Etio-pathological Profiling of Digestive System Affections in Chicken

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(2016-V-340-M)

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Dedicated To

MY PARENTS

Sher-e-Kashmir
University of Agricultural Sciences & Technology of Kashmir
Division of Veterinary Pathology
Shuhama Campus Srinagar – 190006
Certificate – I

This is to certify that the thesis entitled, **"Etio-pathological profiling of Digestive System Affections in Chicken"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Sciences in Veterinary Pathology**, to the **Faculty of Veterinary Sciences & Animal Husbandry, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir** is a record of bonafide research work carried out by **Dr. Rehab Altaf (Regd. No. 2016-V-340-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that information received during the course of investigation has duly been acknowledged.

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ABSTRACT

Present study was aimed to study nature and occurrence of patho-morphological alterations of digestive system in chicken viz-a-viz inflammatory and non-inflammatory conditions and to investigate occurrence and pathology of spontaneously occurring specific disease conditions of digestive system in chicken. Necropsy of 7657 broiler chicken in 201 outbreaks revealed GIT lesions in 95.85% birds including 83.64% with specific and 12.21% non-specific disease conditions. Highest proportionate mortality occurred in age group 2-3 weeks (39.14%) followed by 0-1 week (21.46%), 3-4 weeks (18.11%), 4-5 weeks (12.21%) and 1-2 weeks (9.08%). Also, the proportionate mortality was significantly higher in spring (38.03%) followed summer (33.05%), autumn (15.05%), and winter (13.87%). The commercial strain (CS) showed a significant effect with overall case prevalence ranging from 51.58% in CS10 to 100% in CS8 and CS9. Case prevalence of gross liver lesions was 87.79% including 76.10%

specific and 11.69% non-specific lesions. Mortality with liver affections was significantly higher in age group 2-3 weeks (38.31) followed by 0-1 (22.66%), 3-4 (17.60%), 4-5 (12.65%), and 1-2 weeks (8.79%). Seasonal occurrence was highest in spring (92.41%) and winter (90.68), followed by autumn (87.41%) and summer (81.43%). Grossly lesions were evident in one or more segments of GIT. Jejunum was involved in significantly higher number of cases (81.96%) followed by ileum (81.20%), duodenum (74.06%), proventriculus (42.13%), caecum (38.34%) and caecal tonsils (24.14%). Proventricular lesions included congestion, haemorrhage, thickened/oedematous mucosa and catarrhal exudate. Lesions observed in different segments of intestines included varied degree of congestion, hemorrhages, ulcers, thickened oedematous mucosa, and presence of catarrhal exudate. Evaluation of different lesions across different segments of GIT, by Severity Score Analysis based on weighed mean score of different conditions in different segments indicated that catarrhal exudation was the most frequent type of response observed in GIT (LSRS= 30), followed by mucosal thickening (LSRS=28), mucosal congestion (LSRS=25) and haemorrhage (LSRS=23). Mucosal denudation and ulceration appeared to be the least frequent or rare response with LSRS of 9 and 12 respectively. Case prevalence for gross liver lesions was 87.79%. Lesions observed included varied degree of congestion, hemorrhages, fibrinous perihepatitis, necrosis and haematoma with relative case prevalence of 56.41%, 16.02%, 23.19%, 4.3% and 0.074% respectively. No pathological gross lesions were observed in pancreas. Occurrence of gross bursal lesions was 35.11% and included varied degree of swelling, mucosal thickening/oedema, catarrhal exudation, serous exudation, congestion, hemorrhages, and bursal core with relative case prevalence of 30.02%, 76.34%, 62.98%, 3.46%, 40.88, 26.97 and 1.64%, respectively. The specific diseases/disease conditions associated with lesions in digestive system include intussusception, intestinal torsion, omphalitis, colibacillosis, salmonellosis, IBD, ND, Low Pathogenic Avian Influenza, aspergillosis, coccidiosis, gout, and ascites.

Key words: Chicken, Digestive System, Pathology, Diseases

Signature of Student
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Chapter – 1

INTRODUCTION

The domestic chicken, *Gallus gallus domesticus*, with a global population exceeding 40 billion individuals per year (Muir *et al*., 2008) has a unique status as both 'the model' and 'the system' - chickens are common model organisms for human biological research and also comprise an economically valuable global protein industry. Commercial poultry production is one of the flourishing ventures of animal production in India. India has vast resource of livestock and poultry, which plays a vital role in improving the socio-economic conditions of rural masses. Poultry production in India has taken a quantum leap in the last four decades, emerging from an unscientific farming practice to commercial production system with state of the art technological interventions. Currently egg production is around 82.93 billion in 2015-16. The poultry meat production is estimated to be about 3.26 million tonnes. The current per capita availability of eggs is around 66 eggs per annum. The state of Jammu and Kashmir has also shown a substantial growth in poultry industry during the past decade and occupies 17th position in India with a population of approx. 8.2 million (19^{th} livestock census 2012). However, the profitability of this industry is being continuously threatened by various biotic and abiotic factors challenging health and hence production and survivability of the birds. Out of the various impediments encountered by the poultry industry, the changing disease scenario assumes utmost importance warranting continuous vigil and prompt reporting system in place.

During the last few decades, methods of poultry husbandry have changed considerably. The emphasis on intensive rearing has predisposed birds to various infectious and non-infectious disease conditions. Poultry industry suffers enormous economic losses on account of underperformance and mortality of birds and increased management and medication costs due to these conditions.

The GI tract acts as a major site of potential exposure to pathogens and irritants. Consequently, the health condition of the GI tract plays significant role in achieving optimum productivity and welfare in poultry production. The health of GI tract affects feed digestion, nutrient absorption and metabolism, energy and protein utilization, immune response and disease resistance (Yegani and Korver, 2008). Because of the large surface area, accurate assessment of potential effects of any pathogen or irritant warrants a thorough gross examination and sampling of focal lesions.

Enteric disorders are one of the most important groups of diseases affecting poultry and are continuing to cause high economic losses in the many areas worldwide due to increased mortality rates, decreased weight gain, increased medication costs, and increased feed conversion rates (Hafez, 2011). In a study done for system wise mortality, the digestive system disorders were found to be the main cause of poultry mortality (30.88%) accounting for immense economic losses (Hooda, 2009). The etiology of disturbed health vis-à-vis digestive system is complex involving abiotic and biotic agents (Reynolds, 2003). Several pathogens (viruses, bacteria and parasites) are incriminated as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms (multi-causal) or with non-infectious causes such as feed and/or management related factors (Hafez, 2011). Under field conditions, however, it is difficult to determine whether the true cause of enteric disorders in poultry is of infectious or non-infectious origin. Besides the pathogens and irritants directly affecting digestive system, it may be secondarily involved in systemic diseases.

The common conditions affecting gastro-intestinal tract include Colibacillosis, Salmonellosis, Necrotic Enteritis, Coccidiosis, Ranikhet disease, etc. These infections lead to heavy chick morbidity, mortality and lowered egg or meat production (Ahmad *et al*., 2012). Enteropathogenic *Escherichia coli* having the ability to cause attaching and effacing lesions have been isolated and

characterized from chicken (Alonso *et al.*, 2016) but their impact on health of the birds has not been investigated. Of the many parasitic diseases that affect birds, coccidiosis results in overall 51.38% mortality in the poultry industry worldwide (Cocciforum, 2007). Necrotic enteritis (NE) in chickens is a globally important welfare and economic problem caused by overgrowth of a commensal *C. perfringens*. This overgrowth also results in changes not only of the digestive tract environment, but also its bacterial ecology and histopathology (Cooper *et al.*, 2009). The global cost of coccidiosis to the poultry industry has been estimated to exceed $2 billion per annum (Fornace *et al*., 2013).

Out of the gastrointestinal tract derangements in fowl, intestinal intussusception, intestinal volvulus, proventricular intussusception and herniation of the intestine through the post-hepatic septum into the ventral hepatic cavities have been reported. Intestinal derangements rarely occur in fowl. There is paucity in reports about pathology and pathogenesis of intestinal derangements in Aves (Haridy *et al*., 2010).

In addition to the importance of intestinal health for maximising the health, welfare, and performance of poultry, and the devastating impacts of intestinal health issues in poultry for producers, the food safety concerns for consumers assume a great importance. Infact, much of the research is oriented to address the latter concern (Sultana *et al.*, 2014). Until recently, intestinal health issues were seen as a handful of known infectious agents leading to a set of severe and identifiable named diseases. There is however an emerging area which depicts intestinal health as a more complex and multifaceted system than previously known. Recent progress in technology suitable for microbial community analysis has evolved our understanding of the chicken intestinal microbiome. It is now understood that shifts in the composition of microbial communities can occur. These shifts can result in a series of implications, including: disease, welfare, environmental, and food safety concerns. Minor shifts in intestinal microbial balance can result in a wide continuum of disease

presentations ranging from severe to mild clinical, subclinical or asymptotic. Differential diagnosis of poultry intestinal health issues may be challenging and is important for applying appropriate treatment options (Roberts *et al.,* 2015). Further, the alterations in GIT morphology and microbiome have gained importance as an indicator for evaluating the health implications of various growth promoters, probiotics and toxicants under experimental setups (Adibmoradi *et al.*, 2006; Cao *et al.*, 2012; Lillehoj *et al.*, 2016; Teng *et al.*, 2017).

Liver and pancreas are two important glands associated with digestive tract maintaining the body's homeostasis. Liver is a principle accessory digestive gland, responsible for proper digestion, metabolite detoxification, phagocytosis of particulate material in the splanchnic circulation and metabolism of proteins, fats, and carbohydrates and thus is a softer target for various infectious, nutritional, metabolic, toxic and neoplastic diseases, either primarily or secondarily. The diseases primarily affecting the liver in chicken include inclusion body hepatitis, hydropericardium syndrome and lympho-proliferative diseases viz. Marek's disease and lymphoid leukosis. Other diseases affecting liver include visceral gout, aspergillosis, colibacillosis, salmonellosis, non-specific hepatitis, omphalitis, fatty liver, and liver rupture. (Mir *et al.,* 2005 ; Gupta *et al.,* 2007; Nazir *et al.,* 2012) . Besides liver pathology is noted in almost all cases of enteric affections.

These infections lead to pathological changes in various systems of the birds so it is pertinent to study these changes, aiding to diagnosis. A better understanding of the pathomorphological alterations occurring in digestive tract and associated organs in different infectious and non- infectious diseases / disease conditions in poultry shall be helpful in better evaluation of the pathophysiological process. The knowledge about occurrence and significance of subtle histomorphological alterations in GIT and associated structures should be viewed as critical in comparative evaluations under experimental setups. Also,

standard quantitative approaches are warranted for maintaining intra- and inter-experimental harmony in reporting such changes and assessment of their impact. Such approaches call due consideration of species, strain, sex, and age, as well as temporal harmony in trimming of tissues for histopathology. However, at the same time it has to be flexible to accommodate variations on case-to-case basis as dictated by subjective evaluation and such variations have to be documented and weighed as per requirements.

Therefore, the study was envisaged with the following objectives.

OBJECTIVES

1. To study nature and occurrence of gross patho-morphological alterations of digestive system in chicken.
2. To conduct histopathological profiling of inflammatory and non-inflammatory conditions of digestive system in chicken.
3. To study occurrence and pathology of spontaneously occurring specific disease conditions of digestive system in chicken

Chapter – 2

REVIEW OF LITERATURE

Present study was aimed at characterizing the gross and histopathological lesions of digestive system including GIT and associated strictures viz liver, pancreas, and bursa and to investigate occurrence and pathology of specific disease conditions with primary or secondary involvement of digestive system in chicken. Hence an attempt has been made to review the available literature under different headings and sub-headings.

2.1. Spatial Distribution of Digestive System affections in Chicken

In chicken the development of digestive system continues in the post hatch period and is completed in 2nd week post hatch. It undergoes morphological, biochemical and molecular changes which are greatly influenced by exposure and nature of nutrition (Yegani and Korver, 2008). Besides influencing the normal development, it tremendously alters the microbiome structure of GIT which in turn influence the morphological structure and immunological status of GIT.

Katoch *et al.* (2004) studied an outbreak of necrotic enteritis in 6-7 week old broilers in a government poultry farm in Himachal Pradesh. The 309 birds out of 480 broilers were found to be dull, depressed, anorectic, and passed loose semi-formed bloody droppings.The morbidity rate was 64.37% and case fatality rate was 39.16% over a period of 10 days.

Khaton, (2008) studied the prevalence of colibacillosis in layer chickens. Sixty-five cloacal swabs from apparently healthy birds and 55 swabs of liver (n=15), lung (n=15) and intestine (n=25) from 30 dead birds were collected in sterile nutrient broth. *Escherichia coli* was isolated from 83% of cloacal swabs of apparently healthy chickens and 87% of samples from dead birds.

Ahmed *et al.* (2009) examined a total of 199 broiler chickens for presence of infectious diseases in a study at Kapasia in Gazipur and recorded, colibacillosis 104 (52.26%), mycoplasmosis 25 (12.56%), salmonellosis 02 (1.01%), omphalitis 23 (11.56%), coccidiosis 09 (4.52%), gumboro 22 (11.06%), mycotoxicosis 11 (5.53%) and mixed infection of Gumboro & Coccidiosis 03 (1.51%). It was concluded that colibacillosis is a major threat for broiler production and hence adversely affects poultry rearing.

Islam *et al.* (2009) examined a total of 251 chickens, dead or sick, brought to the FDIL (field disease investigation laboratory), Guibandha during the period from July 2005 to June 2006 for disease diagnosis, The occurrence of parasitic diseases was the highest (88.4%) followed by bacterial diseases (28.3%), viral diseases (27.1%), non-infectious diseases (16.30%) and aspergillosis (1.6%). Bacterial diseases were significantly ($p<0.05$) higher in winter as well as in rainy season compared to summer season. Occurrence of pasteurellosis was significantly ($p<0.05$) higher in winter and rainy season compared to summer season. Parasitic diseases were significantly ($p<0.01$) higher in winter (97.2%) compared to summer (83.3%). The occurrence of coccidiosis was 88% and it was significantly ($p<0.01$) higher in winter (97.2%) compared to summer (82.2%). Egg bound was present among 10% birds and it was significantly ($p<0.05$) lower in rainy season compared to summer season.

Uddin *et al.* (2010) investigated prevalence of diseases in different poultry farms of some selected areas at Narsingdi district of Bangladesh based on clinical history, clinical signs and symptoms prior to death, lesions observed after postmortem examination of dead birds and isolation and identification of causal agents. Examination of a total 1263 dead and sick birds revealed highest prevalence of infectious bursal disease (IBD) (24.96%) followed by chronic respiratory disease (CRD)/mycoplasmosis (9.87%), new castle disease (ND) (8.92%), aspergillosis (7.98%), salmonellosis (7.68%), coccidiosis (7.32%), colibacillosis (5.70%), ascites (5.45%), omphalitis (2.64%), deficiency

disorders/stress (1.34%), necrotic enteritis (0.40%), infectious coryza (0.32%), fowl cholera (0.24%), and infectious bronchitis (0.24%) in that order. In general, the highest number of cases were recorded in the age group of 8-20 days (42.64%), followed by 21-35 days age group (35.76%), 0-7 days age group (16.12%), 36-60 days age group (1.52%) and >60 days age group (3.96%) of poultry. Distribution and proportionate incidence revealed that the poultry diseases occur mostly in rainy season (47.09%), followed by summer (27.53%) and the least in winter season (25.38%).

Ruban and Fairoze, (2011) reported that *Salmonella* was prevalent in a wide range (25-65%) from different parts of chicken meat marketed in Banglore while 15.91% prevalence of *S. enteritidis* was reported from Namakkal .

Ahmed *et al.* (2012) conducted a study on the prevalence of diseases, important causes of mortality in broiler and layer flocks and their interaction with high altitudes as influenced by different climatic conditions. The total cases recorded were 28694, 21896 broilers and 6825 layers. The prevalence was 17.38% for coccidiosis and 18.61% for colibacillosis in broilers; and 22.12% for ND, 12.48% for coccidiosis and 11.36% for colibacillosis in layers. On the basis of altitude, the occurrence of these diseases were colibacillosis 18.53%, and coccidiosis 17.00% in broilers, ND 17.26% in layers above 4000 feet height; colibacillosis 18.78%, and coccidiosis 18.28% in broilers and ND 28.58%, coccidiosis 20.83%, and colibacillosis 16.30% in layers below 4000 feet .Other diseases diagnosed during the period included enteritis (uncharacterised), omphalitis and hydropericardium syndrome.

Jatau *et al*. (2012) reported that the 33.3% of all the collected chicken intestines had coccidia infection, with specific prevalence rates of 44.3% in layers, 37.1% in broilers and 18.6% in indigenous chickens. The 80.7% of the infected layers had unapparent coccidia infection, while 12.9 and 6.5% had low and severe grades infections, respectively. More so, 84.6% of the infected indigenous chickens had in apparent infection, while only 7.69% each had low

grade and severe infections. All the seven *Eimeria* species of chickens were identified with overall prevalences of: *E. maxima* (58.6%), *E. acervulina* (47.1%), *E. mitis* (30.0%), *E. brunetti* (28.6%), *E. tenella* (22.9%) and *E. praecox* (8.6%). Mixed *Eimeria* species infections were common among the sampled chickens with overall prevalence 61.4%.

Jha *et al*. (2012) recorded the causes of death of 6149 chickens submitted for postmortem examination in Jharkhand, India. Colibacillosis (24.93%), yolk sac infection (12.09%), infectious bursal disease (2.82%), lymphoid leukosis (1.02%), marek's disease (0.13%), coccidiosis (3.83%) were observed. Colisepticemia, oophoritis and hepatitis were the main causes of death.

Jinu *et al.* (2014) reported total *Salmonella* prevalence rate of 5.88% from chicken in Bareilly, Uttar Pradesh and the major serotypes were *S. typhimurium* and *S. enteritidis*.

Babaca, (2015) surveyed 17 farms in and around Erbil City (Iraq) with a history of outbreaks of new castle disease (ND) during late spring, summer and winter of 2008. Birds were vaccinated with Lasota strain. They found that fowls 1-8 weeks of age were susceptible to ND and that the mortality rate was 8.1% in broilers.

Sharma *et al.* (2015) conducted a study on the prevalence of chicken coccidiosis in Jammu division in both organized and backyard chickens.A total of 720 faecal samples were collected from both organized farms and backyard poultry (unorganized) sector of Jammu. The overall prevalence of 39.58% was recorded and five *Eimeria* species were identified viz., *E. tenella, E. necatrix, E. maxima, E. acervulina* and *E. mitis. E. tenella* was the predominant species in both organized and unorganized farms. Higher prevalence of 53.61% in unorganized (backyard poultry birds) as compared to organized birds (25.55%) was recorded. The prevalence was highest in monsoon from both organized and unorganized farms.

El Sayed *et al*. (2017) conducted a study to determine the pathological findings of intestine and liver of chickens at Alexandria province. The livers and intestines were taken from different breeds of 100 chickens and were subjected to bacteriological, parasitological and pathological examination. The bacteriologic results revealed that *E.coli* and *Salmonella gallinarum* were the predominant isolated bacteria from hepatic tissues of all examined breeds. In case of intestinal samples, *E.coli* was the main isolated bacteria followed by *Clostridium perfringens*. Hepatic necrosis was the chief hepatic lesion recorded in white broilers (29.41%), saso (35.71%) and balady breeds (30.34%). Moreover, necrotic enteritis was the major intestinal lesion recorded in saso, balady and white broilers at frequency of (81.25%), (68.75%), (67.80%), respectively. Finally, it could be concluded that colibacillosis was the most prevalent disease affecting different chicken breeds at Alexandria province. In white broilers, *Clostridium perfringens* was the most common bacteria accompanying necrotic enteritis and coccidiosis was also widespread among the chickens of this breed.

Umer *et al*. (2017) studied the prevalence of colibacillosis in commercial broiler chicken. Colibacillosis constituted significant component of mortality among broiler chicken of all age groups ranging from 23.634% to 29.845% with overall mean of 26.357%. The most prevalent serogroup of *E. coli* observed was O76 (15.59 %).

2.2. Pathology of Digestive System affections in Chicken

Hafeeji *et al.* (2000) carried out aetiopathological investigation of *Salmonella gallinarum* infection in broilers. Liver showed marked enlargement, congestion, white grey necrotic foci and necrotic patches that were distributed uniformly on their surfaces. In some cases typhlitis was seen. Histopathologically, mild to moderate congestion and haemorrhages, focal to diffuse areas of coagulative necrosis and MNC infiltration in parenchyma were noticed. Occasionally, septic emboli were observed in hepatic parenchyma.

Tafti and Karima, (2000) observed ascites syndrome in 34 commercial broiler chickens of breeder strain in Shiraz area, Iran. Gross changes included dark breast muscle, marked abdominal distention and presence of clear yellow fluid with fibrin clots in the abdominal cavity. Congestion in liver, kidneys and intestines was also noticed. Histopathologically, dilatation of sinusoids, atrophy and degeneration of hepatocytes, marked thickening of capsule and fatty change of liver were observed.

Prasanna *et al.* (2001) reported histopathological and ultra-structural changes in small intestines and liver of chicken with experimentally induced fowl typhoid. Small intestines showed degeneration and desquamation of epithelium, goblet cell hyperplasia, infiltration of heterophils and mononuclear cells. Liver showed changes of vacuolar degeneration, necrosis and focal infiltration of mononuclear cells.

Luengo *et al.* (2001) reported degeneration and necrosis of lymphocytes of bursal follicles with condensation and margination of nuclear chromatin of lymphocytes, three days after IBDV challenge. Bursal cells were widely separated due to accumulation of intercellular oedematous fluid and infiltration of macrophages and heterophils. Seven days after challenge, the lesions were more severe and disseminated. Frequently lipid droplets were seen in the cytoplasm of macrophages, lymphoid cells and reticular cells.

Pazhanivel *et al.* (2002) made an investigation on Newcastle disease (ND) outbreaks in and around Namakkal, Tamil Nadu. Clinical signs included depression, weakness, loss of appetite, dehydration, cyanosis of comb and wattle, greenish watery diarrhoea, nasal and eye discharges. Gross lesions were petechial hemorrhages and stray ulcers with raised borders in the mucosa of proventriculus, intestines, pneumonic lungs, and hemorrhages in trachea, air sacs, brain and spleen.

Gupta *et al.* (2002) reported an outbreak of combined aflatoxicosis and ochratoxicosis with visceral gout in poultry. Gross changes included congested lungs, enlarged pale liver and kidneys and distended ureters with chalky white deposits on kidneys, liver, pericardium, lungs and serosal surfaces of GIT and airsacs. Microscopically, liver showed congestion, vacuolar degeneration of hepatocytes, biliary hyperplasia and focal mononuclear cell infiltration.

Ahmad *et al*. (2003) reported unilateral and bilateral urolithiasis in domestic fowl. Gross changes included deposition of chalky white precipitates on the surface of heart, liver, lungs, proventriculus, spleen, gizzard and intestines. Microscopically, liver revealed congestion, degenerative changes and dilatation of sinusoids and mononuclear cell infiltration.

Shivchandra *et al*. (2003) experimentally induced HPS in broilers to evaluate the gross and histopathological changes. Grossly, pale and enlarged liver with round borders and multiple pinpoint haemorrhages on the surface, hydropericardium, atrophied thymus and bursa were observed. Microscopically, liver showed congestion and sinusoidal dilatation, degeneration and focal areas of necrosis along with disorganisation of hepatic lobules. Large round basophilic intranuclear inclusion bodies and lymphoid aggregates were noticed in perivascular areas of liver.

Goyal *et al*. (2004a) conducted a study on ascites syndrome of broilers 1-6 weeks of age in Ludhiana. Abdominal distension with fluid, mottled, fatty and shrunken livers with thickened capsule was observed. Microscopically, marked degenerative changes, varying degrees of venous congestion , fibrosis and pseudo-lobulation in the liver was observed.

Goyal *et al.*(2004b) recorded gross lesions in different organs including liver in all the poultry birds and quails at PAU, Ludhiana. In cases of hepatitis, grossly liver appeared enlarged, mottled, pale, fatty, congested, hemorrhagic and showed pinpoint necrotic foci. Microscopically, it revealed varying degree of

congestion, haemorrhage, mild to severe hepatic degeneration with disrupted architecture, fatty changes, multiple necrotic foci and mild to severe infiltration of mononuclear cells, heterophils and a few eosinophils. Colibacillosis was grossly characterized by mottled, fatty liver, mild fibrinous perihepatitis and pericarditis. Microscopically, liver revealed congestion, mild fatty changes, hepatic degeneration, mild perihepatitis and focal to diffuse areas of necrosis with infiltration of mononuclear cells.

Goyal, (2004) recorded 26.03% prevalence of hepatic lesions associated with various diseases of poultry. Diseases affecting the liver were colibacillosis, IBD, non-specific hepatitis, omphalitis, fatty liver, liver rupture and infarction, gout and ascites syndrome. In ascites syndrome, chronic perihepatitis, fibrosis and pseudo-lobulation of liver were seen. Visceral form of gout was recorded in all age groups affecting mostly the surface of visceral organs.

Sultan *et al.* (2004) conducted an experiment on four groups of broiler chicken of 50 birds each which received dietary supplement of turmeric (*Curcuma longa*) at concentrations of 0.0, 2.5, 5.0 and 10% respectively. Liver sections from birds of all groups showed parenchymal and portal infiltration of mononuclear cells and hyperaemia of portal vessels. Dilatation of bile ducts, mild proliferation of biliary epithelium and periportal hepatocyte degeneration was noticed only in three groups.

Khan *et a*l. (2004) found that yolk retention and yolk sac infection was an important cause of death in chicken. Yolk sac infection of bacterial origin is most important factor in slowing down the rate of yolk absorption. Other factors that contributed included post hatch starvation, type of initial feed, brooding temperature, prolonged exposure to hatcher environment.

Aengwanich *et al*. (2004) conducted an experiment to investigate pathological changes in broilers under chronic heat stress. Fifteen, twenty-eight day old birds were kept at 33 + 10 ^{0}C environmental temperature for 21 days.

Macroscopic and microscopic lesions of heart, lung, liver and kidney were examined. Yellow and pale livers were observed in 4 out of 15 broilers (26.67%). Liver cells showed fatty degeneration with dilation of sinusoids of all broilers. Besides, necrosis with heterophils and lymphocytes was observed in some parts of the liver, especially in the centritubular region.

Nakatani *et al*. (2005) studied epidemiology, pathology, and immunohistochemistry in layer hens affected with H5N1 highly pathogenic avian influenza (HPAI), in Japan. The farm, which had a total of 34,640 chickens, experienced up to 43.3% mortality. Clinically, the affected chickens exhibited mortality without apparent clinical signs. Histologically, hepatocytic necrosis; necrosis of ellipsoids and follicles with fibrin in the spleen; necrosis of acinar cells in the pancreas; and necrosis of lymphoid tissues in intestinal lamina propria were seen. Occasionally, degeneration of smooth muscle fibres in the cecum was noted. Immunohistochemically, influenza virus antigens were detected in the liver and spleen, heart, intestine, gizzard, proventriculus, and oviduct.

Cortes *et al.* (2005) reported omphalitis associated with aspergillosis in four cases of commercial turkey poults ranging in age from 3 to 9 days old. In two cases, the mycotic agent present in the yolk sac were isolated and identified as *Aspergillus fumigatus*. In the other two cases, the fungi were also identified as *Aspergillus* sp. The fungi present were further confirmed to be of the genus *Aspergillus* by immunohistochemistry.

Mir *et al*.(2005) carried out an investigation in an outbreak of gout in a flock of Kashmir favorella maintained under intensive management system. Both visceral and articular gout was observed simultaneously. Heart, liver, spleen, lungs, air sacs and serosal surfaces of the intestines along with proventriculus and gizzard had whitish frosty appearance. Histological examination revealed urate tophi deposits in the kidneys, liver, spleen, lung and joints. Liver showed focal

hepatitis and haemorrhages associated with urate deposits especially in subscapular region.

Hossain *et al.* (2006) studied the pathology of pullorum disease, fowl typhoid and paratyphoid infection in dead chickens. Grossly, the liver was enlarged and congested , there was catarrhal inflammation in the intestines. Old raised hemorrhages in the caecal tonsils and congested deformed ova were the other important findings. Microscopically, the sections of the liver showed congestion, hemorrhages, focal necrosis with infiltration of mononuclear cells. The intestinal mucosa exhibited congestion, hemorrhages and infiltration of plasma cells, heterophils and macrophages.

Otto *et al.* (2006) studied the intestinal tract etio-pathology associated with runting and stunting syndrome (RSS) in Northern Germany with a special focus on rotaviruses (RVs). Severe villous atrophy was seen in chicks with RSS. Lesions were distributed in the middle-to-distal small intestine. In addition, RV particles were observed in intestinal contents of flocks with RSS.

Gangane *et al*. (2006) conducted studies on experimental colibacillosis in chicks. The chicks from infected group showed petechiae on epicardium, congestion and enlargement of liver with necrotic foci, congestion of spleen and kidneys, intestines were severely congested with catarrhal exudation in lumen. The histopathological examination revealed centrilobular congestion, cellular swelling, diffused necrobiotic changes in liver, and goblet cell hyperplasia and catarrhal exudate in the lumen of the intestine, besides lesions in other organs.

Jayanthi *et al.* (2007) observed enlarged and distended caeca in coccidiosis cases. In some cases ballooning up of caecal pouches with hemorrhagic contents, necrotic material and caseous or reddish brown caecal cores with the presence of large numbers of oocysts were observed. The caecal mucosa revealed desquamation of the superficial layers with the presence of developmental stages of the parasite, schizonts and merozoites in epithelial cells and infiltration of numerous oocysts.

Razik *et al*. (2007) studied occurrence of avian aspergillosis in a private poultry farm at Ismailia, Egypt. *Aspergillus flavus* and *A. fumigatus* was isolated from infected lungs and liver. Grossly, lungs were congested and hemorrhagic and liver was pale. Histopathological lesions were characterised by presence of granuloma , necrotic foci with fungal mycelia in the lungs and liver.

Sodhi *et al*. (2007) conducted biochemical and histopathological studies in hepatic lipidosis in chicks. The hepatic lipidosis affected livers were enlarged in size, pale in colour and fragile. While cutting the liver, fat stuck to the knife. The hepatocytes had varying sized fat vacuoles (adipocytes), which were evident as cells in which the nucleus was seen, but pushed to the margin.

Supartika *et al*. (2007) observed a subclinical necrotising granulomatous hepatitis in normal broilers routinely slaughtered in the Netherlands. Grossly, livers were enlarged, had a firm consistency, and revealed multifocal necrotic spots. Microscopically, more than half of livers showed a granulomatous reaction in addition to the necrosis. A large proportion of the livers revealed growth of *Escherichia coli, Bacteroides* sp., *Lactobacillus* sp. *Staphylococcus* sp., and *Streptococcus* sp. and often had mixed infections.

Nakamura *et al*. (2008) inoculated specific-pathogen-free chickens with H5N1 highly pathogenic avian influenza (HPAI) viruses. The chickens inoculated intravenously with the viruses died within 26 hrs after inoculation. Histologically, minimal focal necrosis of hepatocytes with fibrinous thrombi in sinusoids, mild necrosis of splenic ellipsoids with fibrinous exudation, minimal necrosis of the brain, mild necrosis of epidermal cells of the comb was seen. Virus antigens were seen in the sinusoidal endothelial cells and hepatocytes in the liver, capillary endothelial cells of the spleen, capillary endothelial cells and myocytes in the heart and the pancreatic acinar cells. The chickens inoculated by natural infectious routes died within 1–4 days after inoculation. Macroscopically, some chickens had hemorrhages in the conjunctiva, oedematous swelling of the face and wattles,

hydropericardium, hemorrhages of the proventriculus and bursa of Fabricius, increased secretion of tracheal mucus.

Sood *et al*. (2009) studied the prevalence of coccidiosis in poultry birds in R.S. Pura, Jammu. Blood tinged mucous exudate clinging to mucosa of intestine and ulceration of mucosa with haemorrhages in different parts of intestine were observed. Histopathologically, intestinal sections were severely inflamed with increased thickness of intestinal wall and infiltration of MNCs. Gamonts and schizonts were numerous in the enteric epithelial cells as well as lumen along with exudate. Multifocal areas of epithelial cell denudation, goblet cell hyperplasia with congestion, haemorrhage and oedema in submucosal area was also seen.

Goyal *et al.* (2009) noticed inclusion body hepatitis (IBH) and hydropericardium syndrome (HPS) in broilers of 1-6 weeks of age. Grossly, livers were generally enlarged and mottled with a few necrotic foci on the surface. Additionally, hydropericardium was observed in HPS cases. Intranuclear inclusion bodies (basophilic, eosinophilic or both) as well as hyper-chromatic nuclei were observed in affected hepatocytes along with degenerative changes, besides marked granular or vacuolar degeneration in the myocardium. IBH and HPS were observed in concurrence with Marek's disease and Aflatoxicosis.

Chowdhury *et al*. (2009) examined a total 4372 broiler and layer birds to identify the different forms of colibacillosis in commercial broiler and layer birds in Chittagong region of Bangladesh. Among them, 1893 (70.87%) broiler birds were diagnosed as affected with colibacillosis. The most frequent form of colibacillosis was omphalitis, airsacculitis, pericarditis, perihepatitis and peritonitis. In 30.48% birds, different forms of colibacillosis were recorded.

Patra *et al.* (2010) observed the *Eimeria tenella* infection in broiler chicken in Mizoram. Post-mortem examination revealed distended caeca filled with bloody faeces and mucoid debris with haemorrhages on the mucosa.

Histopathological studies revealed haemorrhages, oedema, necrosis and sloughing of caecal epithelium.

Mor *et al.* (2010) analysed the epidemiological data on IBD obtained from 483 broiler chicken flocks in Haryana. Overall morbidity, cumulative mortality and case fatality rate were recorded as 4.54%, 2.34% and 51.69%, respectively. Clinically, affected birds were dull, depressed, had ruffled feathers and suffered from diarrhoea. At necropsy, the gross lesions were observed mainly in bursa of Fabricius and in thigh and breast muscles. Maximum cases (52.80%) were observed in birds 21-30 days of age followed by 33.13% cases in the age group of 31-40 days.

Eldaghayes *et al*.(2010) investigated an outbreak of gout in a growing layer farm in Libya. Gross lesions composed of deposition of chalky white material covering the pericardium and liver surfaces.

Kumar *et al.* (2010) experimentally induced mycotoxicoses in broiler chickens by feeding 1 ppm aflatoxin (AF) and 20 ppm cyclopiazonic acid (CPA) from 0 to 28 days of age to evaluate the gross and histopathological changes. Grossly, AF and AF-CPA fed birds showed enlargement, yellowish discoloration of the liver while the CPA fed birds showed enlargement and congestion. The CPA and AF-CPA fed birds showed thickening of crop and necrosis and thickening of proventricular mucosa. Histopathologically, degenerative and necrotic changes were observed in the liver, kidneys, intestine, pancreas, heart, pectoral muscle, spleen and bursa of Fabricius of all toxin fed birds. Besides, hyperplastic changes were also observed in the crop, proventriculus and gizzard in the CPA fed birds. The lesions were more marked in the AF-CPA group.

Omer *et al*. (2010) recorded the outbreak of colibacillosis in broilers in Kassala State, Eastern Sudan. Overall 6.8% mortality was recorded in broiler flocks. Diagnosis was made on the basis of case history, clinical signs, postmortem findings and laboratory examination.

Tonu *et al*. (2011) studied the pathogenicity of *E.coli* in birds. Gross examination showed congestion, haemorrhages with excess mucus on the luminal surface of duodenum. Microscopically, the duodenum showed severe infiltration of leukocytes, heterophils, lymphocytes and macrophages in the sub-mucosa.

Daryoush *et al.* (2011) studied pathological lesions occurring in the intestines of dead fowls suffering with enteritis at Tabriz poultry clinics in Iran and reported that the occurrence (78.57%) was highest in broilers. The occurrence of enteritis showed significant co-relation with sex and age. Most severe cases were seen in males 4-6 weeks of age and in the females 1-3 weeks of age. Histopathologically, enteritis could be categorized as catarrhal, hemorrhagic and necrotic.

Dolka *et al*. (2012) estimated the prevalence of histopathological lesions in the different organs of commercial chickens in Poland. Out of a total of 189 cases, 66.7% of the affected cases were broiler chickens. The gastrointestinal tract especially liver was found to be the most frequently affected site with regard to the presence of histopathological lesions. In 29% of the cases of hepatic injury, pathognomonic lesions associated with inclusion body hepatitis were found. Also, proventriculitis and gizzard lesions were seen in many cases. Inclusions in the epithelial cells within the proventriculus were also noticed in many cases.

Renu *et al.* (2012) conducted gross and histopathological studies of chicken with *E.coli* infection. The lesions observed were thick fibrinous layer on all the visceral organs, necrotic foci on the liver, severe enteritis, omphalitis, fibrinous perihepatitis, perivasculitis, goblet cell hyperplasia, reticuloendothelial cell hyperplasia, pericarditis and myocarditis.

Nazir *et al.* (2012) encountered presence of salmonellosis in different commercial broiler farms of Srinagar district and adjoining areas. Grossly, hepatomegaly, bronze discoloration of liver, congestion and necrotic foci on liver was noticed. Histopathological lesions in liver comprised of congestion, haemorrhages, areas of necrosis, reticular endothelial hyperplasia along with infiltration of MNCs and heterophils. Intestinal changes comprised of congestion

of mucosal vessels along with marked hyperplasia of goblet cells and infiltration of heterophils and mononuclear cells in the lamina propria of villi.

Rahman *et al.* (2012) determined the prevalence of avian influenza virus using rapid antigen detection kit from field samples of poultry in Bangladesh. The cloacal swabs were collected from 10 randomly selected birds which included broilers, layers, native chickens and ducks in four different districts or areas. A total of 160 field samples were successfully tested. They recorded that prevalence of AIV in broiler bird was 32.5% respectively.

Davis *et al.* (2012) studied hepato-renal pathology associated with nutritional and metabolic diseases in chickens. Ascites syndrome was observed in 13 chickens with accumulation of clear yellowish fluid in the abdominal cavity. Microscopically, liver revealed multifocal areas of necrosis and disorganisation of hepatic cords. Intertubular hemorrhages and degeneration was seen in kidney sections.

Nasrin *et al*. (2012) identified *E.coli*, *Salmonella* sp. and *Staphylococci* sp. in yolk sac contents from cases of omphalitis in chicks in Bangladesh.

Bhalerao *et al.* (2013) observed that the maximum mortality in 3-4 week old birds occurred due to *E.coli* infection in Hisar. Gross pathological examination revealed congestion in various organs, accumulation of fibrin on the liver and heart. Microscopically, there was fibrinous pericarditis, myocarditis, fibrinous perihepatitis, fatty changes in hepatocytes, interstitial pneumonia, necrosis and depletion of lymphocytes in spleen and enteritis

Kumari *et al.* (2013) conducted detailed patho-microbiological studies on *Salmonella gallinarum* infection in broiler chickens in Haryana. Mortality pattern revealed that maximum mortality occurred in 1-2 week aged birds. 23 *Salmonella* isolates were identified, out of which 19 samples were identified as *S. gallinarum* and 4 samples as *S. enteritidis*. Pathological lesions observed included bronze

discoloration of liver and necrotic foci on liver. Microscopically, liver revealed aggregation of heterophils, lymphocytes and macrophages.

Kumar *et al*. (2013) conducted pathological studies on natural cases of poultry carcasses to study the incidence and pathological lesions of *Escherichia coli* infection. Colibacillosis was noticed in different age groups but maximum mortality was evident in birds of 3-4 weeks age. *Escherichia coli* was isolated from blood and liver samples in 86.6% cases. Gross pathological changes included congestion in various organs, accumulation of fibrin on the liver and heart. Histopathologically, there was fibrinous pericarditis, myocarditis, fibrinous perihepatitis, hepatitis and fatty changes in hepatocytes, interstitial pneumonia, necrosis and depletion of lymphocytes in spleen and enteritis.

Majed *et al.* (2013) reported that most common clinical signs in IBD infected chicks were oedema of the head, face, and wattles, twisted neck and paralysis, greenish diarrhoea, cessation of egg production, soft-shelled eggs and death. The post-mortem findings were haemorrhages in the thigh/pectoral muscles, enlarged, oedematous and hyperaemic bursa with bloody or mucoid contents or its atrophy in chronic cases and haemorrhage in the junction between gizzard and proventriculus.

Amare *et al.* (2013) conducted a study to determine the prevalence of yolk sac infection and to identify yolk sac infection-associated bacteria. A total of 290 dead chicks of White Leghorn and Rhode Island Red breeds of 1 to 7 days of age were necropsied; yolk sac samples from these chicks were cultured and the bacteria were isolated and identified on biochemical tests. Overall, a prevalence of 33.10% (96/290) was found. A total of 170 bacterial isolates were found, of these *Escherichia coli* (51.2%) was the most frequently isolated bacteria followed by *Staphylococcus aureus* (23.5%) and *Proteus mirabilis* (22.9%). Statistically significant association was established between the chick mortality and the bacterial isolates. Yolk sac infection mortality was highly correlated with *E. coli* isolation.

Arif *et al.* (2015) collected serum samples from 570 broilers from 52 poultry farms of Quetta to determine the seroprevalence of avian influenza virus by Enzyme Linked Immunosorbent Assay (ELISA). The sero-positivity of avian influenza virus was recorded to be 14.03% in broilers. All the positive sera of boilers determined by ELISA were further tested by using H5, H7 and H9 specific strains antigen through haemagglutination inhibition and haemagglutination tests. Only H9 was recognised from the sera of broilers.

Noiva *et al.* (2015) detected proventricular necrosis virus from outbreak of transmissible viral proventriculitis associated with runting stunting syndrome in 25-28 day old broiler chickens. At necropsy, enlarged proventriculus with diffusely pale serosa and thickened walls were seen. Microscopically, degeneration and necrosis of the epithelium of the proventricular glands, glandular hyperplasia and formation of lymphoid nodules within the glandular parenchyma was noticed.

Ali and Ali (2015) collected 50 broiler birds from Basra Province which were showing lesions of fibrinous perihepatitis, fibrinous pericarditis and airsacculitis. Bacteriological examination revealed presence of *E.coli* infection in 46% birds. The isolates belonged to the serogroup O78: K80.

Ahad *et al.* (2015) found that the prevalence of coccidiosis in broilers in Kashmir Valley was 29.87%. Microscopic examination revealed the presence of severe enteritis and presence of coccidian oocysts in intestinal epithelium. Coccidiosis was most prevalent in autumn followed by summer, spring and winter season.

Singh *et al.* (2015) included gross, histopathological and immunopathological approaches in their study for the diagnosis of IBD. A total of 33 samples were collected from the six different poultry farms of Ludhiana. Macroscopic changes seen in bursa included swelling, hemorrhages and atrophy. Rarefaction of bursal follicles with intermittent infiltration of lympho-mononuclear cells and chronic cystic changes were seen. Microscopically, bursa

showed prominent fibrotic and atrophic changes, infiltration of MNCs along with chronic cystic changes.

Sultana *et al.* (2015) studied the pathological lesions of aspergillosis in commercial broiler chickens at Chittagong district and recorded an overall 6.14% incidence of aspergillosis. Highest incidence (8.22%) was observed in rainy season and lowest (3.16%) in winter but moderate (5.16%) in summer season. Occurrence of disease was higher (8.27%) in age between 6-10 days and lower (4.11%) in age between 0-5 days.

Nunez *et al.* (2016) recorded the signs of enteric disorders in broiler chickens. Grossly, curving of duodenal loop and intestines filled with liquid and gaseous content were observed. Histopathologically, pancreatic atrophy and enteritis characterised by fusion of intestinal villi, hyperplasia of lymphoid follicles, haemorrhage in the lamina propria and infiltration of lymphocytes and plasma cells was seen.

Kumar *et al.* (2016) reported that prevalence of Newcastle disease in commercial broiler farms at Bochaganj Upazila of Dinajpur district was 5.35%. Grossly, severe haemorrhages in caecal tonsils and on surface near junction of proventriculus and gizzard were observed. Mortality in non-vaccinated and vaccinated broiler flocks was 20.76% and 4.6% respectively.

Brar *et al*. (2017) reported the occurrence of severe enteric form of Newcastle disease virus infections in backyard poultry birds. Grossly, ulcers in intestine, haemorrhages in proventriculus and caecal tonsils, congestion and haemorrhages in liver were noticed. Microscopically, severe haemorrhages in the intestine, degeneration, necrosis of the intestinal villi and fatty changes in hepatocytes were observed. On immunohistochemistry, Newcastle disease viral antigens were found to be localised in the necrotic cells of epithelium of proventriculus, gizzard, liver and intestine.

Chapter – 3

MATERIALS AND METHODS

1. Study Protocol

The study protocol included screening of mortalities from different chicken flocks for affections of digestive system. Information was collected from the farmers for working out possible epidemiological correlation. Following thorough postmortem examination, samples were collected from different parts of digestive tract and associated organs (liver & pancreas) for histopathology, etiopathological studies and histochemistry.

2. Study Material and Sampling Area:

Samples comprised of mortalities from various poultry farms operating in Srinagar, and adjoining areas brought to Division of Veterinary Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology Kashmir, for post-mortem examination during April 2017 to June 2018. Also, farm visits were made for clinical examination in cases of outbreaks of GIT affections.

3. Study Parameters:

1. Nature and occurrence of Gross Lesions:

At necropsy all areas of GIT and associated structures including mouth, oesophagus, crop, proventriculus, gizzard, different parts of intestine, bursa of Fabricius, liver, and pancreas were examined for presence and nature of lesions. Lesions were categorized as primary (affecting only given organ) or secondary (associated with systemic disease). Descriptive characters of the lesion were noted including type, extent, distribution, solitary or presence of multiple types of lesions, etc., for classification and scoring.

2. Histopathological characterization:

Specimens from different areas of GIT and associated structures were collected from 100 outbreaks and 3 to 5 birds from each outbreak. For trimming

of tissues, Registry of Industrial Toxicology Animal-data – Guides for organ sampling published for rat and mice was extrapolated and modified wherever needed to suit for avian species. For preservation, double fixation first with aqueous Bouin's solution for 4 hours followed by maintenance in 10% buffered formalin was adopted. Tissues were processed following routine paraffin embedding technique. 3-5 µm sections were cut and stained with Harris Haematoxylin and Eosin method (Bancroft and Gamble, 2002). INHAND (International Harmonization of Nomenclature and Diagnostic Criteria) recommendations for lesions in rats and mice were extrapolated for descriptive characterization of lesions. In order to perform quantitative lesion scoring, sequential sectioning was performed with five (5) sections per block taken after every 25 sections and multiple fields per slide were screened.

3. Lesion scoring:

The lesions scoring was done as per Mann *et al*., 2012.

Numerical score	Description	Definition
0	Within normal limits	Tissues considered to be normal under the conditions of the study
1	Slight	The lesion is easily identified but of limited severity.
2	Moderate	The lesion is prominent but there is significant potential for increased severity
3	Severe	Degree of change occupies the majority of the organ.

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4. Pathogen Identification and Characterization in Specific Disease Conditions:

Tentative diagnosis of specific disease conditions was arrived at necropsy and confirmation was done following cytological examination, isolation and characterization, or in-situ pathogen or pathogonomonic lesion demonstration. Also, PCR based confirmation was done using genus specific primers**.**

4.1. Isolation and Identification of *E. coli*:

Representative samples from intestines were inoculated into nutrient broth and incubated at 37^{o}C for 24 hours. The bacterial growth in the nutrient broth was re-inoculated on MacConkey agar plates (HiMedia, Mumbai, India) and the plates were incubated at 37^{o}C for 24 hours. The lactose fermenting colonies on MacConkey plates were re-inoculated on Eosin Methylene Blue agar (HiMedia, Mumbai, India). The *Escherichia coli* colonies typically showing metallic sheen were transferred to the nutrient agar slants and stored at 4°C for further characterization. Identification of isolates was further carried out using standard morphological and biochemical tests including Grams staining and IMViC tests, followed by genus specific PCR confirmation by targeting 16S rDNA gene (Table 1).

4.2. Isolation and Identification of *Salmonella*:

The representative samples were initially inoculated into Tetrathionate broth, after overnight incubation the cultures were allowed to grow on Brilliant green agar (BGA). Salmonella produced pink, small circular and smooth colonies on BGA. From pure culture of BGA, Gram's staining was performed to observe *Salmonella* organism. Under compound light microscope the organism was identified as Gram-negative, rod shaped organisms. The pink colonies that were identified as Gram-negative rods were characterized for S*almonella* by inoculating on Xylose lysine desoxycholate agar (XLDA) plates. On XLDA, the *Salmonella* isolates produced red colonies with black centres. Identification of isolates was further carried out using standard morphological and biochemical

tests including Grams staining and IMViC tests followed by genus specific PCR confirmation by targeting 16S rDNA gene (Table 1).

4.3. Isolation and Identification of *Clostridium perfringens* type A:

The samples were subjected to pre-enrichment in Difco™ cooked meat medium and incubated anaerobically in 3.5 litre anaerobic jar with GasPak™Anaerobe Container System at 37ºC for 24 hrs. Enriched samples were streaked on sulphite polymixin sulphadiazine agar plates and the plates were incubated anaerobically at 37ºC for 24hrs. After incubation suspected colonies were sub-cultured on the SPS agar plates until they were free from contaminating bacteria. Confirmation of the isolates was done by PCR amplification of cpa gene (Table 1).

4.4. Fungal (*Aspergillus*)Isolation and Identification:

The suspected samples were impressed directly on Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (0.05 mg/ml). Cultures were incubated aerobically at 25 ºC and 37 ºC for 5–7 days. Whitish-grey colonies revealed *Aspergillus fumigatus*. Wet mount slides were prepared using lactophenol blue staining method for identification on morphological basis.

4.5. Isolation and Identification Newcastle Disease Virus:

For virus isolation tissue samples were pooled and homogenised using sterile mortar and pestle to make a suspension in 5 ml phosphate-buffered saline (PBS). The suspension was filtered through syringe filter and streptozotocin and penicillin G were added. About 200 µL of the sample was inoculated into 10-day-old embryonated chicken eggs through allantoic sac route of inoculation (Alexander, 2004). The eggs were incubated at 37ºC for six days and candled twice daily. Any death during the first 24 hours of incubation was considered non-specific and discarded. All the embryos that died or survived till day 6 post-inoculation were chilled at 4ºC for one hour. The allantoic fluid was aspirated carefully and stored in sterile screw-capped vials and stored at-70ºC and later used

for haemagglutination test using chicken RBC following the standard procedure (Alexander, 2004).and confirmation by PCR by targeting F-gene.

4.6. **Isolation and Identification of *Eimeria* (Coccidiosis):**

Faecal samples were examined by direct smear examination and concentration method using standard technique for presence of characteristic oocysts. Pooled samples were cultured in 2.5% potassium dichromate for sporulation and examined for sporocysts and sporozoites.

5. Polymerase Chain Reaction for pathogen confirmation

5.1. Isolation of bacterial genomic DNA

Pure culture of *E. coli* and salmonella isolates were inoculated in nutrient broth (HiMedia, Mumbai, India) and incubated at 37° C. After overnight growth, 1 ml broth culture was transferred to 1.5 ml micro centrifuge tubes and centrifuged at 10000×g for 10 min. The supernatant was discarded, 100 µl of sterile PBS was added to the pellet and tubes were vortexed gently. For *Clostridium perfringens* isolated colonies from agar plates were suspended in 1.5ml micro-centrifuge tubes containing 100 µl of distilled water by gentle vortexing. The samples were then boiled for 10 min, cooled on ice for 5 min and centrifuged again at 10000xg for 1 min. Two µl of supernatant was directly used as template for each PCR reaction.

To check the purity and concentration of the DNA samples, the OD was taken at 260nm and 280nm in UV spectrophotometer (Eppendorf, Hamburg, Germany) using the following formula as:

Purity = OD at 260/OD at 280

Concentration = OD at 260 x dilution factor x 50µg/ml

5.2. Polymerase chain reaction conditions for *E. coli*

PCR confirmation of E.coli isolates was done by targeting specific 16S rDNA gene and the isolates were also screened for the presence of *iss*, virulence genes. The details of primer sequences used and target product size are given in

Table 1. The PCR assays were carried out in sterile 0.2 ml PCR tubes. For 16S rDNA amplification the reaction mixture consisted of 200µm deoxynucleotide triphosphate (dNTP) mix, 1X PCR buffer, 1.5mM $MgCl_2$, 20 pmol of each primers, 2.5U Taq DNA polymerase (Fermentas, Life Sciences) and DEPC treated nuclease free water. Finally, 5 µl DNA template was added. Nuclease free water and APEC isolate maintained in The Division were used as negative and positive controls, respectively.PCR amplification was done with initial denaturation at 94ºC for 45 seconds, annealing at 64ºC for one minute and extension at 72ºC for two minutes followed by final extension at 72ºC for 10 minutes.

For iss gene amplification the reaction mixture consisted of 2.0µl template DNA, 2.5µl of 10x buffer, 0.2 µl of 25mM dNTP mix, 1.5 U of Taq DNA Polymerase (Fermentas, Life Sciences) and Nuclease free water(NFW). The concentration of $MgCl_2$ was 1.5 mM. Nuclease free water and APEC isolate maintained in The Division were used as negative and positive controls, respectively. The PCR assays were performed in Master-cycler gradient (Eppendorf, Hamburg Germany).The cyclic conditions for PCR were initial denaturation for 3 minutes at 94°C, followed by 26 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 min and extension at 72°C for 1 min. The final extension was carried out at 72°C for 10 min.

5.3. Polymerase chain reaction conditions for *Salmonella*

PCR confirmation of *Salmonella* isolates was done by targeting specific 16S rDNA gene. The details of primer sequences used and target product size are given in Table 1.The PCR assays were carried out in sterile 0.2 ml PCR tubes. Each PCR mixture of 25µl consisted of 2.0µl template DNA, 2.5 of 10x buffer, 1.5 µl $MgCl_2$ (1.5mM), 0.2 µl of 25mM dNTP mix, 0.5µl of each primer, 0.5µl of Taq DNA Polymerase (2.5 U) (Fermentas, Life Sciences) and Nuclease free water(NFW). Sterilised distilled water and known *Salmonella* isolate maintained

in our Division were used as negative and positive controls, respectively. The PCR assays were performed in Master-cycler gradient (Eppendorf, Hamburg Germany).

The cyclic conditions for PCR were initial denaturation for 1minutes at 94°C, followed by 33 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 2 min. The final extension was carried out at 72°C for 10 min.

5.4. Polymerase chain reaction conditions for C*lostridium perfringens*

PCR confirmation of *Clostridium perfringens* isolates was done by amplification of cpa gene. The details of primer sequences used and target product size are given in Table 1. The PCR assays were carried out in sterile 0.2 ml PCR tubes. Each PCR mixture of 25µl consisted of 3.0µl template DNA, 2.5 of 10x buffer, 0.2 µl of 25mM dNTP mix, 1.0 U of Taq DNA Polymerase (Fermentas, Life Sciences) and Nuclease free water(NFW). The concentration of $MgCl_2$ was 2.0 mM. Sterilised distilled water and a confirmed isolate maintained orDivision were used as negative and positive controls, respectively. The PCR assays were performed in Master-cycler gradient (Eppendorf, Hamburg Germany).

The cyclic conditions for PCR were initial denaturation for 15 minutes at 95°C, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 49°C for 90 sec and extension at 72°C for 90 sec. The final extension was carried out at 72°C for 10 min.

5.5. Reverse-Transcriptase Polymerase chain reaction conditions for Newcastle Disease Virus (NDV)

The presence of NDV in the tissue sample suspension and allantoic fluid was further reconfirmed by RT-PCR. In brief, RNA extraction was performed using TRIzol Reagent as per the protocol described by Sambrook and Russell (2001). The extracted RNA was subjected to cDNA synthesis using cDNA synthesis kit (Promaga), followed by PCR amplification of 356 bp genome fragment containing F gene using cDNA as template and a primer

pair(5′GCAGCTGCAGGGATTGTGGT3′)&(5′TCTTTGAGCAGGAGGATGTT G3′) (Nanthakumar *et al*. 2000). The following thermal profile was used: initial denaturation and activation of Taq polymerase at 94ºC for 3 minutes followed by 32 cycles of PCR with denaturation at 94ºC for 45 seconds, annealing at 58ºC for 45 seconds, extension at 72ºC for one minute, and final extension at 72ºC for 10 minute.

5.6. Electrophoresis and documentation

Agarose gel (1.5% & 2.0% w/v) was made by heating the appropriate amount of agarose (Sigma Aldrich, St. Louis, USA) with 70 ml 1× tris acetate EDTA (TAE) buffer in a 500 ml Erlenmeyer flask. The flask was cooled to 60 °C and ethidium bromide (EtBr) added to the final concentration of 0.5 µg/ml. The warm agarose solution was poured into a plastic holder with a suitable comb (with 0.5-mm or 1-mm wells) and allowed to completely set at room temperature for 30 min. The comb was removed and the gel mounted on electrophoresis tank (Vari-Gel Maxi System, SCIE-PLAS Ltd., Cambridge, England) and the tank filed with 1×TAE buffer. Amplified PCR products were mixed with 6×loading buffer and loaded in separate wells on the submerged gel. Standard molecular weight marker (Fermentas Life Sciences) was also loaded in one well. The voltage 1-5V/cm was applied across the gel until the bromophenol blue migrated to appropriate distance. The gel was removed and visualized under ultraviolet illumination and photographed with Gel Documentation System (Ultra Cam Digital Imaging, Ultra.Lum.Inc., Claremont, CA).

Table-1: Details of primers used along with amplicon size

Pathogen	Target Gene	Primer	Oligonucleotide sequence	Products Size (bp)	Reference
E. coli	16S rDNA gene	ECO-f	GACCTCGGTTTAGTTCACAGA	585	Candrian *et al*., 1991; Amit-Romanch *et al*.,2004
		ECO-r	CACACGCTGACGCTGACCA		
	ISS	ISS-F	GTGGCGAAAACTAGTAAAAC AGC	762	Tivendale *et al.* (2004)
		ISS-R	CGCCTCGGGGTGGATAA		
Salmonella Sp	16S rDNA gene	S16S-F	TGT TGT GGT TAA TAA CCG CA	574	Lin and Tsen (1996)
		S16S-R	CAC AAA TCC ATC TCT GGA		
Clostridium Perfringens	cpa-gene	Cpa-F	GCTAATGTTACTGCCGTTGA	324	Van asten *etal*. (2009)
		Cpa-R	CCTCTGATACATCGTGTAAG		
Newcastle Disease Virus	F-gene	NDV-F	GCAGCTGCAGGGATTGTGGT	356	Nanthakumar *et al*. 2000
		NDV-R	TCTTTGAGCAGGAGGATGTTG		

6. Histochemical and Special Staining

The parallel sections from various lesions were stained by specific techniques for demonstration histochemical changes, specific cells and pathogens viz. Alcian Blue-PAS technique for characterization and distribution of neutral and acid mucopolysaccharides; PAS for demonstration of glycogen in liver; Toludine method for demonstration of mast cell reaction; Masson's trichrome stain for demonstration of connective tissue; Brown & Brenn method for demonstration of Gram positive / negative bacteria; Ziehl-Neelsen method for acid fast bacteria and Grocott`s Methanamine Silver method for fungi(Luna, 1968, Bancroft and Gamble, 2002).

4. Statistical analysis

Data regarding mortalities including case prevalence and proportionate mortalities, was presented as *per cent*. Data was analyzed by non-parametric analysis viz Z-test for two proportionate data and Chi-square test for multi-proportionate data(Snedecor and Cochran, 1994).

For lesion scoring a new test was developed and named "**Lesion Severity Rank Scoring**". The method is briefly described as under

LSRS Method:

Various lesions like congestion, haemorrhage, ulcer, mucosal thickening, mucosal denudation in six segements of GIT viz proventriculus, duodenum, jejunum, Ileum, CDE and CT were counted, and assigned a score/weight based on severity. The sum total of the weighed scores was worked out for each lesion in a particular segment of GIT and called as Weighed Lesion score.

The six lesions in a particular segment of GIT were now ranked as I, II, III, IV, V and VI as per the weighed lesion score. Ranks assigned to different lesions across different segements were counted to obtain rank count for each lesion. Ranks were assigned a score as 6, 5, and 4,3,2,1 for I, II, III, IV, V and VI respectively.

Severity rank score for a particular lesion was calculated as

$$LSRS = \sum(RC \times RS)$$

Where

LSRS = Lesion Severity rank score for a particular lesion

RC = Rank count for that lesion

RS = Score assigned to ranks

Mean severity rank score was calculated as

$$MSRS = LSRS/N_R$$

Where

MSRS = Mean Severity Rank Score

LSRS = Lesion Severity Rank Score

N_R = Total number of ranks

Chapter – 4

EXPERIMENTAL FINDINGS

In present study, a total of 7657 broiler chicken of different age groups from a total of 201outbreaks were necropsied and examined for presence of lesions in GIT, and associated structures including bursa of Fabricius, liver, and pancreas.

4.1. Spatial Distribution of Digestive System Affections in Chicken

4.1.1. Spatial Distribution of Gastrointestinal Tract Affections in Chicken

4.1.1.1. Occurrence of Gastrointestinal Tract Affections in Chicken

Out of a total of 7657 chicken carcasses necropsied, gross GIT lesions were observed in 7339 birds giving a case prevalence of 95.85%. Significantly higher number of cases with GIT lesions was associated with some specific disease condition (6404) with case prevalence of 83.64% and proportionate mortality of 87.25%. The solitary GIT lesions were observed in 935 cases with case prevalence of 12.21% and proportionate mortality of 12.74% (Table 2).

Table 2: Occurrence of GIT affections among broiler chickens.

Nature	Mortality associated with GIT affections		
	No.	Case Prevalence	Proportionate Mortality
		N=7657*	N=7339**
Solitary GIT affections	**935**	**12.21**	**12.74**
Specific disease conditions with GIT involvement	**6404**	**83.64**	**87.25**
Total GIT affections	**7339**	**95.85%**	

*N=Total Number of Necropsies Performed
**N=Total number of cases with GIT affections

4.1.1.2. Effect of Age on Gastrointestinal Tract Affections in Chicken

Similar trend was observed in different age groups. Age wise studies revealed a significant effect on proportionate mortality as well as occurrence of GIT affections. The proportionate mortality was significantly higher in age group 2-3 weeks (39.14%) followed by 0-1 week (21.46%), 3-4 weeks (18.11%), 4-5 weeks (12.21%) and 1-2 weeks (9.08%) in that order. The occurrence of GIT affections calculated as a function of mortality in given age group revealed that overall occurrence was significantly higher in age group 0-1 weeks (98.35%) followed by 1-2 weeks (96.97%). 2-3 weeks (95.36%) and 3-4 weeks (95.16%), which were comparable among themselves; whereas significantly lower occurrence was observed in age group 4-5 weeks (93.15%). Almost similar trend was observed for GIT affection as part of specific disease condition with occurrence of 95.49% in 0-1 week chicken which was significantly higher than 92.08% and 90.55% observed in 1-2 and 3-4 week chickens respectively, which were comparable but significantly higher than 80.53% in 4-5 week and 72.93% in 2-3 week chickens, which also differed significantly among themselves. An almost reverse trend was observed for solitary GIT affections wherein the occurrence was significantly higher in 2-3 week chickens (22.42%) followed by 4-5 weeks (12.62%), then 1-2 and 3-4 weeks (4.89% & 4.61% respectively); and significantly lower in 0-1 week chickens (2.86%).

Age, also, showed a significant effect on proportionate distribution of overall, solitary or disease associated GIT affections. The overall occurrence and disease associated occurrence of GIT affections was significantly higher in age group 2-3 weeks followed by 0-1, 3-4, 4-5, and 1-2 weeks with respective proportionate mortalities 38.94% & 34.13%; 22.02% & 24.50%; 17.99% & 19.61%; 11.87% & 11.76%; and 9.18% & 9.99%. The proportionate mortality with solitary GIT affections was significantly higher in 2-3 week chickens (71.87%) followed by 4-5 week (12.62%), then 3-4 and 0-1 week (6.84% & 5.03% respectively) and lowest in 1-2 weeks (3.64%) (Table 3).

Table 3.: Effect of age on occurrence of GIT affections in broiler chicken

Age (in weeks)		Proportionate Mortality	Solitary GIT affections		Specific disease conditions with GIT involvement		TOTAL	
		N	n1	%	n2	%	n.	%
0-1 week	n`	1643	47	2.86^{A}	1569	95.49^{D}	1616	98.35^{C}
	%`	**21.46^{d}**	**5.03^{b}**		**24.50^{d}**		**22.02^{d}**	
1-2 weeks	n`	695	34	4.89^{B}	640	92.08^{C}	674	96.97^{B}
	%`	**9.08^{a}**	**3.64^{a}**		**9.99^{a}**		**9.18^{a}**	
2-3 weeks	n`	2997	672	22.42^{D}	2186	72.93^{A}	2858	95.36^{B}
	%`	**39.14^{e}**	**71.87^{d}**		**34.13^{e}**		**38.94^{e}**	
3-4 weeks	n`	1387	64	4.61^{B}	1256	90.55^{C}	1320	95.16^{B}
	%`	**18.11^{c}**	**6.84^{b}**		**19.61^{c}**		**17.99^{c}**	
4-5 weeks	n`	935	118	12.62^{C}	753	80.53^{B}	871	93.15^{A}
	%`	**12.21^{b}**	**12.62^{c}**		**11.76^{b}**		**11.87^{b}**	
TOTAL	**N`**	**7657**	**935**		**6404**		**7339**	

% Case prevalence calculated as a function of total mortality in given age group (N)
**%`** Proportionate mortality distribution as a function of total number of cases in all age groups (N`)

4.1.1.3. Effect of Season on Gastrointestinal Tract Affections in Chicken

The proportionate mortality was significantly higher in spring (38.03%) followed summer (33.05%), autumn (15.05%), and winter (13.87%). The occurrence of GIT affections calculated as a function of mortality in given season and as a function of total mortality with GIT affections revealed significantly higher overall occurrence in spring (97.35% & 38.63%) followed summer (96.91% & 33.42%). Autumn (94.87% & 14.89%) and winter (90.20% & 13.05%). The occurrence of GIT affection as part of specific disease condition was significantly higher in spring (89.77%) followed by autumn (82.29%) and winter (80.89%) and lowest in summer (78.35%). However, proportionate

distribution was highest in spring (40.82%) followed by summer (30.97%), autumn (14.80%) and winter (13.41%). The differences were significant except for autumn and spring which were comparable. Solitary GIT affections revealed significantly higher occurrence in summer (18.57%) followed by autumn (12.59%). Significantly lower occurrence was recorded in winter (9.32%) and spring (7.59%), which were statistically comparable. The proportionate distribution of solitary GIT affections revealed significantly higher occurrence in summer (50.27%), followed by spring (23.64%), autumn (15.51) and winter (10.59%) (Table 4).

Table 4.: Effect of season on occurrence of GIT affections in broiler chicken

Season		Total necropsies done	Solitary GIT affections		Specific disease conditions with GIT involvement		TOTAL	
		N	n1	%	n2	%	n	%
Spring	n`	2912	221	7.59%^{A}	2614	89.77^{C}	2835	97.35^{C}
	%`	**38.03^{d}**	**23.64^{c}**		**40.82^{c}**		**38.63^{d}**	
Summer	n`	2531	470	18.57%^{C}	1983	78.35^{A}	2453	96.91^{C}
	%`	**33.05^{c}**	**50.27^{d}**		**30.97^{b}**		**33.42^{c}**	
Autumn	n`	1152	145	12.59%^{B}	948	82.29^{B}	1093	94.87^{B}
	%`	**15.05^{b}**	**15.51^{b}**		**14.80^{a}**		**14.89^{b}**	
Winter	n`	1062	99	9.32%^{A}	859	80.89^{B}	958	90.20^{A}
	%`	**13.87^{a}**	**10.59^{a}**		**13.41^{a}**		**13.05^{a}**	
TOTAL	**N`**	**7657**	**935**		**6404**		**7339**	

% Case prevalence calculated as a function of total mortality in given season (N)
**%`** Proportionate mortality distribution as a function of total number of cases in all seasons (N`)

4.1.1.4. Effect of Commercial Strain (Chick Type) on Gastrointestinal Tract Affections in Chicken

Mortality consisted of a total of ten different commercial broiler chick strains. The commercial strain (CS) showed a significant effect on proportionate mortality, and case prevalence as well as proportionate mortality associated with GIT affections (Table 5). The overall case prevalence of GIT affections calculated as a function of total mortality of a given strain, ranged from 51.58% in CS10 to 100% in CS8 and CS9. The case prevalence was significantly higher, but comparable among themselves, in strains CS1 (98.04%), CS2 (98.93%), CS6 (98.32%), CS8 (100%), and CS9 (100%). Case prevalence of disease associated GIT affections ranged from 31.57% in CS10 to 100% in CS8; and for solitary GIT affections from 0.00% in CS8 to 23.71% in CS3. The proportionate mortality among the strains ranged from 1.24% in CS-10 to 46.53% in CS1. Overall, disease associated proportionate mortality with GIT affection was highest in CS1 (47.60% & 46.11% respectively) and lowest in CS10 (0.67% & 0.62%, respectively). The proportionate mortality with solitary GIT affections was also highest in CS1, but no case was observed in CS8.

Table 5 : Effect of commercial strain (chick type) on occurrence of GIT affections in broiler chicken

Strain		Proportionate Mortality	Solitary GIT affections		Specific disease condition with GIT involvement		TOTAL	
		N	n1	%	n2	%	n	%
CS1	n`	3563	540	15.16^{D}	2953	82.88^{C}	3493	98.04^{CD}
	%`	**46.53^{h}**	**57.75^{g}**		**46.11^{h}**		**47.60^{g}**	
CS2	n`	843	86	10.2^{CD}	748	88.73^{D}	834	98.93^{D}
	%`	**11.01^{g}**	**9.20^{e}**		**11.68^{g}**		**11.36^{f}**	
CS3	n`	620	147	23.71^{E}	454	78.06^{B}	601	96.94^{C}
	%`	**8.10^{ef}**	**15.72^{f}**		**7.09^{e}**		**8.19^{e}**	
CS4	n`	678	79	11.65^{D}	524	77.29^{B}	603	88.94^{B}
	%`	**8.85^{f}**	**8.45^{e}**		**8.18^{f}**		**8.22^{e}**	
CS5	n`	502	37	7.37^{C}	418	83.27^{C}	455	90.64^{B}
	%`	**6.56^{d}**	**3.96^{d}**		**6.53^{de}**		**6.20^{d}**	
CS6	n`	597	7	1.17^{AB}	580	97.15^{E}	587	98.32^{CD}
	%`	**7.80^{e}**	**0.75^{b}**		**9.06^{f}**		**8.00^{e}**	
CS7	n`	452	12	2.65^{B}	398	88.05^{D}	410	90.71^{B}
	%`	**5.90^{d}**	**1.28^{bc}**		**6.21^{d}**		**5.59^{d}**	
CS8	n`	179	0	0^{A}	179	100^{F}	179	100^{D}
	%`	**2.34^{c}**	**0.00^{a}**		**2.80^{c}**		**2.44^{c}**	
CS9	n`	128	18	14.06^{D}	110	85.93^{CD}	128	100^{D}
	%`	**1.67^{b}**	**1.93^{c}**		**1.72^{b}**		**1.74^{b}**	
CS10	n`	95	9	9.47^{CD}	40	31.57^{A}	49	51.58^{A}
	%`	**1.24^{a}**	**0.96^{bc}**		**0.62^{a}**		**0.67^{a}**	
TOTAL	**N`**	**7657**	**935**		**6404**		**7339**	

% Case prevalence calculated as a function of total mortality in given strain (N)
**%`** Proportionate mortality distribution as a function of total number of cases in all the strains (N`)

4.1.2. Spatial Distribution of Liver affections in Chicken

4.1.2.1. Occurrence of Liver Affections in Chicken

Out of a total of 7657 chicken carcasses necropsied, gross liver lesions were observed in 6722 birds giving a total occurrence of 87.79%. Significantly higher number of cases with liver lesions, 76.10% (5827/7657) was associated with some specific disease condition. The solitary liver lesions were observed in 11.69% (895/7657) cases(Table 6).

Table 6: Occurrence of liver affections among broiler chickens.

Nature	Mortality associated with liver affections		
	No.	Case Prevalence	Proportionate Mortality
		N=7657*	N=6722**
Solitary liver affections	**895**	**11.69**	**13.31**
Specific disease conditions with liver involvement	**5827**	**76.10**	**86.68**
Total GIT affections	**6722**	**87.79**	

*N=Total Number of Necropsies Performed
**N=Total number of cases with GIT affections

4.1.2.2. Effect of Age on Liver Affections in Chicken

Age wise studies revealed a significant effect on occurrence of liver affections (Table 7). The occurrence of liver affections calculated as a function of mortality in given age group revealed that overall occurrence was significantly higher in age group 0-1 weeks (92.69%) and 4-5 weeks (90.90%) when compared with other age groups but differed non significantly from each other. The age groups 1-2, 2-3, and 3-4 weeks differed non significantly from each other with the

occurrence of 85.04%, 85.91% and 85.29% respectively. Almost similar trend was observed for liver affection as part of specific disease condition with occurrence of 91.84% in 0-1 week chicken which was significantly higher than other age groups. The occurrence of 72.81%, 73.01% and 75.99% observed in 1-2, 2-3, and 3-4 weeks chickens respectively, was comparable but significantly higher than 60.96% in 4-5 week chickens. In case of solitary liver affections, highest occurrence was observed in age group 4-5 weeks (29.95%) followed by significantly lower occurrence in the age group 2-3 weeks (12.91%) and 1-2 weeks (12.23%), which were comparable among themselves but differed significantly from 3-4 weeks (9.3%) chickens. Lowest occurrence was observed in 0-1 weeks (0.85%) chickens. Age, also, showed a significant effect on proportionate distribution of overall, solitary or disease associated liver affections. The overall and disease associated proportionate mortality with liver affections was significantly higher in age group 2-3 weeks (38.31% & 37.55%) followed by 0-1 (22.66% & 25.90%), 3-4 (17.60% & 18.09%), 4-5 (12.65% & 9.78%), and 1-2 weeks (8.79% & 8.68%). The proportionate mortality with solitary liver affections was significantly higher in 2-3 week chickens (43.24%) followed by 4-5 week (31.28%), 3-4 (14.41%), 1-2 weeks (9.50%) and 0-1 week (1.56%) with all the values differing significantly from each other .

4.1.2.3. Effect of Season on Liver Affections in Chicken

The occurrence of liver affections revealed significant differences between seasons. The occurrence of liver affections calculated as a function of mortality in given season revealed significantly higher rates in spring (92.41%) and winter (90.68), followed by autumn (87.41%) and summer (81.43%). The overall proportionate mortality calculated as a function of total mortality with liver affections revealed highest occurrence in spring (40.03%) followed by summer (30.66%). Comparatively lower occurrence was observed in autumn (14.98%) and winter (14.33%) which were comparable among themselves. The occurrence of liver affection as part of specific disease condition was significantly higher in

spring (89.15%) followed by winter (84.84%), autumn (70.92%) and summer (59.77%). However, proportionate distribution was significantly high in spring (44.55%) followed by summer (25.97%). Lower proportionate mortality was observed in winter (15.46%) and autumn (14.02%), which were statistically comparable. Solitary liver affections revealed significantly higher occurrence in summer (21.65%) followed by autumn (16.49%). Significantly lower occurrence was recorded in winter (5.84%) and spring (3.26%), which were statistically comparable. The proportionate distribution of solitary liver affections revealed significantly higher occurrence in summer (61.23%), followed by autumn (21.23%), spring (10.61%), and winter (6.93%) (Table 8).

Table 7: Effect of age on occurrence of Liver affections in broiler chicken

Age (in weeks)		Total necropsies done	Solitary liver Affections		Specific disease conditions with liver involvement		Total	
		N	n1	%	n2	%	n	%
0-1 week	n`	1643	14	0.85^{A}	1509	91.84^{C}	1523	92.69^{B}
	%	**21.46^{d}**	**1.56^{a}**		**25.90^{d}**		**22.66^{d}**	
1-2 weeks	n`	695	85	12.23^{C}	506	72.81^{B}	591	85.04^{A}
	%	**9.08^{a}**	**9.50^{b}**		**8.68^{a}**		**8.79^{a}**	
2-3 weeks	n`	2997	387	12.91^{C}	2188	73.01^{B}	2575	85.91^{A}
	%	**39.14^{e}**	**43.24^{e}**		**37.55^{e}**		**38.31^{e}**	
3-4 weeks	n`	1387	129	9.3^{B}	1054	75.99^{B}	1183	85.29^{A}
	%	**18.11^{c}**	**14.41^{c}**		**18.09^{c}**		**17.60^{c}**	
4-5 weeks	n`	935	280	29.95^{D}	570	60.96^{A}	850	90.9^{B}
	%	**12.21^{b}**	**31.28^{d}**		**9.78^{b}**		**12.65^{b}**	
TOTAL	N`	**7657**	**895**		**5827**		**6722**	

% Case prevalence calculated as a function of total mortality in given age group (N)
**%`** Proportionate mortality distribution as a function of total number of cases in all age groups (N`)

Table 8: Effect of season on occurrence of Liver affections in broiler chicken

Season		Total necropsies done	Solitary liver affections		Specific disease conditions with liver involvement		TOTAL	
		N	n1	%	n2	%	n	%
Spring	n`	2912	95	3.26^{A}	2596	89.15 D	2691	92.41 C
	%	**38.03^{d}**	**10.61^{b}**		**44.55^{d}**		**40.03^{c}**	
Summer	n`	2531	548	21.65 D	1513	59.77 A	2061	81.43 A
	%	**33.05^{c}**	**61.23^{d}**		**25.97^{c}**		**30.66^{b}**	
Autumn	n`	1152	190	16.49 C	817	70.92 B	1007	87.41 B
	%	**15.05^{b}**	**21.23^{c}**		**14.02^{a}**		**14.98^{a}**	
Winter	n`	1062	62	5.84^{B}	901	84.84 C	963	90.68 C
	%	**13.87^{a}**	**6.93^{a}**		**15.46^{b}**		**14.33^{a}**	
TOTAL	N`	**7657**	**895**		**5827**		**6722**	

% Case prevalence calculated as a function of total mortality in given season (N)
**%`** Proportionate mortality distribution as a function of total number of cases in all seasons (N`)

4.1.2.4. Effect of Commercial Strain (Chick Type) on Liver Affections in Chicken

Evaluation of mortality pattern from ten different commercial broiler chick strains for occurrence of liver affections revealed significant effect on case prevalence as well as proportionate mortalities (Table 9). The overall case prevalence, disease associated prevalence, and solitary prevalence of liver affections calculated as a function of total mortality of a given strain, ranged from

12.63% to 94.54%; 12.63% to 86.94%; and 0.00% to 27.88%, respectively in different strains. Significantly lower overall and disease associated case prevalence was noted in CS10 (12.63% each) whereas no case of solitary liver affection was observed in CS3, CS8 and CS10. The proportionate distribution of mortality with liver affections also showed similar trend. Overall, disease associated proportionate mortality with liver affections was highest in CS1 (50.03% & 49.39% respectively) and lowest in CS10 (0.18% & 0.21%, respectively). The proportionate mortality with solitary liver affections was also highest in CS1, but no case was observed in CS3, CS8 and CS10.

4.2. Pathology of Digestive System in Chicken

4.2.1. Pathology of Gastrointestinal Tract in Chicken

4.2.1.1. Gross Pathological Lesions observed in Gastrointestinal Tract in Chicken

Out of a total of 7339 cases with grossly evident lesions in one or more segments of GIT, jejunum was involved in significantly higher number of cases (81.96%) followed by ileum (81.20%), duodenum (74.06%), proventriculus (42.13%), caecum (38.34%) and caecal tonsils (24.14%) (Table 10). Rarely lesions were also observed in colorectum. No gross lesions were evidenced in oral cavity (buccal mucosa, tongue, oropharynx), oesophagus and crop (Plate 1A-C).

4.2.1.1.1. Proventricular Lesions

Lesions observed in proventriculus included varied degree of congestion, hemorrhages, thickened oedematous mucosa and presence of catarrhal exudate with respective case prevalence of 3.62%, 9.43%, 29.91, and 41.34% (Table 10). Similarly the occurrence calculated on the basis of 3092 cases showing proventricular lesions was 8.60%, 22.38%, 70.99%, and 98.12% respectively (Table 11). The differences were statistically significant.

Table 9: Effect of commercial strain (chick type) on occurrence of liver affections in broiler chicken

Commercial Strain		Mortality	Solitary liver affections		Specific disease conditions with liver involvement		Total	
		N	n1	%	n2	%		%
CS1	n`	3563	485	13.61^{D}	2878	80.77^{E}	3363	94.39^{E}
	%`	**46.53^{h}**	**54.19^{f}**		**49.39^{i}**		**50.03^{h}**	
CS2	n`	843	95	11.27^{D}	602	71.41^{D}	697	82.68^{C}
	%`	**11.01^{g}**	**10.61^{d}**		**10.33^{h}**		**10.37^{g}**	
CS3	n`	620	0	0^{A}	539	86.94^{F}	539	86.94^{D}
	%`	**8.10^{ef}**	**0.00^{a}**		**9.25^{g}**		**8.02^{f}**	
CS4	n`	678	189	27.88^{E}	452	66.66^{BC}	641	94.54^{E}
	%`	**8.85^{f}**	**21.12e**		**7.76^{f}**		**9.54^{g}**	
CS5	n`	502	29	5.78^{C}	387	77.09^{DE}	416	82.87^{CD}
	%`	**6.56^{d}**	**3.24^{c}**		**6.64^{e}**		**6.19^{e}**	
CS6	n`	597	72	12.06^{D}	430	72.03^{D}	502	84.09^{CD}
	%`	**7.80^{e}**	**8.04^{d}**		**7.38^{ef}**		**7.47^{f}**	
CS7	n`	452	12	2.65^{B}	328	72.57^{D}	340	75.22^{B}
	%`	**5.90^{d}**	**1.34^{b}**		**5.63^{d}**		**5.06^{d}**	
CS8	n`	179	0	0^{A}	125	69.83^{CD}	125	69.83^{B}
	%`	**2.34^{c}**	**0.00^{a}**		**2.15^{c}**		**1.86^{c}**	
CS9	n`	128	13	10.16^{CD}	74	57.81^{B}	87	67.97^{B}
	%`	**1.67^{b}**	**1.45^{b}**		**1.27^{b}**		**1.29^{b}**	
CS10	n`	95	0	0^{AB}	12	12.63^{A}	12	12.63^{A}
	%`	**1.24^{a}**	**0.00^{a}**		**0.21^{a}**		**0.18^{a}**	
Total	**N`**	**7657**	**895**		**5827**		**6722**	

% Case prevalence calculated as a function of total mortality in given strain (N)

**%`** Proportionate mortality distribution as a function of total number of cases in all the strains (N`)

Table 10: Distribution Gross pathological lesions observed in GIT of broiler chicken (N=7339)

Gross pathology	Proventriculus		Duodenum		Jejunum		Ileum		Caecum		Caecal Tonsils	
Total affected cases	3092	**42.13**	5435	**74.06**	6015	**81.96**	5959	**81.20**	2814	**38.34**	1772	**24.14**
Congestion	266	**3.62**	4999	**68.12**	6001	**81.77**	5959	**81.20**	2257	**30.75**	1006	**13.71**
Haemorrhage	692	**9.43**	930	**12.67**	1187	**16.17**	997	**13.58**	593	**8.08**	775	**10.56**
Ulcers	0	**0**	459	**6.25**	519	**7.07**	492	**6.7**	117	**1.59**	197	**2.68**
Mucosa Thickened / Oedematous	2195	**29.91**	4973	**67.76**	5691	**77.54**	5663	**77.16**	318	**4.33**	1458	**19.87**
Mucosal denudation/ core formation	0	**0**	0	**0**	0	**0**	0	**0**	242	**3.3**	0	**0**
CATARRHAL EXUDATES	3034	**41.34**	5430	**73.99**	5887	**80.22**	5859	**79.83**	448	**6.1**	330	**4.5**

Table 11: Segment-wise distribution Gross pathological lesions in broiler chicken

Gross pathology	Proventriculus (N=3092)		Duodenum (N=5435)		Jejunum (N=6015)		Ileum (N=5959)		Caecum (N= 2814)		Caecal Tonsils (N=1772)	
	n	%	n	%	n	%	n	%	n	%	n	%
Congestion	266	8.60	4999	91.98	6001	99.77	5959	100.00	2257	80.21	1006	56.77
Haemorrhage	692	22.38	930	17.11	1187	19.73	997	16.73	593	21.07	775	43.74
Ulcers	0	0.00	459	8.45	519	8.63	492	8.26	117	4.16	197	11.12
Mucosa Thickened / Oedematous	2195	70.99	4973	91.50	5691	94.61	5663	95.03	318	11.30	1458	82.28
Mucosal denudation/ core formation	0	0.00	0	0.00	0	0.00	0	0.00	242	8.59	0	0.00
CATARRHAL EXUDATES	3034	98.12	5430	99.91	5887	97.87	5859	98.32	448	15.92	330	18.62

Congestion: Degree of mucosal congestion was mild, moderate and severe. The mucosa appeared light to bright pink. In most of the cases it was associated to

mucosal swelling and exudation. Occasionally it was also associated with glandular hemorrhages (Plate 1D-H).

Haemorrhage: Proventricular haemorrhages were invariably observed on mucosal surface and varied markedly in their pattern and severity. Glandular or non-glandular haemorrhages were noted. Non-glandular haemorrhages were linear or ecchymotic. Glandular haemorrhages frequently involved tips of the mucosal glands and extended radially towards base of the glands. The extent ranged from few focal haemorrhages involving few glands to almost complete matting pattern involving the mucosal glands. In certain cases haemorrhages were marked but restricted to proximal (oesophageo-proventricular) or distal (proventriculo-ventricular) junctions. In most of the cases, the hemorrhagic glands were prominent. In some cases of severe mucosal hemorrhages, serosal surface appeared to be congested (Plate 2).

Thickened / oedematous mucosa: Varying degree of mucosal thickening was observed. It was invariably associated with increased exudation. In moderate to severely affected cases the glands were prominent (Plate 2).

Catarrhal Exudate: The proventricular lumen contained small quantities to profuse stingy mucoid exudate adhering to mucosa; in some cases the underlying mucosa was congested, or hemorrhagic, but invariably thickened (Plate 2).

4.2.1.1.2. Intestinal Lesions

Lesions observed in different segments of intestines included varied degree of congestion, hemorrhages, ulcers, thickened oedematous mucosa, and presence of catarrhal exudate. Congestion was noted in significantly higher number of cases in jejunum (81.77%) and ileum (81.20%) followed by duodenum (68.12%), caecum (30.75%) and lowest in caecal tonsils (13.71%). Similarly, haemorrhage was observed in significantly higher number of cases in jejunum (16.17%), followed by ileum (13.58%), duodenum (12.67%), caecal tonsils (10.56%) and caecum (8.08%). Highest number of cases had ulcers in jejunum

(7.07%), followed by ileum (6.70%), duodenum (6.25%), caecal tonsils (2.68%) and caecum (1.59%). Mucosal thickening was observed in 77.54% and 77.16% cases in jejunum and ileum, respectively, and were significantly higher than duodenum (67.76%), caecal tonsils (19.87%) and lowest in caecum (4.33%), which also differed significantly. Presence of catarrhal exudate was observed in 80.22% and 79.83% cases in jejunum and ileum, followed by 73.99% cases in duodenum. However, it was seen only in 6.1% cases in caecum and 4.5% cases in caecal tonsils. Necrosis with mucosal denudation and core formation was additionally noted in caecum with case prevalence of 3.3% (Table 10).

Evaluation of the occurrence of the different gross lesions as a function of total number of cases showing lesions in a particular intestinal segment revealed presence of catarrhal exudate in 99.91% affected duodenum which was significantly higher than 91.98% cases with congestion and 91.50% with thickened mucosa. A significantly lower number of cases showed presence of haemorrhage (17.11%) and ulceration (8.45%) in duodenal mucosa. In jejunum and ileum, the significantly predominant lesion observed was congestion (99.77% & 100%, respectively) followed by presence catarrhal exudate (97.87% & 98.32%, respectively), and thickening of mucosa (94.61% & 95.03% respevtively); haemorrhage (19.73% & 16.73%, respectively) and mucosal ulcers (8.63% and 8.26% respectively). In caecum, also, significantly higher occurrence was noted for congestion (80.21%), followed by haemorrhage (21.07%), presence of catarrhal exudate (15.92%), mucosal thickening (11.30%), necrosis with denudation and core formation (8.59%) and lowest for ulcers (4.16%). Caecal tonsils showed thickened mucosa in 82.28% cases, followed by congestion (56.77%), haemorrhage (43.74%), catarrhal exudate (18.62%) and ulcers in 11.12% cases. The differences were statistically significant (Table 11).

Congestion: Congestion usually involved more than one segment of the intestine and was also associated with other lesions in one or other part of the tract. In general, congestion varied in severity, involving a segment partially or

completely with mucosa appearing light to bright or dark pink in colour. In severely affected cases, congestion was evident from serosal surface of the intestines and the mesenteric vessels were variedly distended with blood. The luminal contents varied in consistency/nature and were normal, fluid / watery or mucoid in nature (Plate 3).

Haemorrhage: Haemorrhages were observed in one or more segments of intestine, and involved a segment either partly or completely. The nature of haemorrhage varied markedly in their pattern and severity. Mostly petechial haemorrhages were seen but ecchymosis and frank haemorrhages with bloody exudate in lumen were also seen. The latter were mostly seen in caecum. The hemorrhagic mucosa appeared bright red in colour(Plate 3,4)

Thickened / oedematous mucosa: Varying degree of mucosal thickening was observed giving a velvety appearance. It was invariably associated with increased exudation with thick mucous exudate. In some cases, it was associated with congestion and/or petechial haemorrhages (Plate 3,4).

Catarrhal Exudate: The exudate in the intestines appeared thin to thick stingy mucoid in nature, filling the intestinal lumen and in some cases causing segmental or uniform distention of the intestines (Plate 3).

4.2.1.1.3. Severity Score Analysis

Evaluation of different lesions across different segments of GIT, by Severity Score Analysis based on weighed mean score of different conditions in different segments, indicated that catarrhal exudation was the most frequent type of response observed in GIT (LSRS= 30), followed by mucosal thickening (LSRS=28), mucosal congestion (LSRS=25) and haemorrhage (LSRS=23). Mucosal denudation and ulceration appeared to be the least frequent or rare response with LSRS of 9 and 12 respectively (Table 12).

Table 12 : Lesion Severity Rank Score different lesions observed in various segments of gastrointestinal tract of broiler chickens

CONDITION	Score	Number	Weighed score	Number	Weighed score	Number	Weighed score	Number	Weighed score	Number	Weighed score	Number	Weighed score	LSRS
	PROVENTRICULUS			DUODENUM		JEJUNUM		ILEUM		CAECUM		CAECAL TONSILS		
CONGESTION (W=1)	1	156	156	2306	2306	3191	3191	2955	2955	508	508	258	258	
	2	70	140	1545	3090	1565	3130	1634	3268	1137	2274	530	1060	
	3	40	120	1148	3444	1245	3735	1370	4110	612	1224	218	654	**25**
	TWS		416		8840		10056		10333		4006		1972	
HAEMORRHAGES (W=3)	1	201	603	411	1233	583	1749	278	834	108	324	206	618	
	2	312	1872	343	2058	389	2334	565	3390	373	2238	315	1890	
	3	179	1611	176	1584	215	1935	154	1386	112	1008	254	2286	**23**
	TWS		4086		4875		6018		5610		3570		4794	
ULCERS (W=3)	1	0	0	314	1256	143	572	157	628	34	136	61	244	
	2	0	0	95	760	267	2136	251	2008	60	480	97	776	
	3	0	0	50	600	109	1308	84	1008	23	276	39	468	**12**
	TWS		0		2616		4016		3644		892		1488	
MUCOSA THICKENED /	1	579	1158	1534	3068	1727	3454	1812	3624	93	186	451	902	
	2	1198	4792	2156	8624	2451	9804	2391	9564	176	704	749	2996	

OEDEMATOUS (W=2)	3	418	2508	1283	7698	1513	9078	1460	8760	49	294	258	1548	**28**
	TWS		8458		19390		22336		21948		1184		5446	
MUCOSAL DENUDATION/ CORE FORMATION (W=4)	1	0	0	0	0	0	0	0	0	86	344	0	0	**9**
	2	0	0	0	0	0	0	0	0	112	896	0	0	
	3	0	0	0	0	0	0	0	0	44	528	0	0	
	TWS		0		0		0		0		1768		0	
CATARRHAL EXUDATES (W=2)	1	678	1356	1328	2656	1448	2896	1298	2596	58	116	84	168	**30**
	2	1567	6268	2543	10172	2716	10864	2636	10544	239	956	159	636	
	3	789	4734	1559	9354	1723	10338	1925	11550	151	906	87	522	
	TWS		12358		22182		24098		24690		1978		1326	
W: Weight; TWS : Total Weighed Score														

4.2.1.2. Histopathological Lesions observed in Gastrointestinal Tract in Chicken

Histopathological examination of proventriculus revealed degeneration and necrosis of epithelium of mucosal folds and sub mucosal proventricular glands, congestion, haemorrhages and infiltration of heterophils in lamina propria and sub mucosa (Plate 5).Intestinal sections revealed congestion, haemorrhages in lamina propria, necrosis of epithelium, hyperplasia of goblet cells, oedema and inflammatory cells mainly heterophils in acute catarrhal enteritis. Necrotic enteritis characterised by severe necrosis of villous epithelium, presence of fibrino-necrotic exudate and infiltration of heterophils in large numbers was seen. Sub acute enteritis was characterised by thickened mucosal wall due to infiltration of heterophils, lymphocytes and plasma cells in the lamina propria and sub mucosa. Chronic enteritis was characterised by infiltration of mononuclear cells predominantly lymphocytes, macrophages and few plasma cells in sub mucosa. Regeneration characterised by hyperplasia of crypt epithelium and presence of mitotic figures was also seen (Plate 6).

4.2.2. Pathology of Liver in Chicken

4.2.2.1. Gross Pathological Lesions observed in Liver in Chicken

Out of a total of 7657 chicken carcasses necropsied, gross liver lesions were observed in 6722 birds giving a total occurrence of 87.79%. Lesions observed in liver included varied degree of congestion, hemorrhages, fibrinous perihepatitis, necrosis and haematoma with relative case prevalence of 56.41%, 16.02%, 23.19%, 4.3% and 0.074% respectively (Table 13).

Congestion: Grossly the congestion varied in severity and distribution and was classified as mild, moderate and severe with proportionate distribution of 27.27%, 51.5% and 21.23%, respectively (Table 13). Generally the livers were enlarged and bright pink to cherry red colour. In severe cases there was rounding of edges.

The large veins were engorged and dilated with large amount of blood. Large amount of dark blood exuded on cut section and the cut surfaces were mottled in appearance. Congestion of liver was also a feature associated with salmonellosis wherein livers were bright pink to dark tan in appearance (Plate 7).

Haemorrhages: Grossly mild, moderate to severe haemorrhages were observed with proportionate distribution of 26.28%, 62.4% and 11.33%. In most of the cases liver was enlarged with rounded edges and dark red in colour. The pattern of haemorrhages varied in different cases and occurred as minute pin point sized petechiae, ecchymotic, paint brush or suffusions. In some cases the haemorrhages involved all lobes of liver whereas in others only focal areas of haemorrhages were observed. In cases when whole liver was involved, large amount of blood exuded from the cut surface (Plate 7).

Fibrinous perihepatitis: Grossly, fibrinous perihepatitis varied from mild, moderate to severe with proportionate occurrence of 16.55%, 53.5% and 29.96% respectively.The livers were swollen with rounded borders and were invariably covered with thick granular layer of dirty white to yellowish white cheesy fibrinous exudates(Plate 7).

Table 13: Gross Pathological Lesions observed in liver in Chicken

Condition	**Occurrence**		**Proportionate Distribution**					
	Total (N=6722)		**Mild**		**Moderate**		**Severe**	
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
Congestion	3792	56.41	1034	27.27	1953	51.5	805	21.23
Haemorrhage	1077	16.02	283	26.28	672	62.40	122	11.33
Fibrinous perihepatitis	1559	23.19	258	16.55	834	53.50	467	29.96
			Focal		**Multifocal**		**Diffuse**	
Necrosis	289	4.3	48	16.61	218	75.43	23	7.96
			Solitary		**Multiple**			
Haematoma	5	0.074	5	100	0	0		

Necrosis: Grossly, necrotic areas in the liver varied from focal, multifocal to diffuse with proportionate distribution of 16.61%, 75.43% and 7.96% respectively. The necrotic areas appeared as pale white, greyish or yellowish discoloured areas standing out distinctly from the surrounding normal tissue. In most of the cases the areas were multifocal. In a few cases either focal or diffuse areas of necrosis were also observed on the surface of liver. Necrotic areas were usually surrounded by a brighter/reddish zone. Necrotic lesions were mostly associated with areas of haemorrhage. Focal necrotic areas were often associated with the presence of petechiae at the periphery of the liver(Plate 7).

Haematoma: In all the cases the haematomas occurred singly irrespective of their location and appeared as a large solitary blood filled elongated sac like structure on the outer boarder of the left lobe of the liver mainly. In all the cases on cut section, large blood clot exuded from the sac(Plate 7).

4.2.2.2. Histopathological Lesions observed in Liver in Chicken

Histomorphologically, congestion of liver was generally characterized by engorgement of blood vessels including sinusoids. On the basis of severity, congestion was scored as mild with low grade or focal vascular engorgement, moderate involving wider areas, and severe with wide spread engorgement of veins and sinusoids resulting in their dilation. Generally together with congestion hepatocytes appeared swollen and their corners were rounded without any inflammatory reaction around the site. In few cases oedematous fluid separating the hepatic cells was also observed. In few cases atrophy of hepatic cells were also evident.

Histologically, haemorrhage was characterized by extravasation of large amount of erythrocytes in to the liver parenchyma. The areas varied in size and appeared either single involving small areas or multiple involving areas. However most of the cases hemorrhagic areas were multifocal and involved whole of the liver parenchyma. At places the areas of haemorrhage completely replaced the

hepatocytes. Haemorrhage was mostly prominent around the central vein. The hepatocytes in the area adjacent to haemorrhage revealed generally degenerative changes characterized by cellular swelling with hazy cytoplasm, nuclear pyknosis, karyorhexsis and karyolysis. At places binucleated hepatocytes were also evident. Haematoma was characterized by extravasation of large amount of erythrocytes replacing large volume of liver parenchyma.

Vacuolar change was noted with individual hepatic cells revealing presence of numerous small vacuoles which appeared clear spaces within an eosinophilic mesh with haematoxylin and eosin.

Hepatosis was characterized by swollen hepatocytes. Cytoplasm of hepatocytes had a granular appearance with increased eosinophilia. Disruption and disorientation of hepatocytes was generally evident in most of the cases. At places hepatocytes appeared rounded. Frequently sinusoids were compressed. Degeneration was especially marked in the perivascular region which stood out distinctly from the adjoining areas. In some cases cytoplasm revealed small clear vacuoles especially in the perinuclear region.

Focal areas of hepatic necrosis, varying in size, characterised by infiltration of inflammatory cells together with degeneration and necrosis of hepatocytes were noted in liver parenchyma. Large areas of coagulative necrosis were seen in most of the areas. Hepatic sinusoids were generally congested in such areas. The necrotic hepatocytes were characterised by presence of eosinophilic cytoplasm together with nuclear changes which included pyknosis, karyorhexis, and karyolysis. These necrotic areas at places replaced the liver parenchyma completely. The necrotic areas were frequently surrounded by large number of infiltrating cells predominantly heterophils and occasionally few mononuclear cells. In a few cases fibrous tissue proliferation was also noted adjacent to these necrotic areas.

Acute hepatitis was characterized by focal to diffuse infiltration of leukocytes predominantly heterophils along with few mononuclear cells which were mostly prominent in periportal area. At places the infiltration was so severe

that the infiltrating cells completely replaced liver parenchyma. In a few cases, the leukocytes both heterophils and few mononuclear cells were present in the form of aggregates in the liver parenchyma(Plate 8A-E).

4.2.3. Pathology of Pancreas in Chicken

No pathological gross or histopathological lesions were observed in pancreas (Plate 8H).

4.2.4. Pathology of Bursa in Chicken

4.2.4.1. Gross Pathological Lesions observed in bursa in Chicken

Out of a total of 7657 chicken carcasses necropsied, gross bursal lesions were observed in 2688 birds giving a total occurrence of 35.11%. Lesions observed in bursa included varied degree of bursal swelling, mucosal thickening/oedematous, catarrhal exudation, serous exudation, congestion, hemorrhages, and bursal core with relative case prevalence of 30.02%, 76.34%, 62.98%, 3.46%, 40.88, 26.97 and 1.64%, respectively (Plate 9,10).

Bursal swelling: Bursa appeared to be variedly enlarged in size and frequently associated with congestion evident from marked discoloration on serosal surface. The proportionate occurrence of mild, moderate to severe bursal swelling was 13.63%, 44.36%, and 42.01% respectively.

Mucosal thickening: The proportionate occurrence of mild, moderate, and severe thickening of bursal mucosa was 20.61%, 40.98% and 38.40%, respectively. The bursal folds appeared oedematous often associated with congestion and mucoid exudation.

Catarrhal exudate: Grossly, catarrhal exudate appeared thin to thick stingy mucoid in nature. The proportionate occurrence of mild, moderate, and severe catarrah was 12.17%, 33.79% and 54.05%, respectively.

Serous exudate: Varying degree of serous exudation was noted with proportionate occurrence of22.58%, 35.48% and 41.94% for mild, moderate and severe cases respectively.

Table 14: Gross Pathological Lesions observed in bursa in Chicken

Condition	Case Prevalence (N=2688)	Proportionate Distribution					
		Mild		Moderate		Severe	
		No.	%	No.	%	No.	%
BURSAL SWELLING	807	110	13.63	358	44.36	339	42.01
	30.02	1.5		4.88		4.62	
MUCOSA THICKENED / OEDEMATOUS	2052	423	20.61	841	40.98	788	38.4
	76.34	5.76		11.46		10.74	
CATARRHAL EXUDATES	1693	206	12.17	572	33.79	915	54.05
	62.98	2.81		7.79		12.47	
SEROUS EXUDATE	93	21	22.58	33	35.48	39	40.63
	3.46	0.29		0.45		0.53	
CONGESTION	1099	201	18.29	322	29.3	576	52.41
	40.88	2.74		4.39		7.84	
HAEMORRHAGES	725	128	17.66	248	34.21	349	48.14
	26.97	1.74		3.38		4.75	
BURSAL CORE	44	9	20.45	14	31.82	21	47.73
	1.64	0.12		0.19		0.29	

Congestion: Grossly, congestion varied from mild, moderate to severe with the prevalence of 18.29%, 29.3% and 52.41% respectively. The mucosa appeared light to bright or dark pink in colour. In severely affected cases, congestion was evident from serosal surface.

Haemorrhages: Grossly, haemorrhages varied from mild, moderate to severe with the prevalence of 17.65%, 34.21% and 48.14%, respectively. The haemorrhages were ecchymotic and frank haemorrhages with bloody exudate were also seen. The hemorrhagic mucosa appeared reddish-brown in colour.

Bursal core: Cheesy bursal core formation was observed with varying degrees of bursal enlargement. The core formation was classified as mild, moderate and severe with proportionate occurrence of 20.45%, 31.82% and 47.73% respectively.

4.3. Occurrence and Pathology of Specific Diseases / Disease Conditions of Digestive System in Chicken

4.3.1. Occurrence and Pathology of Intussusception

Occurrence: Intussusception was observed only in 1 case in the age group of 3-4 weeks with occurrence of 0.02%.

Gross pathology: Necropsy revealed intussusception within the jejunal segment with the proximal part telescoping into the distal part which showed a marked distension below the site of intussusception with fluid contents. The mesenteric and serosal blood vessels were congested.The serosal surface of the intestine revealed multiple diffuse petechial hemorrhages and necrotic foci, predominantly, in the proximal intestinal segments. The lumen of the intestine at the vicinity of intussusception was characterized by necrotic enteritis with denuded mucosa. When the intussuscepted segments were incised, atypical triple layer pattern was noted with a portion of the upper part of the jejunum invaginating into its distal part. The lumen of invaginated portion contained hemorrhagic content amidst necrotic debris (Plate 11A).

Histopathology: Microscopic examination of transmural sections in the area of intussusception revealed triple layered intestinal wall arranged in a typical pattern viz serosa-mucosa-mucosa-serosa-serosa-mucosa-lumen. Varying degrees of

congestion, haemorrhages and necrosis were observed in different levels, but were in general more severe in the interior most layer. Section from the portion near the proximal portion i.e. near point of intussusception revealed moderate mucosal congestion and haemorrhage, necrosis of villi with denudation, but crypts were intact. The innermost mucosal layer revealed necrotic enteritis with complete destruction of mucosa and the necrotic debris forming the core within the lumen. Necrotic changes were also observed in the submucosa, muscularis and serosal layers in the telescoped portion. All the layers showed marked cellular infiltration consisting predominantly mononuclear cells admixed with scant polymorphs in the mucosa. Sections from the deeper portions revealed marked necrotic and hemorrhagic enteritis. The interior lumen was completely filled with hemorrhagic necrotic debris. Both the segments of invaginated loop i.e. inner most and middle layer, revealed complete mucosal necrosis with denudation and haemorrhage. Even the serosal surfaces were markedly congested. The mucosa of outermost layer also revealed necrosis and loss of villi but crypts appeared intact. The intestinal portions above and below the area of intussusception revealed mucous degeneration with marked acid mucopolysaccharide activity (Plate 11B-H).

4.3.2. Occurrence and Pathology of Intestinal Torsion

Occurrence - Torsion was observed only in 3 cases in the age group of 2-3 weeks with occurrence of 0.05%.

Gross pathology - The clinically affected birds showed signs of abdominal enlargement, dog-sitting posture and reluctance to move. The birds showed twisted areas in the small intestines. The twisted loop of intestine appeared severely congested or hemorrhagic and was greatly distended with gas admixed with bloody mucus. Intestinal wall appeared to be thin and transparent. The mesenteric vessels supplying to the loop of intestine were markedly congested and appeared stout and dark in colour (Plate 12A,B).

Histopathology: Microscopic examination of intestinal sections proximal and distal to the loop involved in torsion revealed vascular congestion and predominantly mononuclear infiltration with few polymorphs. Mucosa revealed degeneration and for most part necrosis of tips of villi was seen. Section from the loop involved in torsion revealed marked vascular congestion in serosal layer and congestion associated with severe haemorrhage in mucosa and submucosa. Diffuse necrosis of the mucosa including villi as well as crypts was observed. Denudation of mucosa with hemorrhagic debris was seen in the lumen. Pockets of haemorrhage separated by necrotising mucosal elements with cellular infiltration were seen (Plate 12C,D).

4.3.3. Occurrence and Pathology of Omphalitis

Occurrence – Out of a total of201 flocks screened in different age groups omphalitis was observed in 9 (4.48%)flocks. The condition was observed only in the age groups 0-1 and 1-2 weeks with flock level incidence of 24.14% and 6.89% respectively. The total mortality due to omphalitis in the 9 affected with a total flock strength of 30550 birds, was 0.30%. The per cent mortality was higher in flocks affected in 0-1 week age (0.34%) when compared with flocks affected in 1-2 week age (0.17%)(Table 15).Out of a total of 9 outbreaks 3, 4, 1, and 1 outbreaks were observed in spring, summer, autumn and winter, respectively, with seasonal flock level occurrence of 4.0%, 5.88%, 2.86%, and 4.35%, respectively (Table 16).

Table 15: Effect of age on occurrence of Omphalitis in Chicken

Age group	Total no. of flocks screened	Flocks with Omphalitis				
		Flocks affected		Total flock strength	Mortality due to Omphalitis	
	N	n	%		No.	%
0-1 week	29	7	24.14%	24150	82	0.34%
1-2 weeks	29	2	6.89%	6400	11	0.17%
2-3 weeks	81	-	-	-	-	-
3-4 weeks	47	-	-	-	-	-
4-5 weeks	15	-	-	-	-	-
Total	**201**	**9**	**4.48%**	**30550**	**93**	**0.30%**

Table 16: Effect of season on occurrence of Omphalitis in Chicken

Season	Total no. of flocks screened	Flocks with Omphalitis				
		Total no .of flocks affected		Total flock strength	Mortality due to omphalitis	
	N	n	%		No.	%
Spring	75	3	4%	12100	38	0.31%
Summer	68	4	5.88%	14450	28	0.19%
Autumn	35	1	2.86%	2000	7	0.35%
Winter	23	1	4.35%	2000	20	1%
Total	**201**	**9**	**4.48%**	**30550**	**93**	**0.3%**

Clinical Signs: Clinically, the affected chicks appeared lethargic with ruffled feathers and hurdled near the heat source. They showed disinclination towards feed and water. Their abdomen appeared to be distended and pendulous and were generally sitting on their hocks.

Gross pathology – Umbilical area appeared to be inflamed and greenish red in colour. Yolk sac of birds was distended resulting in pendulous abdomen. The sac revealed marked congestion of the superficial blood vessels and inflammation. Unabsorbed and hemorrhagic yolk sac was found to be attached to intestine through a stalk. The contents of the unabsorbed yolk sac were yellow in colour and foul smelling. In some chicks, the yolk content was caseous and inspissated. Liver was enlarged, congested or pale yellow in colour. Intestine showed congestion and catarrhal exudate in the lumen (Plate 13A,B).

Histopathology: the transmural sections of yolk sac revealed marked vascular congestion and diffuse infiltration of inflammatory cells comprising of heterophils admixed with mononuclear cells. Macrophages and few plasma cells were frequently seen. Pockets of necrotic areas consisting of individualised and necrotising cells were seen. Liver revealed sinusoidal congestion and consequent dilatation. Hepatocellular degeneration characterized by cellular swelling was consistently seen. Individualisation of hepatocytes and hepatocellular necrosis was also seen. Varying degree of inflammatory changes were seen in all parts of intestines. Enteritis was characterized by predominant mononuclear cells and few heterophils infiltrating into lamina propria. Degeneration and necrosis of mucosa with denudation of apical portions of the villi and presence of desquamated necrotic epithelium and inflammatory cells in the lumen (Plate 13C-H) was present.

4.3.4. Occurrence and Pathology of Colibacillosis

Occurrence – Out of a total of201 flocks screened in different age groups colibacillosis was observed in 39 (19.40%)flocks. The condition was observed in all the age groups. The flock level incidence was highest in age group 4-5 weeks (33.34%), followed by 2-3 weeks (27.16%), 3-4 weeks (19.15%), 0-1 week (6.90%) and 1-2 weeks (3.45%) in that order. The total flock strength of the flocks with mortality due to colibacillosis was 114820 and mortality due to colibacillosis was 1493 (1.30%). Mortality in different age groups ranged from 0.54% to 1.52%.

(Table 17).Out of a total of 39 outbreaks 17, 15, 3, and 4 outbreaks were observed in spring, summer, autumn and winter, respectively, with seasonal flock level occurrence of 22.67%, 22.06%, 8.57%, and 17.39%, respectively. Seasonal mortality due to colibacillosis was highest during autumn (3.57%) followed by winter (1.69%), summer (1.23%) and least in spring (0.81%)(Table 18).

Clinical signs: The affected birds generally had history of respiratory signs ranging from snicking to laboured breathing or gasping, and was frequently associated with diarrhoea. Clinically the birds were dull and depressed, with inappetence.

Bacterial characterization: Impression smears from liver, spleen, heart, and smears prepared from pericardial fluid, when stained with Gram's stain revealed Gram negative coco-bacillary organisms. The organisms were capsulated as evidenced by Wright's and methylene blue stain. Following culture *E. coli* was characterized by rose pink coloured colonies on Mac Conkey agar indicating fermentation of lactose and greenish colonies with metallic sheen on Eosin Methylene Blue agar. The smears prepared from culture revealed Gram-negative, short rods arranged singly or in pairs. Biochemically the isolates were positive for Indole and Methyl Red test while negative for Voges-Proskauer and Citrate Utilization test. PCR of 16S rDNA gene resulted in amplification of characteristic 585 bp confirming E. coli (Plate 15).

Table 17: Effect of age on occurrence of colibacillosis in Chicken

Age group	Total no. of flocks screened	Flocks with Colibacillosis				
		Flocks affected		Total flock strength	Mortality due to Colibacillosis	
	N	n	%		No.	%
0-1 week	29	2	6.9%	4600	25	0.54%
1-2 weeks	29	1	3.45%	2300	35	1.52%
2-3 weeks	81	22	27.16%	66030	932	1.41%
3-4 weeks	47	9	19.15%	23640	331	1.4%
4-5 weeks	15	5	33.34%	18250	170	0.93%
Total	**201**	**39**	**19.40%**	**114820**	**1493**	**1.3%**

Table 18: Effect of season on occurrence of colibacillosis in Chicken

Season	Total no. of flocks screened	Flocks with Colibacillosis				
		Total no .of flocks affected		Total flock strength	Mortality due to colibacillosis	
	N	n	%		No.	%
Spring	75	17	22.67%	49522	401	0.81%
Summer	68	15	22.06%	45780	563	1.23%
Autumn	35	3	8.57%	10588	378	3.57%
Winter	23	4	17.39%	8930	151	1.69%
Total	**201**	**39**	**19.40 %**	**114820**	**1493**	**1.3%**

Gross pathology

The carcasses were usually dark colored and dehydrated. The characteristic gross pathological finding was fibrinous polyserositis with presence of varying amounts of gelatinous fibrin layer on all the visceral organs

particularly on liver and heart. Liver appeared to be swollen with rounded edges. In most of the severe cases the fibrin layer was thick and covered whole liver giving shaggy appearance, and in heart resulted in adherence between parietal and visceral layers of pericardium giving a typical“bread and butter” appearance. Gallbladder was often distended with bile. In some cases heart revealed petechial and ecchymotic haemorrhages. Spleen frequently showed congestion and dark discolouration along with multiple necrotic foci. Kidneys were congested and hemorrhagic and in some cases nephrosis was evident. Lungs appeared to be congested and oedematous. In some cases areas of consolidation were seen.Mild to moderate congestion of trachea with varying quantities of dirty catarrhal exudate in lumen was seen. The airsacs appeared opaque and were thickened with fibrinous mass.Intestines, from serosal surface, appeared congested with dirty dark red discoloration. Mesenteric blood vessels were engorged with blood. Lumen of the intestines was invariably filled with fluid mucous contents. Mucosal surface was congested and had velvety appearance. Bursal folds were oedematous, and varied quantities of catarrhal exudate was present in the lumen (Plate14).

Histopathology

Proventriculus : Revealed necrosis of epithelium of mucosal folds, oedema, congestion and severe infiltration of inflammatory cells mainly heterophils in lamina propria and underlying sub mucosa.

Intestines : Intestines in general revealed enteritis characterized by infiltration of heterophils and lymphocytes. It was associated with necrosis desquamation of epithelial mucosa of villi. In duodenum, there was severe infiltration of leukocytes, mainly heterophils, lymphocytes and macrophages in the lamina propria and sub-mucosa.The serosal layer of the duodenum showed thickening due to severe congestion. Goblet cell hyperplasia was observed in the areas with

intact epithelium. In some cases, focal necrosis of villi and mucosal folds was also evident (Plate 16A-F).

Heart: Heart revealed mild to severe thickening of pericardium with fibrinous exudate and predominantly mononuclear infiltration admixed with heterophils. In severe cases the pericardium was excessively thickened and revealed eosinophilic necrotic areas containing heterophils in different stages of degeneration. Leukocytic infiltration consisting of admixture of heterophils and mononuclear cells was also observed in epicardium and myocardium. In some cases eosinophils, macrophages, and plasma cells were seen. In some cases degeneration characterized by eosinophilia and cytoplasmic vacuolation, and disruption of myocardial fibres was also seen. Blood vascular congestion was invariably seen (Plate 16G-H).

Liver: Vascular congestion especially congestion and dilatation of hepatic sinusoids and hepatocellular degeneration with cellular swelling was a consistent feature. Frequently vacuolar changes and nuclear pyknosis were seen. There was hyperplasia of Kupffer cells. Focal areas of necrosis were seen in some cases. Focal hepatitis with mononuclear cell infiltration was seen especially near portal area either with or without hepatocellular necrosis. Necrotic areas were evident with presence of macrophages, lymphoid cells, plasma cells and other inflammatory cells. In some cases, severe inflammatory changes were observed near the blood vessels. Variable degree of thickening of Glisson's capsule with fibrinous perihepatitis was seen. Fibrin was deposited around blood vessels and perivascular fibrosis was also seen at few places which was demonstrated by Masson's trichome stain. Frequently capsule revealed focal to diffuse areas of necrosis with heterophilic infiltration associated with basophilic areas suggestive of bacterial colonies. Gram negative bacteria could be demonstrated by Brown and Brenn stain (Plate17A-E).

Trachea, Lungs and air sacs: Trachea revealed varying degrees of congestion, degeneration and denudation of mucosa and frequently submucosal oedema and leukocytic infiltration. Lungs revealed congestion of blood vessels in parabronchi and interstitial septa. Frequently haemorrhages were seen. Invariably, heterophil and mononuclear cell infiltration was seen in parabronchi, mesobronchi and metabronchi. In severe cases bronchopneumonia with exudate consisting predominantly of necrotic cell debris and heterophils in prabronchial lumen was seen. Bacteria could be demonstrated in the tissues following staining with Brown and Brenn method. Air sacs were diffusely thickened with predominantly heterophilic infiltration.

Kidneys: Kidneys revealed varying degrees of congestion, haemorrhage and mild degeneration of tubular epithelium. Focal infiltration of leucocytes consisting of admixture of mononuclear cells and heterophils was seen. Increased cellularity of glomeruli was additionally noted.

Pancreas: Pancreas revealed various degree of congestion and hemorrhages. Hyper-activity of the cells along the ductal epithelium was evident.

Spleen: Spleen revealed varying degrees of congestion and haemorrhage. Frequently thickening of blood vessels and focal heterophil infiltration was seen. Depletion of lymphocytes was observed in the white pulp which was accompanied by reticular cell proliferation. Besides, multifocal necrosis was also seen.

Thymus: Thymus revealed mild to severe congestion together with mild depletion of lymphocytes and also, discrete eosinophilic necrotic areas in the thymic follicles.

Bursa of Fabricius : Bursa of Fabricius revealed haemorrhages, and mild to moderate depletion of lymphocytes in bursal follicles. The inter-follicular space

appeared to be thickened due to fibroblast proliferation and mononuclear cells infiltration (Plate17 H).

4.3.5. Occurrence and Pathology of Salmonellosis

Occurrence – Out of a total of201 flocks screened in different age groups salmonellosis was observed in 17(8.46%)flocks. The condition was observed in the 0 to 3 weeks age. The flock level incidence was highest in age group 0-1 week (34.48%), followed by 1-2 weeks (20.69%), and2-3 weeks (1.23%%) in that order. The total flock strength of the flocks with mortality due to salmonellosis was 60940 and mortality due to salmonellosis was 947 (1.55%). Mortality in different age groups ranged from 0.58% to 2.06%. (Table 19). Out of a total of 17 outbreaks 11, 3, 2, and 1 outbreak were observed in spring, summer, autumn and winter, respectively, with seasonal flock level occurrence of 14.67%, 4.41%, 5.71%, and 4.35%, respectively. Seasonal mortality due to salmonellosis was highest during autumn (3.22%) followed by spring (1.50%), winter (1.10%) and least in summer (1.02%) (Table 20).

Clinical signs: Clinically the birds were dull and depressed with inappetence. Frequently birds had loose droppings with pasty vent and sat with eyes closed.

Table 19: Effect of age on occurrence of salmonellosis in Chicken

Age group	Total no. of flocks screened	Flocks with Salmonellosis				
		Flocks affected		Total flock strength	Mortality due to Salmonellosis	
	N	n	%		No.	%
0-1 week	29	10	34.48%	32540	671	2.06%
1-2 weeks	29	6	20.69%	23900	250	1.05%
2-3 weeks	81	1	1.23%	4500	26	0.58%
3-4 weeks	47	-	-	-	-	-
4-5 weeks	15	-	-	-	-	-
Total	**201**	**17**	**8.46%**	**60940**	**947**	**1.55%**

Table 20: Effect of season on occurrence of salmonellosis in Chicken

Season	Total no. of flocks screened	Flocks with Salmonellosis				
		Total no .of flocks affected		Total flock strength	Mortality due to Salmonellosis	
	N	n	%		No.	%
Spring	75	11	14.67%	41180	619	1.5%
Summer	68	3	4.41%	12100	124	1.02%
Autumn	35	2	5.71%	5660	182	3.22%
Winter	23	1	4.35%	2000	22	1.1%
Total	**201**	**17**	**8.46%**	**60940**	**947**	**1.55%**

Bacterial characterization: Impression smears from liver and spleen, when stained with Gram's stain revealed Gram negative coco-bacillary organisms. Following culture *Salmonella* was characterized by pink, small circular and smooth colonies on Brilliant green agar (BGA) and red colonies with black centres on Xylose lysine desoxycholate agar (XLDA) plates. The smears prepared from culture revealed Gram-negative, rods shaped organisms. Biochemically the isolates were negative for Indole and Methyl Red test while as positive for Voges-Proskauer and Citrate Utilization test. PCR of 16S rDNA gene resulted in amplification of characteristic 574 bp confirming *Salmonella* sp.(Plate18E-H).

Gross Pathology: Liver was congested and swollen and invariably showed bronze discolouration. Frequently liver revealed mottling with multiple necrotic foci. Lungs were mildly congested. Intestines, in general were congested and invariably revealed hemorrhagic areas in different segments(Plate 18 A-D).

Histopathology(Plate19):

Liver: Severe congestion and hemorrhages along with hepatitis characterized by leukocytic infiltration at perivascular areas were observed. Multiple necrotic foci were noticed with kupffer cell hyperplasia in hepatic parenchyma. At places, degeneration of hepatocytes, with focal aggregation of heterophils, lymphocytes and macrophages were present around necrotised areas. Hydropic vacuolation in cytoplasm of hepatocytes along with infiltrated leucocytes was also observed in a few cases.

Pancreas: Congestion, hemorrhages along with mild degenerative changes and leukocytic infiltration in acinar cells and interlobular connective tissue were seen.

Spleen: Congestion and hemorrhages were evident in some cases along with presence of multiple necrotic foci on the surface. There was severe depletion of lymphoid cells in white pulp along with reticuloendothelial cell hyperplasia.

Heart: Severe non suppurative myocarditis with leukocytic infiltration mainly of heterophils, lymphocytes and macrophages was evident in some cases. Severe degeneration and fragmentation of myocardial muscle fibres was exhibited in most of the cases with leukocytic infiltration**.** In addition, fibrinous pericarditis with infiltration of heterophils, lymphocytes and macrophage was also observed in some cases.

Kidneys: Glomeruli were contracted and there were degenerative changes in renal tubules. Infiltration of the mononuclear cells was less evident and degenerative changes in the tubules were noticed. Cloudy swelling of tubular epithelial cells along with vacuolation was also prominently present.

Bursa of Fabricius: There was mild depletion of lymphoid tissue in bursal follicles along with inter-follicular fibrosis. Appearance of reticuloendothelial cells was also observed in degenerating follicles. Necrosis of some of the follicles along with infiltrated leucocytes.

Proventriculus: Proventriculus revealed degeneration of proventricular glands and denudation of the apical epithelium into the lumen. In a few cases leukocytic infiltration was seen.

Intestine: Desquamation of mucosal epithelium resulting into denuded villi was observed and lumen was filled with necrotic mass. Secretory glands were atrophied at some places due to severe infiltration of heterophils and mononuclear cells and presence of exudate. Congestion and hemorrhages were common in the lamina propria but in some cases, it was extending upto the muscularis and serosal layers along with infiltrated leucocytes.Goblet cell hyperplasia and focal fibroblastic connective tissue proliferation between the glands was present in many cases.

4.3.6. Occurrence and Pathology of Infectious Bursal Disease

Occurrence – Out of a total of201 flocks screened in different age groups IBD was observed in 24(11.94%)flocks. The condition was observed in the 1 to 4 weeks age. The flock level incidence was highest in age group 2-3 week (17.28%), followed by 3-4 weeks (14.89%), and 1-2 weeks (10.34%%) in that order. The total flock strength of the flocks with mortality due to IBD was 75100 and mortality due to IBD was 927 (1.23%). Mortality in different age groups ranged from 0.68% to 1.47% (Table 21). Out of a total of 24 outbreaks 6 each were observed in spring, summer, autumn and winter, with seasonal flock level occurrence of 8.0%, 8.82%, 17.14%, and 26.09%, respectively. Seasonal mortality due to IBD was highest during winter (3.05%) followed by summer (0.99%), spring (0.76%) and least in autumn (0.67%) (Table 22).

Clinical signs: Affected birds appeared dull, depressed, anorectic had ruffled feathers and were suffering from yellowish white or greenish yellow diarrhoea.

Gross Pathology: Darkening and discolouration of thigh and breast muscles along with presence of paint brush like haemorrhages was noticed in the affected birds. Bursa of Fabricius was observed to be enlarged, oedematous and had haemorrhages on its serosal and mucosal surfaces. In some cases catarrhal or creamy exudate was present in the lumen whereas in some cases, lumen was filled with cheesy material forming the core. Kidneys were congested and swollen. Intestines showed enteritis characterised by congestion and oedema especially in duodenum (Plate 20).

Histopathology (Plate21-22):

Liver: Liver showed moderate congestion, and degeneration of hepatocytes. Areas of coagulative necrosis and periportal infiltration of mononuclear cells were consistently seen.

Pancreas: Pancreas showed mild vascular congestion.

Table 21: Effect of age on occurrence of Infectious Bursal Disease in Chicken

Age group	Total no. of flocks screened	Flocks with IBD				
		Flocks affected		Total flock strength	Mortality due to IBD	
	N	n	%		No.	%
0-1 week	29	-	-	-	-	-
1-2 weeks	29	3	10.34%	5000	34	0.68%
2-3 weeks	81	14	17.28%	49700	730	1.47%
3-4 weeks	47	7	14.89%	20400	163	0.8%
4-5 weeks	15	-	-	-	-	-
Total	201	24	**11.94%**	**75100**	**927**	**1.23%**

Table 22: Effect of season on occurrence of Infectious Bursal Disease in Chicken

Season	Total no. of flocks screened	Flocks with IBD				
		Total no .of flocks affected		Total flock strength	Mortality due to IBD	
	N	n	%		No.	%
Spring	75	6	8%	22500	172	0.76%
Summer	68	6	8.82%	18000	179	0.99%
Autumn	35	6	17.14%	20100	134	0.67%
Winter	23	6	26.09%	14500	442	3.05%
Total	**201**	**24**	**11.94%**	**75100**	**927**	**1.23%**

Spleen: Microscopically the spleen revealed marked congestion.Severe lymphoid depletion and multifocal areas of necrosis were prominent changes. In severely

affected cases the areas of necrosis had coalesced to form a large coagulated necrotic mass. Reticuloendothelial cell hyperplasia was observed with infiltration of the infiltratory cells in the adenoid sheath. A thickening of arteries and arterioles with thickening of the splenic capsule and vascular congestion was observed in several cases.

Bursa of Fabricius: Histopathological evaluation of bursa of Fabricius showed marked bursal lymphoid necrosis and depletion both in clinical and subclinical cases. Severe lymphoid necrosis and depletion was observed both in the medulla and cortex of the follicles. Severe oedema with heavy infiltration of heterophils and macrophages was observed. The lymphoid cells in the cortex and medulla were severely reduced with vacuolation and cysts, which contained clear to pinkish fluids. The medulla was devoid of lymphoid elements with degeneration of inter-follicular connective tissue. There was degeneration and loss of columnar epithelial tuft. Some of the cases revealed inter-follicular and intra-follicular oedema, inter-follicular connective tissue proliferation and congestion. In severe cases Bursal tissue revealed necrosis of lymphoid elements with pyknotic nuclei with presence of both degenerating and normal heterophils and severe fibrosis.

Proventriculus: Degeneration of proventricular glands along with congestion was observed. The lumen was filled with necrosed denuded mass.

Intestines: Enteritis was seen in most of the cases characterised by congestion, haemorrhages and necrosis of villi with infiltration of inflammatory cells mainly lymphocytes and a few heterophils in lamina propria.

4.3.7. Occurrence and Pathology of Newcastle Disease

Occurrence – Out of a total of201 flocks screened in different age groups ND was observed in 5(2.49%)flocks. The condition was observed in the 2 to 5 weeks age. Out of 5 out breaks 3 were observed in age group 4-5 weeks. The total flock strength of the flocks with mortality due to IBD was 23500 and mortality due to

ND was 547 (2.32%). Mortality in different age groups ranged from 0.60% to 2.85% (Table 23). All the 5 outbreaks were observed in summer with seasonal flock level occurrence of 76.35%. Seasonal mortality due to ND was 2.33% (Table 24).

Gross pathology - Post mortem examination revealed congestion and hemorrhages in trachea, lung, liver and heart. Characteristic glandular haemorrhages were seen in proventriculus. Intestines revealed marked congestion, and haemorrhages in the caecal tonsils. Button like ulcers were observed in different segments of intestines including duodenum, jejunum, and ileum. Ulcers were occasionally seen in caecal tonsils. Spleen was enlarged and mottled. Multiple petechiae were seen in the epicardium, surface of the proventriculus fat and serosal surface of peritoneum (Plate 23).

Histopathology (Plate 24-25):

Liver: Severe congestion and foci of inflammatory cells were observed in the hepatic parenchyma. Hepatocytes showed mild to moderate fatty change.

Spleen: Congestion along with lymphoid depletion and multi-focal necrosis, were observed in spleen. At places apoptosis of lymphocytes was evident.

Kidney: Tubular degeneration and necrosis associated with mild interstitial haemorrhage was a consistent feature.

Lungs: Lungs revealed severe congestion, hemorrhages, and inflammatory cell infiltration within the lung parenchyma.

Bursa: Bursal revealed moderate to marked lymphoid depletion. Frequently necrosis was observed within the follicles.

Proventriculus - Proventriculus showed hemorrhages and necrotising lesions. Severe haemorrhages were seen within the mucosal ridges of the proventriculus. Individualisation of the proventricular cells as also observed.

Table 23: Effect of age on occurrence of Newcastle Disease in Chicken

Age group	Total no. of flocks screened	Flocks with ND				
		Flocks affected		Total flock strength	Mortality due to ND	
	N	n	%		No.	%
0-1 week	29	-	-	-	-	-
1-2 weeks	29	-	-	-	-	-
2-3 weeks	81	1	1.23%	2000	12	0.6%
3-4 weeks	47	1	2.13%	4500	50	1.11%
4-5 weeks	15	3	20%	17000	485	2.85%
Total	**201**	**5**	**2.49%**	**23500**	**547**	**2.32%**

Table 24: Effect of season on occurrence of Newcastle Disease in Chicken

Season	Total no. of flocks screened	Flocks with ND				
		Total no .of flocks affected		Total flock strength	Mortality due to ND	
	N	n	%		No.	%
Spring	75	-	-	-	-	-
Summer	68	5	7.35%	23500	547	2.33%
Autumn	35	-	-	-	-	-
Winter	23	-	-	-	-	-
Total	**201**	**5**	**2.49%**	**23500**	**547**	**2.33%**

Intestines - Degeneration and sloughing of epithelial cells covering tips of villi, and mononuclear infiltrations in the lamina propria were observed. Severe ulcerative lesions involving whole thickness of intestines with necrosis of the ulcerative lesion was seen.

4.3.8. Occurrence and Pathology of suspected Low Pathogenic Avian Influenza

Occurrence – The suspected case of Avian Influenza was observed in the age group of 3-4 weeks with mortality of 0.02%.

Gross pathology - The affected bird showed the presence of oedema, swelling and hemorrhagic spots on the head and leg shanks, and necrotic foci and cyanosis of the comb and wattles. Hemorrhagic spots were also observed on proventriculus. Severe swelling was observed in kidneys, spleen and pancreas with the presence of diversified hemorrhagic and necrotic foci in the latter (Plate 26).

Histopathology (Plate 27-29):

Liver: Hepatocytic necrosis was markedly evident giving a "honey comb" appearance to the liver, besides infiltration of heterophils, lymphocytes and macrophages in the sinusoids and portal areas. Additionally, congestion of the hepatic sinusoids and parenchymatous haemorrhage and hemosiderosis were observed, together with desquamation of the bile duct epithelium.

Pancreas: Mild Congestion in the pancreas was observed.

Spleen: There was severe diffuse lymphoid necrosis and depletion. Multifocal areas of coagulative necrosis, congestion and haemorrhages, and hyperplasia of phagocytic cells were also seen.

Trachea: There was desquamation of the lining epithelium with extensive haemorrhage. Lamina propria revealed congestion and infiltration of lymphocytes, macrophages, plasma cells and heterophils.

Lungs: There was severe congestion in the para-bronchial region. Also, interstitial pneumonia with thickening of the interlobular septa with inflammatory

cells infiltration and oedema was seen. The cytoplasm of the pneumocyte of the air capillaries was laden with hemosidrin pigment.

Heart: There was severe necrosis and destruction of the myocardium with high infiltration of heterophils and macrophages in between the destructed myocytes and in the epicardium. Besides vascular congestion and mononuclear cell infiltration were noted.

Kidney: Severe congestion of glomeruli of kidney was observed along with tubular degeneration and necrosis. Additionally individualisation of cells with pyknosis and chromatolysis of the nucleus was frequently observed. There was occasional presence of granular casts which was demonstrated by PAS+ive reaction.

Bursa: Lymphocyte depletion along with necrosis and apoptosis of lymphoid elements and hyperplasia of macrophages was observed.

Proventriculus: Proventriculus showed hemorrhages and necrotising lesions along with congestion.

Intestines: Duodenum showed degeneration and sloughing of villi. Lymphohistiocytic infiltration in submucosa.Widespread epithelial necrosis was also observed. Ileum showed marked infiltration of mononuclear cells.

4.3.9. Occurrence and Pathology of Aspergillosis

Occurrence: Out of a total of201 flocks screened in different age groups aspergillosis was observed in 3(1.49%)flocks. The condition was observed in the 1 to 2 weeks age group. The total flock strength of the flocks with mortality due to aspergillosis was 9700 and mortality due to aspergillosis was 36 (0.37%) (Table 25).Out of 3 outbreaks 2 were observed in summer and 1 in autumn with seasonal mortality of 0.36% and 0.4%respectively (Table 26).

Table 25: Effect of age on occurrence of aspergillosis in Chicken

Age group	Total no. of flocks screened	Flocks with Aspergillosis				
		Flocks affected		Total flock strength	Mortality due to aspergillosis	
	N	n	%		No.	%
0-1 week	29	1	3.45%	3000	12	0.4%
1-2 weeks	29	2	6.89%	6700	24	0.36%
2-3 weeks	81	-	-	-	-	-
3-4 weeks	47	-	-	-	-	-
4-5 weeks	15	-	-	-	-	-
Total	**201**	**3**	**1.49%**	**9700**	**36**	**0.37%**

Table 26: Effect of season on occurrence of Aspergillosis in Chicken

Season	Total no. of flocks screened	Flocks with aspergillosis				
		Total no .of flocks affected		Total flock strength	Mortality due to aspergillosis	
	N	n	%		No.	%
Spring	75	-	-	-	-	-
Summer	68	2	2.94%	6700	24	0.36%
Autumn	35	1	2.86%	3000	12	0.4%
Winter	23	-	-	-	-	-
Total	**201**	**3**	**1.49%**	**9700**	**36**	**0.37%**

Mycological examination: Wet mount preparations from crushed fungal lesions revealed fungal hyphae. Whitish-grey colonies revealed *Aspergillus fumigatus* were seen following culture on Sabouraud Dextrose Agar (SDA). Characteristic morphology of *Aspergillus fumigatus* was confirmed in lactophenol blue stained wet mount preparations (Plate 30 E-G).

Gross pathology - Pulmonary lesions characterized by multiple hard cream to yellow colored, circumscribed plaques a few mm to several cm in diameter were seen throughout the lungs surface, inside the lungs, scattered in ventral surface of sternum and air passages. The plaques were also found in the air sacs, liver and intestines. Lung parenchyma was consolidated and single or multiple necrotic areas are visible on cut surfaces of lungs (Plate 30 A-D).

Histopathology:

Lungs: The microscopical examination showed vascular congestion and peri-vascular oedema. The normal architecture of the lung was replaced by disseminated granulomatous foci. The centre of the granulomatous foci contained necrotic cellular debris along with fungal hyphae, surrounded by rims of heterophils, lymphocytes, macrophages and multi-nucleated giant cells (Plate31).

Liver: The normal architecture of the liver was replaced by disseminated granulomatous foci. Fungal septate hyphae were demonstrated in the inflammatory necrotic mass.

Intestines: Intestines revealed varying degrees of congestion and mild degeneration and denudation of mucosal epithelium. Fungal granulomas were discretely attached to serosal surface without involving intestinal wall.

4.3.10. Occurrence and Pathology of coccidiosis

Occurrence: Coccidiosis was observed in 13 flocks and was mostly prevalent in the age group of 3-4 weeks. An overall flock level occurrence of 6.47% and mortality of 0.73% was observed.

Table 27: Effect of age on occurrence of coccidiosis in Chicken

Age group	Total no. of flocks screened	Flocks with coccidiosis				
		Flocks affected		Total flock strength	Mortality due to coccidiosis	
	N	n	%		No.	%
0-1 week	29	-	-	-	-	-
1-2 weeks	29	-	-	-	-	-
2-3 weeks	81	6	7.41%	15550	99	0.64%
3-4 weeks	47	6	12.77%	17700	143	0.81%
4-5 weeks	15	1	6.67%	2000	15	0.75%
Total	**201**	**13**	**6.47%**	**35250**	**257**	**0.73%**

Laboratory Diagnosis:

Scrapings taken from the mucosa of intestine from suspected cases followed by direct examination of wet mount smears under microscope, revealed presence of *Eimeria* oocysts. Following sporulation each oocyst contained four sporocysts and each sporocyst two sporozoites.Most of the cases of caecal coccidiosis had a co-incidence with necrotic enteritis which was confirmed by isolation and PCR confirmation of *Clostridium perfringens*. PCR amplification of cpa gene resulted in characteristic 324 bp product. Scanning Electron microscopy **(**SEM) revealed the presence of coccidian oocysts within the intestinal lumen along with severe denudation of the intestinal villi (Plate 32 G,H; 33 A-C).

Table 28: Effect of season on occurrence of coccidiosis in Chicken

Season	Total no. of flocks screened	Total no .of flocks affected		Total flock strength	Mortality due to Coccidiosis	
	N	n	%		No.	%
Spring	75	4	5.33%	7150	79	1.1%
Summer	68	6	8.82%	19000	136	0.72%
Autumn	35	2	5.71%	7000	24	0.34%
Winter	23	1	4.35%	2100	18	0.86%
	201	**13**	**6.47%**	**35250**	**257**	**0.73%**

Gross pathology: The birds showed lesions of diarrhoea and hemorrhagic enteritis.The vent was found soiled with faeces. Ballooning of intestines was observed. Petechial hemorrhages on caeca were another common feature observed during postmortem examination of such birds. Mucosa of the caeca was thickened and distended with caecal core and the lumen was filled with fluid, blood and tissue debris. Liver, breast muscles, eyes and bursa also appeared pale due to anaemia (Plate 32 A-F; 33 D-G).

Histopathology(Plate34-37):

Liver: Degenerative and necrotic changes were observed in the liver along with some areas of congestion and hemorrhages. Peri-portal congestion along with infiltration of inflammatory cells was evident.

Spleen: There was severe diffuse lymphoid necrosis and depletion. In some cases multifocal areas of coagulative necrosis, congestion and haemorrhages, lymphoid depletion and hyperplasia of phagocytic cells were observed.

Bursa: Congestion along with lymphoid depletion and mononuclear cells infiltration was mostly observed. Thickening of the inter-follicular space due to fibroblast proliferation was observed and demonstrated by Masson's trichome stain.

Intestines: Intestinal section typically showed severe hemorrhagic caecal core formation. Presence of developing schizonts and oocysts in villi crypts and sub mucosal glandular epithelium caused severe degeneration and necrosis of the epithelium and secretory glands. Hyperactivity of the goblet cells was observed with increased acid mucopolysaccharide activity. Desquamated and necrotised cells admixed with coccidian oocyst were present in the lumen as debris. There was severe infiltration of macrophages, plasma cells, lymphoid cells along with heterophils in the lamina propria. Severe congestion of sub-mucosal blood vessels, fibrosis of sub-mucosal glandular epithelium harbouring coccidian life-cycle stages and also shedding of oocysts in the glandular lumen was observed.

4.3.11. Occurrence and Pathology of Gout

Occurrence – Out of a total of201 flocks screened in different age groups gout was observed in 13 (6.47%) flocks. The condition was observed in the 1 to 4 weeks age. The flock level incidence was highest in age group 1-2 week (24.14%). The total flock strength of the flocks with mortality due to gout was 51100 and mortality due to gout was 268 (0.52%). Mortality in different age groups ranged from 0.22% to 3.34% (Table 29). Out of a total of 13 outbreaks 2, 3, 5, and 3 outbreaks were observed in spring, summer, autumn and winter respectively, with seasonal flock level occurrence of 2.67%, 4.41%, 14.29%, and 13.04%, respectively. Seasonal mortality due to gout was highest during winter (1.47%) (Table 30).

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Table 29: Effect of age on occurrence of Gout in Chicken

Age group	Total no. of flocks screened	Flocks with gout				
		Flocks affected		Total flock strength	Mortality due to gout	
	N	n	%		No.	%
0-1 week	29	1	3.45%	3000	100	3.34%
1-2 weeks	29	7	24.14%	31000	129	0.42%
2-3 weeks	81	4	4.94%	15100	33	0.22%
3-4 weeks	47	1	2.13%	2000	6	0.3%
4-5 weeks	15	-	-	-	-	-
Total	**201**	**13**	**6.47%**	**51100**	**268**	**0.52%**

Table 30: Effect of season on occurrence of Gout in Chicken

Season	Total no. of flocks screened	Flocks with gout				
		Total no .of flocks affected		Total flock strength	Mortality due to gout	
	N	n	%		No.	%
Spring	75	2	2.67%	5700	21	0.37%
Summer	68	3	4.41%	15500	41	0.26%
Autumn	35	5	14.29%	17900	30	0.17%
Winter	23	3	13.04%	12000	176	1.47%
Total	**201**	**13**	**6.47%**	**51100**	**268**	**0.52%**

Gross pathology

The kidneys and ureters were extensively distended and puffed-up with urates. Whitish chalky deposits of uric acid were present on other visceral organs like liver and heart. Highly congested and swollen kidneys, together with presence of

ecchymotic hemorrhages on the surface were observed in few cases. There was massive deposition of white chalky urates on heart (Plate 38 A-E).

Histopathology (Plate 38F-H; 39 A-C):

Liver: The hepatic vessels were severely congested and peri-portal areas were generally infiltrated with leucocytes. Fatty changes were also evident in peri-portal areas. The diffuse necrotic areas involving most parts of hepatic lobules were seen. These necrotic lesions revealed large amounts of bluish stained amorphous material in their centre representing presence of urates. Sinusoids were mostly congested. Disintegration of hepatic architecture and infiltration with heterophils was also noted.

Kidneys: Tubules were degenerated and filled with urates. Medullary tubules were dilated and filled with eosinophilic masses. Urate crystals appeared as pink colour radiating amorphous masses in the form of tophi. Also, there were multiple necrotic areas in the cortex. At some places epithelial hyperplasia and obliteration of tubules was also evident.

Spleen: Congestion, haemorrhage with lymphoid tissue depletion and reticular cells hyperplasia was observed at certain places in white pulp. Uric acid tophi were present in all over spleen.

Intestines: Intestines of birds revealed haemorrhage & congestion. Villi were degenerated and lumen was filled with cellular debris.In some cases, lamina propria was infiltrated with leucocytes and goblet cells generally revealed hyperplasia .

4.3.12. Occurrence and Pathology of Ascites

Occurrence – Out of 201 flocks, ascites was observed in 12 flocks (5.97%) in the age group 1 to 4 weeks. Highest number of outbreaks were observed in age group 3-4 weeks (14.89%). The flock strength of affected flocks was 35700 with 318

(0.89%) deaths due to ascites. Mortality among different age groups ranged from 0.74% to 1.03% (Table 31). Seasonal flock level occurrence was highest in spring (10.67%) where as mortality was higher during summer (2.11%) and autumn (2.15%) (Table 32)

Gross pathology: Straw coloured transudate occupied all chambers of coelomic cavity. In most of the cases, fluid was coagulated forming gelatinous masses, loose or adhering to viscera. Marked congestion of intestines was a consistent feature. Often diffuse haemorrhages were seen in duodenum and jejunum. Frequently mucosa presented a velvety appearance. Varying degrees of congestion and haemorrhages was seen in liver which was associated with swelling of liver with rounding of edges (Plate 40).

Histopathology (Plate 41) :

Liver: Liver revealed moderate to severe congestion of sinusoids and central vein, and hepatocellular degeneration with vacuolar changes. Focal mononuclear infiltration was often seen especially in the perivascular region. Occasionally diffuse mononuclear cell infiltration was evident. Kupffer cell proliferation was noted which was more prominent in sub-capsular region.

Lungs: Lungs showed congestion of interstitial vessels and blood capillaries, focal haemorrhages and interstitial oedema. Pulmonary arterioles appeared thickened with medial cystic dilatations. Frequently perivascular leukocytic infiltration, consisting of heterophils admixed with mononuclear cells, was observed.

Table 31: Effect of age on occurrence of ascites in Chicken

Age group	Total no. of flocks screened	Flocks with ascites				
		Flocks affected		Total flock strength	Mortality due to ascites	
	N	n	%		No.	%
0-1 week	29	-	-	-	-	-
1-2 weeks	29	2	6.89%	4300	32	0.74%
2-3 weeks	81	3	3.7%	6200	64	1.03%
3-4 weeks	47	7	14.89%	25200	222	0.88%
4-5 weeks	15	-	-	-	-	-
Total	**201**	**12**	**5.97%**	**35700**	**318**	**0.89%**

Table 32: Effect of season on occurrence of ascites in Chicken

Season	Total no. of flocks screened	Flocks with ascites				
		Total no .of flocks affected		Total flock strength	Mortality due to ascites	
	N	n	%		No.	%
Spring	75	8	10.67%	25700	166	0.65%
Summer	68	2	2.94%	3500	74	2.11%
Autumn	35	1	2.86%	2000	43	2.15%
Winter	23	1	4.35%	4500	35	0.78%
Total	**201**	**12**	**5.97%**	**35700**	**318**	**0.89%**

Kidneys: Kidneys revealed marked vascular congestion and haemorrhages, degeneration of tubular epithelium and focal nephritis.

Pancreas: In general, pancreas did not reveal any significant histomorphological alterations in either exocrine glands or islets of Langerhans, except occasional mild to moderate vascular congestion.

Spleen: Spleen showed mild to severe vascular congestion, moderate to marked depletion of lymphoid cells and multifocal areas of necrosis.

Bursa: Bursa revealed moderate to severe vascular congestion, haemorrhages, interstitial oedema and moderate to severe depletion of lymphoid cells

Intestines - Proventriculus revealed mild to severe vascular congestion and oedema in submucosa and mucosa. Occasionally mononuclear cell and heterophil infiltration was evident in mucosa. Intestines revealed marked vascular congestion and extensive oedema in mucosa and submucosa. Frequently, mucous degeneration of goblet cells and infiltration of heterophils and mononuclear cells in lamina propria were seen.

Chapter – 5

DISCUSSION

Occurrence of Gastrointestinal Tract Affections in Chicken

In present study a total of 7657 broiler chicken of different age groups from a total of 201outbreaks were necropsied and examined for presence of lesions in GIT, and associated structures including bursa of Fabricius, liver, and pancreas. Out of a total of 7657 chicken carcasses necropsied, gross GIT lesions were observed in 7339 (95.85%) birds including 83.64% with some specific disease condition and 12.21% solitary GIT lesions.GIT is influenced by a multitude of direct and indirect factors which not only modulate its first line of defence but also have structural and functional implications. It is a major site of potential exposure to pathogens and irritants (Reynolds, 2003; Yegani and Korver, 2008). In a study done for system wise mortality, the digestive system disorders were found to be the main cause of poultry mortality (30.88%) accounting for immense economic losses (Hooda, 2009). The etiology of disturbed health vis-à-vis digestive system is complex involving abiotic and biotic agents (Reynolds, 2003). Several pathogens (viruses, bacteria and parasites) are incriminated as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms (multi-causal) or with non-infectious causes such as feed and /or management related factors (Hafez, 2011). Under field conditions, however, it is difficult to determine whether the true cause of enteric disorders in poultry is of infectious or non-infectious origin. Besides the pathogens and irritants directly affecting digestive system, it may be secondarily involved in systemic diseases.

Age had significant effect on GIT affections with highest proportionate mortality in age group 2-3 weeks (39.14%) followed by 0-1 week (21.46%), 3-4 weeks (18.11%), 4-5 weeks (12.21%) and 1-2 weeks (9.08%). Age, also, showed

a significant effect on proportionate distribution of overall, solitary or disease associated GIT affections. In poultry age is one of the important determinants for susceptibility to various disease conditions. It corroborates well with the observation that majority cases were associated with specific disease conditions. Minor shifts in intestinal microbial balance has been implicated for a wide continuum of disease presentations ranging from severe to mild clinical, subclinical or asymptotic (Roberts *et al.,* 2015). Further exposures to various stress factors especially in terms of probiotics, feed additives, supplements, medications etc. during different periods of rearing modulate the mortality pattern and associated conditions. Generally the mortality observed is lower during early brooding period except for occasional early spike due to transportation stress, environmental stress and sudden death syndrome (Aengwanich and Simaraks, 2004; Behra *et al.*, 2009).Early access to feeding or its deprivation greatly modulates morphology and functional capacity of GIT which in turn influences the pathobiome structure and overall gut health and disease conditions (Bigot *et al.*, 2003; Maiorka *et al*., 2003; Potturi *et al*., 2005)

The proportionate mortality was significantly higher in spring (38.03%) followed summer (33.05%), autumn (15.05%), and winter (13.87%). While season has been found as one of the most important epidemiological factor with respect to diseases like respiratory affections, ND, etc., less number of farms operational in Kashmir during winter and managemental implications may be additional factors determining mortality pattern. Stress factors play a critical role in determining susceptibility to and complicacies associated with infections at any point in time (Ipek and Sahan, 2006; Wideman *et al*., 2013).

The commercial strain (CS) showed a significant effect on proportionate mortality, and case prevalence as well as proportionate mortality associated with GIT affections.The overall case prevalence of GIT affections calculated as a function of total mortality of a given strain, ranged from 51.58% in CS10 to 100% in CS8 and CS9. The case prevalence was significantly higher, but comparable

among themselves, in strains CS1 (98.04%), CS2 (98.93%), CS6 (98.32%), CS8 (100%), and CS9 (100%). The differences among the commercial strains may be attributed to genetic factors which have a direct impact on both immunocompetence and metabolism, which in turn determine susceptibility to various diseases (Sharma, 2003). Besides, the hatchery management practices and transportation conditions may greatly influence the chick performance.

Occurrence of Liver Affections in Chicken

The gross liver lesions were observed in 6722 (87.79%)birds including 76.10% associated with some specific disease condition and 11.69% solitary liver lesions. The incidence and occurrence reported by various workers from different areas vary widely (Pearson *et al.*, 1978; Joshi and Baghwat, 1995; Handharyani, 2001; Aengwanich *et al*., 2004).

The overall and disease associated proportionate mortality with liver affections was significantly higher in age group 2-3 weeks (38.31% & 37.55%) followed by 0-1 (22.66% & 25.90%), 3-4 (17.60% & 18.09%), 4-5 (12.65% & 9.78%), and 1-2 weeks (8.79% & 8.68%). The proportionate mortality with solitary liver affections was significantly higher in 2-3 week chickens (43.24%) followed by 4-5 week (31.28%), 3-4 (14.41%), 1-2 weeks (9.50%) and 0-1 week (1.56%) with all the values differing significantly from each other. Liver pathology is an integral component of specific disease conditions which in turn show strong age and season association in chicken. Besides, the nutritional and toxicological factors especially early medication greatly influences liver pathology. Transportation, housing and management stress also play a critical role in determining pathophysiological status of liver. Broiler birds being reared under high metabolic stress, succumb to liver failure either primarily or secondary to cardiac failure (Wideman *et al*., 2013). The quality of nutrients and mycotoxins may be regarded as important factors in non-specific hepatic conditions (Lakkawar *et al.*, 2017). Liver frequently suffers from hypoxia due to metabolic stress (Wideman *et al*., 2013). The occurrence of seasonal liver affections

revealed significantly higher rates in spring (92.41%) and winter (90.68), followed by autumn (87.41%) and summer (81.43%). The overall case prevalence, disease associated prevalence, and solitary prevalence of liver affections calculated as a function of total mortality of a given strain, ranged from 12.63% to 94.54%; 12.63% to 86.94%; and 0.00% to 27.88%, respectively in different strains.This may be attributed to management factors and associated stress, besides variation in the seasonal disease prevalence (Roussan *et al.*, 2008; Uddin *et al.*, 2010; Itoo *et al.*, 2013)

Pathology of Gastrointestinal Tract in Chicken

Grossly, jejunum was involved in significantly higher number of cases (81.96%) followed by ileum (81.20%), duodenum (74.06%), proventriculus (42.13%), caecum (38.34%) and caecal tonsils (24.14%). Proventricular lesions included congestion, haemorrhage, thickened/oedematous mucosa and catarrhal exudate. Lesions observed in different segments of intestines included varied degree of congestion, hemorrhages, ulcers, thickened oedematous mucosa, and presence of catarrhal exudate. Evaluation of different lesions across different segments of GIT, by Severity Score Analysis based on weighed mean score of different conditions in different segments, indicated that catarrhal exudation was the most frequent type of response observed in GIT (LSRS= 30), followed by mucosal thickening (LSRS=28), mucosal congestion (LSRS=25) and haemorrhage (LSRS=23). Mucosal denudation and ulceration appeared to be the least frequent or rare response with LSRS of 9 and 12 respectively. GIT is a highly vascular system functioning in a dynamic phase, providing for absorption of nutrients as well as evasion of injurious agents (Korver, 2006). Its mucous membrane is in continuous contact with ingesta as well as harbours microbiome (Gabriel *et al*., 2006). There is also significant diversity in bacterial populations among different parts of the GI tract and population densities tend to increase from the proximal to distal GI tract (Richards *et al*., 2005). Each region of the GI tract develops its own unique microbial profile, and this community becomes more

complex as chickens age (Gong *et al*., 2002a,b; Van der Wielen *et al*., 2002; Guan *et al*., 2003; Lu *et al*., 2003; Amit-Romach *et al*., 2004).Any alteration in diet, physical texture of feed and infectious agents alter the physiological state of gut(Apajalahti, 2004; Engberg *et al*., 2004). Direct irritation and need for increased circulation leads to vascular congestion, which in turn may cause oedema when hypoxia prevails. Besides the increase in mucous production by intestinal glands appears to be the first line of defence in evading an irritant. The gut associated lymphoid tissue plays an important role in immunity but may itself be affected as in case of Newcastle disease. Thus some of the lesions observed in GIT constitute a non-specific defence mechanism and associated implications, whereas some lesions observed *viz*. proventricular haemorrhages, haemorrhages and ulcerations in GALT including caecal tonsils may be associated with specific diseases. Also, the general response may actually be associated with prevalent specific pathogens. The pathogens following enteric route of infection, e.g., *E. coli, Salmonella, GIT parasites, etc.,*cause varying degrees of pathomorphological and pathophysiological alterations in GIT.

Pathology of Liver in Chicken

Out of a total of 7657 chicken carcasses necropsied, gross liver lesions were observed in 6722 birds giving a total occurrence of 87.79%. Lesions observed in liver included varied degree of congestion, hemorrhages, fibrinous perihepatitis, necrosis and haematoma with relative case prevalence of 56.41%, 16.02%, 23.19%, 4.3% and 0.074% respectively. Liver and pancreas are two important glands associated with digestive tract maintaining the body's homeostasis. Liver is a principle accessory digestive gland, responsible for proper digestion, metabolite detoxification, phagocytosis of particulate material in the splanchnic circulation and metabolism of proteins, fats, and carbohydrates and thus is a softer target for various infectious, nutritional, metabolic, toxic and neoplastic diseases, either primarily or secondarily (Lakkawar *et al.*, 2017). The diseases primarily affecting the liver in chicken include inclusion body hepatitis, hydropericardium syndrome and lympho-proliferative diseases *viz*. Marek's

disease and lymphoid leukosis. Other diseases affecting liver include visceral gout, aspergillosis, colibacillosis, salmonellosis, non-specific hepatitis, omphalitis, fatty liver, and liver rupture. (Mir *et al.,* 2005*;* Gupta *et al.* 2007; Nazir *et al.,* 2012).besides liver pathology is noted in almost all cases of enteric affections.

Pathology of Pancreas in Chicken

No pathological gross lesions were observed in pancreas. Histopathologically, mild congestion was seen. Involvement of pancreas in pathology of biotic or abiotic causes have been rarely reported.

Pathology of Bursa of Fabricious in Chicken

The gross bursal lesions were observed in 2688 birds giving a total occurrence of 35.11%. Lesions observed in bursa included varied degree of bursal swelling, mucosal thickening/oedematous, catarrhal exudation, serous exudation, congestion, hemorrhages, and bursal core with relative case prevalence of 30.02%, 76.34%, 62.98%, 3.46%, 40.88, 26.97 and 1.64%, respectively.

Occurrence and Pathology of Specific Diseases/Disease Conditions of Digestive System in Chicken

The specific diseases/disease conditions associated with lesions in digestive system include intussusception, intestinal torsion, omphalitis, colibacillosis, salmonellosis, IBD, ND, Low Pathogenic Avian Influenza, aspergillosis, coccidiosis, gout, and ascites aspergillosis, coccidiosis, gout, and aspergillosis, coccidiosis, gout, and ascites .The etiology of disturbed health vis-à-vis digestive system is complex involving abiotic and biotic agents (Reynolds, 2003). Several pathogens (viruses, bacteria and parasites) are incriminated as possible causes of enteric disorders either alone (mono-causal), in synergy with different other microorganisms (multi-causal) or with non-infectious causes such as feed and /or management related factors (Hafez, 2011). Under field conditions,

however, it is difficult to determine whether the true cause of enteric disorders in poultry is of infectious or non-infectious origin. Besides the pathogens and irritants directly affecting digestive system, it may be secondarily involved in systemic diseases. The common conditions affecting gastro-intestinal tract include colibacillosis, salmonellosis, necrotic enteritis, coccidiosis, ranikhet disease, etc (Ahmad *et al*., 2012).

Intestinal derangements rarely occur in fowl. However, intestinal intussusception, intestinal volvulus, proventricular intussusception and herniation of the intestine through the post-hepatic septum into the ventral hepatic cavities have been reported. There is paucity of reports about pathology and pathogenesis of intestinal derangements in Aves (Haridy *et al.*, 2010). In the present study, intussusception was observed only in 1 case in the age group of 3-4 weeks with mortality of 0.02%. Palanivelu *et al*. 2014 recorded a case report on the intussusception of jejunum in pullet naturally infected with coccidiosis. Rajkhowa *et al.* (2012) reported intestinal coccidiosis with intussusception in Giriraja chicken. Olasemi *et al.* 2011 also reported a case report on jejunal intussusception associated with necrotic enteritis in a flock of pullets in Nigeria. Intussusception of intestines in poultry is a rare condition mostly seen in laying hens and replacement pullets. Increased incidence is seen 7-10 days after restriction feeding in replacement pullets. It occurs often as a result of hyper active gut. In poultry, the contributing factors include high parasitic load particularly due to *Eimeria necatri*x and overgrowth of disease causing bacteria e.g., *E.coli*, *Clostridium* and *Campylobacter*. Lakshman *et al*. (2004) reported torsion of intestines in emu (*dromeius novehollondiae*) chick.

Borah *et al*. (2017) recorded incidence of omphalitis, colibacillosis, IBD, necrotic enteritis, Newcastle disease and brooder pneumonia as 13.40%, 11.11%, 10.58%, 6.35%, 4.59% and 3.70%, respectively from birds in Assam. Jha *et al*. (2012) reported colibacillosis (24.93%) to be the most prevalent in broilers followed by yolk sac infection (12.09%), infectious bursal disease (2.82%), and

coccidiosis (3.83%) in Jharkhand, India. Ahmed *et al.* (2009) however, found the occurrence of colibacillosis IBD, omphalitis, and coccidiosis in Bangladesh to be much higher at 52.26%, 11.06%, 11.56% and 4.52%, respectively. In the present study, the maximum mortality was seen in cases of Newcastle disease (2.32%) followed by salmonellosis (1.55%), colibacillosis (1.3%), IBD (1.23%), ascites (0.89%), coccidiosis (0.73%), gout (0.52%), aspergillosis (0.37%), omphalitis (0.30%), torsion (0.05%), intussusception (0.02%), and avian influenza (0.02%). Ahmed *et al.* (2009) reported that highest mortality was due to colibacillosis (52.26%) followed by omphalitis (11.56%), IBD (11.06%), coccidiosis (4.52%), mixed infection of IBD & coccidiosis (1.51%) and salmonellosis (1.01%) at Kapasia in Gazipur district. Balachandran *et al.* (2013) reported that maximum mortality was caused by coccidiosis(18%) followed by new castle(14%) and necrotic enteritis (13%) among commercial laying chicken in and around Namakkal district. Similarly, Uddin *et al.* (2010) recorded the prevalence of infectious bursal disease (24.96%) followed by new castle disease (8.92%), aspergillosis (7.98%), salmonellosis (7.68%), coccidiosis (7.32%), colibacillosis (5.70%), ascites (5.45%), and omphalitis (2.64%) at Narsingdi district of Bangladesh. Ahmed *et al.* (2012) recorded the prevalence of 17.38% for coccidiosis and 18.61% for colibacillosis in broilers; and 22.12% for ND, 12.48% for coccidiosis and 11.36% for colibacillosis in layers.

In the present study, omphalitis was observed only in 0-2 weeks age group with total occurrence and mortality of 4.48% and 0.30% respectively throughout the year. Jha *et al.* (2012) recorded occurrence of omphalitis with respective mortality of 12.09% in Jharkhand, India. Ahmed *et al.* (2009) recorded an occurrence of 11.56% of omphalitis in Kapasia, Gazipur. Amare *et al.* (2013) recorded an overall prevalence of 33.10% of omphalitis in white leghorn and rhode island red breeds of 1 to 7 days of age. The gross and histopathological lesions observed were almost similar type of lesions were earlier described by Gross (1964); Khan *et al.* (2004); Ahmed *et al.* (2009); Tonu *et al.* (2011);

Amare *et al.* (2013) and Jalob *et al.* (2015).

In the present study, colibacillosis was a cause of mortality in all age groups with occurrence of 19.40% and mortality of 1.3%. *E.coli* was observed throughout the year. Similarly, Umer *et al*. (2017) recorded the prevalence of colibacillosis among broiler chicken of all age groups ranging from 23.634% to 29.845% with overall mean of 26.357% throughout the year in Kashmir Valley. Matin *et al*. (2017) recorded 0.84% prevalence of colibacillosis in broilers in Mymensingh district of Bangladesh. During the period from July 1996 to June 1997, *Escherichia coli* infections accounted for 17.23% of total poultry disease outbreaks in parts of Haryana as investigated by Jindal *et al.* (1999). Dave *et al.* (2004) recorded 5.12% mortality due to colibacillosis in Gujarat. Sarpe *et al.* (2009) recorded 11.34 per cent mortality due to *E. coli* infection in Parbhani, Maharashtra. *E. coli* was reported as the major cause of mortality in growers during a study on mortality pattern in poultry in Haryana by Renu *et al.* (2009). Out of 4771 chicks within the age group of 1-3 weeks, 17.6 per cent (839) mortality due to *E. coli* was reported in Bhubaneswar (Behra *et al.*, 2009). Lutful Kabir, (2010) reported that avian colibacillosis is the major bacterial disease problem in the poultry industry world-wide which constitute a major public health burden and represents a significant cost in many countries. *E. coli* associated lesions, similar to present finding have been recorded by workers all over the world (Moharana *et al*. 1993; Gangane *et al*. 2006 ; Tonu *et al*., 2011; Renu *et al*. 2012; Kumar *et al*. 2013; and Azeem Riaz *et al.* 2016).

In the present study, salmonellosis was observed in chicks up to 3 weeks of age with an overall occurrence of 8.46% and mortality of 1.55%. Ruban and Fairoze, (2011) recorded that *Salmonella* was prevalent in a wide range (25-65%) from different parts of chicken meat marketed in Bangalore while 15.91% prevalence of *S. enteritidis* was reported from Namakkal. Jinu *et al.* (2014) reported total *Salmonella* prevalence rate of 5.88% from chicken in Bareilly, Uttar Pradesh. Kumari *et al.* (2013) recorded that maximum mortality occurred in 1-2

week aged birds. The pathological alterations observed are in concurrence with those of Hafeeji *et al.* (2000), Prasanna *et al. (*2001); Hossain *et al.*(2006); Nazir *et al.* (2012); Kumari *et al*. (2013) and El Sayed *et al*.(2017).

In the present study, IBD had an occurrence and mortality of 11.94% and 1.23% respectively. Disease was recorded throughout the year. Jha *et al*. (2012) recorded the overall incidence of IBD to be 2.82% in Jharkhand whereas Bhutia and Singh (2016) recorded the 15.13% incidence of disease in Mizoram. Mor *et al.* (2010) recorded an overall morbidity, cumulative mortality and case fatality rate as 4.54%, 2.34% and 51.69%, respectively in Haryana. Anjum (1994) reported an acute form of IBD with high mortality (35.80%) in Pakistan. Lesions observed in the present study were strongly in agreement with the study of Okoye and Uzuokwu, 2001; Eterradossi and Saif, 2008; Mor *et al.* 2010; Majed *et al.* 2013; and Singh *et al*. 2015.

In the present study, ND had an occurrence and mortality of 2.49% and 2.32% respectively. Disease was recorded only during the summer season. Babaca, (2015) recorded a mortality rate of 8.1% in and around Erbil City, Iraq . Ahmed *et al*. (2012) recorded an occurrence of 17.26% in layers above 4000 feet height and 28.58%in layers below 4000 feet.Lesions observed in the present study were strongly in agreement with the study of Pazhanivel *et al.* (2002); Alexander *et al.* (2004); Saif *et al.* (2008); Kumar *et al.* (2016); Brar *et al*. (2017)

A suspected case of avian influenza was observed in the age group of 3-4 weeks of age. Nakatani *et al.* (2005) recorded a mortality of 43.3% in layer hens affected with H5N1 highly pathogenic avian influenza (HPAI), in Japan. These findings are in concurrence with those of Nakatani *et al* . (2005); Nakamura *et al*. (2008); Ali *et al*. (2015); Aslam *et al*. (2015) .

In the present study, aspergillosis was observed with an overall occurrence of 1.49% and mortality of 0.37% respectively only in summer and autumn season mostly in the age group of 0-2 weeks. Borah *et al*. (2017) recorded an incidence

of 3.70% of brooder pneumonia from birds in Assam. Sultana *et al*. (2015) recorded an overall 6.14% incidence of aspergillosis in Chittagong district. Singh *et al.* (2003) found the occurrence of aspergillosis in Punjab to be much lower at 0.14%. Mahajan *et al*. (1994), recorded a mortality of 7.69% of brooder pneumonia in Hisar.

Of the many parasitic diseases that affect birds, coccidiosis results in overall 51.38% mortality in the poultry industry worldwide (Cocciforum, 2007). Necrotic enteritis (NE) in chickens is a globally important welfare and economic problem caused by overgrowth of a commensal *C. perfringens*. This overgrowth also results in changes not only of the digestive tract environment, but also its bacterial ecology and histopathology (Cooper *et al*., 2009). The global cost of coccidiosis to the poultry industry has been estimated to exceed $2 billion per annum (Fornace *et al*., 2013). Branton *et al*. (1987) observed that use of wheat ground with a hammer mill (finely ground) increased mortality to 28.9%, but a roller mill-ground wheat diet (coarsely ground) resulted in a mortality of 18.1%. Mortalities were also associated with a combined occurrence of necrotic enteritis and coccidiosis.

In the present study, ascites was observed in 1-4 week old birds with total occurrence and mortality of 5.97% and 0.89% respectively. Itoo (2013) reported 4.00% to 16.66% mortality due to ascites in 12 of 186 broiler flocks screened in Kashmir. Buragohain and Kalita (2010) reported that in Mizoram (India), ascites syndrome (34.3%) was the main cause of mortality, significantly more than colibacillosis (19.23%). Bhat (2008) reported that occurrence of ascites was 11.72% among broilers reared in Kashmir. Ascites has been recognised as an important cause of mortality from 3rd week of rearing because of increased metabolic stress (Rajkhowa, 2004; Singh *et al*., 2006). Tafti and Karima(2000); Goyal *et al*. (2004); Dixit *et al*. (2005); Davis *et al.* (2012) and Bhalerao *et al*. (2013) reported changes identical to our findings in chickens suffering from ascites syndrome.

In the present study, gout was observed in almost all age groups except4-5 weeks with total occurrence and mortality of 6.47% and 0.52% respectively throughout the year. Singh *et al.* (2003) found the occurrence of gout in Punjab to be at 0.06%. Itoo *et al.* (2013) recorded occurrence of gout with respective mortality of 8.7% in and around Srinagar. Gupta *et al.* (2002) ; Ahmad *et al*. (2003); Mir *et al*.(2005); Eldaghayes *et al*.(2010) reported identical changes in chickens suffering from gout.

Chapter – 6

SUMMARY AND CONCLUSION

The GI tract acts as a major site of potential exposure to pathogens and irritants. Consequently, the health condition of the GI tract plays significant role in achieving optimum productivity and welfare in poultry production. The health of GI tract affects feed digestion, nutrient absorption and metabolism, energy and protein utilization, immune response and disease resistance. Enteric disorders are one of the most important groups of diseases affecting poultry and are continuing to cause high economic losses in many areas worldwide due to increased mortality rates, decreased weight gain, increased medication costs, and increased feed conversion rates. Liver and pancreas are two important glands associated with digestive tract maintaining the body's homeostasis. Liver is a principle accessory digestive gland, responsible for proper digestion, metabolite detoxification, phagocytosis of particulate material in the splanchnic circulation and metabolism of proteins, fats, and carbohydrates and thus is a softer target for various infectious, nutritional, metabolic, toxic and neoplastic diseases, either primarily or secondarily. Present study was aimed at characterizing the gross and histopathological lesions of digestive system including GIT and associated structures *viz* liver, pancreas, and bursa and investigating occurrence and pathology of specific disease conditions with primary or secondary involvement of digestive system in chicken.

Samples comprised of mortalities from various poultry farms operating in Srinagar, and adjoining areas during April 2017 to June 2018. Also, farm visits were made for clinical examination in cases of outbreaks of GIT affections. At necropsy all areas of GIT and associated structures including mouth, oesophagus, crop, proventriculus, gizzard, different parts of intestine, bursa of Fabricius, liver, and pancreas were examined for presence and nature of lesions. Lesions were

categorized as primary (affecting only given organ) or secondary (associated with systemic disease). Descriptive characteristics of the lesion were noted including type, extent, distribution, solitary or presence of multiple types of lesions, etc., for classification and scoring. Specimens from different areas of GIT and associated structures were collected from different outbreaks and 3 to 5 birds from each outbreak were necropsied. Double fixations done, first with aqueous Bouin's solution followed by 10% buffered formalin, and the samples were processed following routine paraffin embedding technique for histopathology. Tentative diagnosis of specific disease conditions was arrived at necropsy and confirmation was done following cytological examination, isolation and characterization, or in-situ pathogen or pathogonomonic lesion demonstration. Also, PCR based confirmation was done using genus specific primers**.**

In present study, a total of 7657 broiler chicken of different age groups from a total of 201 outbreaks were necropsied and examined for presence of lesions in GIT, and associated structures including bursa of Fabricius, liver, and pancreas. Out of a total of 7657 chicken carcasses necropsied, gross GIT lesions were observed in 7339 (95.85%) birds including 83.64% with some specific disease condition and 12.21% solitary GIT lesions. Age had significant effect on GIT affections with highest proportionate mortality in age group 2-3 weeks (39.14%) followed by 0-1 week (21.46%), 3-4 weeks (18.11%), 4-5 weeks (12.21%) and 1-2 weeks (9.08%). Age, also, showed a significant effect on proportionate distribution of overall, solitary or disease associated GIT affections. The proportionate mortality was significantly higher in spring (38.03%) followed summer (33.05%), autumn (15.05%), and winter (13.87%). The commercial strain (CS) showed a significant effect on proportionate mortality, and case prevalence as well as proportionate mortality associated with GIT affections. The overall case prevalence of GIT affections calculated as a function of total mortality of a given strain, ranged from 51.58% in CS10 to 100% in CS8 and CS9. The case prevalence was significantly higher, but comparable among themselves, in strains CS1 (98.04%), CS2 (98.93%), CS6 (98.32%), CS8 (100%), and CS9 (100%).

The gross liver lesions were observed in 6722 (87.79%) birds including 76.10% associated with some specific disease condition and 11.69% solitary liver lesions.The overall and disease associated proportionate mortality with liver affections was significantly higher in age group 2-3 weeks (38.31% & 37.55%) followed by 0-1 (22.66% & 25.90%), 3-4 (17.60% & 18.09%), 4-5 (12.65% & 9.78%), and 1-2 weeks (8.79% & 8.68%). The proportionate mortality with solitary liver affections was significantly higher in 2-3 week chickens (43.24%) followed by 4-5 week (31.28%), 3-4 (14.41%), 1-2 weeks (9.50%) and 0-1 week (1.56%) with all the values differing significantly from each other. The occurrence of seasonal liver affections revealed significantly higher rates in spring (92.41%) and winter (90.68), followed by autumn (87.41%) and summer (81.43%). The overall case prevalence, disease associated prevalence, and solitary prevalence of liver affections calculated as a function of total mortality of a given strain, ranged from 12.63% to 94.54%; 12.63% to 86.94%; and 0.00% to 27.88%, respectively in different strains. Grossly lesions were evident in one or more segments of GIT. Jejunum was involved in significantly higher number of cases (81.96%) followed by ileum (81.20%), duodenum (74.06%), proventriculus (42.13%), caecum (38.34%) and caecal tonsils (24.14%). Proventricular lesions included congestion, haemorrhage, thickened / oedematous mucosa and catarrhal exudate. Lesions observed in different segments of intestines included varied degree of congestion, hemorrhages, ulcers, thickened oedematous mucosa, and presence of catarrhal exudate.Evaluation of different lesions across different segments of GIT, by Severity Score Analysis which was based on weighed mean score of different conditions in different segments, indicated that catarrhal exudation was the most frequent type of response observed in GIT (LSRS= 30), followed by mucosal thickening (LSRS=28),mucosal congestion (LSRS=25) and haemorrhage (LSRS=23). Mucosal denudation and ulceration appeared to be the least frequent or rare response with LSRS of 9 and 12 respectively.

Out of a total of 7657 chicken carcasses necropsied, gross liver lesions were observed in 6722 birds giving a total occurrence of 87.79%. Lesions

observed in liver included varied degree of congestion, hemorrhages, fibrinous perihepatitis, necrosis and haematoma with relative case prevalence of 56.41%, 16.02%, 23.19%, 4.3% and 0.074% respectively.

No pathological gross lesions were observed in pancreas. The gross bursal lesions were observed in 2688 birds giving a total occurrence of 35.11%. Lesions observed in bursa include varied degree of bursal swelling, mucosal thickening/oedematous, catarrhal exudation, serous exudation, congestion, hemorrhages, and bursal core with relative case prevalence of 30.02%, 76.34%, 62.98%, 3.46%, 40.88, 26.97 and 1.64%, respectively. The specific diseases/disease conditions associated with lesions in digestive system included intussusception, intestinal torsion, omphalitis, colibacillosis, salmonellosis, IBD, ND, Low Pathogenic Avian Influenza, aspergillosis, coccidiosis, gout, and ascites.

Conclusions

- Digestive system affection constitute a significant component of disease conditions in broiler chickens with case prevalence of 95.85% for GIT, 87.79% for liver.

- The occurrence of solitary affections is significantly lower (GIT :12.21%; Liver: 11.69%) and majority of the cases are associated with specific disease conditions (GIT: 83.64%; Liver: 76.10%).

- Age, season and commercial chick type has significant impact on occurrence of digestive system affections in chicken.

- Jejunum was involved in significantly higher number of cases (81.96%) followed by ileum (81.20%), duodenum (74.06%), proventriculus (42.13%), caecum (38.34%) and caecal tonsils (24.14%).

- Proventricular lesions included congestion, haemorrhage, thickened/oedematous mucosa and catarrhal exudate.

- Lesions observed in different segments of intestines included varied degree of congestion, hemorrhages, ulcers, thickened oedematous mucosa, and presence of catarrhal exudate.

- Severity Score Analysis revealed that catarrhal exudation was the most frequent type of response observed in GIT (LSRS= 30), followed by mucosal thickening (LSRS=28), mucosal congestion (LSRS=25) and haemorrhage (LSRS=23).

- Mucosal denudation and ulceration appeared to be the least frequent or rare response with LSRS of 9 and 12 respectively. Case prevalence for gross liver lesions was 87.79%.

- Lesions observed in liver included varied degree of congestion, hemorrhages, fibrinous perihepatitis, necrosis and haematoma with relative case prevalence of 56.41%, 16.02%, 23.19%, 4.3% and 0.074% respectively.

- No pathological gross lesions were observed in pancreas.

- Occurrence of gross bursal lesions was 35.11% and included varied degree of swelling, mucosal thickening/oedematous, catarrhal exudation, serous exudation, congestion, hemorrhages, and bursal core with relative case prevalence of 30.02%, 76.34%, 62.98%, 3.46%, 40.88, 26.97 and 1.64%, respectively.

- The specific diseases/disease conditions associated with lesions in digestive system include intussusception, intestinal torsion, omphalitis, colibacillosis, salmonellosis, IBD, ND, Low Pathogenic Avian Influenza, aspergillosis, coccidiosis, gout, and ascites.

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CERTIFICATE

Certified that all the corrections/amendments as suggested by External Examiner **Dr. Shilpa Sood, Associate Professor Department of Veterinary Pathology, SKUAST-J** during Viva-Voce examination held on **12 February 2019** have been incorporated in the manuscript entitled **"Etio-pathological Profiling of Digestive System Affections in Chicken"** submitted by **Dr. Rehab Altaf (Regd. No. 2016-V-340-M).**

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