histomoniasis accounted for one percent. Nutritional and metabolic diseases namely fatty liver syndrome accounted for 20.5 percent, gout (nine percent) and ascites syndrome (6.5 percent). Aflatoxicosis accounted for two percent, hepatic congestion (11 percent) and non-specific hepatosis and hepatitis (6.5 percent).

The percentage incidence of various conditions associated with renal lesions included IBD 38.5 percent, Marek's disease 21.5 percent, nephroblastoma 0.5 percent , gout nine percent, fatty liver kidney syndrome 3.5 percent, aflatoxicosis 2 percent, vascular disturbances 17 percent and non specific nephrosis and nephritis 8 percent.

Aflatoxin residues from the tissues were detected only in two cases, which had aflatoxin contamination in the feed at the rate of 2 and 2.5 ppm.