

**PATHOLOGICAL INVESTIGATION OF DISEASES IN BROILER AT
DINAJPUR DISTRICT**

174
1.11.10

144
01.11.10

A THESIS

BY

MD. ABDUS SALAM

REGISTRATION NO. 0905078

SESSION: 2009-2010

SEMESTER: MARCH- AUGUST, 2010



MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY



**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

AUGUST, 2010

**PATHOLOGICAL INVESTIGATION OF DISEASES IN BROILER AT
DINAJPUR DISTRICT**

A THESIS

BY

MD. ABDUS SALAM

REGISTRATION NO. 0905078

SESSION: 2009-2010

SEMESTER: MARCH- AUGUST, 2010



Submitted to the

**Department of Pathology and Parasitology
Faculty of veterinary and Animal Science
Hajee Mohammad Danesh Science and Technology University
in partial fulfillment of the requirements
for the degree of**

MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

AUGUST, 2010

**PATHOLOGICAL INVESTIGATION OF DISEASES IN BROILER AT
DINAJPUR DISTRICT**

A THESIS

BY

MD. ABDUS SALAM

REGISTRATION NO. 0905078

SESSION: 2009-2010

(SEMESTER: MARCH- AUGUST, 2010)



Approved as to style and content by

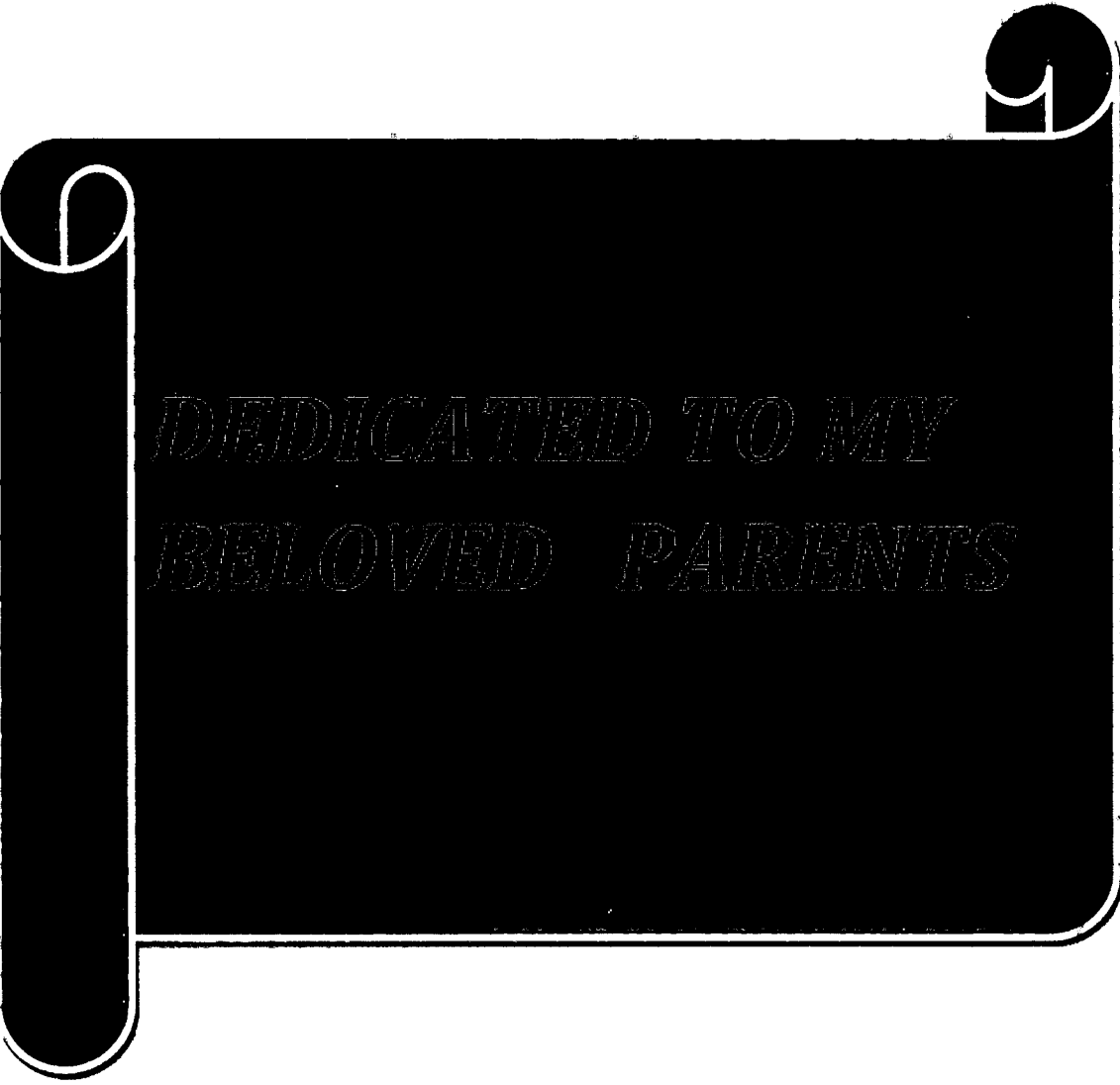
(Dr. S. M. Harun-ur-Rashid)
Supervisor

(Dr. Md. Nazrul Islam)
Co- Supervisor

(Dr. S. M. Harun-ur-Rashid)
Chairman
Examination committee
&
Chairman

**DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

AUGUST, 2010



*DEDICATED TO MY
BELOVED PARENTS*

Acknowledgement

All praises are to almighty Allah Who enables the author to complete the research work successfully and to submit the thesis leading to Master of Science (MS) degree in Pathology.

The author expresses heartfelt respect, gratitude and sincere appreciation to his research supervisor Dr. S. M. Harun-ur-Rashid, Associate Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, for his scholastic guidance, supervision, constructive criticisms and constant inspiration during the entire period of the study as well as the research work.

Immense indebtedness, heartfelt gratitude and sincere appreciation are extended to author's co-supervisor Dr. Md. Nazrul Islam, Assistant Professor, Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, for his valuable advice, exclusive suggestions and provisions of facilities and supports needed to complete this research work.

Profound respect and gratitude are expressed by the author to Dr. Sheikh Arafatur Rahman, Lecturer, Department of Pathology and Parasitology (HSTU) for co-operation extended during the course of the study.

The author expresses his cordial thanks to the laboratory technicians and other staffs of the Department of Pathology and Parasitology (HSTU).

The author wishes to express his sincere indebtedness to his parents for their blessing and encouragement throughout the course of the study.

Thanks and appreciations are also extended to the author's friends and well wishers.

The author
August, 2010

ABSTRACT

The prevalence of diseases in broiler encountered in commercial broiler farms at Dinajpur district of Bangladesh was pathologically investigated. The course of the study was one year from September, 2009 to August, 2010 under the Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The diagnosis of different disease conditions was done based on history, clinical signs, characteristic gross morbid lesions and histopathological study. In the present investigation, a total of 264 dead birds were collected for necropsy from different broiler farms. The highest proportional incidence of the diseases in broiler in the present investigation was recorded 25.75% which was due to Infectious bursal disease (IBD). This was followed by Newcastle disease (17.72%), Colibacillosis (14.01%), Coccidiosis (8.71%), MC complex (8.71%), Salmonellosis (7.57%), Aspergillosis (4.92%), Deficiency disorders (4.92%), Aflatoxicosis (4.54%) and Miscellaneous disease conditions (3.40%). The findings indicate that Infectious bursal disease (IBD) is the major disease problem in broiler farming at Dinajpur.

Key words: Broiler diseases, clinical findings, gross pathology, histopathology

CONTENTS

CHAPTER	TITLE	PAGE
	ACKNOWLEDGEMENT	v
	ABSTRACT	vi
	CONTENTS	vii
	LIST OF TABLES	ix
	LIST OF FIGURES	x
	ABBREVIATIONS AND SYMBOLS	xii
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	4
III	MATERIALS AND METHODS	24
	3.1. Experimental areas	24
	3.2. Research duration	24
	3.3. Experimental chickens	24
	3.4. Tentative and/or final diagnosis	24
	3.5. Farm management	24
	3.6. Clinical signs and relevant histories	28
	3.7. Pathological studies	29
IV	RESULTS	34
	4.1. Viral Diseases	38
	4.2. Bacterial Diseases	39
	4.3. Mycoplasmal Diseases	40
	4.4. Fungal diseases	41



CONTENTS (CONTD.)

CHAPTER	TITLE	PAGE
	4.5. Protozoal diseases	42
	4.6. Deficiency disorders	42
	4.7. Miscellaneous disease conditions	43
V	DISCUSSION	53
	5.1. Incidences of diseases in broiler	54
	5.1.1. Infectious bursal disease (IBD)	54
	5.1.2. Newcastle disease (NCD)	55
	5.1.3. Salmonellosis	56
	5.1.4. Colibacillosis and MC complex	56
	5.1.5. Coccidiosis	56
	5.1.6. Aspergillosis and Aflatoxicosis	57
	5.1.7. Deficiency disorders	57
	5.1.8. Miscellaneous disease conditions	57
VI	SUMMARY AND CONCLUSIONS	59
	REFERENCES	61

LIST OF TABLES

TABLE NO.	TITLE	PAGE
1	Collection date of dead birds related to disease in broiler	25
2	Prevalence of diseases in broiler at Dinajpur district and their proportional incidences (%)	35

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
1	Prevalence of diseases in broiler at Dinajpur district.	36
2	Proportional incidences (%) of diseases in broiler at Dinajpur district	37
3	Hemorrhage in thigh muscle (Infectious bursal disease)	44
4	Hemorrhage in the pectoral muscle (Infectious bursal disease)	44
5	A Hemorrhage in proventriculus (Newcastle disease)	45
	B Hemorrhage & ulcers in intestine & caecal tonsil (Newcastle disease)	45
	C Congestion in lung (Newcastle disease)	45
6	A & C Formation of cream color fibrinous layer (perihepatitis) over the liver (MC Complex)	46
	B Lung become severely dark spotted (MC Complex)	46
7	Haemorrhage and discoloration of caeca (Coccidiosis)	47
8	Small white caseous nodules in the lung (Aspergillosis)	47
9	Enlarged and fatty liver (Aflatoxicosis)	48
10	Thickened unabsorbed yolk sac (Colibacillosis)	48
11	Enlarged and congested liver (Salmonellosis)	48

LIST OF FIGURES (CONTD.)

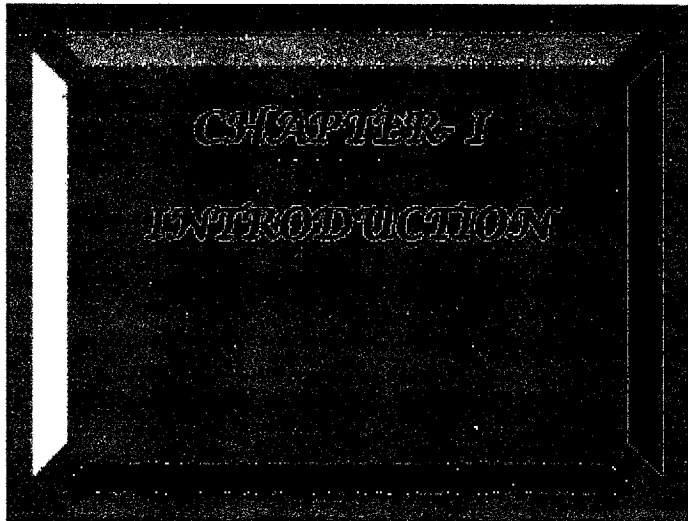
FIGURE NO.	TITLE	PAGE
12	Infiltration of reactive cells with mild hepatocellular destruction in liver stained with H & E-40X (MC Complex)	49
13	Infiltration of reactive cells as well as destruction of muscular cells in heart stained with H & E-40X (MC complex)	49
14	Focal hepatocytic necrosis with reactive cell infiltration in liver stained with H & E-40X (Salmonellosis)	50
15	Infiltration of inflammatory cells with severe enteroepithelial cells in intestine stained with H & E-40X (Newcastle disease)	50
16	Severe hemorrhage in muscle septa stained with H & E- 40X (Infectious bursal disease)	51
17	Moderate depletion of lymphoid cells with infiltration of reactive cells in the inter-follicular space of bursa stained with H & E-40X (Infectious bursal disease)	51
18	Presence of nodular mass in lung stained with H & E-10X (Aspergillosis)	52
19	Coccidian oocysts in intestinal epithelia stained with H & E-10X (Coccidiosis)	52

LIST OF ABBREVIATIONS AND SYMBOLS

°c	:	Degree Centigrade
µm	:	Micrometer
ml	:	Milliliter
As	:	Ascites syndrome
Cg	:	Control group
cfu	:	Colony forming unit
eg	:	Experimental group
<i>et al.</i>	:	And his associates
etc.	:	Etcetera
ed.	:	Edition
F.F.Y.P	:	Fifth Five Year Plan
Hps	:	Hydropericardium syndrome
hrs.	:	Hours
H & E stain	:	Hematoxylin and Eosin stain
IBD	:	Infectious bursal disease
MC	:	Mycoplasma-colibacillosis
No.	:	Number
NCD	:	Newcastle disease
P.i.	:	Post infection
Vsmc	:	Vascular smooth muscle cell

13

14



15

16

CHAPTER I

INTRODUCTION

Poultry industry is the most promising sector in Bangladesh which will not only generate employment opportunity but also encourage the unemployment youths to take up this type of farming as a means of self-employment. Now a day, the poultry farming is a popular enterprise in every body. This poultry is providing animal protein for our large people.

Protein is the most important constituent of humans' food. Animal protein is required for promotion of growth, disease resistance, body maintenance, mental development etc. We get animal protein from milk and its by-products, meats, eggs and fishes. Poultry meat contributes approximately 38% of the total animal protein supplied in Bangladesh (FAO, 1999). Broiler meat is one of the major sources of animal proteins. It is calculated that in our country total demand of meat per year is 6.40 million metric tons but production is only 0.62 metric tons, i.e., deficit is around 90.31%. In 1998-99, meat production was 515 thousand metric tons and estimated target till 2010 is 1177 thousand metric tons (DLS, 1999).

Development of poultry industry may meet up the existing meat shortage. Broiler raising is an important part of poultry industry and broiler meat is becoming popular day by day. For the improvement of national health status and socio-economic condition of the population of our country, establishment of broiler farm should be increased. Fortunately, in the recent year, poultry raising have become a growing and prospective industry in our country.

Many commercial broiler and layer farms are being setup every year. About 50,000 poultry farms and 26,000 duck farms have already been setup in private sector in addition to the government farms (F.F.Y.P., 1998).

Rural communities could raise broiler farm in less cost than other farms. So, broiler farming is the most suitable and cheapest way to fulfill the target of demand of meat. Moreover, national health will be enhanced with improvement of socio-economic condition of the poor people. Scientific breeding, feeding, management and disease control are the key points of success in poultry improvement programme. One of the major constraints in the development of poultry industry is the outbreak of infectious and non-infectious diseases which cause about 30% mortality of chicks in every year (Ali, 1994). So, in national interest, emphasis is to be given to check the mortality of chickens. A thorough knowledge about pathogenesis and pathology of the diseases is a prerequisite in proper diagnosis of malady, as well as, in the prevention and control of the diseases.

The outbreaks of various diseases are directly or indirectly related to the management status or biosecurity of the farms. So, emphasis should be given to improve the management or biosecurity of the farm to check the mortality of chickens. It is remembered that vaccination and treatment is not the substitution of the proper management. Most of the poultry diseases of our country are managerial origin, and a close relationship exists between the management of the farm or flock and disease prevalence of that particular flock.

A thorough knowledge about the epidemiology, pathogenesis and pathology of a particular disease is the first and fundamental factor for proper diagnosis of malady, as well as, in the prevention, control and eradication of the disease.

Among the various diagnostic procedures, necropsy undoubtedly remains in the key role for the detection of diseases needed for taking instant therapeutic measures that can be the effective attempt for saving from a devastating condition.

Objectives

- i. To study the incidences of diseases in broiler encountered at Dinajpur district of Bangladesh
- ii. To study the gross morbid lesions and histopathological changes of different organs in diseases of broiler encountered at present study

Goals

- ❖ Diagnosis of diseases in broiler of different origins

CHAPTER III
REVIEW OF
LITERATURES

CHAPTER II

REVIEW OF LITERATURE

The available literatures, pertinent to the present study, were reviewed in the following paragraphs.

Jungerr (1964) observed the pathological lesions of the Newcastle disease. The most prominent changes were dark red or purple red hemorrhagic lesions associated with some necrosis and hemorrhage in the intestinal wall, especially in the posterior half of the duodenum and in the jejunum and ileum and also caecal tonsil. In later stage, there was thickening of the intestinal wall which bulges inwardly. In respiratory form, the primary lesions were found in the respiratory tract. Serous or catarrhal exudates were present in the nasal passage, larynx and trachea.

Siccardi and Pomeroy (1964) found that pathogenic strains of *E. coli* apparently caused no growth depression or other adverse effect when confirmed to the digestive tract.

Helmboldt and Garner (1964) studied the histologic features of infectious bursal disease and noted that the changes were most severe in the cloacal bursa. There were depletion and necrosis of lymphocytes in the medullary area of the bursas follicles. In some places, lymphocytes were replaced by heterophils, pyknotic debris and hyperplastic reticulo-endothelial cells.

There were also hemorrhages, severe edema, hyperemia and marked accumulation of heterophils.

Domermuth et al. (1967) described a case of *Mycoplasma gallisepticum* induced salpingitis of chickens and turkeys.

Smith and Hamilton (1970) reported a field case of aflatoxicosis in broilers. They found aflatoxin in the toxic feed and reproduced the syndrome by feeding the suspect feed to control chicks. They also described the lesions of aflatoxicosis which were pale comb, shank and bone marrow; enlarged pale and fatty liver, enlarged spleen and pancreas; and regressed bursa of Fabricius. They also found that a major portion of the increase in size of liver was the result of lipid deposits.

Kutubuddin (1973) performed a pathologic investigation on the causes of mortality of birds at Bangladesh Agricultural University poultry Farm. He observed ascariasis in 32%, coccidiosis in 14.66%, avian leukosis in 13.33%, salmonellosis in 12.00%, fowl cholera in 10.66%, aspergillosis in 9.33% and infectious bronchitis in 8.00% cases.

Sarker (1976) recorded the prevalence of avian diseases in Bangladesh Agriculture University Poultry farm, Mymensingh. A total of 300 birds were examined, out of which fowl cholera was diagnosed in 34 cases, salmonellosis in 30, fowl typhoid in 12, pullorum disease in 3, *E. coli* infection in 15, leukosis in 33, coccidiosis in 36, round worm infection in 76, aspergillosis in 20, favus infection in 4. In addition fowl pox and vitamin deficiency were recorded in large number of birds.

Haider *et.al.* (1980) examined a total of 600 broiler chickens collected from different poultry farms in and around Lahore, Pakistan, during July, 1977 to January, 1978. The most common nematodes infection included *Ascaridia galli* and *Heterakis gallinae*. Among cestodes *Subutura brumpti* and *Raillietina letragona* were the most followed by *R. cesticellus* and *choanotaenia infundibulum*. The prevalence of infection increased from 50% at 6 weeks old to 69% at 10 weeks and older.

Bhargava and Prior (1983) the incidence of Salmonella contamination in ten Saskatchewan broiler flocks varying in size from 6 200 to 14 000 was investigated from February, 1977 to April, 1979. Prior to the initial chick placement, brooding equipment, feed, water and fresh litter samples were found to be free of Salmonellae. Samples obtained from the clean and disinfected processing plant equipment before the commencement of daily operation were negative except the isolation for *Salmonella anatum* from the fingers of the defeathering machine in flock 4. There was no evidence of Salmonella contamination in flocks 5, 6, 8 and 10. The incidence of Salmonella was lower when cloacal swabs were taken from day old chicks fasted for 48 hours than for the same groups of chicks when carcasses were blended in nutrient broth (flocks 7 and 9). The blending of such chicks appears to be a more critical test. The serotypes isolated from eviscerated birds were the same as those isolated from used litter samples. *Salmonella saintpaul* was isolated from a water sample at 53 days in flock 1 and the same serotype was recovered from the intestinal contents and skin of eviscerated birds. *Salmonella typhimurium* was recovered from the eviscerated birds and

neck samples in flock 3. In flock 4, *S. saintpaul* and *S. anatum* were isolated from 13% of the eviscerated birds sampled. *Salmonella thompson*, *Salmonella agona* and *Salmonella heidelberg* were recovered from 61%, 5% and 1%, respectively, of the processed carcasses sampled in flock 7.

Chishti et al. (1985) reported postmortem examination on 43, and 18 chickens infected-with *Salmonella pullorum* and *S. gallinarum*, respectively. The changes of liver due to *Salmonella pullorum* infection included bronze discoloration 75%, mottling 25%, haemorrhagic 60%, and necrotic foci 11 %; while in the spleen mottling 37%, hemorrhages 13% and necrotic foci in 25% cases. Histologic alteration in the liver showed thickening of the capsule, congestion, degeneration and necrotic changes while the spleen showed similar changes in addition to hemorrhages. In infection due to *S. gallinarum*, the liver showed bronze discoloration 60 %, mottling and hemorrhage 45 % each, and necrotic foci 10 %; while in the spleen mottling 40 %, hemorrhage 25% and necrotic foci in 45% cases. The histopathologic alteration in the liver and spleen were similar to those in *Salmonella pullorum* infection but fatty degeneration was also observed.

Pramanik and Bhattacharya (1987) examined 779 dead chickens from three rural districts of West Bengal, India, over a period of 18 months. They found that 171 deaths were due to infectious bursal disease, 85 to coccidiosis, 72 to Newcastle disease, 69 to salmonellosis, 50 to avian encephalomyelitis and 46 to aflatoxicosis. Death of 30 birds due to fowl pox was also observed even though these birds were vaccinated against fowl pox, as well as, Newcastle disease.

Lukert and Hitchner (1988) described that infectious bursal disease affected birds showed dehydration with darkened discoloration of the pectoral muscles. Hemorrhages were frequently found in the thigh and pectoral muscles. Bursa of Fabricius became swollen, edematous and haemorrhagic.

Yoder (1988) reported that in *Mycoplasma gallisepticum* infection, the gross lesions consist primarily of catarrhal exudate in nasal and paranasal passages, trachea, bronchi and air sacs. Air sacs frequently contained caseous exudate, although there were present in a 'beaded' or lymphofollicular appearance. In severe cases of typical air sac disease in chickens there was fibrinous or fibrinopurulent perihepatitis and pericarditis along with massive air sacculitis. Microscopically, there were pneumonic areas in the lungs along with lymphofollicular changes and granulomatous lesions.

Gross (1988) described the pathology of colibacillosis which were thickened air sacs often contain caseous exudates on the respiratory surface. Panophthalmitis characterized by hypopyon, pericarditis and perihepatitis, salpingitis and in more acute cases acute septicemia were noted. Microscopically the earliest lesions consisted of edema and infiltration of heterophils. Mononuclear phagocytes were very common with giant cells lining the margins of necrotic areas. There was much fibroblastic proliferation and accumulation of vast numbers of necrotic heterophils in caseous exudates.

Kamal (1989) conducted a pathologic investigation on the mortality of chicken in Bangladesh Agricultural University Poultry Farm. A total 311 chickens of different ages and breeds were autopsied. The diagnosed diseases included Newcastle diseases (18.65%), salmonellosis (4.82%), Coccidiosis (17.36%), Aspergillosis (10.61%), Ascariasis (6.75%), Fowl cholera (6.43%), Fowl typhoid (4.82%), Lymphoid leukosis (1.93%), Avian tuberculosis (0.8%) and Nodular worm diseases (0.8%). The mortality rate was highest (47.91%) in birds above 1-3 weeks of age and in respect of breeds such rate was highest (51.71%) in Starcross and lowest (2.80%) in indigenous chickens.

Banerjee et al. (1994) reported an outbreak of neurotropic velogenic Newcastle diseases at cormorants in Michigan. The most common and consistent gross lesions included edema of the eyelids and peri-ocular tissues, pulmonary edema and congestion, marked splenomegaly, hepatic necrosis and scattered hemorrhages in caecal tonsil and visceral organs. Histologically, the main changes were severe lymphocytic meningo-encephalitis, myelitis, splenic lymphoid necrosis and hemorrhage.

Park et al. (1994) conducted epidemiological, pathological and microbiological studies on 12 naturally occurring cases of necrotic enteritis (*Clostridium perfringens*) in chicks. Clinical signs were depression, decreased appetites, reluctant to move, diarrhea, ruffled feathers and death within several hours. Gross lesions seen in the infected chicks were distension of the intestine with gas, thickened mucosa and formation of a thick pseudomembrane in the intestine. The liver was friable with yellowish brown color and in some cases had demarcated necrotic foci. Histopathology

showed severe necrosis of the intestinal mucosa and attachment of numerous large bacilli to the mucosal surface of the necrotic villi.

Islam *et al.* (1995) conducted pathologic characterization of a very virulent local isolate of infectious bursal disease virus. For this purpose, five weeks old chicks were infected orally with the virus. The infection resulted in 100 per cent mortality. Drowsiness, anorexia and white diarrhea were the main clinical manifestations, which appeared by day 3 to 4 post infection (p.i.). Few infected birds died on day 4 or 5 p.i. while others became progressively weak, emaciated and dehydrated before death between day 7 and 10 p.i. acute deaths were associated with edematous swelling of the bursa and hemorrhages in the proventriculus, intestine and caecal tonsils. These lesions were, however, absent in the birds which died on day 7 p.i. or later, but the bursa was atrophied. Severe lymphoid depletion, necrosis and hemorrhages in the bursa, spleen and caecal tonsils were the major microscopic lesions.

Bhattacharjee *et al.* (1996) conducted investigation on diseases of chickens at the Central Disease Investigation Laboratory, Dhaka, over a period of one year. A total of 8947 birds were examined of which diagnosis was confirmed in 83.08% cases (14.36% remains undiagnosed due to lack of facilities and 2.56% materials were found unfit for laboratory examination). The major diseases of chickens encountered were Viral diseases (Infectious bursal disease-10.99%, Ranikhet-4.80%); Bacterial diseases (Colibacillosis-10.61%, salmonellosis-9.28%, omphalitis-8.92% and Fowl cholera-1.98%); Parasitic diseases (Coccidiosis-9.40%, Nematode and Cestode infection-2.45%); Fungal diseases (aspergillosis-5.11%, Mycotoxicosis-1.79%) and disease associated with malnutrition (8.22%) and faulty management (5.11%).

Rahman *et al.* (1996) observed eight outbreaks of infectious bursal disease of chickens in Central Poultry Farm at Mirpur, Dhaka over a period of 11 months. The age of affected chicks varied from 2-16 weeks, mortality ranged from 7.2 to 16.73% with unchanged morbidity at 100%. Coccidiosis, Salmonellosis and Colibacillosis were common sequelae. Course of the disease was 8 to 12 days. Vaccination of chicks with a live vaccine of intermediate strain given at 14 to 21 days in drinking water was unsuccessful; however, vaccination through ocular route with same vaccine at 18 to 28 days successfully controlled the outbreak.

Islam *et al.* (1998) conducted necropsy of chickens in the Department of Pathology, Bangladesh Agricultural University, Mymensingh, during a period of 20 months from January 1997 to October, 1998. Single or multiple samples from 139 outbreaks of diseases in 69 small or medium scale commercial poultry farms were received from Mymensingh and neighboring districts. Thirteen diseases were diagnosed on the basis of history. The diseases included mycoplasmosis-collibacillosis complex with a relative occurrence of 20.9%, Newcastle disease 17.2%, infectious bursal diseases 16.00%, primary *E. coli* and Salmonella infection of chickens 11.0%, pullorum disease 9.2%, coccidiosis 8.0%, aspergillosis 4.03%, ulcerative enteritis 1.8%, fowl cholera 1.2%, necrotic enteritis 0.6%, non-specific pneumonia and non-specific enteritis 2.5% and 1.2% respectively, and various nutritional deficiencies 6.1%. Outbreak of two or three concurrent diseases was also noticed on 21 occasions. In general, the occurrence of diseases was relatively in chicken's upto 8 weeks of age and infectious bursal disease, coccidiosis

and aspergillosis were confined to this age group only. The authors demanded that the recent emergence of mycoplasmosis-colibacillosis complex and outbreaks of Newcastle diseases and infectious bursal disease in vaccinated flocks deserve further investigation.

Sam and Baruah (1998) described the gross lesions of IBD. The bursa was enlarged, grayish white, edematous and covered by slimy material, in some cases hemorrhage were marked. The liver and kidneys were enlarged and hemorrhagic and sometimes pale. Sometimes hemorrhages found in the lungs and at the junction of proventriculus and gizzard. Hemorrhages particularly in the thigh and breast muscles were detected. Congestion and hemorrhages in the intestine was also noticed. Histologically, the bursa of Fabricious revealed reduction of lymphoid cells in the follicles, medullary necrosis with accumulation of homogenous eosinophilic mass and depletion of follicles with mild to moderate infiltration of inflammatory cells were also noticed. Degeneration and necrosis of lining tubular epithelium, focal and diffuse infiltration of inflammatory cells with hemorrhages were found in the kidney. Diffuse hemorrhages and congestion were recorded in the lung and thymus with depletion of lymphoid cells in the thymus.

Wahyuwardani *et al.* (2000) perform a field surveys and showed that the prevalence rate of stunting syndrome in broiler varied between 0.1% to 50%. Clinical signs were noted as ununiformity of body size in a flock of chicken, stunted and or runted of body weight gain and protrusion of wing feather. Pathological changes were hyperaemic thymus and atrophied thymus and pancreas. The packed cell volume level did not show direct link to the affected stunting or runting syndrome.

Mahmoud and Moussa (2000) studied a total of 120 chicks with *E. coli*, which were either diseased or which died recently, were collected from 21 broiler flocks representing 130 000 birds from different localities in North Sinai province. The clinical signs and gross pathological findings varied according to the type of microorganism. Microscopically, the liver of birds demonstrated focal coagulative necrosis and congestion of hepatic blood vessels. The heart of infected birds displayed fibrinous pericarditis represented by congestion, fibrin threads, fibrinous tissue proliferation with leukocytic infiltration, and the spleen of infected birds showed focal coagulative necrosis. The intestines of infected birds also demonstrated desquamation of the villus epithelium with leukocytic infiltration in the mucosa and submucosa. The antibiogram for isolated *E. coli* strains revealed that these were highly sensitive to gentamicin followed by norfloxacin, colistin sulfate and neomycin, respectively.

Khodakaram and Dadras (2001) reported that following the subcutaneous injection of oil-emulsified Newcastle disease vaccine, 400 out of 40 000 chicks (5-week-old) in a broiler breeder farm showed severe local reactions at the posterior part of their neck, head and face. Postmortem examination of the affected chicks showed thickening and hardening of the subcutaneous tissues of the affected areas. Moreover, many white to grayish foci about few mm up to one cm in diameter were seen in the subcutaneous tissues of the neck. In histopathological sections, granulomatous dermatitis and granulomatous myositis were diagnosed at the site of injection. Granulomas had a typical structure consisted of central cystic cavity and debris of necrotic cells that were surrounded by giant cells with numerous nuclei. Around these lesions,

myxomatous connective tissue and mononuclear inflammatory cells including macrophages, lymphocytes, plasma cells and few heterophils were present. Cysts with inflammatory reactions, scattered giant cells and fibroplasia were observed in the tissues between granulomas.

Alhyali and Aljubory (2001) conducted the study to examine the histopathological lesions of the lymphatic organs (caecal tonsils, spleen and thymus gland) and endocrine glands (testes, oviduct, thyroid, uropygial and adrenal glands) associated with infectious bursal disease (IBD) in the 50 broiler chickens. Histopathological findings showed hydropic degeneration, coagulative necrosis, oedema, lymphocytic infiltration with hyperplasia and atrophy of the examined organs. The pathological findings in the testes, oviduct, thyroid, uropygial and adrenal glands were recorded for the first time and added as a new pathological lesions of IBD.

Chamanza *et al.* (2001) was studied a total of 52 cases comprising 368 chickens including formalin-fixed specimens. Gross and microscopic lesions were common in the pancreas and the intestines. Over 80% of the birds had pancreatic lesions characterized by fibrosis, while 75% had thin, dilated and mucus-filled intestines associated with villi sloughing and cellular infiltration in the duodenum and goblet cell hyperplasia in the jejunum. The severity of stunting was associated with intestinal bacterial infections, management systems, and the time during the course of the study period. Severe stunting and rickets were present during the first quarter of the study period, suggesting a vertical transmission during that period, whereas milder stunting was seen as the disease incidence subsided, suggesting a horizontal transmission and a gradual build-up of immunity by broiler parents.

Chang (2002) reported that blood parameters in chickens with staphylococcal arthritis were studied. The total number of heterophils and lymphocytes in experimental group (EG) broilers increased within 7 days after inoculation and was significantly different from that of control group (CG) broilers. The number of monocytes in EG broilers at 3 days after inoculation was significantly higher than that in CG broilers. The lymphocyte transformation rate was significantly higher in EG broilers than in CG broilers at 3, 5 and 7 days after inoculation, reaching a maximum of 70.30%. The serum antibody level slowly increased during the study.

Sarkozy *et al.* (2002) performed the study with experimental fowl cholera that was induced in 60 healthy 10 week old broilers by intramuscular inoculation with approximately 80 colony-forming units (cfu) of *Pasteurella multocida* X-73 strain and with approximately 70 cfu of *P. multocida* P-1059 strain. This method of infection proved to be useful for evaluating the efficacy of anti-microbial medication, by measuring mortality, weight gain, pathological responses and frequency of re-isolation of *P. multocida*. The efficacies of two different dosing methods, continuous and pulse dosing, were compared. Using the continuous-dosing method, norfloxacin was administered to drinking water at 100 mg/l for 5 days in chickens. Efficacies were slightly improved compared with pulse dosing at 15 mg/kg bodyweight for the same length of time.

Rath *et al.* (2002) observed pathological changes in broiler chickens with ascites syndrome consisted of marked abdominal distention due to accumulation of straw coloured fluid. There were congestion and oedema in

the liver, kidney, heart and lungs. The heart revealed right ventricular dilatation with hypertrophy of right ventricular wall. In addition, degeneration and/or necrosis of hepatic cells and renal tubular epithelium and depletion of bursal follicles were observed.

Ismail (2002) studied with a total of 50 intestinal samples of broiler chickens (3-6 weeks of age) that were collected from private chicken farms in Mosul Province, Iraq. These farms were not using coccidiostats in feed. The most prominent gross pathological changes were ballooning, thickening of the intestinal wall and haemorrhages in the mucosal layer. Histological changes were seen in the intestines in the form of necrosis of mucosa and haemorrhages in the lamina propria, with infiltration of inflammatory cells especially lymphocytes and macrophages. Direct microscopic examination of intestinal contents revealed that 80% of samples were positive to *Eimeria brunettii*, *C. perfringens* was isolated from 50% of the samples.

Rahman et al. (2003) a pathological investigation was carried out at three different broiler farms around the Bangladesh Agricultural University, Mymensingh from day-old chicks up to marketing. The diseases were diagnosed both grossly and microscopically as aspergillosis, salmonellosis, choline deficiency, yolk sac infection, mycoplasma-colibacillosis complex, hydropericardium hepatitis syndrome, infectious bursal disease and pneumonia, with the case fatality rate of 77.97, 73.91, 66.67, 56.25, 50.00, 36.36, 33.33 and 18.57%, respectively. In farm no. 1, yolk sac infection, mycoplasmosis-colibacillosis complex and infectious bursal disease were diagnosed pathologically, with the morbidity and mortality rate of 2.00, 1.50, 2.25, 1.25, 1.00 and 0.50%, respectively. In farm no. 2, yolk sac infection,

salmonellosis, aspergillosis, infectious bursal disease, mycoplasmosis-colibacillosis complex, hydropericardium hepatitis syndrom, pneumonia and choline deficiency were confirmed both grossly and microscopically, their morbidity and mortality rate were 1.11, 5.10, 12.00, 0.44, 0.88, 2.67, 0.67, 0.67, 0.44, 3.75, 10.00, 0.44, 0.22, 0.88, 0.22 and 0.44%, respectively. In farm no. 3, pneumonia, yolk sac infection, mycoplasmosis-colibacillosis complex, aspergillosis and hydropericardium hepatitis syndrome were diagnosed by gross and microscopic examination, their morbidity and mortality rate were 0.87, 0.65, 1.96, 1.08, 4.56, 0.22, 0.22, 1.09, 0.22 and 1.74%, respectively. The findings indicate that aspergillosis is the major disease problem in broiler farming in Mymensingh.

Rahman and samad (2003) diagnosis of disease was made on the basis of history, necropsy findings, isolation and identification of causative bacteria and other laboratory tests. Diseases were associated with Salmonellosis (21.99%), Colibacillosis (8.40%), Avian mycoplasmosis (13.65%), Infectious bursal disease (11.20%), Newcastle disease (10.34%), Coccidiosis (6.23%) and Deficiency disorders (3.43%) were recognized as major diseases associated with mortality in commercial chickens in Bangladesh. It may be concluded from the study that both the single and concurrent infections are associated with high mortality rate in commercial chickens in Bangladesh.

Aengwanich and Simaraks (2003) observed the effect of long term heat stress on pathological changes of liver in broilers .The results revealed yellow and pale livers in 4 out of 15 broilers (26.7%) were observed. In all cases, the majority of hepatocytes demonstrated cytoplasmic microvesiculation with

dilation of sinusoids. In addition, hepatocytic necrosis with inflammatory cells was observed in some centrilobular region.

Islam *et al.* (2003) a total of 1352 sample of either dead or sick birds were brought from different Upazillas of Sylhet region. Diagnosis of different disease conditions was made on the basis of the history, age of birds, clinical signs, gross and microscopic lesions. The diagnosed diseases included Infectious Bursal disease (IBD) (24.26%), Newcastle Disease (ND) (6.73%), Infectious Bronchitis (0.29%), Omphalitis (2.81%), Fowl Cholera (0.44%), Salmonellosis (6.73%), Colibacillosis (5.17%), Necrotic enteritis (0.44%), Aspergillosis (17.53%), Infectious Coryza (0.37%), Chronic respiratory disease (CRD)/Mycoplasmosis(5.32%),Coccidiosis(9.46%),Deficiency disorders/stress condition (1.03%) and Miscellaneous diseases (2.04%). In general, the highest number of cases were recorded in the age group of 8-21 days (42.60%), followed by 22-35 days age group (26.62%), 0-7 days age group (26.10%), 36-60 days age group (1.03%) and over 60 days age group (3.62%) of Poultry. Distribution and proportionate incidence of poultry disease of Bangladesh reveals that the poultry diseases occur mostly in rainy season (56.36%), followed by summer (28.11%) and the least in winter season (15.53%).

Sharad Kumar and Gupta (2003) studied with Biochemical and pathological changes in experimentally induced hydropericardium syndrome were in broiler chickens by injecting HPS virus infected liver inoculum subcutaneously. The characteristic gross and histopathological lesions in HPS group were hydropericardium, hepatitis, myocarditis, perivascular oedema, depletion of lymphocytes in lymphoid organs and heterophilic and

mononuclear cells infiltration in interstitial tissue of kidneys. The lesions in lymphoid organs reflect the immunosuppressive effect of the disease.

Shivachandra *et al.* (2004) studied the effects of aflatoxin feeding and serotype-4 infection on the pathogenesis of HPS in broiler chicks. Chicks in the combined group showed marked clinical signs, early onset of high mortality rate (56.66%), increased quantity of pericardial fluid, more conspicuous intranuclear inclusion bodies in hepatocytes, a severe degree of degenerative changes in the liver, heart and kidney's and depletion of lymphocytes in the spleen, thymus, bursae and caecal tonsils, in comparison to the groups with one treatment.

Olkowski *et al.* (2005) observed the Pathological and functional changes in the left ventricle and atrium play a significant role in the pathogenesis of ascites in broilers. Severe dilation of the left atrium and pulmonary veins seen on postmortem examination, as well as regurgitant blood flow in the left atrium, demonstrated by Doppler study in ascitic birds, provide evidence that chronically elevated pressure in the left atrium is involved in the etiology of pulmonary hypertension and ascites in fast-growing broilers.

Andrade *et al.* (2006) observed the lesions of cellulitis in broiler chickens were described and compared with that caused by *Escherichia coli* isolates. In the samples with positive for *E. coli*, the main gross changes were skin thickness and irregularity, colour alteration and yellow detached plaques in the subcutaneous tissue. Microscopically, fibrinous plaques of cellular debris in the subcutaneous tissue with inflammatory infiltration of histiocytes or infiltration and multinucleated giant cells and fibrosis were observed.

Raj Wali *et al.* (2007) On the basis of history and detailed postmortem pathological lesions, the prevalence of IBD in district Peshawar was 7.75% and was highest in town 3 of Peshawar district (8.085%). The mortality and morbidity rates were found to be 6.38% and 1.35%, respectively. In district Peshawar the highest mortality rate was recorded in town 1 of district Peshawar with 7.037% and morbidity was highest in town 2 with 1.847%. The major macroscopic lesions observed in this study were hemorrhages on thigh and breast muscles, kidneys were found swollen and principal lesions were in the bursa of Fabricious which appeared inflamed, edematous, hyperemic and finally hemorrhagic and atrophied. Four weeks old broilers were highly susceptible to IBD (38%) followed by 3rd week (28%) and 5th week (28%) and no clinical case was found positive for IBD in first two weeks of age.

Alves and Percira (2007) this work is an epidemiological study of case control, evaluating 128 broiler chickens, between 40 and 48 days old, under sanitary inspection, vaccinated against the DIB in the 14th day of age (through drinking water). The case group consists of 68 broiler chickens with cellulitis and the control group consists of 60 healthy animals. The bursals, when collected, were measured in bursometer and registered in accordance with its scale. After the macroscopic examination were collected skin fragments, both injured and not injured, as well as bursal fragments, fixed with 10% phormol, in order to finish the histopathology, and also for the and processing by the established techniques, as well as included in paraffin and stained with hematoxilin and eosin, at the Pathological Anatomy Service Professor Jefferson Andrade dos Santos (UFF). A score has been created for

the histopathological analysis of the injuries with the following criteria: score 0 - without injury; score 1 - discrete hyperplasia and lymphoid follicles rarefaction; score 2 - edema, inflammatory infiltrate, follicle necrosis, severe lymphoid rarefaction and follicle discrete atrophy; 3 - presence of epithelial and follicle cysts, severe follicle atrophy and fibrosis. The statistical analysis was made with the Qui-square Kruskal-Wallis tests and Linear Regression. The bursometer analysis has demonstrated that: in case group - 28 broiler chickens with bursae the diameter 10 mm (size 3); 37, with diameter 13 mm (size 4); three, with diameter 16 mm (size 5); and in control group - 26 broiler chickens with bursae the diameter 10 mm (size 3); 31, with diameter 13 mm (size 4); three, with diameter 16 mm (size 5). Through microscopical analysis we registered 1 bursal with score 0; 3 with score 1; 25 with score 2; and 99 with score 3. There was statistical difference ($p < 0.05$) between the case and control groups in relation to the diameter and to the scores of bursal injuries that we had obtained. Yellowish injuries under the skin in plaques in the subcutaneous of broiler chickens must be considered as cellulitis and a criterion of the carcass condemnation and favored by the presence of more serious injuries (score 3) in the bursae.

Wang et al. (2007) observed that pulmonary vascular remodelling, mainly characterized by arterial medial thickening, is an important pathological feature of broiler ascites syndrome (AS). Since vascular smooth muscle cells (VSMC) form the major cellular component of arterial medial layer, they speculate that VSMC proliferation is one of the causes of pulmonary arterial medial thickening in ascitic broilers. Proliferation of VSMC may facilitate pulmonary vascular remodelling and have a pivotal role in AS induced by low ambient temperature.

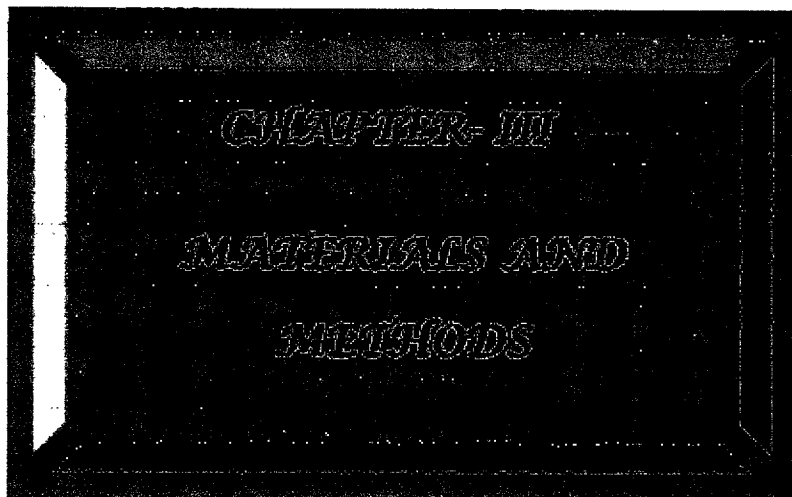
Islam and Schumacher (2007) studied with or without maduramicin (coccidiostat) containing feeds that were offered for 35 days to 160 newly hatched female broiler chicks to know the effect of semduramicin on growth performance as well as carcass and sensory characteristics of meat after 5 to 8 days withdrawal of semduramicin. Blood parameters, enzyme profiles and pathological study did not give evidence for any adverse influence of maduramicin. The mduramicin group had slightly higher body weight at slaughter after 5-8 days withdrawal. Nevertheless, there were no treatment effects on hot carcass weight, viscera, dressing percentage, edible portions (breast muscles, haunch), fat and skin portions. However, the aroma/flavour of carcass in the maduramicin group was improved, probably due to a slightly higher intramuscularly fat content in meat.

Hussein and Alattar (2007) reported that in 12-day-old broiler chicks were experimentally infected with *E. coli* at a concentration of 0.1 ml suspension via the air sac this dose contained 1×10^8 cfu/ml of *E. coli*. After being infected for 12 hours mortality of chicks already occurred and after 72 hours, histopathological examination showed thickening of the muscle fibers in the air sac, heart and lungs and necrosis of the liver bacterial count also increased after 72 hours.

Barnali Chandra *et al.* (2008) observed in the *E. coli* infected birds showed enlarged and congested liver covered with thick yellow/white sero-fibrinous membrane. Heart and other visceral organs were also covered by the sero-fibrinous membrane. Intestine was congested and oedematous. Lungs were also congested. In histopathological study, the liver showed extensive

haemorrhage, necrosis of hepatic cells and thickening of liver capsule, cellular infiltration and fatty changes. In intestine, there was congestion, severe necrosis of villi and intestinal gland, cellular infiltration and oedema of submucosa. Heart showed congestion, cellular infiltration and thick pericardium. Lungs showed congestion and emphysematous changes. Spleen was congested and necrosed with thick splenic capsule.

Lutful Kabir (2009) avian colibacillosis and salmonellosis are considered to be the major bacterial diseases in the poultry industry world-wide. Colibacillosis and salmonellosis are the most common avian diseases that are communicable to humans. This article provides the vital information on the epidemiology, pathogenesis, diagnosis, control and public health concerns of avian colibacillosis and salmonellosis. A better understanding of the information addressed in this review article will assist the poultry researchers and the poultry industry in continuing to make progress in reducing and eliminating avian colibacillosis and salmonellosis.



CHAPTER III

MATERIALS AND METHODS

3.1. Experimental areas

A total of 264 dead and sick birds were collected from the different regions such as Ranir bonder, Doshmile, Boroil, West shibrumpur, Ranigong, Ranipur, Noshipur, Gopalgong, Birol and Parbatipur of Dinajpur district. Then the necropsy and histopathology were carried out in the laboratory of the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.2. Research duration: From September, 2009 to August, 2010.

3.3. Experimental chickens

The chickens of different small scale broiler farms were considered as experimental chickens.

3.4. Tentative and/or final diagnosis of diseases

The diagnosis of different disease conditions were based on history, clinical signs and characteristic gross as well as histopathological alterations.

3.5. Farm management

The managemental systems of different farms were different. It was varied from farmer's experience, personal knowledge, efficiency and capacity.

Table-1: Collection date of dead birds related to diseases in broiler

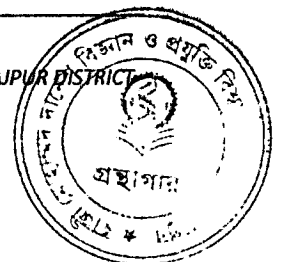
Duration	No. of farms	No. of dead birds	Name of diseases	No. of diseases
01 September to 30 September	06	15	Infectious bursal disease Colibacillosis	8 7
01 October to 31 October	05	18	Newcastle disease Mycoplasma- colibacillosis complex Deficiency disorders	7 6 5
01 November to 30 November	05	23	Salmonellosis Infectious bursal disease Coccidiosis	6 12 5
01 December to 31 December	04	16	Newcastle disease Collibacillosis Mycoplasma- colibacillosis complex	7 6 3
01 January to 31 January	06	27	Infectious bursal disease Mycoplasma- colibacillosis complex Miscellaneous disease condition Coccidiosis	10 8 5 4

Collection date of dead birds related to diseases in broiler (Contd.)

Duration	No. of farms	No. of dead birds	Name of diseases	No. of diseases
01 February to 28 February	08	28	Infectious bursal disease Colibacillosis Deficiency disorder Miscellaneous disease conditions	11 8 5 4
01 March to 31 March	06	24	Newcastle disease Aspergillosis Aflatoxicosis Mycoplasma-colibacillosis complex	11 4 6 3
01 April to 30 April	07	28	Infectious bursal disease Colibacillosis Salmonellosis Mycoplasma-colibacillosis complex	11 6 8 3
01 May to 15 May	05	22	Newcastle disease Infectious bursal disease coccidiosis	11 7 4

Collection date of dead birds related to diseases in broiler (Contd.)

Duration	No. of farms	No. of dead birds	Name of diseases	No. of diseases
16 May to 31 May	07	18	Newcastle disease Colibacillosis Aspergillosis	6 7 5
01 June to 15 June	04	11	Infectious bursal disease Mycoplasma- colibacillosis complex Deficiency disorder	4 3 4
16 June to 30 June	03	07	Salmonellosis Aspergillosis Coccidiosis	3 2 2
01 July to 15 July	04	12	Infectious bursal disease Newcastle disease Aflatoxicosis	3 4 3
16 July to 31 July	05	16	Colibacillosis Aspergillosis Coccidiosis	6 2 7



Collection date of dead birds related to diseases in broiler (Contd.)

Duration	No. of farms	No. of dead birds	Name of diseases	No. of diseases
01 August to 08 August	04	11	Infectious bursal disease	3
			Deficiency disorder	2
			Colibacillosis	3
			Aflatoxicosis	3
09 August to 15 August	03	06	Infectious bursal disease	2
			Salmonellosis	3
			Deficiency disorder	1

3.6. Clinical signs and relevant histories

The clinical signs exhibited by the individual bird during illness were recorded in detail in a prescribed form provided by the respective poultry firm's owner / attendant. Besides, breed, age and sex of the bird, the name of the hatchery from where day old chicks were collected, rearing system, types of supplied feed, name of feed additives and vitamin-minerals premix, date of outbreak occurred, number of birds affected, number of birds died , and treatment measures if taken etc. were also recorded.

3.7. Pathological studies

3.7.1. Gross pathology

The pathological study was carried out in the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The postmortem examination in all the cases was performed as soon as the dead birds were collected. At necropsy, gross tissue changes were observed and recorded carefully. The representative tissue samples containing lesions were fixed in 10 percent formalin for histopathological studies.

3.7.2. Histopathological study

During necropsy, various organs having gross lesions were collected, preserved at 10% formalin, processed, sectioned and stained for histopathological studies following a standard procedure (Luna, 1968).

Equipment and appliances

- Samples: Liver, heart, thigh muscle, bursa of Fabricius, intestine etc.
- 10% formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- H & E stain
- Water bath
- Microtome

- Microscope
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX)

Tissue processing

1. Collection of Tissue: During tissue collection, the following points were taken into consideration-

- The tissues were collected in condition as fresh as possible.
- Normal and diseased tissues were collected side by side.
- The thickness of the tissues were as less as possible (5 mm approximately).

The following tissues were collected from the broiler in the Histopathology Laboratory of Department of Pathology and Parasitology, HSTU, Dinajpur, for histopathological examination.

- Liver
- Spleen
- Intestine
- Lungs
- Thigh muscle
- Trachea
- Bursa of Fabricius
- Heart etc.

2. Fixation

Fixative (10% formalin) was added by 10 folds of the tissue size and weight. It is important to consider that a fixative should not be too toxic to its handler, and it should not damage the tissue being preserved.

3. Washing

The tissues were trimmed into a thin section and washed over night in running tap water to remove formalin.

4. Dehydration

Dehydration by ascending ethanol series to prevent shrinkage of cells as per following schedule-

- ❖ 50% alcohol - 1 hour
- ❖ 70% alcohol - 1 hour
- ❖ 80% alcohol - 1 hour
- ❖ 95% alcohol - 1 hour
- ❖ absolute alcohol - three changes (one hour for each change)

5. Cleaning

Cleaning by chloroform for 3 hours to remove ethanol (1 and half hr in each of 2 chloroform jar).

6. Impregnation

Impregnation was done in melted paraffin (56- 60°C) for 3 hours.

7. Embedding

Paraffin blocks containing tissue pieces were made using templates and melted paraffin.

8. Sectioning

Then the tissues were sectioned with a microtome at 5-6 μ m thickness. The sections were allowed to spread on luke warm water bath (40-45° C) and taken on a glass slide. A small amount of gelatin was added to the water bath for better adhesion of the section to the slide. The slides containing sections were air dried and stored in cool place until staining.

ROUTINE HEMATOXYLIN AND EOSIN STAINING

Preparation of Ehrlich's hematoxylin solution

❖ Haematoxylin crystal	4.0 gm
❖ Alcohol, 95%	200 ml
❖ Ammonium or potassium alum	6 gm
❖ Distilled water	200 ml
❖ Glycerine	200 ml
❖ Glacial acetic acid	20 ml

Hematoxylin is dissolved in the alcohol and the alum is dissolve in distilled water and mixed thoroughly. After these are in complete solution the glycerin and acetic acid are added.

Preparation of eosin solution

❖ Eosin Y, water soluble	1.0 gm
❖ Distilled water	20 ml
❖ Dissolved and added 95% alcohol	80 ml

Eosin was dissolved in water and then 80 ml of 95% alcohol was added.

Working eosin solution

❖ Eosin stock solution	1 part
❖ Alcohol, 80%	3 parts

0.5ml of glacial acetic acid was added to 100 ml of working eosin solution just before use.

Procedure of Routine Hematoxylin and Eosin Staining

1. Deparaffinization in Xylene

- ❖ The sectioned tissues were deparaffinized in three changes of xylene (three minutes in each change).

2. Rehydration in descending grades of alcohol

- ❖ Absolute alcohol - three changes (three minutes for each change)
- ❖ 95% alcohol - two minutes
- ❖ 80% alcohol - two minutes
- ❖ 70% alcohol - two minutes

3. Dipping with distilled water for 10 minutes.

4. The tissues were stained with Ehrlich's hematoxylin for 2-10 minutes.

5. Washing in running tap water (10-15 min)

6. Dipping in lithium carbonate (few dips)

7. Staining in Eosin solution for 1 min.

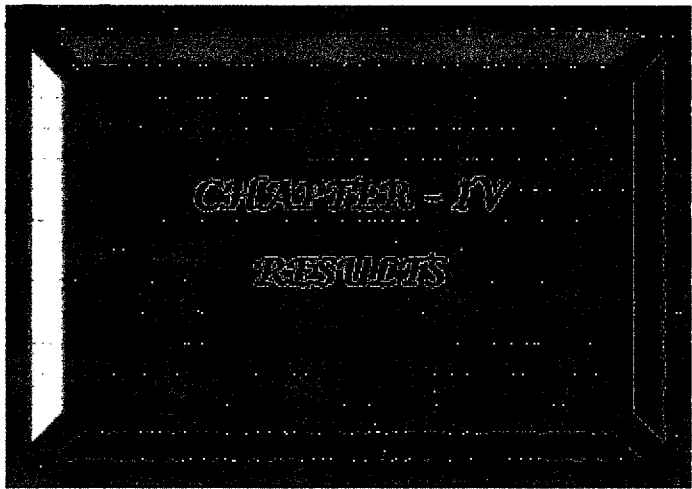
8. Dehydration in ascending grades of alcohol as follows:

- ❖ 95% alcohol - three changes (2-4 dips for each change)
- ❖ Absolute alcohol - three changes (2-3 minutes for each change)

9. Cleaned in xylene: three changes (five minutes for each change).

10. Tissues were mounted with cover slip by using suitable mounting media (DPX) or Canada Balsum.

11. Observation of the tissues under microscope using low and high magnification.



CHAPTER IV

RESULTS

The present study on pathological investigation of diseases in broiler encountered at Dinajpur district detected a number of maladies responsible for morbidity and mortality of chickens in different broiler farms during the period from September, 2009 to August, 2010. The proportional incidences of those disease conditions are shown in Table-2 and Figure-1&2.

For the convenience of description, the recorded disease conditions were classified as follows

- ❖ Viral diseases
- ❖ Bacterial diseases
- ❖ Mycoplasmal disease
- ❖ Fungal diseases
- ❖ Protozoal disease
- ❖ Deficiency disorders
- ❖ Miscellaneous disease condition.

Table-2. Prevalence of diseases in broiler at Dinajpur district and their proportional incidences (%).

Sl. No.	Tentative and/ or final diagnosis	Number of dead birds for necropsy	Proportional incidences (%)
1	Infectious bursal disease	68	25.75
2	Newcastle disease	46	17.72
3	Salmonellosis	20	7.57
4	Colibacillosis	37	14.01
5	Mycoplasma-colibacillosis complex	23	8.71
6	Aspergillosis	13	4.92
7	Aflatoxicosis	12	4.54
8	Coccidiosis	23	8.71
9	Deficiency disorder	13	4.92
10	Miscellaneous disease condition	9	3.40
Total		264	100%

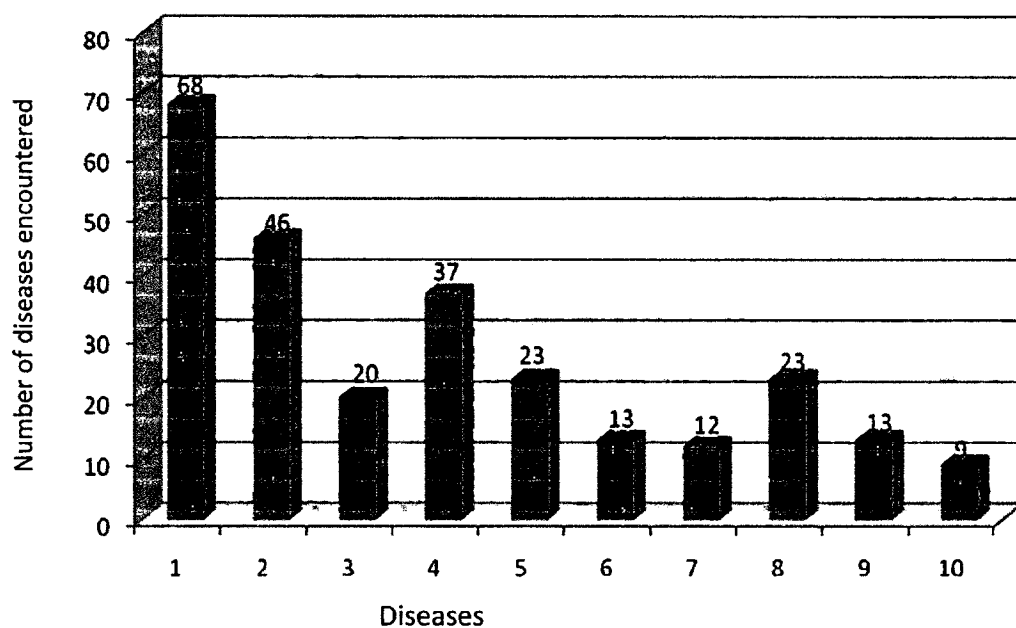


Figure-1. Prevalence of diseases in broiler at Dinajpur district

1. Infectious bursal disease 2. Newcastle disease 3. Salmonellosis
4. Colibacillosis 5. Mycoplasma-colibacillosis complex 6. Aspergillosis
7. Aflatoxicosis 8. Coccidiosis 9. Deficiency disorders
10. Miscellaneous disease conditions.

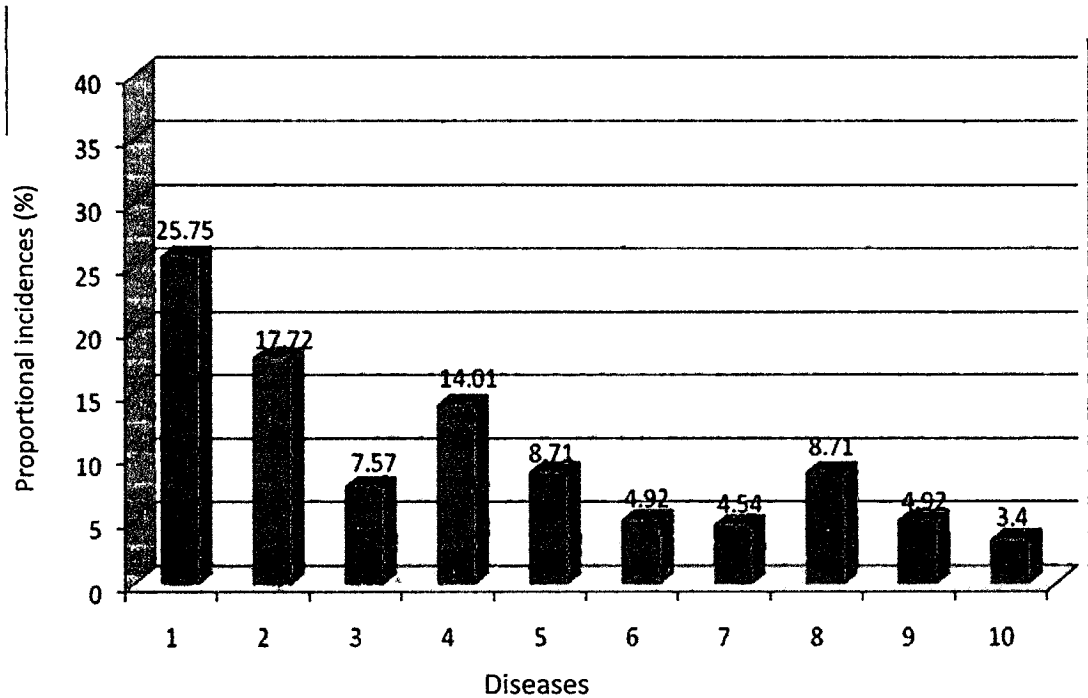


Figure-2. Proportional incidences (%) of diseases in broiler at Dinajpur district

1. Infectious bursal disease
2. Newcastle disease
3. Salmonellosis
4. Colibacillosis
5. Mycoplasma-colibacillosis complex
6. Aspergillosis
7. Aflatoxicosis
8. Coccidiosis
9. Deficiency disorders
10. Miscellaneous disease conditions.

4.1. Viral Diseases

1. Infectious bursal disease

The present investigation detected a total of 68 (25.75%) cases of infectious bursal disease of broiler birds from 264 dead broiler birds (Table-2). The clinical signs were recorded during the physical visit of broiler flocks and the farmers complaints were also emphasized, and these were whitish or watery diarrhea, sometime bloody droppings, soiled vent feathers, vent picking, trembling, severe prostration and sudden death.

Gross lesions

The affected birds exhibited hemorrhages in the thigh and pectoral muscles (Figure-3 & 4).

Microscopic lesions

Microscopically, the section of the bursa showed moderate depletion of lymphoid cells with infiltration of reactive cells in the inter-follicular space (Figure-17). Severe hemorrhage was found in muscle septa (Figure-16).

2. Newcastle Disease

A total of 46 (17.72%) cases of newcastle disease of which 264 dead broiler birds (Table-2). The affected birds exhibited respiratory distress, coughing, gasping and anorexia. In some cases, paralysis of the legs and /or wings and torticollis were found. Greenish or whitish-greenish diarrhea often blood stained was also observed.

Gross lesions

Grossly, dark red or purple red hemorrhagic lesions associated with necrosis were found in the intestinal wall especially in the posterior half of the duodenum and the jejunum, ileum and caecal tonsils (Figure-5B). Hemorrhages were also found in the glandular surface of the proventriculus and sometime in the gizzard (Figure-5A). Congestion was found in the lung (Figure-5C).

Microscopic lesions

Microscopically, Section of the intestine exhibited extensive infiltration of inflammatory and mononuclear cells with severe enteroepithelial cells (figure-15).

4.2. Bacterial Diseases

1. Salmonellosis

The investigation recorded a total of 20 (7.57%) cases of salmonellosis of which 264 dead birds (Table-2). The affected chicks exhibited weakness, poor growth and inappetance. Chalky white excreta sometimes stained with greenish brown adhered to the vent. Labored breathing and gasping were observed. The adult affected birds showed depression, anorexia, diarrhea and dehydration. There was drop in feed consumption.

Gross lesions

The liver was fragile, enlarged and congested (Figure-11).

Microscopic lesions

The section of the liver showed focal hepatocytic necrosis scattered with reactive cells infiltration (Figure-14).

2. Colibacillosis

This study revealed a total of 37 (14.01%) cases of colibacillosis of which 264 dead broiler birds (Table-2). In most cases, chicks were found dead without showing significant clinical signs. However, few birds were reported to be lethargic, dehydrated and found depressed with poor growth performance.

Gross lesions

Thickened unabsorbed yolk sacs were found which often hemorrhagic (Figure-10).

4.3. Mycoplasmal Diseases

1. Mycoplasma-colibacillosis complex (MC complex)

This study revealed a total of 23 (8.71%) cases of Mycoplasma-colibacillosis complex (MC complex) of which 264 dead broiler birds (Table-2). In most cases, the birds exhibited tracheal rales, nasal discharges, coughing, anorexia and poor weight gain.

Gross lesions

Formation of cream color fibrinous layer (perihepatitis) was observed over the liver. (Figure-6A & C). Dark spotted was found in the lung (Figure-6B).

Microscopic lesions

In the section of liver, mild hepatocellular destruction with reactive cells infiltration was observed (Figure-12). In the section of heart, destruction of muscular cells with reactive cell infiltration were observed (Figure-13).

4.4. Fungal Diseases

1. Aspergillosis

The present investigation detected 13 (4.92%) of cases aspergillosis from 264 investigated dead broiler birds (Table-2). Affected birds showed dyspnea, gasping and accelerated breathing. Most of these birds showed emaciation, ruffled feathers and depression.

Gross lesions

Most of the birds were found emaciated and cachectic at necropsy. Whitish nodules of different sizes and shapes were found mainly in the lungs (Figure-8).

Microscopic lesions

Histopathologically, sections of the lung showed nodular mass in the parenchyma (Figure-18).

2. Aflatoxicosis

This study revealed a total of 12 (4.54%) cases of aflatoxicosis from 264 investigated dead broiler birds (Table-2). Affected birds exhibited anorexia, ruffled feathers, listlessness, lethargy, lameness and death.

Gross lesions

Liver was enlarged, fatty or grayish, and friable (Figure-9).

4.5. Protozoal diseases**1. Coccidiosis**

This study revealed a total of 23 (8.71%) cases of coccidiosis from 264 investigated birds (Table-2). In most cases, the birds exhibited droopiness, depression, ruffled feathers and bloody diarrhea.

Gross lesions

All the birds died due to caecal coccidiosis showed characteristic lesions in the caeca. The affected caeca were swollen and hemorrhagic, the walls appeared thickened and firmer in consistency; the lumen filled with blood tinged exudates (Figure-7).

Microscopic lesions

Numerous oocysts were observed in the intestinal epithelia (Figure-19).

4.6. Deficiency disorders

The present investigation recorded a total of 13 (4.92%) cases in which birds were died due to vitamin deficiency and/or mineral and nutritional deficiency disorders (Table-2). In severe cases, affected birds showed a marked edema of the subcutaneous tissue. The edema fluid which was greenish-blue, viscous materials accumulated under the skin and could easily see through the skin. This condition was selected as exudative diathesis and was due to vitamin E deficiency. In 6 cases, affected birds showed paralysis of the muscles evidenced by their characteristic star grazing position.

Retraction of the head was due to paralysis of muscles of the neck. At necropsy no significant gross lesions were observed. The affected birds respond to oral administration of thiamine within a few hours and so the condition was detected as thiamine deficiency disorder.

In 2 cases, affected chicks did not walk except when forced to. Necropsy of the affected sacrificed birds showed no marked abnormalities. Supplementation of riboflavin to the affected chicks resulted the marked improvement of the disorders and the condition was detected as thiamine deficiency disorder.

In 3 cases, the affected birds showed anemia and marked hemorrhage on the breast, legs, wings, and in the abdominal cavity. The whole lengths of the intestine were hemorrhagic and congested. In some bird, accumulation of edema fluid in abdominal cavity occurred. This condition was suspected as vitamin K deficiency disorder.

4.7. Miscellaneous disease conditions

The present investigation detected a total 9 cases in which birds were died due to miscellaneous causes (Table-2). Out of 9 cases, birds of 4 cases died due to environmental stresses induced by extreme hot or cold. At necropsy, there was no apparently gross lesion except history of the stress recorded from the owner.

In five cases birds died duo to ammonia intoxication. There was history of extensive ammonia production in the poultry shed and lack of sufficient ventilation.

Postmortem lesions of different diseases in broiler

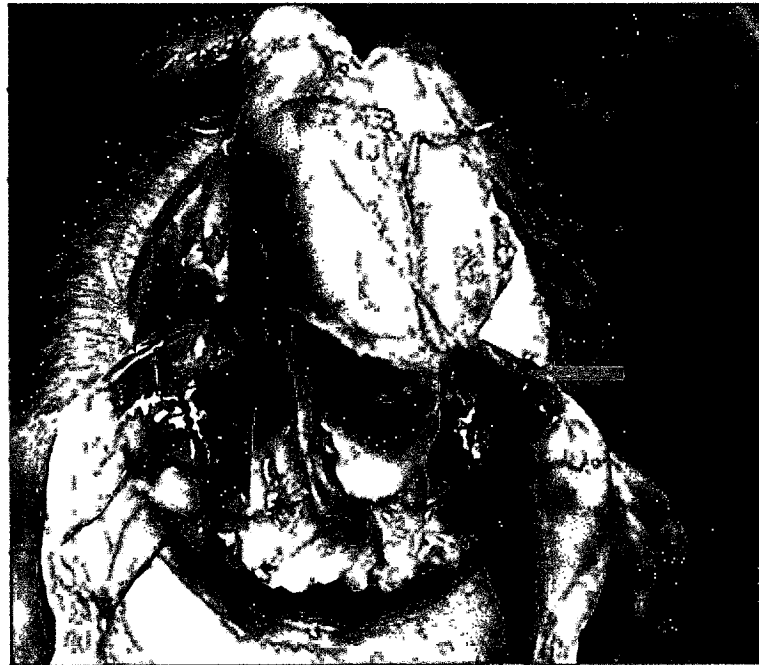


Figure-3: Hemorrhage in thigh muscle (Infectious bursal disease)



Figure-4: Hemorrhage in the pectoral muscle (Infectious bursal disease)

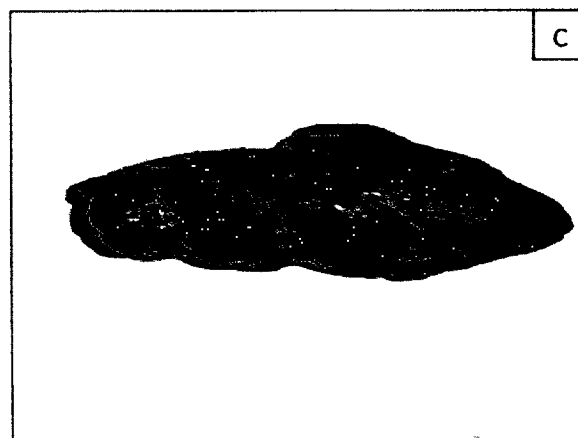
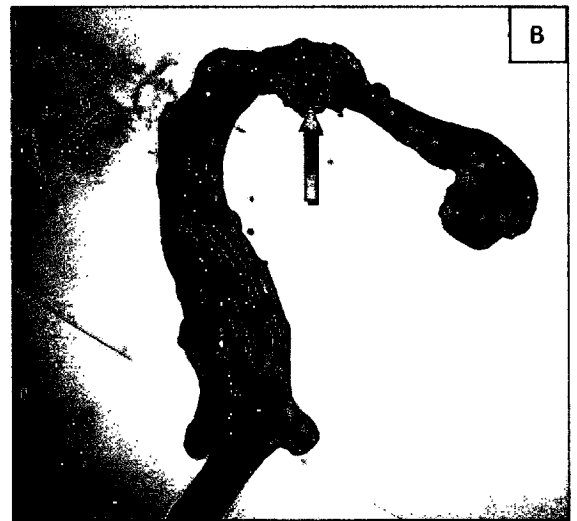
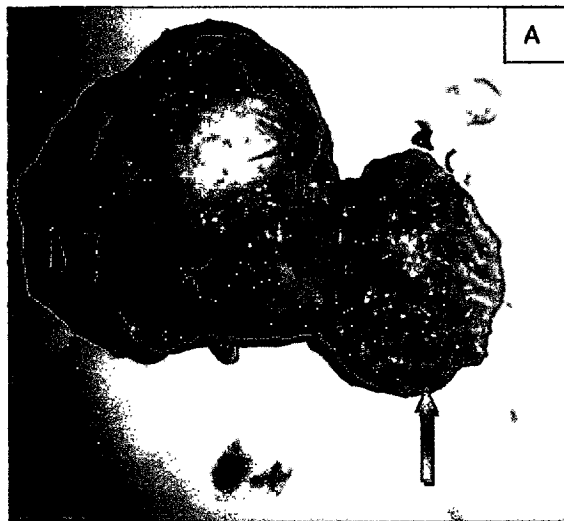


Figure-5 (Newcastle disease)
(A) Hemorrhage in proventriculus.
(B) Hemorrhage & ulcers in intestine & caecal tonsil.
(C) Congestion in lung.

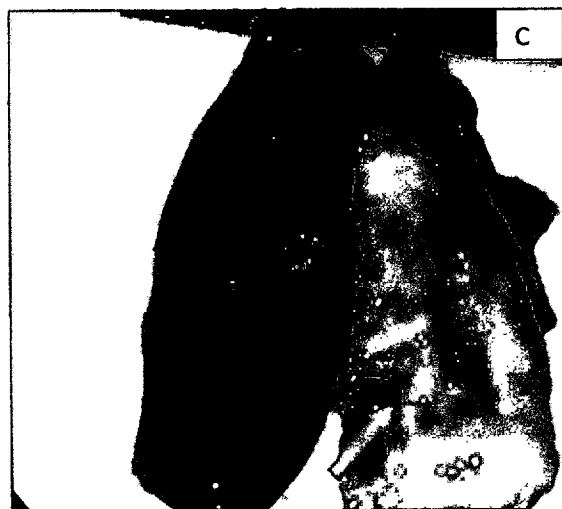
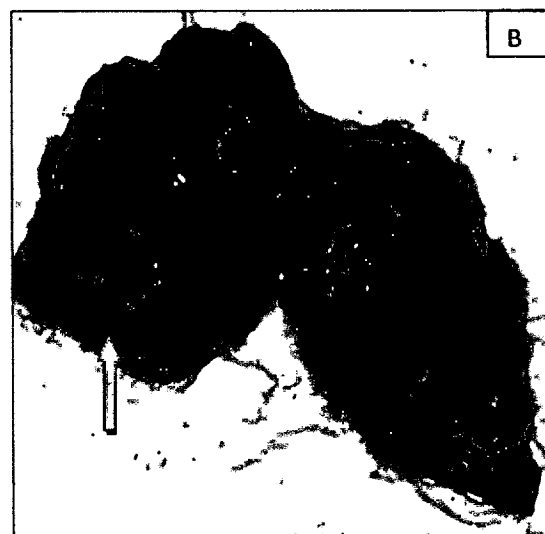


Figure-6 (MC complex)
 (A) & (C) Formation of cream color fibrinous layer (perihepatitis) over the liver.
 (B) Lung become severely dark spotted.

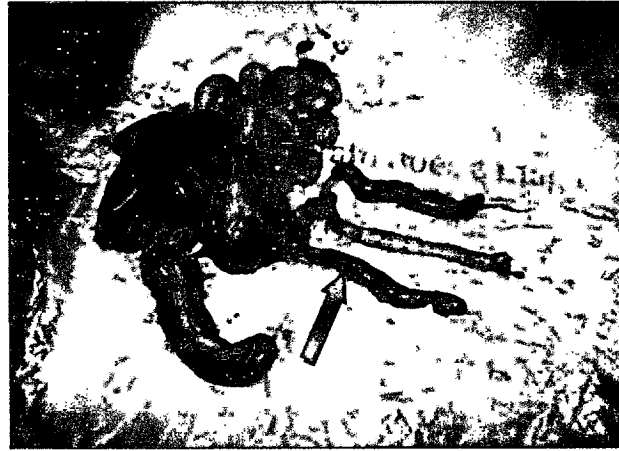


Figure-7: Haemorrhage and discoloration of caeca (Coccidiosis)

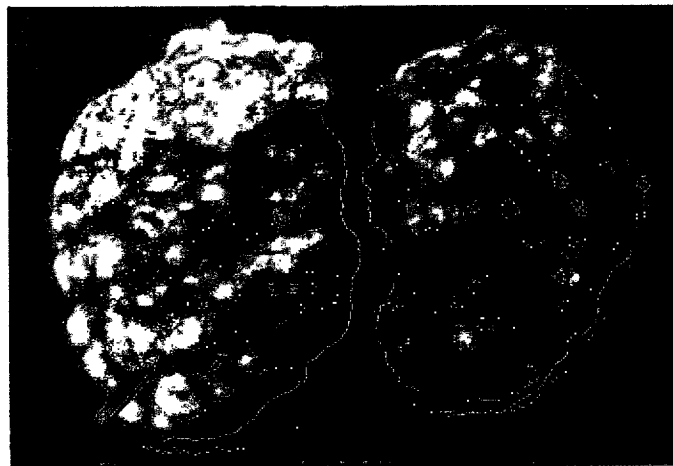


Figure-8: Small white caseous nodules in the lung (Aspergillosis)

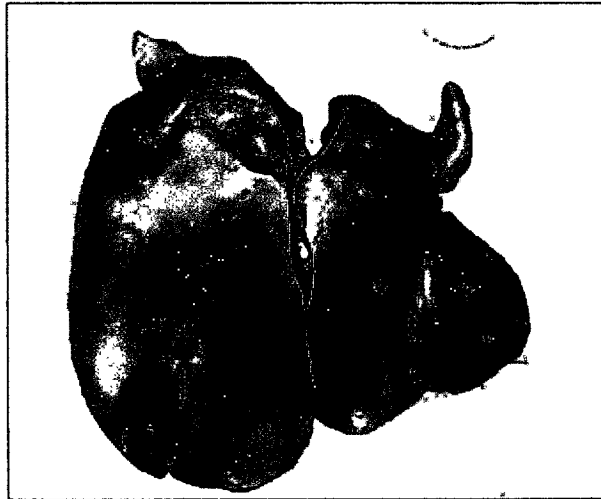


Figure-9: Enlarged and fatty liver (Aflatoxicosis)



Figure- 10: Thickened unabsorbed yolk sac (Colibacillosis).

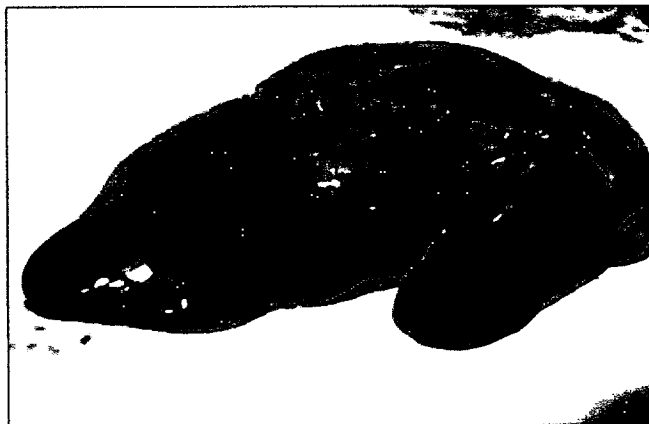


Figure-11: Enlarged and congested liver (Salmonellosis)

Histopathological changes of different organs in diseases of broiler

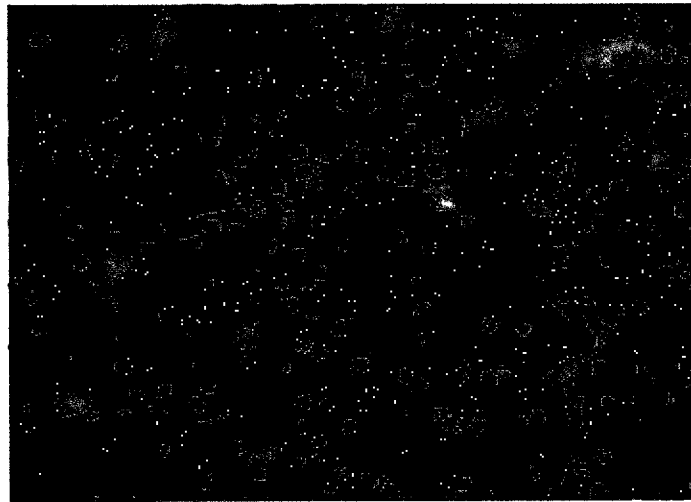


Figure-12: Infiltration of reactive cells with mild hepatocellular destruction in liver stained with H & E-40X (MC Complex)

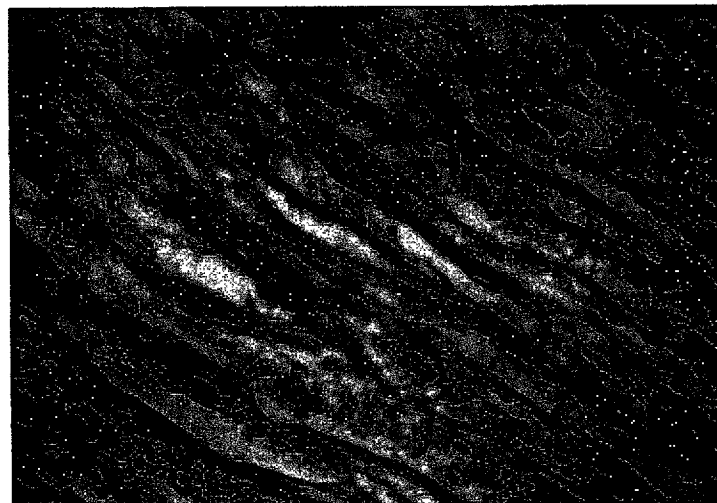


Figure-13: Infiltration of reactive cells as well as destruction of muscular cells in heart stained with H & E-40X (MC Complex)

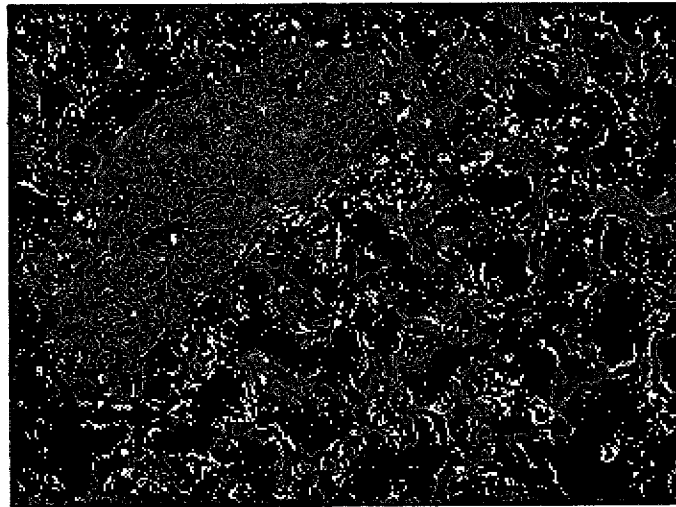


Figure-14: Focal hepatocytic necrosis with reactive cell infiltration in liver stained with H & E-40X (Salmonellosis).

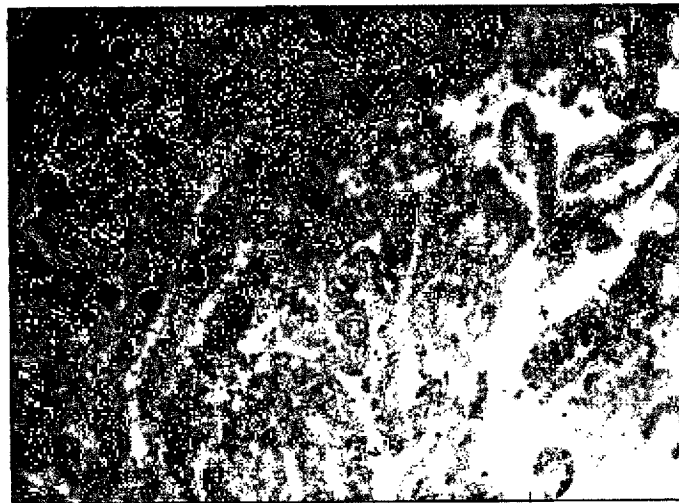


Figure-15: Infiltration of inflammatory cells with severe enteroepithelial cells in intestine stained with H & E-40X (Newcastle disease)

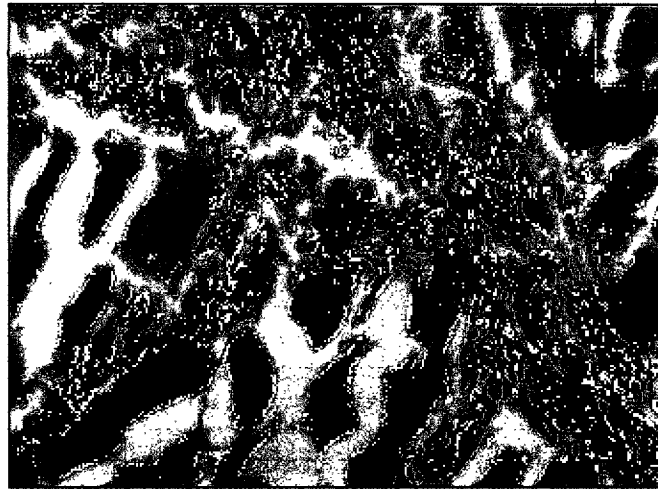


Figure-16: Severe hemorrhage in muscle septa stained with H & E- 10X
(Infectious bursal disease)

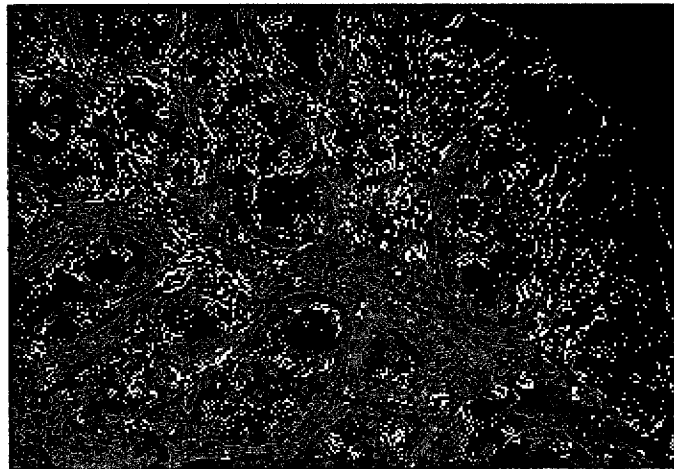


Figure-17: Moderate depletion of lymphoid cells with infiltration of reactive cells in the inter-follicular space of bursa stained with H & E-10X (Infectious bursal disease)



Figure-18: Presence of nodular mass in lung stained with H & E-10X
(Aspergillosis)

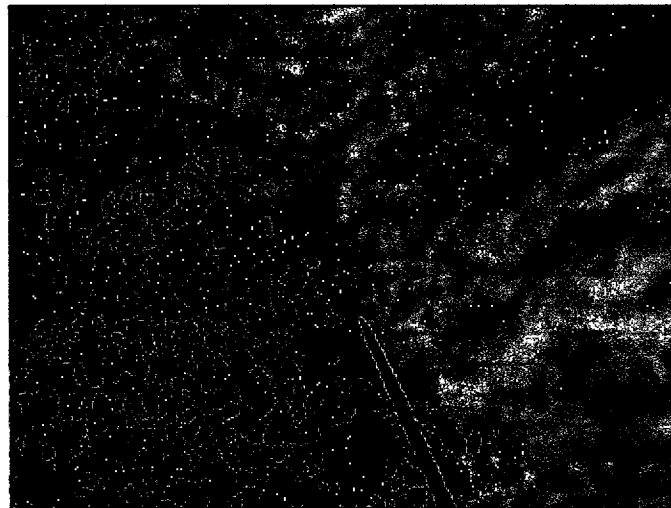
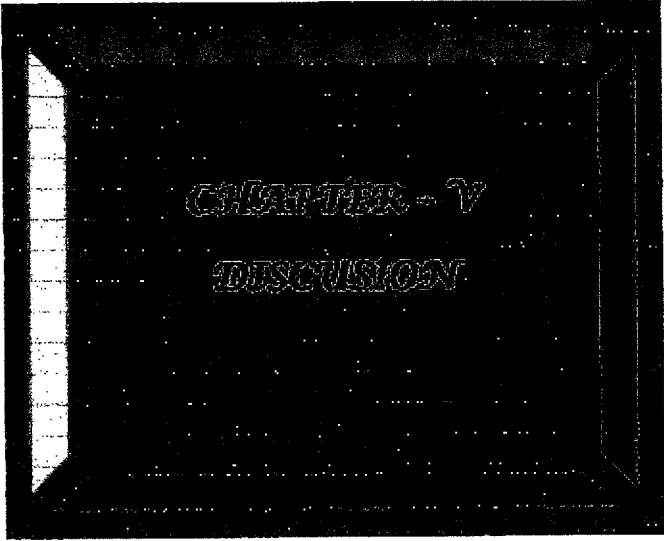


Figure-19: Coccidian oocysts in intestinal epithelia (Coccidiosis)



CHAPTER V

DISCUSSION

Pathological investigation of diseases in broiler at Dinajpur was studied and a group of viral, bacterial, mycoplasmal, fungal, protozoal, deficiency disorder and miscellaneous disease conditions were encountered during my observation from September, 2009 to August, 2010.

One of the major constrains in the development of poultry industry in Bangladesh is the outbreaks of various diseases which cause about 30 % mortality of chickens in every year (Ali, 1994). So, to render the broiler industry sustainable emphasis should be given to check the mortality of chickens. The prevalence of diseases in a particular area depends on various factors like geo-climatic condition, biological barriers, immunization status, social awareness etc. A thorough knowledge about the prevalent of diseases in an area, their epidemiology including morbidity and mortality patterns, pathogenesis and pathology of the disease is the prerequisite in proper diagnosis of the maladies, as well as, in the prevention and control strategies of the diseases. Mass immunization against a particular disease without knowing about the prevalence of that disease in the area cause not only economic loss in term of vaccination cost but also stress the bird, making them more susceptible to other diseases. Moreover, emphasis should be given first in the prevention and control of those maladies that cause heavy mortality and/or reduce production performance of the chickens. On the other hand, incidence of a malady may be reduced in a particular area in course of time and another disease may appear.

In the present investigation, a total of 264 dead birds were collected for necropsy from different broiler farms of Dinajpur. Although the total number of birds necropsized were 264 but it did not only reflect the similar number of birds affected, because in a flock more than one bird could be affected and / or died but only few of them were collected . So, the values obtained in the present investigation reflect the total picture of the broiler diseases of Dinajpur district.

5.1. Incidences of diseases in broiler

The highest proportional incidence of the diseases in broiler in the present investigation was recorded 25.75%, which was due to Infectious bursal disease (IBD) (Table-2). This was followed by Newcastle disease (17.72%), Colibacillosis (14.01%), Coccidiosis (8.71%), Mycoplasma-colibacillosis complex (8.71%), Salmonellosis (7.57%), Aspergillosis (4.92%), Deficiency disorders (4.92%), Aflatoxicosis (4.54%) and Miscellaneous disease conditions (3.40%).

5.1.1. Infectious bursal disease (IBD)

The proportional incidence of IBD in present study was 25.75% in which Islam (2003) recorded 24.26% and also Bhattacharjee *et al.* (1996) recorded (10.99%) diagnosed at the Central Disease Investigation Laboratory, Dhaka and Islam *et al.* (1998) recorded 16.0% due to this malady. In present study, the clinical sign of IBD as whitish or watery diarrhea which was similar to those described by Cosgrove (1962). The present study found the gross morbid lesions as hemorrhage in thigh and pectoral muscle which were similar to those described by Sam and Baruah (1998) and Lukert *et al.* (1988). The histopathology of infectious bursal disease in

present study exhibited mild depletion of lymphoid follicular cells of bursa which was similar described by Sam and Baruah (1998) and Helmboldt (1964). The present finding indicates that IBD infection has still continuing with enhance virulence in our country and cause highest mortality in broiler chickens.

5.1.2. Newcastle disease (NCD)

The proportional incidence of NCD in present study was 17.72% in which Islam *et al.* (2003), Rahman and Samad (2003), Islam *et al.* (1998), Bhattacharjee *et al.* (1996) and Kamal (1989) recorded NCD in 6.73%, 10.34%, 17.20%, 4.80% and 18.65% respectively. The present finding would indicate that NCD in commercial flocks is still a major threat to the poultry industry inspite of availability and the use of ND vaccine. In the present study, the clinical sign of NCD as paralysis of legs and wings recorded which were similar to those described by Ressang (1961). In present study, the gross morbid lesion of NCD as hemorrhage in caecal tonsil which was similar to those described by Banerjee *et al.* (1994), Jungherr (1964) and Crawford (1930). Kutubuddin (1973) and Sarker (1976) did not observe NCD in birds of Bangladesh Agricultural University Poultry farm. These findings indicate that the prevalence and incidence of this disease may vary from time to time. Immunization procedure, if not adopted properly, may influence the incidence rate of the malady. It is usually said that NCD is under control now a days in an organized broiler farms but the present findings do not indicate this.

5.1.3. Salmonellosis

The proportional incidence of Salmonellosis was recorded in 7.57% in the present study. Islam *et al.* (2003), Bhattacharjee *et al.* (1996), Kamal (1989) and Kutubuddin (1973) recorded Salmonellosis in 6.73%, 9.28%, 4.82% and 12.0% respectively from the poultry of Bangladesh. The present finding would indicate that Salmonellosis is a threat to the broiler farms. In the present study, the histopathological changes of liver as necrosis in liver which was similar to those described by Cishti *et al.* (1985).

5.1.4. Colibacillosis and MC complex

In this present study, the proportional incidence of Colibacillosis was recorded in 14.01%. Rahman and Samad (2003), Islam *et al.* (2003) and Bhattacharjee *et al.* (1996) recorded Colibacillosis in 8.40%, 5.71%, and 10.61% respectively. MC complex recorded in present study was 8.71%. Islam *et al.* (1998) designated those two diseases as Mycoplasmosis-colibacillosis complex with relative occurrence of 20.9%.

5.1.5. Coccidiosis

The proportion incidence of Coccidiosis in present study was recorded in 8.71%. Islam *et al.* (2003), Bhattacharjee *et al.* (1996) and Kutubuddin (1973) recorded 9.46%, 9.40% and 14.66% respectively. In this present investigation, incidence of Coccidiosis was lower. The farmers are very much aware of Coccidiosis and other parasitic diseases now-a-days. They usually use coccidiostats routinely.

5.1.6. Aspergillosis and Aflatoxicosis

In this present study, Aspergillosis and Aflatoxicosis were recorded 4.92 % and 4.54% respectively. Islam *et al.* (1998), Kamal (1989) and Kutubuddin (1973), and recorded in case of Aspergillosis were 4.03%, 10.61% and 9.33% respectively. The gross morbid lesion of Aflatoxicosis as enlarged fatty liver in present study recorded which was similar to those described by Smith *et al.* (1970).

5.1.7. Deficiency disorders

Deficiency disorders in present investigation were recorded in 13 (4.92%) in which Rahman and Samad (2003) recorded 3.43%. The deficiency disorders vary from farm to farm depending up on the management system. Moreover, various feed additives are used now-a-day to improve meat qualities. The increasing trend of nutritional deficiency disorders possibly related to indiscriminate use of feed additives or other antibiotics in the organized broiler farms.

5.1.8. Miscellaneous disease conditions

In the present study, the proportional incidence of miscellaneous disease conditions was found 3.40%. Islam *et al.* (2003) recorded those as 2.04%.

The above discussion indicates that prevalence and incidence pattern of various diseases have changed in present days. Infectious bursal disease, Newcastle disease and Coccidiosis should get priority in the prevention and control strategies in these days. Most of the recorded diseases are managerial diseases including Salmonellosis, Colibacillosis, Aspergillosis, Aflatoxicosis, Coccidiosis etc. So, emphasis should be given in proper management of the flocks which will reduce the mortality of broiler chicks. Mass immunization with proper vaccines and appropriate maintenance system is necessary to prevent the most devastating diseases. Therefore, various diseases as constraints for the development of broiler farms at Dinajpur district were identified. So, Measures should be taken for the prevention and control of the above mentioned maladies.

CHAPTER - VI
SUMMARY AND
CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

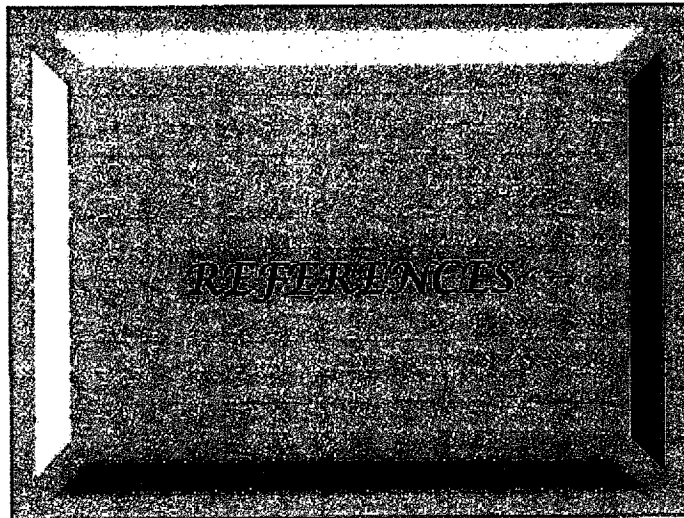
The pathological investigation was carried out on the diseases in broiler occurring in different small scale broiler farms of various regions of Dinajpur, Bangladesh which was conducted during the period from September, 2009 to August, 2010. A total of 264 dead birds were collected for necropsy from different poultry farms. The diagnosis of different disease conditions were based on history, clinical signs, post-mortem lesions and histopathological study. The diagnosed diseases included Infectious bursal disease (25.75%), Newcastle disease (17.72%), Colibacillosis (14.01%), MC complex (8.71%), Salmonellosis (7.57%), Coccidiosis (8.71%), Aspergillosis (4.92%), Aflatoxicosis (4.54%), Deficiency disorders (4.92%) and Miscellaneous disease conditions (3.40%).

In respect to the proportional incidences, the top three diseases were Infectious bursal disease (25.75%), Newcastle disease (17.72%), and Colibacillosis (14.01%).

From the above facts and findings, it could be concluded that

- ❖ Proper immunization procedure against these diseases should be under taken to prevent the mortality of broiler birds. Moreover, causes of immunization failure against the IBD deserve further investigation.

- ❖ Managemental diseases like salmonellosis, colibacillosis, aflatoxicosis, deficiency disorders, coccidiosis etc. also resulted in high mortality in broiler of Dinajpur. So, managemental procedures should be emphasized.
- ❖ Farmers are to be trained up before set up broiler farms.
- ❖ The incidence rate of diseases in broiler should be monitored time to time to adopt proper prevention and control strategies, and to treat the flock if required.
- ❖ To make social awareness about different diseases in broiler to recover economic loss.



REFERENCES

- Aengwanich, W., Simaraks, S. (2003). Effect of long term heat stress on pathological changes of liver in broilers. *Journal of the Thai Veterinary Medical Association*, 54 (1/2): 57-62.
- Ahmed, M.S., Anjaneyulu, Y., Lakshman, M., Rao, S.V.R. (2003). Urolithiasis in broiler breeders a pathological study. *Indian Journal of Veterinary Pathology*, 27 (1): 63-64.
- Alhyali, H.M., Aljubory, K.H. (2001). Pathology of infectious bursal disease in the broiler chicken. *Iraqi Journal of Veterinary Sciences*, 14 (1): 29-36.
- Ali, M.J. (1994). Current Status of veterinary Biologics Production in Bangladesh and their quality control. Proceeding of the BSVER symposium held on July 28, 1994 at NIPSOM auditorium, Mohakhali, Dhaka, Bangladesh.
- Alves, F.M., Percira, V.L. (2007). Cellulitis associated with lesions of bursa of Fabricius from broiler under sanitary inspection. *Rivista Brasileria de ciercia veterinaria*, 14 (1):23-27.
- Amin, M.R., Islam, A., Trisha, A., Das, A. (2006). Retrospective study of some poultry diseases at Gaibandha district in Bangladesh. *Bang. J. Vet. Med.*, 7 (1):239-247.

- Andrade, C.L., Ferreira, G.B, Franco, R.M., Nascimento, E.R.D., Tortelly, R. (2006). Pathological alterations and identification of *Escherichia coli* as causal agent of avian cellulitis in inspectioneted broilers chickens from a slaughterhouse of Sao Paulo. *Revista Brasileira de Ciencia Veterinaria*. 13 (3): 139-143.
- Banerjee, M., Reed, W.M., Fitzgerald, S.D. and Panigrahy, B. (1994). Neurotropic velogenic Newcastle disease in cormorants in Michigan. Pathology and virus characterization. *Avian Diseases*, 38 (4): 873-878.
- Barnali Chandra, Niyogi, D., Ghosh, C.K., Shukla, V.K., Joshi, R.K., Jana, S., Mukhopadhyay, S.K. (2008). Incidence and pathomorphology of spontaneous *Escherichia coli* infection in broiler birds in two districts of West Bengal. *Journal of Interacademia*, 12(4): 521-524.
- Bhargava, K. K., Neil, J. B., Prior, M.G. (1983). Incidence of salmonella contamination in broiler chickens in Saskatchewan. *Can. J. Med.*, 47 (1): 27-31.
- Bhattacharjee, P.S., Kundu, R.L., Biswas, R.K., Mazumder, J.U., Hossain, E. and Miah, A.H. (1996). A retrospective analysis of chicken diseases diagnosed at the Central Disease Investigation Laboratory, Dhaka. *Bangladesh veterinary Journal*, 30 (3-4):105-113.
- Bertke, E.M. (1955). Pathological effect of Coccidiosis caused by the protozoan parasites *Eimeria tenella* in chicken. Univ. Wisconsin, USA.

- Chishti, M.A., Khan, M.Z. and Irfan, M. (1985). Pathology of liver and spleen in avian salmonellosis. *Pakistan Veterinary Journal*, 5 (4): 157-160.
- Cosgrove, A.S. (1962). An apparently new disease of chickens avian nephrosis. *Avian Diseases*, 785-821.
- Chamanza, R., Dube, M., Zimhunga, T., Zanamwe Sikala, E.T., Chitauro, D., Chipanga, P., Rogers, S. (2001). Pathological findings in broiler chickens with malabsorption syndrome in Zimbabwe. *Zimbabwe Veterinary Journal*, 32 (1/2): 23-31.
- Crawford, M. (1930). Ranikhet disease. Ann. Rpt. Govt. Vet. Surgeon, Colombo, Ceylon.
- Dekock, G. (1954). Studies on the histopathological and pathogenesis of Newcastle disease of fowls in South Africa with special reference to the lymphoid tissue. *Onder stepoort. J. Vet. Res.* 26: 599-620.
- Domermuth, C.H., Gross, W.B. and Dubose, R.T. (1967). Mycoplasmal salpingitis of chickens and turkeys. *Avian Diseases*, 11: 393-398.
- Dyokov, L. (1966). Pathology of salmonellosis with reference to differential diagnosis. *Nauchni. Tr. Visshiya Vet. Med. Inst.* 16: 13-22.
- DLS, (1999). Brochure on Development and Activities. Directorate of Livestock services, Dhaka, Bangladesh.
- F.F.Y.P. (1998). The fifth Five Year Plan. Planning Commission, Ministry of Planning, Government of People's Republic of Bangladesh.

- Islam, M.R., Chowdhury, E.H.; Das, P.M., Dewan, M.L. and Khan, M.S.R. (1995). Pathologic characterization of a very virulent local isolate of infectious bursal disease virus. Proceedings of 1st BSVER Annual Scientific Conference held on 27th March, 1995, at Officer's Training Institute, D.L.S., Savar, Dhaka.
- Islam, M.R., Khan, M.A., Das, P.M. and Bari, A.S.M. (1998) Poultry diseases diagnosed at necropsy in 1997 and 1998 in the Department of Pathology of Bangladesh Agricultural University, Mymensingh. Proceedings of 5th BSVER Annual Scientific Conference held on 3-4 Dec. 1998, at Bangladesh Agricultural University, Mymensingh.
- Islam, M.R., Lucky, N.S., Mostafa, M.G. (2003). A study on occurrence of poultry diseases in Sylhet region of Bangladesh. *International Journal of Poultry science*, 2 (5): 354-356.
- Ismail, H.K. (2002). Pathological study of necrotic enteritis in broiler chickens in Mosul region. *Iraqi Journal of Veterinary Sciences*, 16 (2): 179-183.
- Jungherr, E.L. (1964). Pathogenecity of Newcastle disease virus for the chicken. In. Hanson, R.P. (ed.). Newcastle disease virus. Univ. Wisconsin, Medison pp: 257-272.
- Jungherr, E.L. and Terrel, N. (1946). Observation on the spread of Newcastle disease. Proc. Am. Meet. US Livestock saint. Ass. 50:158-171.

- Kamal, A.H.M. (1989). Pathological investigation on the mortality of chicken in Bangladesh Agricultural University Poultry Farm. M.Sc. Thesis, Dept. of Pathology, Faculty of Vet. Sc., BAU, Mymensingh.
- Khodakaram, A., Dadras, H. (2001). A pathological study on local tissue reactions induced after subcutaneous injection of oil Newcastle disease vaccine in broiler breeder chickens. *Iranian Journal of Veterinary Research*, 2 (2): 163-168.
- Kutubuddin, (1973). Pathological investigation on the cause of mortality of poultry birds in the BAU Poultry Farm. M.Sc. Thesis, Dept. of Pathology, Faculty of Vet. Sci. BAU, Mymensingh.
- Lalita Rani Barman, (2003). An epidemiological and experimental study of Newcastle disease in chickens of Bangladesh. *Bang. J. Vet.*, 5 (4): 56-68.
- Lange, W. (1914). Schimmel pilzerkankungen beim geflugel Deut. Dtsch Tieraerzt Wochenschr, 22: 642-643.
- Lukert, P.D. and Hitchner, S.B. (1988). Infectious bursal disease. Diseases of Poultry, 8th ed. Hofstad, M.S. (ed.) Iowa State University Press, Iowa, U.S.A., p. 570.
- Lutful Kabir, S.M., (2009). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int. J. Environ. Res. Public health*, 5 (4): 12-19.

- Mahmoud, A.E.A., Moussa, H.M.M. (2000). Studies on some aerobic bacterial causing of broilers. *Egyptian Journal of Agricultural Research*, 78 (1, Special Issue):25-34.
- Olkowski, A.A., Abbott, J.A., Classen, H.L. (2005). Pathogenesis of ascites in broilers raised at low altitude, aetiological considerations based on echocardiographic findings. *Journal of Veterinary Medicine Series-A*, 52 (4): 166-171.
- Park, K.Y., Jung, S.D., Yeh, J.G and Seo, I.B. (1994). Studies on the necrotic enteritis of chicken in Korea. *Korean Journal of Veterinary Research*, 34 (3): 593-599.
- Peckham, M.C. (1960). Further studies on the causative organism of ulcerative enteritis. *Avian Diseases.*, 4: 449-456.
- Pramanik, A.K. and Hattacharya, H.M. (1987). Diseases of poultry in three districts of West Bengal affecting rural economy. *Indian Journal of Veterinary Medicine.*, 7 (1): 63-65.
- Rahman, M.A., Samad, M.A. (2003). Pattern and occurrence of single and concurrent diseases associated with mortality in commercial chickens in Bangladesh. *Bang. J. Vet. Med.*, 1 (1):15-20.
- Rahman, M.M., Das, P.M., Bari, A.S.M., Islam, M.R., (2003). Pathological investigation of broiler diseases in some farms of Mymensingh district. *Journal of Animal and Veterinary Advances*, 2 (12):660-665.

- Rahman, M.M., Rahman, A., Islam, A.H.M.N., Miah, A.H., Mazumder, J.U. and Bhattacharjee, P.S. (1996). Observation on outbreaks and subsequent control of infectious bursal disease in the Central Poultry Farm in Bangladesh. *Bang. Vet. J.*, 30 (12):13-17.
- Rani, B.K., Nalini, T.S., Vijayasarithi, S.K., Gowda, R.N.S. (2000). Sequential pathological changes in experimentally induced hydropericardium syndrome (HPS) in broilers. *Indian Journal of Veterinary Pathology*, 24 (2): 94-95.
- Rath, N.C., Chanda, S.K., Rao, A.G., Panda, S.K. 2002. Pathology of Ascites syndrome in broiler chickens. *Indian Journal of Animal Health*, 41(2): 106-108.
- Ressang, A.A. (1961). Newcastle disease in Indonesia, Part-11. Its Symptomatology, gross and microscopic anatomy. *Commun Vet. (Boger Indonesisa)*, 5: 16-37.
- Sarkozy, G., Semjen, G., Laczay, P., Horvath, E. (2002). Treatment of experimentally induced *Pasteurella multocida* infections in broilers and turkeys: comparative studies of different oral treatment regimens. *Journal of Veterinary Medicine Series B*, 49 (3): 130-134.
- Sam, W. and Baruah (1998). Pathology of Infectious bursal disease in broiler chickens. *Indian Journal of animal science*, 68 (11): 1171-1172.
- Sarker, A. J. (1976). The prevalence of avian diseases in Bangladesh Agriculture University Poultry farm. *Bangladesh Veterinary Journal*, 10: 61-66.

- Schlegel, M. (1915). Schimmelpilzerk mankung (Aspergillose) in den lungen. *Beri Muench Tiergerztl Wochenschr*, 31-25.
- Sharad Kumar, Gupta, R.P. (2003). Biochemical and pathological changes in experimentally induced hydropericardium syndrome in broiler chickens. *Haryana Veterinarian*, 42 (5): 56-60.
- Shivachandra, S.B., Sah, R.L., Singh, S.D., Kataria, J.M., Manimaran, K. (2004) .Comparative pathological changes in aflatoxin feed broilers infected with hydropericardium syndrome. *Indian Journal of Animal Sciences*, 74 (6): 600-604.
- Siccardi, F.J. and Pomeroy, B.S. (1964). Effects of *E. coli* on growth rate, morbidity and mortality in monocontaminated and conventional chickens. *Poultry Science*, 43 (5): 1361.
- Smith, J. W. and Hamilton, P.B. (1970). Aflatoxicosis in the broiler chicken. *Poultry Science*, 49 (1): 207-215.
- Talukdar, M.H., Karim, M.J., Rahman, M.T. (2000). Concurrent infection of *Eimeria tenella* with some bacteria and viruses in broiler chicks. *Bangladesh veterinarian*, 17:21-26.
- Wahyuwardani, S., Sani, Y., Parede, L., Syafriati, T., Poeloengan, M. (2000). Stunting or runting syndrome in broiler chickens and its pathological changes. *Journal of Ternak dan Veteriner*, 5 (2): 125-131.

- Wang, J., Qiao, J., Zhao, L.H., Wang, H., Tian, Y., Gao, M., Wang, X. (2007). Proliferation of pulmonary artery smooth muscle cells in the development of ascites syndrome in broilers induced by low ambient temperature. *Journal of Veterinary Medicine Series A*, 54 (10): 564-570.
- Yoder, H.W. (1988). *Mycoplasma gallisepticum* Infection. Diseases of Poultry, 8th edn. Hofstad, M.S. (ed.) Iowa State University Press, Iowa, U.S.A., p. 195.