COMPARATIVE HISTOMORPHOLOGICAL STUDY OF NON-AFFECTED AND AFFECTED LYMPHOID ORGANS BY NEWCASTLE DISEASE (ND) IN BROILER CHICKEN

A Thesis
By

DR. MD. AKTHARUL ALAM
Registration: 1405097
Semester: January-June'15
Session: 2014-2015

MASTER OF SCIENCE (M.S.)
IN
ANATOMY

DEPARTMENT OF ANATOMY AND HISTOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY DINAJPUR-5200

JUNE, 2015

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Submitted to the Department of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University, Dinajpur in partial fulfilment of the requirements for the degree of

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DEPARTMENT OF ANATOMY AND HISTOLOGY
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JUNE, 2015

DEDICATED

TO
MY

BELOVED
ELDER BROTHER

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The Author
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ABSTRACT

Newcastle disease (ND) remains a constant threat to the poultry industry and is a limiting disease for poultry producers worldwide. Newcastle disease (ND) has been considered as one of the important viral diseases.

Poultry production is an easy and efficient way of producing animal protein. With less capital investment relatively more profit could be earned by producing poultry. About 31.5 percent people live under malnutrition (Brad Field, 2010). The average quantity of protein uptake by people is insufficient per head per day where as desirable requirement is decreasing daily per head day by day. At present there are more than about 30,000 commercial broiler and layer farms are supplying 260 thousand metric ton poultry meat and 5.21 billion table eggs per year (Rahman, 2003). The current investment in poultry sector is about 22 billion taka and a total of about 5 million people are working presently in this sector (Rahman, 2003). The poultry population of Bangladesh has increased from around 71 million in 1986 to around 188 million in 2006, an increase of about 164 percent in 20 years (FAO 2008, BBS 2006). Poultry can be an important tool to fight poverty not only for this group of people but also for the distressed women as poultry requires minimum land, short capital and skill. Despite the special emphasis of the state on this sector, the development of poultry industry is seriously threatened by the outbreaks of acute contagious and fatal diseases. Among them Newcastle diseases (ND), also known as Ranikhet diseases, is one of the major problems in the development of poultry industry in Bangladesh. It causes considerable economic losses to the poultry industry due to high mortality, morbidity, stress, decreased egg production and hatchability throughout the world (Alexander, 2000) including Bangladesh (Kafi *et al.*, 2003). ND has been recognized as one of the major problems of the large and small poultry industries in Bangladesh (Islam *et al.*, 1998; Rahman and Samad, 2003) and it causes up to 40-60% of total mortality among poultry population in Bangladesh (Talha *et al.*, 2001). Mortality and morbidity is very high in this case. Occurrence of the disease is reportable and may result in trade restrictions.

The purpose of this study was to observe the histomorphological and histopathological features of the major lymphoid organs of Newcastle Disease affected chicken in Dinajpur

district of Bangladesh. The investigation carried out in different farms of mentioned area. The investigation was conducted from 1st July'14 to 30th June'15. On gross and microscopic examination of lymphoid organs like thymus, spleen, cecal tonsil and bursa fabricious that are collected freshly from different farms was subjected for Newcastle Disease condition and observed these lymphoid organs in chicken affected with Newcastle Disease. Organs were cleaned with normal neutral saline and the changes were recorded.

A total of ten farms (5 days to 35 days old) has been selected where total number of birds were 5000. Birds were divided into ten groups, each group contained 300-700 birds; namely A (N= 400), B (N = 600), C (N-300), D (N-700), E (700), F (N-300), G (N-350), H (N-650), I (N-500), J (N-500).

After gross and microscopic study I found that mortality was 1.52 times higher in the non-vaccinated birds due to NDV. Clinical signs were marked depression, prostration, droopy wings, muscle tremor, greenish white diarrhea, edema of the head, inactive, weak, accompanied by the nervous signs (torticollis) are major. Gross changes were occur like swollen and molted spleen, enlargement of cecal tonsil with hemorrhage, swollen and yellowish bursa, thymic enlargement etc. In the spleen Lymphocytes degeneration, necrosis, nuclear pyknosis etc. and hemorrhages, congestion in mucosa, necrosis etc in caecal tonsil has been observed. In Bursa of Fabricius lymphoid depletion, lymphocytolysis, marked interfollicular fibrosis, glandular transformation of epithelium occurred. Medullary lynphocytolysis in thymus are common histopathological changes has been observed in the thymus.

Overall the performance of ND affected birds were remarkably reduced which is directly influenced the profitability of the farmers.

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ACRONYMS USED

%	:	Percent
&	:	And
@	:	At the rate of
<	:	Less than
>	:	Greater than
±	:	Plus minus
µg	:	Microgram
^{0}C	:	Degree centigrade
BAU	:	Bangladesh Agricultural University
Conc.	:	Concentration
Cu mm	:	Cubic millimeter
D.W.	:	Drinking water
et al.	:	Associates
Fig.	:	Figure
Gm	:	Gram
Hb	:	Hemoglobin
Hr	:	Hour
HSTU	:	Hajee Mohammad Danesh Science and Technology University
i.e.	:	That is
J.	:	Journal
Kg	:	Kilogram
Lit	:	Liter
Ltd.	:	Limited
Mg	:	Milligram
ND	:	Newcastle Disease
NDV	:	Newcastle Disease Virus
No.	:	Number
PBS	:	Phosphate Buffer Solution
Vol.	:	Volume

CHAPTER I

INTRODUCTION

INTRODUCTION

Bangladesh's economy is dependent on agricultural production of which the livestock sector alone contributes significantly. Livestock are important sources of animal protein to the people. They contribute to food security and to socioeconomic well being of many households in the country. Poultry (broiler chicken) is one of the short-cycle species of animals, which are more able to increase in numbers within a shorter time than some of the longer-cycle species of animals. Poultry providing food security and elevating poverty and to improve our socio-economic status.

Newcastle disease (ND) has been considered as one of the important viral diseases of poultry worldwide. It is an acute highly contagious viral disease of various species of birds of all age groups (Alexander, 1997) characterized by respiratory, digestive and nervous signs (Mishra *et al.,* 2000). Virulence of ND strains varies greatly with the host, but breed or genetic stock does not appear to have a significant affection on the susceptibility of chickens to the disease (Cole and Hutt's, 1961; Huang *et al.,* 1971). It causes considerable economic losses to the poultry industry due to high mortality, morbidity, stress, decreased egg production and hatchability throughout the world (Alexander, 2000).

ND has been recognized as one of the major problems of the large and small scale poultry industries in Bangladesh (Islam *et al.,* 1998; Rahman and Samad, 2003) and it causes up to 40-60% of total mortality among poultry population in Bangladesh (Talha *et al.,* 2001). The transmission of NDV occurs through newly introduced birds, selling or giving away sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing (Tu *et al.,* 1998). Due to its worldwide occurrence, ND drew the attention of research workers for an effective control program. Vaccination along with strict bio-security measures are considered as effective means for its control. Therefore, the practice of vaccination became a routine work throughout the world (Rahman *et al.,* 2002). Vaccination as a means to prevent the disease is routinely practiced in Bangladesh (Samad, 2000).

However, outbreaks of ND have been occurring frequently due to failure of vaccination resulted from high level of maternal antibody titre in young chickens which neutralizes vaccine virus and heterogeneous level of antibody titre in adult birds (Rahman *et al.*, 2002). Unfortunately, the prognosis for this disease is poor, with nearly a 100 percent mortality rate in chickens, once infected (Alexander, 1997).

The lymphoid tissues have an independent phylogenic origin, their function being to react to foreign antigens by producing antibodies, thereby, providing adaptive "Immunity ". Adaptive immunity is evidently a very ancient characteristic of vertebrates, first appearing in cyclostomes. It supplements the various non-specific mechanism of resistance including bactericidal enzymes, interferon and phagocytosis; these constitute an "innate immunity" (King, 1975).

The mechanism of adaptive immunity in birds have two components: one is the bursa of Fabricius with germinal centers and plasma cells in various tissues that are strongly interlinking with humoral immunity. The other is the thymus-dependent system which is the thymus and scattered collections of lymphocytes that are related to cellular immun.
Furthermore, lymphoid tissue can be divided into "central" and "peripheral" tissues. The former are believed to be primary sites of development lymphocytes. In birds these are the thymus and bursa as opposed to the thymus alone in mammals. The peripheral or secondary lymphoid tissues apparently depend on the central or primary lymphoid tissues for their origin, development and function. In birds they include lymphoid tissues in the spleen and in the alimentary tract including the cecal tonsils (Getty, 1975; Bach, 1978).

The obvious characteristics of the lymphatic tissues of mammals and birds are densely populated with the lymphocytes. So they are involved with lymphocyte production, immune responses or both of these processes occurring at the same time (Cormack, 1987). The bursa and thymus resemble one another with respect to their epithelial derivation, lymphoid nature, growth during embryonic and early postnatal life and early involution. Due to the fact that these organs begin to involutes before the development of sexual maturity, the account of their structure will be that found in the young chick from hatching to old age (Hodges, 1974).

However, both in birds and animal and even in human beings, lymphomas are not treating in the right way due to lack of people diagnosis and cost management. Moreover chemotherapy treated birds, animals humans, the lymphocytic changes in the major lymphoid organ and lymphoid tissue were not clearly understood. The chicken is an important model for research into the basic features of immunology since its immune system function in a similar way to of human and the chicken embryo allows easy experimental access (Battanddier *et al.,* 1980; Vanio *et al.,* 1995). If there are diseases in the lymphoid organs, irreversible changes are produced in the physiology of the chicken body. So evaluation of the functional state of the lymphoid organs is of immense practical importance, for results of such studies may establish the cause of diseases and determine which type of therapy should be employed.

The present study reveals the gross and microscopic aspect of the lymphoid tissues and in addition attempts were made to bridge the gap between the gross and microscopic anatomy of the lymphoid organs of non-affected and ND affected boiler chickens. The description of the lymphoid organs of chickens had been previously made but the study on the broiler chickens are inadequate and does not contain the particular data of the present study. The great importance of these data is bearing on the functional significance of lymphoid organs of the broiler chicken. It is hoped that the present investigation will be a base line for the study of lymphoid organs of these birds, and also will provide valuable information to poultry immunologist, pathologist, researchers, cell biologist and anatomists.

Objectives:

I. To study the gross and microscopic anatomy of the lymphoid organs (thymus, bursa fabricius, spleen and cecal tonsil) of non affected and ND affected broiler chickens.
II. To study the histopathological features of lymphoid organs (thymus, bursa of fabricius, spleen and cecal tonsil) of non affected and NDV affected broiler chickens.

So the present study investigate the histomorphology of lymphoid organs of ND affected and non affected broiler chicken and that will add to the knowledge in this field.

CHAPTER II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The main aim of the this chapter is to provide a some major review of the previous research conducted so far in regarding to the present conducted study, the number of works directly related to the present study were scanty detailed anatomical study of the lymphoid organs of birds had been studied previously by different researchers.

2.1 Clinical signs

Beard and Hanson (1984), summarized ND into pathotypes, based on clinical signs in chicken as: Viscerotropic velogenic ND, also known as Doyle's form in which, clinical signs often begin with listlessness, increased respiration and weakness, prostration and death. Oedema around the eyes and head may occur. Greenish diarrhoea, muscular tremors, torticolis, paralysis of legs and wings and opisthotonus may occur and mortality may reach 90- 100% in fully susceptible flock (Cynthia, *et al.,* 2005). The neurotropic velogenic form (Beach's form) of ND presents with sudden onset of severe respiration distress, followed by neurologic signs. Morbidity may reach 100% and mortality 50 to 90% (Saif, *et al.,* 2005). Mesogenic strains of ND virus causes respiratory disease with marked drop in of production and the mortality rate is usually low, while the lentogenic virus strain does not cause disease in adult chickens. In young birds, respiratory disease may occur and death may result from secondary bacterial infection.

Alexander *et al.,* (1993) reported that green diarrhea is frequently seen in birds that do not die in early infection and prior to death, muscular tremors, torticulis, paralysis of legs and wings, and opisthotonous may be apparent. Mortality may reaches 100 percent in flocks of fully susceptible chicken through the flock.

Okoye *et al.,* (2000) reported that the signs are sneezing, coughing, nasal discharge in case of ND and signs are indicating involvement of the nervous system include clonic spasm, muscular tremor, torticulis appears in the birds that survive in the initial phase.

2.2 Gross Anatomy

2.2.1 Gross anatomy of thymus

King (1975) reported that the thymus is an organ of fetal and early baby chicken hood. It is an important lymphoid organ responsible for the mechanisms rejecting foreign tissues. With growth of the bird it soon undergoes retrogressive changes into fat and connective tissue.

Getty (1975) observed that, the appearance of the thymus varies considerably with age. The paired thymus of the chicken consists of a series of separate pale red or yellowish irregular lobes. In the adult there were there or eight such lobes, of varying size and shape, extending along each jugular vein as far as the thyroid gland. Thymic tissue sometimes penetrates the parathyroid gland. Upper fifth of the neck of chicken was devoid of lobes. In white Rock males the maximum total weight (the left and right thymus together) of 15.76 gm. was reached at 17 weeks of age. Involution was then progressive, so that in males at 19 months of age it is about 2.2 gm. and in females of similar ages about 0.6 gm., but remnants of all the is including the most cranial ones , persists in birds at least up to 16 months of age.

2.2.2 Gross anatomy of the bursa of Fabricious

The bursa of Fabricius was an asymmetric medial, lymphoepithelial organ which was peculiar to birds. King (1975) reported that it was the second primary lymphoid organ, after the thymus in birds. It was a blind, round to oval, sac like, dorsal diverticulum of the proctodeal wall of the cloaca. The central lumen of the organ was to a great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursa) wall, which resemble villous projections.

Bach (1978) reported that it is primary lymphoid organ whose development was independent of exogenous antigenic stimulation. It was the second lymphoid organ, after the thymus, to appear in birds. It developed from a proliferation of the endodermal epithelium of the dorsal caudal region of the embryonic cloaca at about fifth days of incubation. by the tenth day of incubation the bursa has developed a lumen and formation of the plicae begins. The lumen was lined with a layer of cuboidal to columnar cells thick. About the twelfth day of incubation the primitive undifferentiated epithelial cells lining the lumen gave rise to points of points of epithelial proliferation.

Hodges (1974) reported that at the time of hatching the bursa of the white leghorn chicken was well developed and was growing rapidly. On the day of hatch it weights about 0.04gm. There after growth was rapid, the weight increasing much faster, in proportion , than that of the body weight until the fourth week. At this point it was about 0.42% of the body weight and from then on the rate of growth begins to fall slowly until at 10 weeks.

When bursa was about 0.3% of the body weight, being about 4.25gm. After 10 weeks there was a decline in bursa weight as the organ begins to involutes by 23 weeks of age the bursa was reduced to a remnant. The actual time of involution varies according to strains and although it was originally thought to occur at about 4 month, recent figures give a time of around 2-3 months. Bursa regression was related to increase in testosterone or estrogen levels at puberty. The bursa of Febricious plays a central role in the chicken B cell development.

The bursa was colonized between days 8 and 14 of embryonic development by precursor cells which migrate across the epithelial basement membrane from the epithelial buds which ultimately develop into the lymphoid follicles of the bursa (Cooper, 1965; Glick, 1956, 1977; Befus, 1980; Michael, 1994).

2.2.3 Gross anatomy of the spleen

Hodges (1974) observed in white leghorn chicken, that the spleen were soft, friable, highly vascular, rounded and reddish-brown organ which lies close to right side of the junction between the proventriculus and the gizzard . He further reported that, the spleen was rather variable in size and shape but may be as much as 2 cm in diameter in the adult chicken. Small accessory spleens have been reported as occurring frequently in fowles. At 10 weeks of age the spleen weighted 2.63 gm or 0.2% of the body weight, there after its weight tended to fluctuate. The spleen is at its maximum size at 3-4 month of age of white leg horn. The size and weight of the spleen very at different ages in different breed and in the same breed under different conditions.

The spleens are briefly erythropoietic in the embryo and lymphopoietic through out adult life. It facilitates immune responses to antigens that have gained access to the circulating blood and to dispose of defective blood cells (King, 1975; Cormack, 1987).

2.2.4 Gross anatomy of the Cecal tonsil

Lymphoid foci occur irregularly in the lamina propria or submucosa of the alimentary tract and they are called tonsils. The majority of mucosa-associated lymphoid tissues (MALT), develop in various ways and at various rates. Cecal tonsils were always the most immunologically mature lymphoid organ. In birds, the ceca commonly arise bilaterally at the junction of small and large intestines.

King (1975) the ceca of the white leghorn chicken average 16-18 cm in length. They were large in cailbre toward the blind extremity and are constricted near their origin. The parietal coats are continued from the small intestine. The cecum extend towards the liver and later were doubled on themselves. The mesentery runs between the ceca and passes on the ileum. King (1976) described about lymphoid foci situated irregularly in the lamina propria or submucosa of the alimentary tract and in some regions these have been called tonsils. The most prominent of those are two "Cecal tonsils", each of which is an enlargement on the medical wall of the cecum near the lieocecocolic junction.

Mclelland (1989) stated that the ceca may be classified according to length into long, intermediate, short and vestigial categories. As in most birds, the chicken cecum was long and simple. King 1975 reported that the galliform caeca were generally subdivided into a proximal light red portion or base , a middle portion, the body which bluish green to gray green, is wider and the wall is thinner and the blind tip or apex with fairly thick walls of light red colour (Cormack 1987, Mclelland, 1989).

King (1975); and McLoed (1939) reported that both the body and apex occupy a major part of the cecum, preserving a unique environment which continuously regulates the proliferation of specific microflora and to prevent the infection by foreign organizations. These intestinal lymphoid tissues appear to be dependent partly on the thymus and partly bursa. The cecal tonsils are an important source of antibody, besides sharing the general immune responses, the cecal tonsils and other intestinal lymphoid foci evidently have a local immunological function in relation to bacteria and other antigenic substance in the gut.

2.3 Histology

2.3.1 Histology of thymus

Hodges (1974) state that the chicken thymus was enclosed by a connective tissue capsule. This was comparatively thin and is composed mainly of course collagen fibers together with a few, fine elastic fibers. External to the capsule are considerable amount of loose connective tissue and adipose tissue. Passing inwards from the capsule were numerous fine septa which divide the mass of the gland into lobules. Branching out from the main septa were normally numerous small, short, septules into segments.

Hodges (1974) reported that the lobules were divided into, indistinct cortex and the medulla. The underlying stoma of both cortex and medulla consists of a network of reticular cells and their fibers. These cells possess a round, oval or elongated nucleus with a few fine chromatin granules and one or two nucleoli. In the cortex there were packed large numbers of lymphocytes, mainly small lymphocytes, although medium-sized lymphocytes can also be seen. In normal 2-month old chickens the cortical lymphocyte population fairly uniform in consistency. The cells being about 3.5-4.5 micron in diameter.

Bach (1978) reported that subcapsular cortex of the thymus of white leghorn chicken consisting of large lymphocytes which were less numerous, however, in this zone, the stem cells penetrate and divide, explaining the high incidence of mitosis. He further reported that, the deep cortex stores small lymphocytes. A large number of cortical lymphocytes also die in the deep cortex and he also further observed in his study, that, the medulla contains few lymphocytes with pale cytoplasm and Hassall's corpuscles. Hassall's corpuscles consist of epithelial cell piled and coiled on top of one another. Their size is variable, and they may be cystic, with a central eosinophilic substance plus pyknotic cells. The cells are linked together by demonsomes and show cytoplasmic tonofilaments. The functional significance of Hassall's corpuscles in chicken remains obscure. They could play a role in transferring the humoral products of thymus to the circulation.

The blood supply within the medulla is more abundant than that in the cortex. It consists of a well developed network of arterioles and small veins. Other cells which can be seen within the medulla of chicken thymus were eosinophils, haemocytoblasts and plasma

cells. The germinal centers are normally not found, however, present in occasional cases in the cortex at 14 weeks old birds (Greenwood, 1930; Bradly *et al.*, 1960; Ham *et al.*, 1961; Hodges 1974; Copenhaver *et al.*, 1975; Dellmom *et al.*, 1976; Leeson, 1976; Cormack, 1987, Arey, 1994; Khan 1996, Junqueira, 1998).

2.3.2 Histology of the bursa of fabricius (Cloacal bursa)

Bach (1978) reported that, the mucosa, the musculosa and the serosa of the bursa are in continuity with the corresponding tissues of the intestine. The epithelial surface, like that of the intestine, consists of cylindrical cells, but the bursa has no mucous cells, lymphoepithelial nodules are present in the lamina propria directly under the epithelium. These nodules contain a light medulla and a dark cortex. The medulla contains epithelial cells that form a continuous area in the periphery, which projects into the epithelial coating. The center of the meddulla was less structured; it contains in addition epithelial cells and various other types of cells, including large lymphocytes, plasma cells, reticular cells, marcrophages and granulocytes. Cortex is seperated from the medulla by basement membrane. This membrane permits exchange between the two zones of the lymphoepithelial nodule. The cortex consists mostly of small lymphocytes and plasma cells (Ackerman, 1959; Bradly, 1960; Papermaster, 1962; Gilmore, 1977; Bach, 1978; Odend'hal *et al.*, 1979; Battandier, 1980; Dolfi, 1988; Michael, 1964; Paramithiostis *et al.*, 1994 and khan, 1996).

2.3.3 Histology of the spleen

King (1975) stated that the spleen was enclosed by a capsule, comprising collagen fibers, elastic fibers, a new muscle fibers and fibroblasts. He also observed that, the internal architecture of the spleen in general resembles that of the mammal. About 85 percent of the substance was equally divided into the red and the white pulps. The connective tissue trabeculae were relatively indstinct. The splenic tissue consists of a network of reticular cells and recticular fibers. Superimposed upon this frame work were the two types of splenic tissue, the white pulp and the red pulp. The dominant cell type of the whole spleen was the small to medium lymphocyte. The chicken spleen does not have a

marginal zone with marginal zone macrophages and marginal metallophil (Bradly, 1960; Hodges, 1974; Jeurissen, *et al.*, 1992;).

The red pulp was a loose spongy tissue composed of ramifying cellular cords surrounded by venous sinuses. The cell cords between the sinuses were composed basically of recticular cells, lymphocytes, macrophages and all types of circulating blood cells. Plasma cells may be seen occasionally in the normal spleen but tend to become more abundant in experimental and infective conditions (Hodges, 1974).

Bach (1976) reported, the red pulp plays a role in the capture of antigens that were initially bound to macrophages in the marginal zone and the rest of the red pulp.

Payne (1971) reported that, the white pulp consists of two types of tissue, diffuse lymphoid tissue enveloping the central arteries and their branches, and germinal centers near the central arteries. The diffuse lymphoid tissue of the white pulp was evidently thymus dependent, where as the germinal centers and the plasma cells around them were bursa dependent (Dransfield, 1945; Stiles, 1965; Hoshi, 1972; Dellmom, 1976; Ogata, 1981; Fukuta, 1987; Nicader, 1993; Junqueira, 1998).

2.3.4 Histology of the Cecal Tonsil

Hodges (1974); Del Cacho *et al.*, (1993) stated that the gut-associated lymphoid tissue (GALT) must be richly populated at the cecal mucosa. However, no detailed distribution of lymphoid nodules has been reported in the mucosa of the body and apex, where as well developed accumulated lymphoid nodules, termed the cecal tonsil, and distal lymphoid nodules have both been reported in the base. each consists of a diffuse mass of dense lymphoid tissue in which small lymphocytes predominate and many plasma cells are present together with numerous large circumscribed germinal center. In the mucosa, the linning epithelium is composed of columnar and goblet cells. Both peneth's cells and Russell's bodies were plentiful. The lamina propria is formed by the connective tissue fibres. The mucose forms folds and crypts. Between the folds, glandular crypts dip into the corium. The submucosa was separated from the mucosa by the muscularies mucosa, which was thin at the cecal apex. The muscular coats were an outer longitudinal, 2 middle circular and an in inner longitudinal. The apex shows a great thickening of all three layers of muscle.

Elastic tissue was confined to the tunica media of the blood vessels. The argentophil reticulum was as profuse as in any other part of the digestive tract. The serous coat has many nerve plexuse (Looper, 1929; Dransfield, 1945; Hoshi, 1973; Hodges, 1974; Burns, 1982; Jeurissen, 1989; Calhoum, 1993; Kitagawa, 1996).

2.4 Histopathology

2.4.1 Histopathology of Thymus

O. G. Mohammadamin and T. S. Qubih (2009) stated that on day three of post-challenge, tissue sections from all challenged groups showed histopathological changes. There was lymphocyte depletion in cortex and medulla. Also, thymic sections revealed total depletion and disappearance of lymphocytes in cortex and medulla with only islands of lymphocytes remained in cortex. On day seven of postchallenge, sections of thymus revealed changes similar to those observed in 3 days post challenge.

2.4.2 Histopathology of Bursa of Fabricius

O. G. Mohammadamin and T. S. Qubih (2009) stated that, Bursal sections from negative control group did not reveal any histological changes. On day three post-challenge, sections from challenged groups; revealed reduction in size in bursal follicles, lymphocyte depletion in bursal follicles, formation of intrafollicular glandular structures with enormous proliferation of fibrous connective tissue in interfollicular space. At seven days of post-challenge, sections of bursa from groups revealed changes similar to those observed in 3 days post challenge.

2.4.3 Histopathology of Spleen

O. G. Mohammadamin and T. S. Qubih (2009) stated that on day three post challenge, sections revealed histopathologic changes. Lymphocyte depletion in splenic lymphoid follicles was the most prominent lesion found, the lymphocyte depletion was characterized by fewer lymphocytes than normal. On day seven postchallenge, sections revealed changes similar to those observed in 3 days post challenge.

2.4.4 Histopathology of Cecal Tonsils

O. G. Mohammadamin and T. S. Qubih (2009) stated that on day three post-challenge, microscopical sections from affected groups revealed lymphocyte infiltration in lamina propria as well as infiltration of lymphocytes between mucosal glands.On day seven psot-challenge, sections of cecal tonsils revealed changes similar to those observed in 3 days post challenge.

CHAPTER III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Experimental Birds: Broiler Chicken

3.2 Experimental area and research period:

The broiler chicken were collected from five selected broiler farm wherever NDV vaccine and other managemental program were performed properly in different upazila of Dinajpur district which is almost 40km far away from the University of Hajee Mohammed Danesh Science and Technology, Dinajpur-5200, Bangladesh skillfully from January to June, 2015.

3.3 Experimental Design:

The broiler chickens of Cobb 500 were grouped into ten groups. Each group was consisting of five hundred chickens. Chickens from each group were killed with cervical subluxation. Food and water was with held two hours before killing and body weight of each chickens were recorded for the calculation of the relative weighted of the lymphoid organs.

Just after killings of the chickens the lymphoid organs such as Spleen, Thymus, Bursa-fabricious and Cecal tonsil has been collected both for gross and histopathological studies.

3.4 Gross Anatomical Study:

The gross study included the color, length, weight, diameter and thickness of all lymphoid organs. Relative weights of each of the lymphoid organs were also been calculated adopting following formula according to Federova (1987).

$$\text{Relative wt. of the lymphoid organs} = \frac{\text{Weight of lymphoid organ}}{\text{Live weight of broiler}} \times 100$$

3.5 Microscopic Study:

For the purpose of histopathological studies the tissues of different lymphoid organs of broiler chicken (Spleen, Thymus, Bursa-fabricious and Cecal tonsil) were collected and fixed in the 10% formalin solution for few days. 10% formalin was prepared as follows –

37% formaldehyde 10 ml

Distilled water 90 ml

Then tissues were taken from the formalin and were dehydrated by using ascending graded of alcohol (70%, 80%, 90%, 95%, 100% and a00%). For each grade of alcohol one hour was provided. After dehydrating the tissue it was transferred to the Xylene-1 and Xylene-2 each for ninety minutes for clearing purpose. Then the tissues were infiltrated in the liquid paraffin at 60^{o}C temperature for ninety minutes and it was repeated again. Finally the tissues were embedded in paraffin blocks were made.

The paraffin blocks were cut at 6m thickness using microtome machine (Mu 509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45^{o}C for stretching. Then the sliced tissue was placed on grease free clean glass slide using adhesive like Mayer's egg albumin. Then the glass slides were dried at 37^{o}C temperature for 24 hours in an incubator. After drying the slides the sections were stained with Hematoxylin and Eosin (H & E) stain for general histological study.

Hematoxylin and Eosin (H & E) Stain

Preparation of Harris hematoxylin solution

Hematoxylin crystal----------5gm

Alcohol, absolute----------50cc

Potassium alum----------100gm

Distilled water----------1000cc

Mercuric oxide (red) ----------2.5gm

Hematoxylin was dissolved in alcohol, the alum in water by the aid of heat. After mixing the solutions it was brought to heat. After removal from heat, the mercuric oxide was added slowly. Reheated and was removed immediately and vessel was plunged into basin of cold water until cool. 4 cc glacial acetic acid per 100 cc of solution was added. The solution was added. The solution was filtrated before use.

Preparation of counter stain for hematoxylin stain

stock 1% aqueous eosin solution:

Eosin Y, water watersolube----------10g

Distilled water----------1000g

Glacial acetic acid----------2cc

Working eosin solution:

Eosin, 1% stock solution----------1part

Alcohol,80%----------3part

Staining procedure:

Xylene-1	2minutes
Xylene-2	2 minutes
Absolute alcohol-1	2 minutes
Absolutes alcohol-2	2 minutes
70% alcohol	2 minutes
Running water	2 minutes
Hematoxylin	5 minutes
Running water	15 minutes
Distilled water	5 minutes
Eosin	1 minute
70 Alcohol	5 seconds
80% alcohol	2 minutes
90% alcohol	2 minutes

95% alcohol	2 minutes
Absolute alcohol-1	2 minutes
Absolute alcohol-2	2 minutes
Xylene-1	2 minutes
Xylene-2	2minutes

Mounting in canada balsam

3.6 The sections were stained in order to study:

1. The structure of Spleen, Bursa fabricious, Cecal tonsil, Thymus.
2. Histological development during various period of life.
3. To study the distribution of connective tissue.
4. To study the distribution of lymphocytes.

3.7 Statistical Analysis:

The raw data were decoded, entered and sorted using the MS Excel from various parameters in present study was subjected to statistical analysis. The data were calculated by one factor randomized complete block design using analysis of variance table. Initially the data were sorted and cross cheeked for duplication and/or missing value. The missing values for each variable were recorded (numeric) as to be excluded in the analysis.

3.8 Photographics from selected specimens were prepared and placed in the thesis for better illstrations.

CHAPTER IV

RESULTS

RESULTS

Histopmorphological and histopathological investigation of Newcastle diseases encountered in small scale commercial poultry farms at different upazila of Dinajpur district was studied and different clinical gross and histopathological features were recorded during the study period.

4.1 Clinical Examination

The clinical signs of the birds affected (fig-5&7) with NDV varied from farm to farm. Chicken typically may become marked depression, prostration, droopy wigs, muscle tremor, greenish white diarrhea, edema of the head, inactive, weak, accompanied by the nervous signs (Fig-6) like twisted necks (torticolis) with high mortality reaching 100%. Sometime complete inability to make sound (M.A. Samad, 2000).

4.2 Status of Prevalence and Mortality of the Disease

The study revealed the following actual status of mortality and prevalence of Newcastle diseases virus (NDV) in broiler chicken. Prevalence of NDV at different farms of different region of Dinajpur district (Table-1). A total of 5000 birds from ten farms were examined during the study period from which 2350 birds of four farms were found infected with NDV. The Prevalence of NDV was in 47%.

STable-1: Status of prevalence of ND at different area of Dinajpur District.

Location (Thana)	Name of Farmers	No.of birds	Necropsy done suspected as NDV	NDV found	Result
Parbotipur	Mr. Abul kalam	400	6	5	+ve
Parbotipur	Abdus Sattar	600	7	7	+ve
Chiribander	Syed Ali	300	4	0	-ve
Chiribander	Ramjan Mia	700	5	0	-ve
Sadar thana	Sagor Hossain	700	9	6	+ve
Sadar thana	Rafiul Alam	300	4	0	-ve
Birol	Sahidur Rahman	350	5	0	-ve
Birol	Kawsar Chow	650	9	8	+ve
Kaharole	Kashem Azad	500	6	0	-ve
Kaharole	Ali Akber	500	4	0	-ve

Whereas Table-2 showed that the prevalence is almost 47% among all visited farms.

Table 2: Mortality at different of ND affected commercial broiler farms in Dinajpur District.

Location (Thana)	Name of Farmers	No. of affected birds	Vaccine status	Mortality	Percentage (%)
Parbotipur	Mr.Abul kalam	400	Not given	149	37.25%
Parbotipur	Abdus Sattar	600	Not given	276	46%
Sadar thana	Sagor Hossain	700	Given	189	27%
Birol thana	Kawsay Chow	650	Given	188	28.92%
Total	4 farms	2350		802	34.12%

Above table showing that mortality is lower in vaccinated flock that is 377 birds among 1350 birds (27.92%) than in non vaccinated flock 425 birds among 1000 birds (42.25%).

Fig-1: One of the selected farms

Fig-2: Non-affected flock

Fig-3(a): Non-affected flock

Fig-3(b): Affected flock

Fig-4(a): Non-affected bird

Fig-4(b): Non-affected bird

Fig-5(a): Affected birds with ND

Fig-5(b): Affected birds with ND

Fig-6: Torticolis due to CNS infection by ND

Fig-7(a): Respiratory symptoms of ND affected bird

Fig-7(b): Respiratory symptoms of ND affected bird

4.3 Necropsy Examination

Remarkable swollen spleen with white sub-capsular necrotic foci in the spleen (Fig-8b) of a broiler chicken has been found in maximum cases in necropsy. In the flock, 35% of the birds are died. This type of foci has been observed in all ages of broiler chicken those were affected by Newcastle Disease.

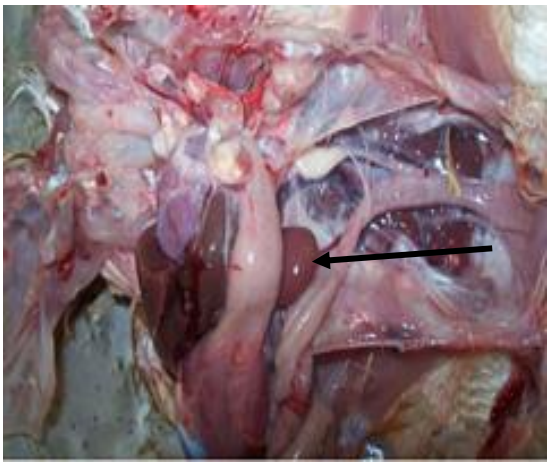

Fig-8(a): Non affected spleen (arrow)

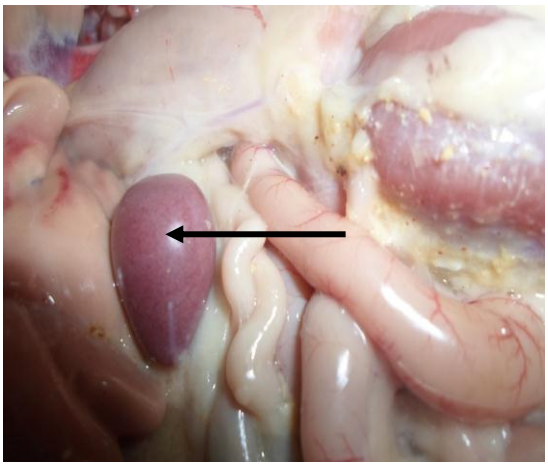

Fig-8(b): Swollen spleen with white sub-capsular necrotic foci. (arrow)

In the viscera of broiler chicken swollen lesions (fig-9b) in the spleen has been observed in necropsy .The spleen has became slightly blackish in color than normal. But such kinds high swollen hasn't been occurring in case of early ages of New Castle Disease affected birds. Spleen has also been found as moulted with visible necrotic foci. (Fig-10b).

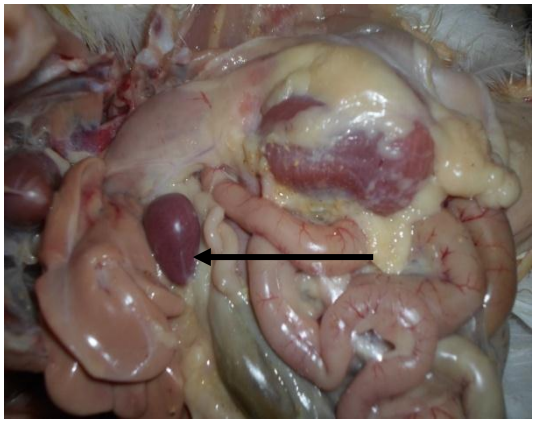

Fig-9(a): Non affected of spleen (arrow)

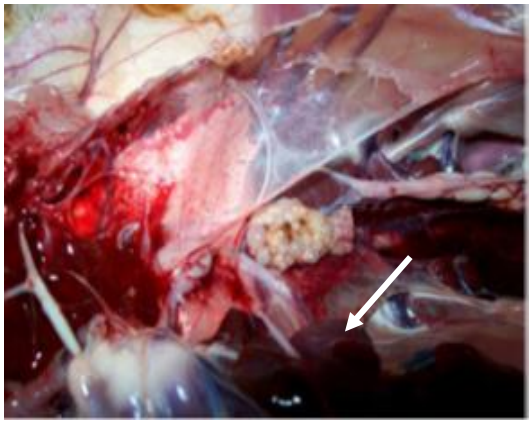

Fig-9(b): Blackish spleen of affected bird (arrow)

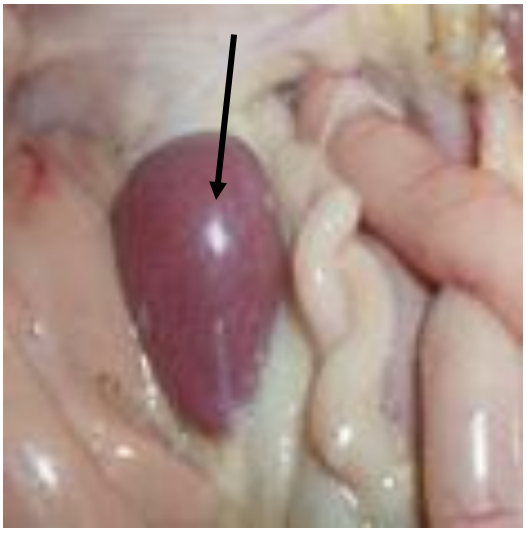

Fig-10(a): Non affected spleen (no necrosis)

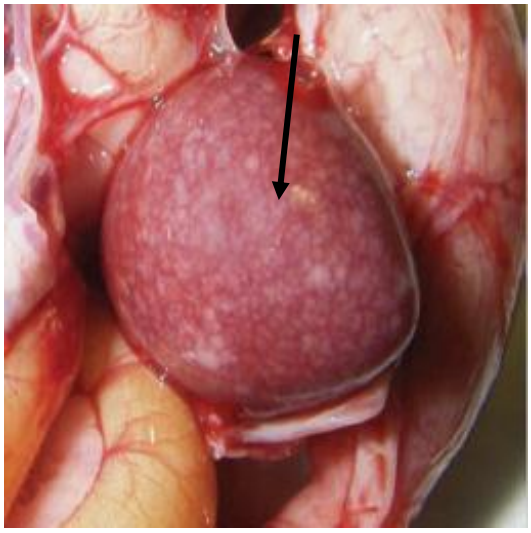

Fig-10(b): Moulted spleen with necrotic foci (arrow)

In some cases bilateral enlargement (Fig-11b; A-1& A-2) of cecal tonsil observed in young chicken. This kinds enlargement has also been observed in adult chicken also. But

the enlargement was highly remarkable in case of adult birds. In addition to these enlargement, hemorrhages (Fig-12b) has also been observed in the cecal tonsils in a broiler chicken that with air sacculitis. Numerous folds observed in the apex of cecal tonsil (Fig-13).

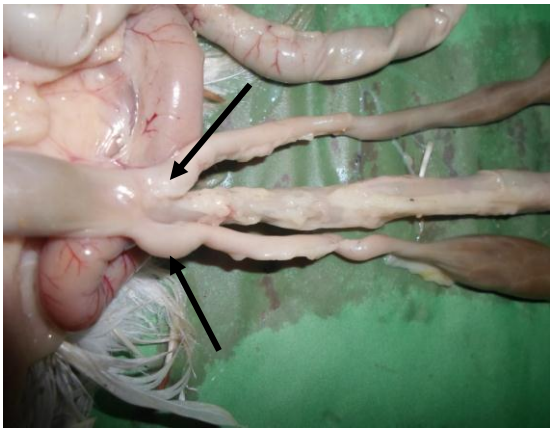

Fig-11(a): Non affected cecal tonsil (arrow)

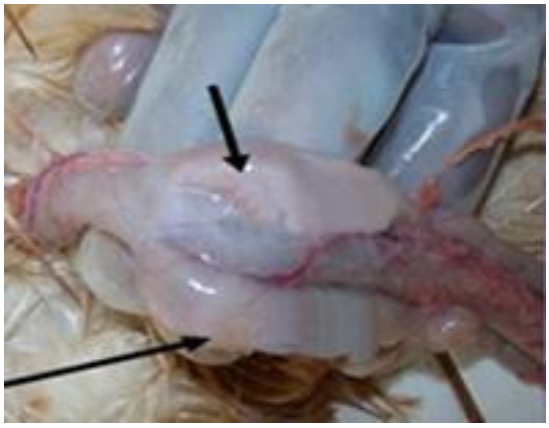

Fig-11(b): Bilateral enlargement (arrow) of cecal tonsil of affected bird

Fig-12(a): Non affected cecal tonsil (arrow)

Fig-12(b): Hemorrhage (arrow) in the cecal tonsil

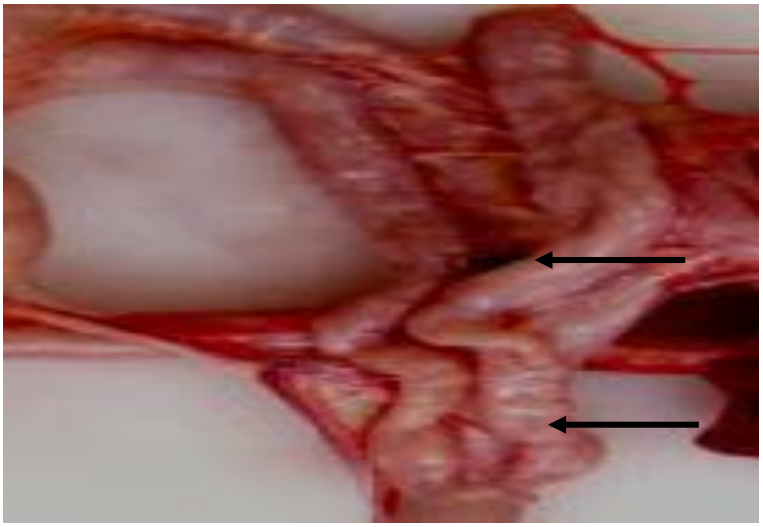

Fig-13: Numerous folds and reddish apex of cecal tonsil (arrows)

The bursa of Fabricius of broiler chicken is a single lympho-epithelial organ which is peculiar to birds. The organ appears as a dorsal median diverticulum of the proctoderm, being smooth and globular in shape and yellowish white in color. When fully developed it consists of a wall surrounding a small, axial, main cavity. The main cavity gives off small diverticula, and also leads into the cloaca through a small median opening in the dorsal wall of the proctodeum. The central lumen of the organ is to a great extent obscured by the presence of about 12 plicae, long folds of the mucous membrane of the bursal wall, which resemble villous projections.

In Busa Fabricious there is remarkable swollen (Fig-15b) has been occurred in adult birds of 30 days of age but not clearly visible in case of early ages of broiler chicken. Color has also been changed from yellowish to slightly reddish when affected by new castle disease virus. When open hemorrhage and yellowish necrotic debris (Fig-16b) like substance has been observed. But after three days of post infection these substance has not been observed without slightly yellowish white in color (Fig-17) which is slighter than during infection.

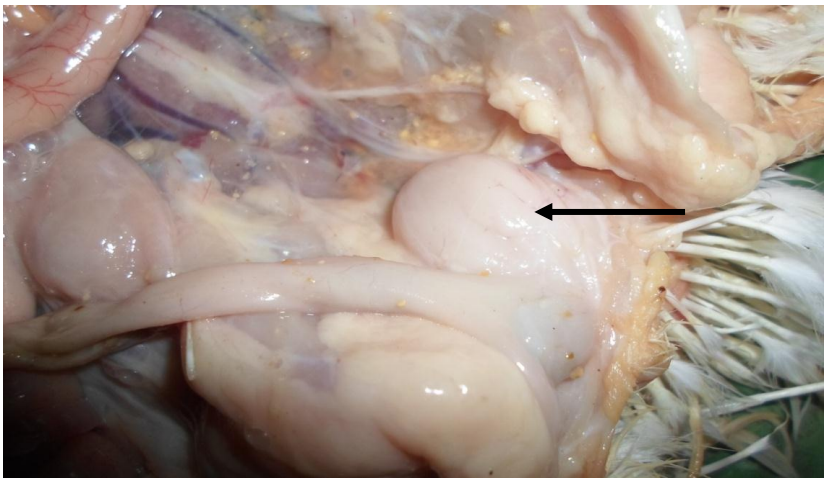

Fig-14: Non affected bursa of fabricius (arrow) of broiler chicken

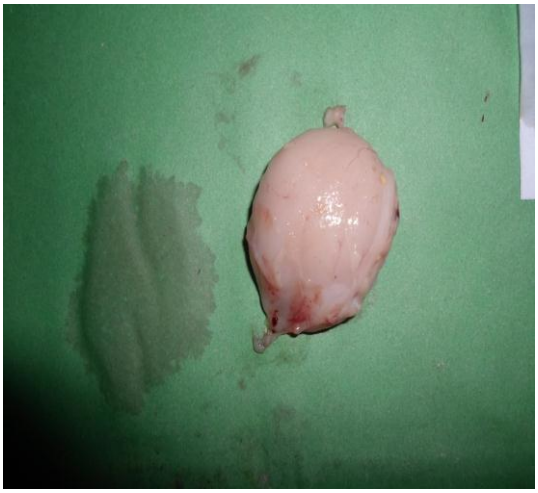

Fig-15(a): Non affected busa fabricius

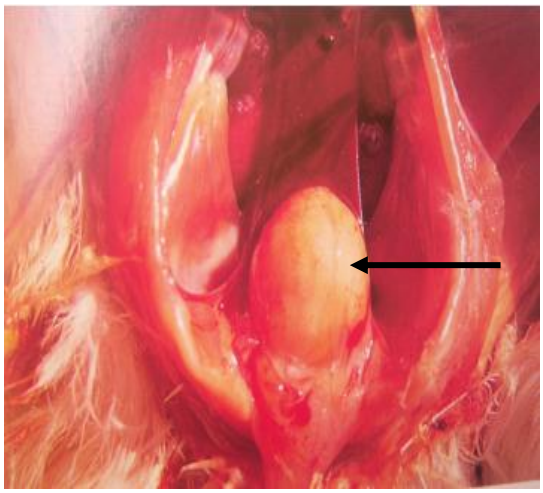

Fig-15(b): Swollen, reddish bursa (arrow) due to ND

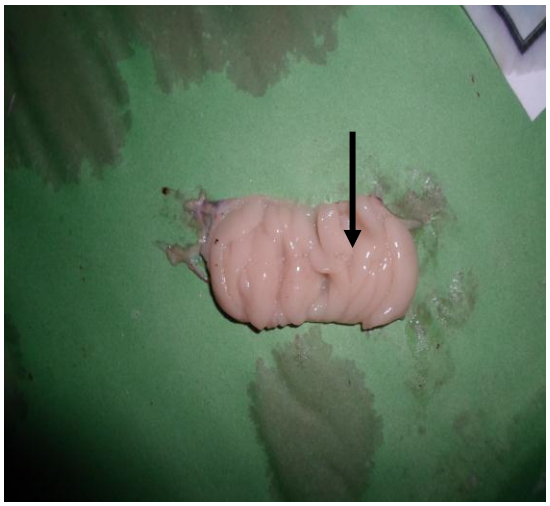

Fig-16(a): Non affected inner layer of bursa (arrow)

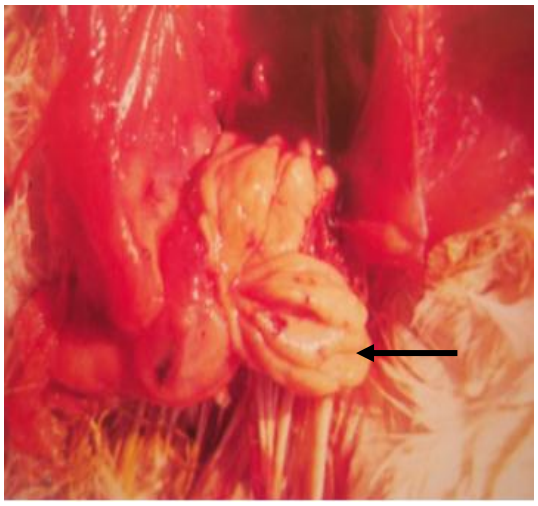

Fig-16(b): Hemorrhage, swollen, debris (arrow) in the bursa

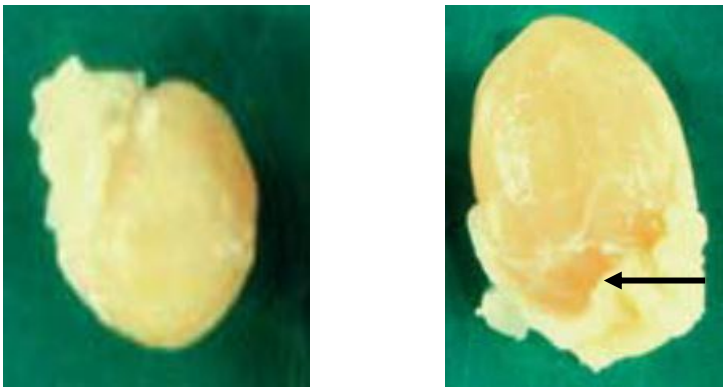

Fig-17: Non affected (left) and affected bursa (arrow in right fig.) after three days post infection

Enlargement of thymus (Fig-18b, 19) is most common and remarkable when affected by NDV. Thymic lobes seemed to have disappeared at first but later, the organ regenerated to its normal size. Atrophied thymus has also been observed.

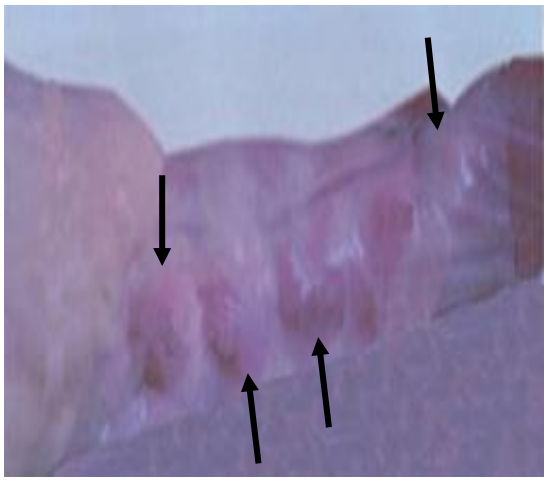

Fig-18(a): Non affected thymus (arrows)

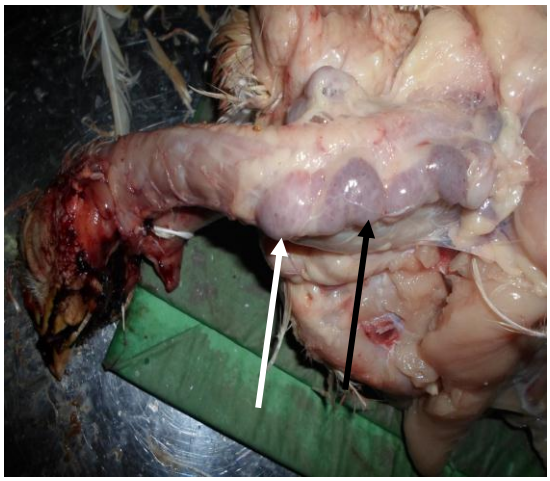

Fig-18(b): Swollen (arrow) thymus

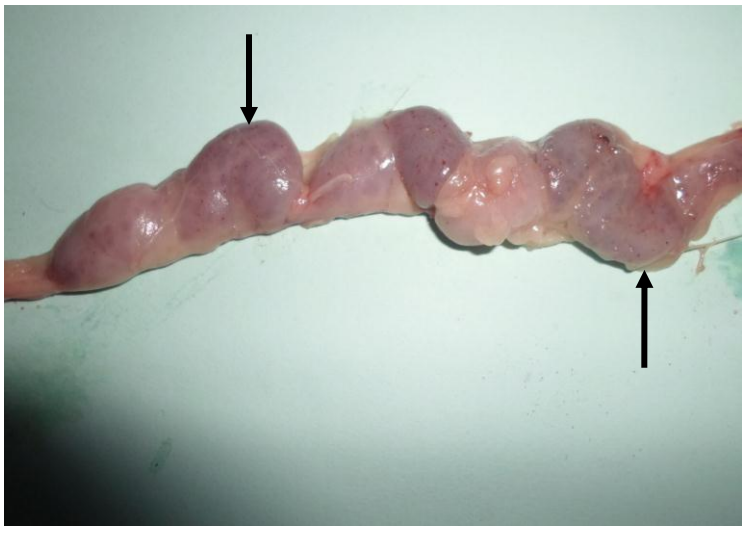

Fig-19: Swollen thymus (arrows) of broiler chicken due to ND

4.4 Histological study in normal structure of Lymphoid organs

In case of adult bird's indistinct demarcation between red and white pulps are diffusely scattered throughout the spleen. Central arteriole slightly visualized. There was a gap around the central arteriole which may be due to shrinkage of blood vessels.

The spleen of the broiler chicken are covered at this by a thick fibrous capsule with very few trabeculae and composed of network of recticular cells within which small medium and large size lymphocytes were diffusely located. It contains sheath arteries and occasionally, lymphatic nodules. Red pulp was formed from venous sinuses and was consisting of anastomosing cords of reticular cells, macrophages, lymphocytes and blood cells. The framework of the splenic tissue consists of a network of reticular cells and reticular fibers. Over 80% of the substance of the spleen was divided about equally between red and white pulp. The collagen and reticular fibers were confined within the arteriolar system of the spleen (Fig-20).

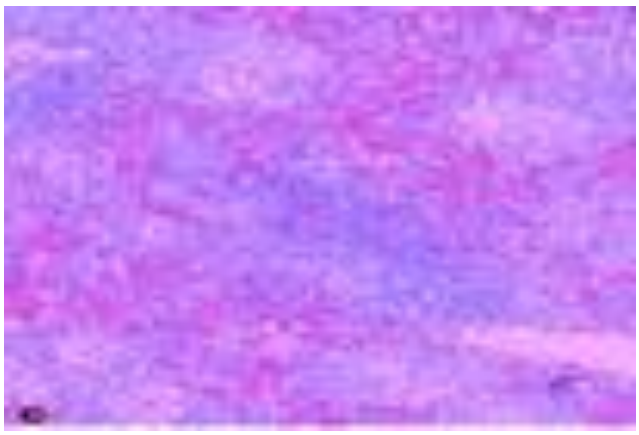

Fig-20: Microscopic structure of Spleen. (H and E, 10x)

In adult birds of 4-5 weeks the bursa are characterized by the presence of tall and thick plicae which is lined by the pseudo stratified and columnar epithelium. Primary lymphoid follicles were spherical or ovoid with no clear central region. Each plicae consist mainly of large number of polyhedral, prominent elongated and square shaped follicles which are closely packed together and separated little bit with very small amounts of connective tissue. The follicles consist of outer cortex and an inner medulla (Fig-21).

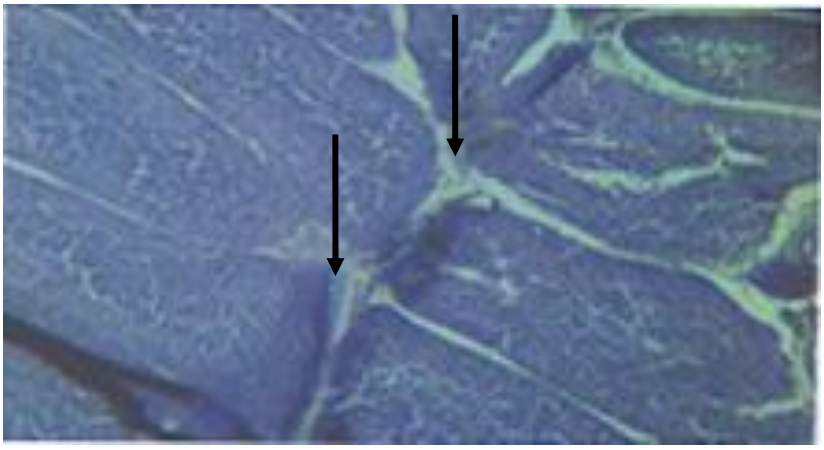

Fig 21: The histological structure of bursa fabricius; showing developed placae (arrows) (H and E, 10x)

All the components of the thymus are developed at the adult stage. The capsules are very thin from which septums arose and divided the thymus into lobules. The cortex is composed of an extensive population of lymphocytes and the medulla has less cell population .The cortex are packed large numbers lymphocytes. The medulla contains far fewer lymphocytes than the cortex and was consequently much more pale staining. Large numbers of Hassall's corpuscles were found which takes eosinophilic stain (Fig.-22).

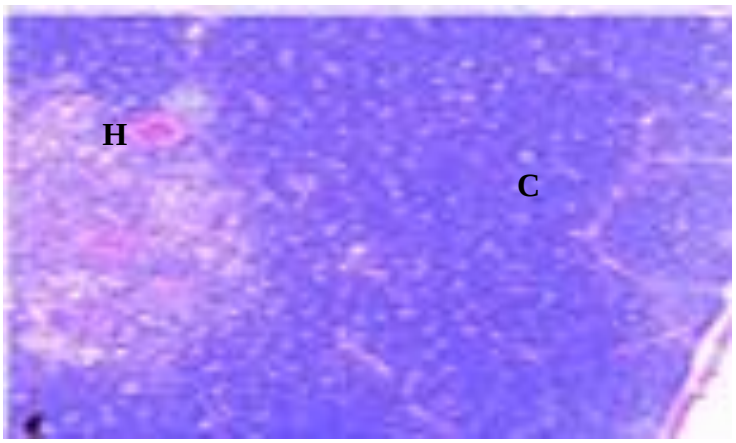

Fig-22: Microscopic structure of thymus; Cortex (C), Hassal's corpuscles (H). (H and E, 10x)

All the components of the cecal tonsil e.g. Villi, Intestinal gland, Submucosa, Muscularis externa and Serosa are shown. Diffuse lymphoid tissue and lymphoid nodules are observed in the mucosa and submucosa. The mucosal fold (MF) were well developed.

Within the Mucosal fold and at the base of the mucosal fold abundant lymphocytes and lymphoid nodules with cortex and medulla where observed (Fig.-23).

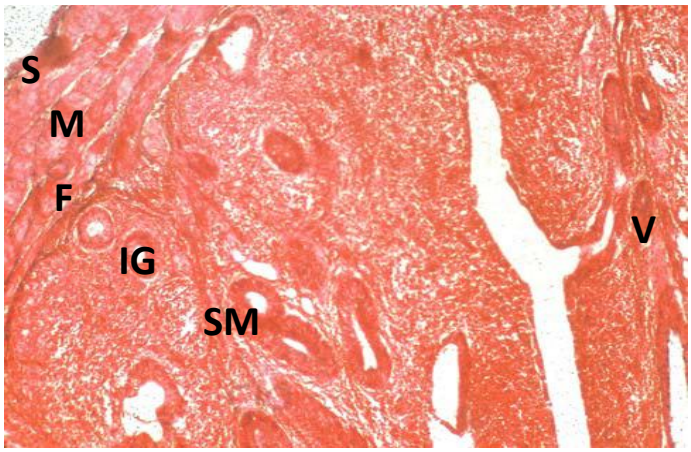

Fig.-23: Histology of ceccal tonsil of the chickens showing Villi (V), Intestinal gland (IG), Submucosa (SM), Muscularis externa (ME) and Serosa (S) at D-28. (H and E, 40x)

4.5 Histopathological findings of ND affected broiler chicken

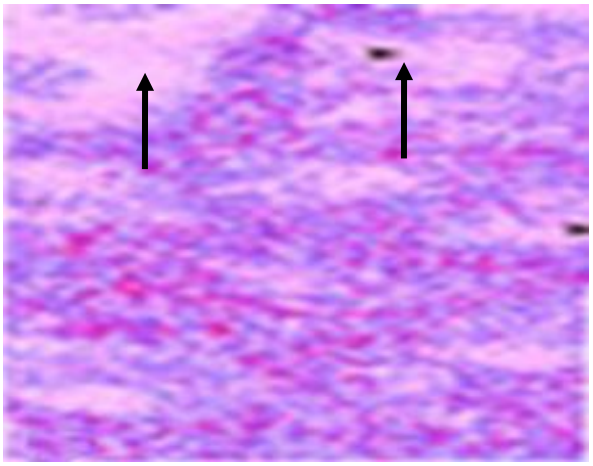

Fig-24(a): Microscopically, the spleen shows multifocal areas of necrosis and fibrin exudation (arrow). (H and E, 10x)

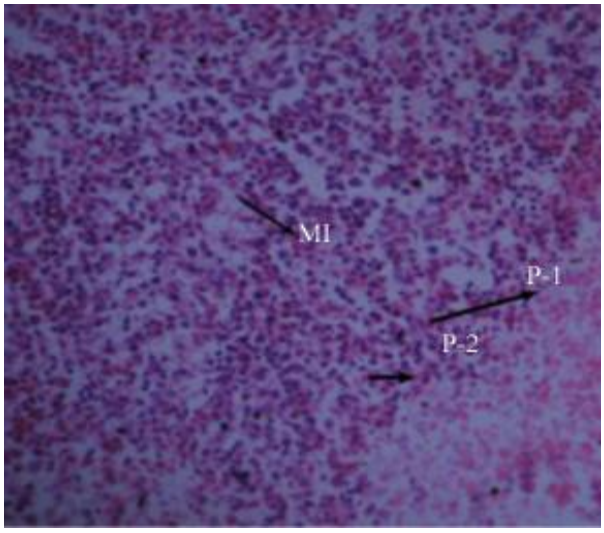

Fig-24(b): Histopathogy of spleen of a broiler chicken that had ND. Note, the necrosis and lymphocytes degeneration in the spleen, nuclear Pyknosis (P-1 & P-2) and Macrophages infiltration in the spleen tissue (MI). Gross lesions noted in the same bird included swollen spleen with necrotic foci in the sub capsular area. (H and E, 40x)

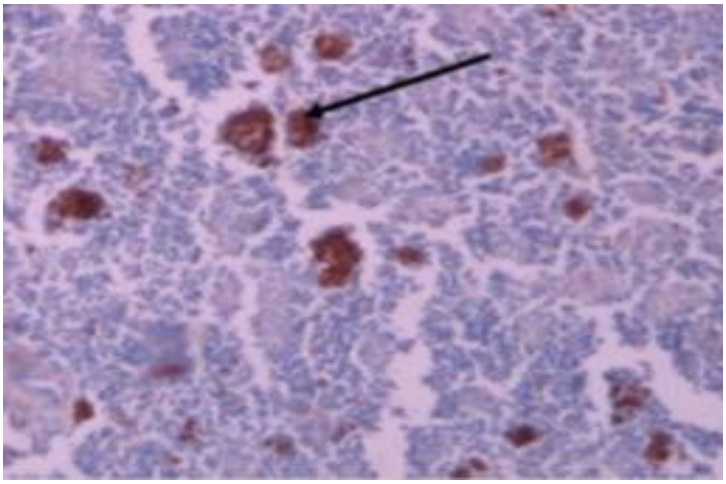

Fig-24(c): Detection of ND in Spleen Section of chicken, positive case. Note the arrows pointing to the brown precipitates. (H and E, 100x)

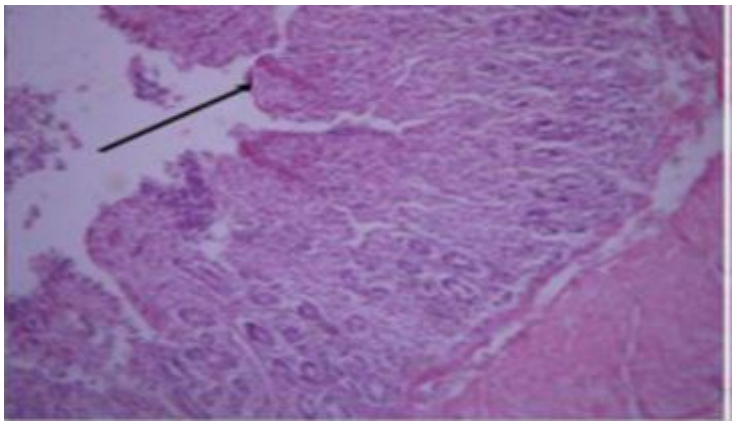

Fig-25(a): Haemorrhages in the cecal tonsils (B2) in a broiler chicken that had air sacculitis. (H and E, 40x)

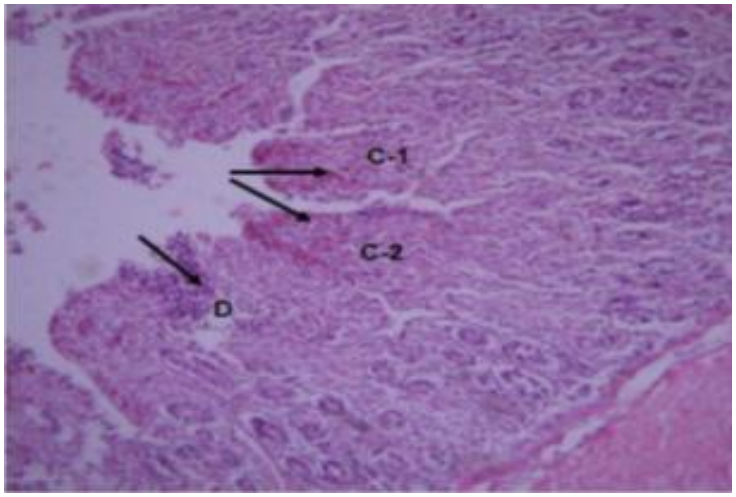

Figure 25(b): Histopathology of caecal tonsil note the haemorrhages and congestion in the mucosa of the tonsils as indicated by the arrows, C-1 and C-2, necrosis and sloughing off of the lymphoid tissues (D). (H and E, 100x)

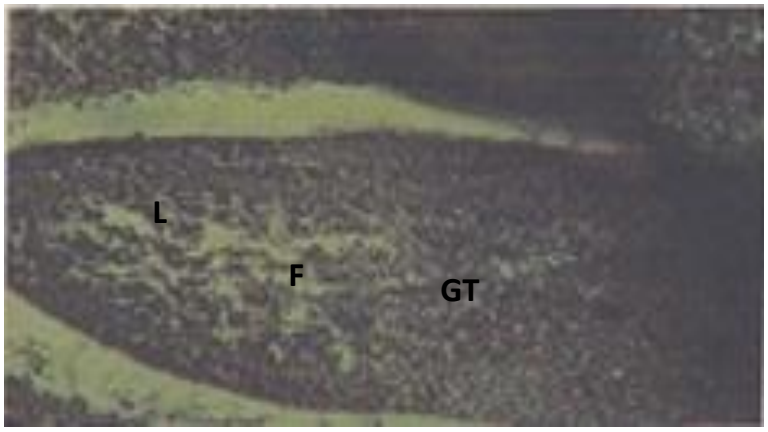

Fig.-26: Four weeks old broiler Bursa of Fabricius showing lymphoid depletion, lymphocytolysis (L), marked interfollicular fibrosis (F), glandular transformation of epithelium (GT). (H and E, 40x)

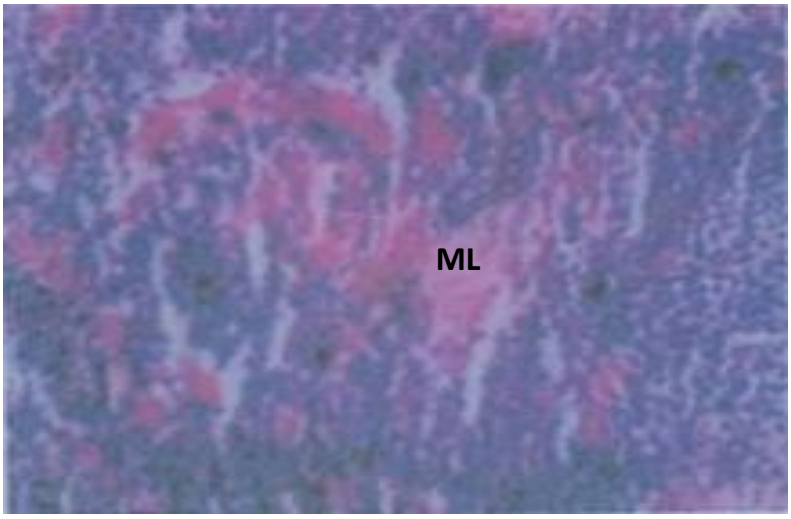

Fig-27: Medullary lymphocytolysis in thymus (A). (H and E, 100x)

CHAPTER V

DISCUSSION

DISCUSSION

In the present study, observation of gross and histopathological structure of lymphoid organs of NDV at small scale commercial broiler farms has been carried out to identify the different structure of the thymus, bursa of fabricius, cecal tonsil and spleen of broiler chicken. Similarities and dissimilarites has been observed in these lymphoid organs of broiler chicken and other previous study related to my study and the results were discussed very briefly.

Although the disease does not have lesions pathognomonic to it, typical lesions are proventricular haemorrhage, most commonly seen in the surface near the junction with the ventriculus, and in the caecal tonsils (Mishra *et al.,* 2000; Okoye *et al.,* 2000). Greenish diarrhea which was also similar with the findings of (Alexander *et al.,* 1993).

Prevalence of NDV at different commercial broiler farms in Dinajpur District are showing- total 10 farms visited in different upazila. Total 59 diseased and dead birds were examined out of which 26 birds were found to be positive for NDV. The Prevalence of NDV was 47% (Table-1) which were almost similar findings reported by Yuguda *et al.,* 2007 stated that (46%) prevalence of Borno state in Nigeria, Salihu *et al.,* 2012 stated that (54.67%) prevalence of Nasarawa state in Nigeria .There is far dissimilarity reported by A.Idi Maikano *et al* 1999, reported that (28.3%) in Niger.

In case of non-vaccinated birds mortality is higher (42.5%) comparatively than vaccinated birds (27.92%) due to NDV. The risk of dying from ND was 1.52 times higher in non-vaccinated birds. Result is similar with (Barman *et al.,* 2010) stated that the risk was 1.5 time higher in non-vaccinated birds than vaccinated birds.

In this observation, the gross pathological lesions were swollen, blackish and molted spleen with necrotic foci (Fig-8b, 9b, 10b).That is similar with (Giovanni Cattoli *et al., 2011)* who stated that swollen and molted spleen with blackish in color with necrotic foci is the most common in case of NDV affected birds. Also similar to the observation of (Banerje *et al.,* 1992) noted in the same bird included swollen spleen with necrotic foci in the sub capsular area.

Bilateral enlargement of cecal tonsil (Fig-11b) has also been observed with slight to severe hemorrhage (Fig-12b) and reddish apex with numerous folds (Fig-13) which is similar to the earlier findings of (Mishra *et al.,* 2000; Okay *et al.,* 2000) who reported that typical lesions are proventricular, most commonly seen in the surface near the junction with the ventriculus and in the cecal tonsil.

In the present study bursa fabricius appeared as swollen (Fig-15b) and slightly yellowish (Fig-16b) in color with necrotic debris .After three days of post infection the color becomes yellowish white (Fig-17). Which is close to the earlier observation of (King, 1975; Hodges, 1974) reported that the color of bursa of fabricius of the broiler chicken becomes yellowish white at day 35.

In my study thymic enlargement has observed with atrophy(Fig-18b,19).Which is not similar to the study of (Afayoa Mathias, 2007; Krishnamoorthy *et al.,* 2007)) who revealed that hemorrhage is most common findings but enlargement occurred only in case of adult stages of birds.

In present study histopathological lesions in the spleen are multifocal areas of necrosis and fibrin exudation (Fig-24a) which is similar to the observation of (Leonardo *et al.,* 1997). The swollen, necrosis and lymphocytes degeneration in the spleen, nuclear Pyknosis and Macrophages infiltration in the spleen tissue has also been observed in my study(Fig-2b) which is similar to the findings of (Banerje, *et al.,* 1992) were; severe the principal alterations observed by lymphocytic meningo-encephalitis and myelitis as well as splenic swollen lymphoid necrosis and hemorrhages. Which IS concurred with the findings of (Kaur *et al.,* 1999 and Malik *et al.,* 2002).

Hemorrhagic ceacal tonsils (Fig-25a), congestion in the mucosa of the tonsil, necrosis and sloughing off of the cecal tissue of broiler chicken (Fig-25b) has been observed in present study. Similar result were also reported by (Jordan, F., M. Patisson *et al.,* 2001; Kamalavenkatesh, 2003).

Present study revealed that Bursa of Fabricius of affected birds are showing lymphoid depletion, lymphocytolysis, glandular transformation of epithelium (Fig-26) which is similar to the findings of (Krishnamoorthy *et al.,* 2007) that are follicles showing scattered lymphoid depletion in the cortex and medulla and mild interfollicular fibrosis

with glandular transformation of epithelium. Which correlated with the findings of (Hoerr *et al.,* 1981; Kubena *et al.,* 1989 and 1990; Narayanaswamy, 1998 and Kamalavenkatesh, 2003).

In my observation medullary lymphocytolysis (Fig-27) has been found in thumus. Which is simiar with the findings of (Krishnamoorthy *et al.,* 2007) where noted that medullary lynphocytolysis is most common in the thymus and it is correlated with the reports of (Hoerr *et al.,* 1981; Niyo *et al.,* 1988; Narayanaswamy, 1998 and Kamalavenkatesh, 2003).

CHAPTER VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSIONS

Newcastle disease is still among the most endemic and fatal poultry diseases that constraint chicken production in the study area (Dinajpur District under Rangpur Division of Bangladesh). Despite the availability of vaccines for Newcastle disease, poultry farmers are continue to experience of losses due to mortalities, drop of weight gain, diagnostic and control costs.

The study was mainly conducted to explore histopathological investigation of ND based on the clinical, gross and histpathological lesions. Overall 10 farms were visited where birds were 5000, 59 birds are examined out of which 26 birds were found to be positive for ND. In above the discussion mortality has been occurred higher due to ND in the non-vaccinated birds.

Marked depression, prostration, droopy wigs, muscle tremor, greenish white diarrhea, edema of the head, inactive, weak, accompanied by the nervous signs like twisted necks (torticulis) with high mortality are major clinical signs. Gross changes are occur like swollen and molted spleen, enlargement of cecal tonsil with hemorrhage, swollen and yellowish bursa, thymic enlargement etc. Lymphocytes degeneration in the spleen, nuclear Pyknosis and Macrophages infiltration, hemorrhagic ceacal tonsils and congestion in the mucosa of the tonsil, lymphoid depletion, lymphocytolysis, glandular transformation of epithelium of bursa, medullary lynphocytolysis in thymus are common histopathological changes.

The study may act as a guide line for the advance research on development aspect of major lymphatic organs. It may also plays as a base line for the future researchers.

In conclusion, I would like to state that vaccination is very important for the broiler chickens which will increase the profitability of farmers by reducing the risks of ND. Because vaccine reducing the mortality of birds. But proper diagnosis of the disease, improved management, bio-security must have to be ensured.

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