# STUDY ON PATHOLOGICAL INVESTIGATION OF NEWCASTLE DISEASE VIRUS (NDV) IN BROILER AT DINAJPUR DISTRICT



A Thesis
By

**RUMANA ISLAM**
Registration No.: 1105127
Semester: July-December, 2012
Session: 2011-2012



**MASTER OF SCIENCE (M.S.)**

**IN**

**PATHOLOGY**

**Department of Pathology and Parasitology**
**Hajee Mohammad Danesh Science and Technology University**
**Dinajpur**

**December, 2012**

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**Submitted to**

*Department of Pathology and Parasitology*
*Faculty of Veterinary and Animal Science*
*Hajee Mohammad Danesh Science and Technology University, Dinajpur*

*In partial fulfillment of the requirements for the degree of*

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**December, 2012**

Dedicated
to MY
Respectable Parents

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***The Author***
***December, 2012***

# ABSTRACT

The study was designed to investigate the pathological conditions of NDV in the small scale commercial broiler farms at different region in Dinajpur district. The duration of experiment was 6 months from July, 2012 to December; 2012.The objectives of the study were clinical sign, gross lesion, microscopic lesion, and the prevalence of the diseases. Different organ mainly proventiculus, intestine were collected, preserved and processed for histopathological examination. The clinical signs were sneezing, caughing, depression, torticolis of neck, white greenish diarrhea, and paralysis of leg. At necropsy severe haemorrhages in the proventiculus, caecal tonsils, intestine and trachea. Histopathologicaly in proventiculus distortion of the normal structure of the tissue, globular destruction, haemorrhage and sever epithelial layer destruction. Total 250 diseased and dead birds (From 35 farms) were examined out of which 135 birds were found to be positive for NDV.The mortality was higher in nonvaccinated than in vaccinated birds

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# ACRONYMS USED

| Abbreviations | Elaborations |
|---|---|
| @ | At the rate of |
| AF | Alantoic fluid |
| DLS | Department of Livestock service |
| g | gram |
| HA | Hemagglutination |
| HI | Hemagglutination Inhibition |
| hr | Hour |
| HSTU | Hajee Mohammad Danesh Science & Technology University |
| mg | Miligram |
| min | Minute |
| ml | Mili liter |
| ND | Newcastle Disease |
| NDV | Newcastle Disease Virus |
| nm | Nanaometer |
| PBS | Phosphate buffered saline |
| RBC | Red blood cell |
| RDV | Ranikhet diseases vaccine |
| & | And |
| i.e. | That is |
| + | Positive |
| - | Negative |
| % | Percentage |
| µl | Microlitre |
| µg | Microgram |

# CHAPTER I
# INTRODUCTION

# CHAPTER I

## INTRODUCTION

In the recent years poultry rising has become a growing and prospective industry in Bangladesh. Poultry rearing can play a vital role in the country like Bangladesh where most of the people are landless, disadvantaged and devoid of formal education or skill to participate in income generating activities. 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.

Newcastle diseases is a deadly viral diseases of poultry due to its high and rapid spreading nature among poultry and other domestic and semi domestic species of birds. A Newcastle disease is caused by Avulovirus, a newly formed genus under Paramyxoviridae (De Leeuw and Peters, 1999; Chang *et al*, 2001; Mayo, 2002). Outbreaks Newcastle disease have a tremendous impact of backyard chickens in developing countries, where these birds are a significant source of protein and tills disease is endemic. In developed countries, where the more virulent forms of the virus have been eradicated, cause significant economic losses during outbreaks. It was a very common disease in the project area in semi-intensive system of rearing. The affected broiler showed varied types of symptoms. These included difficult breathing, coughing, and loss of appetite. Paralysis of the leg and/or wings along with torticollis and incoordination of movement was also notice Greenish diarrhoea was a common feature. Postmortem examination revealed petechial hemorrhages in the proventriculus, hemorrhage in larynx, trachea, heart and stomach (Jungherr, 1964; Alexander and Allan, 1974; Wan *et al.*, 1984). Strains NDV are present in most countries.In many countries there is a wide spectrum of Strains from non-pathogenic to highly virulent. In 2002 outbreaks occurred in Australia and later on in Japan. ND is endemic in our country (Saha *et al.*, 1998). The virus mainly infects birds through their respiratory and gastrointestinal tract (Alexander, 1988). Embryos can be infected if their Shells are contaminated with virus. Depending on the strains of virus and how it reacts it causes huge economic losses to the poultry industry due to its High mortality rate in acute cases. In chicks, mortality rate reaches upto 100% and in adult somewhat lower to extent, about 80-90% (Brandly, 1950).

So, keeping the above in view, the study was undertaken with the following objectives:

1. To study the clinical findings of NCD at Dinajpur district.
2. To study the gross lesions of Newcastle diseases in field outbreaks.
3. To study the microscopic lesions of diseases.
4. To study the prevalence of diseases in birds.

# CHAPTER II

# REVIEW OF LITERATURE

# CHAPTER II

# R EVIEW OF LITERATURE

Newcastle disease (ND) is a highly contagious viral disease of domestic poultry and wild birds. The disease is prevalent worldwide and cause severe economic losses in the poultry industry. The disease is characterized by either gastrointestinal or respiratory or nervous signs. Available literature on ND is reviewed in this chapter.

## 2.1 NEWCASTLE DISEASE

One of the major constrains in the development of poultry industry in Bangladesh is the outbreaks of diseases which causes about 30% mortality of chickens in every year (Ali 1994). Among the infectious diseases, Newcastle disease, popularly known as Ranikhet diseases, is most important. Avian Newcastle diseases highly contagious viral diseases of chickens. Usually the diseases are manifested as a respiratory problem and may cause high mortality rate in young flock. An envelop single standard RNA virus belonging to the family paramyxoviridae causes the diseases.

### 2.1.1 History

Newcastle disease virus (NDV) was the name given by Doyle to a highly contagious viral infection of poultry ,also known as fowl pest, which was first reported on a farm near Newcastle upon Tyne ,UK, in 1926 (Doyle, 1927). Shortly after the reported disease at Newcastle, two further outbreaks occurred in the UK, one in Somerset and other in Staffordshire. At about the same time, a disease with similar symptoms was observed in Java (the capital city now known as Jakarta), Indonesia (Kraneveld, 1926), and shortly there after in other regions of Southeast Asia, notably around the

seaports of the Indians ocean. In India, an outbreak of this new fowl disease was first recorded by Edwards (Edwards, 1928) in 1927 in the poultry farm at Ranikhet. cooper worked on the disease (in Mukteswar, Laboratory, Kumaun) and confirmed that the causative agent was a filter passing virus which was immunologically identical to Newcastle disease virus of England and other countries (Cooper, 1931).He named the disease as 'Ranikhet disease '.In the USA, Newcastle disease was first recognized in California by Beach (Beach, 1942) which was known as pneumoencephalitis.

The causative agent of the disease in Newcastle upon Tyne was identified as a virus that was distinct from fowl plague (avian influenza virus); although the symptoms had some resemblance .It is thought likely that the virus was transported to the port of Newcastle upon Tyne by ship from Southeast Asia. Whatever is its origin; the new disease emerged and rapidly spread throughout the world (Emmersion, 1999; csatary *et al.*, 2000; lorence *et al.*, 2001).

### 2.1.2. Epidemiology

#### 2.1.2.1. Distribution

Strains of NDV are present in most countries .In many countries there is a wide spectrum of strains from non-pathogenic to highly virulent. New Zealand, Papua New Guinea, Fiji and a number of pacific island countries have a non pathogenic strain of virus, but are free from pathogenic strains (OIE, 2000). Birds from these islands should be considered immunologically native with respect to NDV. In 2002 outbreaks occurred in Australia and later on in Japan. ND is endemic in our country (Saha *et al.*, 1998).

Three panzootics of ND have occurred since the disease was first recognized (Alexander, 1988a; Alexander; 1997). By restriction site mapping and sequence analysis of the F gene, Newcastle disease virus (NDV) strains were divided into eight genotypes (Ballagi-pordany *et al.*, 1996; lomniczi *et al.*, 1998; Herczeg *et al.*, 1999). Among these, at least three genotypes (ii, iii, and iv) were involved in first panzootic, genotypes v and vi were considered to be responsible for the second and third panzootics. In addition, it was indicated that the severe outbreaks in weatern Europe (Lomniczi *et al.*, 1998), South Africa and southern Europe (Herczeg *et al.*, 1999) and Taiwan (Yang *et al.*, 1999) in the 1990s were caused by prevalent genotypes vii. Genotype I consists of the virulent strains of NDV, whereas genotype viii appears to be endemic to South Africa during the past few decades (Herczeg *et al.*, 1999).

### 2.1.2.2 Host and age susceptibility

ND occurs in domestic fowl, turkey, pheasants, pigeons, quail, and guinea fowl. Ducks and geese are susceptible but severe disease is rare (OIE,2000).Some wild birds like crows, sparrows, jungle fowls, kites, and vultures can suffer and spread the disease to poultry farms (Chauhan and Roy, 1996).

Psittacines (parrots) are highly susceptible and can excrete virus for long periods (Roy *et al.*, 1998). Kaleta and Baldauf (1998) listed more than 250 species of free living and caged birds that have been infected with Newcastle disease virus .The consequences of these infections vary with the strain of virus and the species of host (Spradbrow,2004).Duck can act as a carrier of NDV. In a village situation in Indonesia, Kingston and Dharsana (1979) found that the virus persisted for one year in a flock of only 300 ducks.

Factor; no real seasonal peaks have been described the host spectrum includes hundreds of species from at least 27 orders. Susceptibility and the clinical course of disease are highly variable between species and apparently depend on the epitopes and the enzymatic status of the host. Birds of all ages are susceptible to infection .although excessive heating may be a triggering.

NDV can infect mammals .Human infection occurs and at least with virulent strains of the virus, which causes severe conjunctivitis (Burnet, 1943) and flu like symptoms. There has been an isolation of NDV again from Indonesia there has been an account of the apparent replication of NDV in rice field crabs (Kingston and Dharsana, 1977).

**2.1.2.3. Transmission**

The virus mainly infects birds through their respiratory and gastrointestinal tract (Alexander,1988b).Embryos can be infected if their shells are contaminated with virus i.e. virus can penetrate the shell after laying (Williams and Dillard, 1968).vertical transmission can occur ,but is rare with velogenic strains because viremic hens usually stop laying .Infected embryos have been reported during naturally occurring infections of laying hens with virulent strains (Lancaster and Alexander, 1975;Beard and Hanson, 1984), but this generally results in the death of the infected embryo during incubation .lentogenic and apathogenic NDV might be egg transmitted via the vitelline membrane .This route of transmission is thought to occur regularly following vaccination with live lentogenic strains (Hitchner B1) (Raszewaska, 1964).

(Pospisil *et al.* 1991) were able to demonstrate the presence of lentogenic virus in chick embryos and young progeny, including day-old chicks, of a

vaccinated laying flock. Although virus can be found in respiratory secretion, the main route of viral shedding is the faeces. This is likely to be the main method of bird to bird spread for a virulent enteric NDV and the pigeon variant virus.

(Alexander *et al.*, 1984).the virus sheds during incubation, the clinical state and for a limited time during convalescence .chickens are infected by aerosols and by ingesting contaminated water or food. the virus may be spread by the wind or insects .it can also settle on equipment and on peoples shoes or clothing and spread to birds (Lancaster ,1966;Alexander ,1988b).

Immune birds can function as carriers and intermittently shed virus persistent infections are limited to weeks or months (http://www.PMV-RH&H.htm).the most common carriers includes free –ranging waterfowl, psittaciformes (parrots, parakeets etc.), some stringformes and Passeriformes. NDV has an affinity for red blood cells, allowing it to spread throughout the host's body (http://www.PMV-RH&H.htm). The incubation period varies from 2-17 days depending upon the species of bird, environment, concurrent infection etc. (Alexander, Huchzermeyer 1993) made the interesting suggestion that NDV may spread amongst village chickens at night rather than during the day when ultraviolet radiation is strong .He also postulated a state of endemic ND that would not dependent on persistent carriers .

### 2.1.2.4. Morbidity and mortality

Newcastle disease virus reacts with avian hosts in various ways .when non – immune domestic chickens encountered highly pathogenic strain of NDV, The common sequel is an acute disease with mortality close to 100% (Fan

*et al.*, 1999; Spradbrow,2004).there are several reports on morbidity and mortality due to ND in various countries .in Philippines morbidity and mortality rate in chickens were 2.05% and 1.55% ,respectively ,in 12 regions of islands (Corpuz and Shortridge 1982),in Sudan 100% morbidity and 80% mortality in pigeon were recorded (Eisa and Omer ,1984).In Faisalabad, Pakistan, mortality ranged from 2-50% in different vaccinated chicken flocks (Siddique *et al.*, 1986).In A.P. of India mortality was 6.31% (Srinivas *et al.*,1983 ) and in northern India morbidity reached up to 100% and mortality up to 60% in pigeon (Manager *et al.*, 1988). Alexander (1997) reviewed the morbidity and mortality due to ND in chickens and reported that morbidity may reach up to 100% and mortality up to 50% in adult birds and 90 % in young chickens. In Bangladesh, ND accounted for 10.24% mortality of total submission of samples for diagnosis during period from July 1998 to October 1999 (Thalha *et al.*, 2001). The Prevalence of NDV was (54.62%) of Borno state in Nigeria (A.D.EL.Yuguda *et al* 2007). In Nigeria of Nasarawa state prevalence was (54.67%) (A.E.Salihu *et al* 2012). Mortality was higher in nonvaccinated than in vaccinated birds. The risk was 1.5 time higher in nonvaccinated birds (L. R. Barman *et al.*2010).

### 2.1.3 Clinical signs

Historically, ND has been a devastating disease of poultry ,and in many countries the disease remains as one of the major problems affecting existing or developing poultry industries (Alexander ,2000) .Clinical signs depend on the strains of virus and severity of the disease .The factors that are important in establishing the severity of the disease are the host species ,age ,immune status, co-infection with other organisms environmental stress, social stress, route of exposure and the virus dose (Mcferran and Mccracken, 1988).In some cases the infection may be inapparent and the affected birds may have no evidence of illness .Some flocks have only mild

respiratory infection indicated by "cold'' like signs over a period of a few days (Johnson *et al.*,1953) .

In young chickens, the earliest and most frequent sign of illness following introduction of NDV is a respiratory involvement that spreads rapidly laboured breathing to frank respiratory distress with open mouthed breathing. Inspiration can be accompanied by a rattling sound. Head shaking, with birds trying to dislodge mucus from the respiratory passages can be a feature. There may be a uni or bilateral mucopurulent conjunctivitis (McFerran *et al.*, 1988).

Green diarrhea is frequently seen in birds that do not die early in infection, and prior to death, muscular tremors, torticollis, paralysis of legs and wings, and opisthotonos may be apparent. Mortality reaches 100 percent in flocks of fully susceptible chickens (Alexander *et al.*, 1993)through the flock. Signs are sneezing, coughing, nasal discharge and Signs indicating involvement of the nervous system include clonic spasm, muscular tremor, torticollis and opisthotonos that appear in the birds that survive the initial phase of disease (Okoye *et al.*, 2000). Other nervous system involvement is marked by paralysis of legs and occasionally the wings (Ressang, 1961).

(Alexander 1997) reviewed the clinical signs of Newcastle disease in chickens due to velogenic viscerotropic Newcastle disease virus (VVNDV) pathotype, which were listlessness, increased respiration and weakness ending with prostration and death .green diarrhea was frequently seen in birds that did not die early in infection ,and prior to death ,muscular tremors, torticollis, paralysis of legs and opisthotonos were found.

The clinical feature caused by the virus responsible for panzootic infection in pigeon includes nervous signs, Diarrhea, Periocular Oedema and bilateral

conjunctivitis (Alexander, 1985; Alexander and Parsons, 1986; Vindevogel and Duchatel, 1988; Kommers *et al.,* 2002). Clinical signs are less severe in turkey (Box *et al.*, 1970).

### 2.1.4 Pathogenesis and Pathology

NDV has an affinity for erythrocytes allowing the virus to be widely distributed throughout the host's body. Dyspnea may be caused by lung congestion and damage to the respiratory centre. Petechiation results from viral adherence and damage to vascular endothelium.

Systemic antibodies are essential elements in protection against ND, whereas, the local antibodies limit the multiplication of NDV at the site of entry. Cytotxic T. lymphocyte against specific NDV was detected in the spleen of vaccinated birds an increase of the number of various leukocyte subsets was noticed in the respiratory tract and the harderian gland, which favours involvement of the local cellular immunity in the defence against NDV infection (kommers *et al.*, 2002). The local lymphoid infiltration are involved in the first defense and the cytotoxic cells clean virus by directly lysing infected target cells at the site of NDV inoculation .Various cell types, mainly T-lymphocytes and macrophages, may be equipped to produce a range of cytokines with antiviral activity and cytokines that stimulate B-lymphocyte to proliferate and differentiate into antibody forming cells responsible for local antibody production against NDV (Al-Garib *et al.,* 2003).

#### 2.1.4.1 Gross lesions

Depending on the strain of virus and how it reacts, post mortem findings are variable .Affected birds typically have haemorrhage in larynx, trachea, and heart and stomach (Jungherr, 2004; Alexander and Allan, 1974; Wan *et al.,*

1984, Koncicki and Rotkieicz, 1988). 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). Haemorrhagic lesions associated with necrosis are found in the intestinal wall, specially in the posterior half of the duodenum, in the jejunum forming button ulcers (Orr and John 1946; Jungherr, 2004; Kianizadeh *et al.*, 2002). The presence of haemorrhagic lesion in the intestine of infected chickens has been used to distinguish velogenic viscerotropic ND virus from non-velogenic ND virus.

Birds with CNS signs may have no gross lesion or only hyperemia of the brain. Air sacculitis may be present even after infection with relatively mild strains, and thickening of the air sacs with catarrhal or caeseous discharge and congestion of lung is often observed (Koncicki and Rotkiewicz ,1988).there are also lymphoid depletion and degeneration in the bursa of fabricius ,spleen and other lymphoid organs (Mishra *et al.*, 2000). Some birds show petechial haemorrhage and oedema in the conjunctiva of lower eyelid (Banerjee *et al.*, 1994; Kommers *et al.*, 2002). Velogenic viral infection of chickens and turkeys in lay usually reveal egg yolk in the abdominal cavity with flaccid, degenerative follicles. The reproductive tract would be haemorrhagic and discolored.

### 2.1.4.2 Histopathology

The histopathology of NDV infections varied as the clinical signs and gross lesions and can be greatly affected by the same parameters .In addition to the strain of the virus and the host, the method of infection may also be of paramount importance. Histological examination may show congestion and haemorrhages in lung, trachea and peritracheal tissue. There may be

degenerative lesions in kidneys, Myocardium and liver. In the proventriculus proventricular glands were already present extending throughout the lamina propria during development. The lamina propria, tunica submucosa, tunica muscularis and tunica serosa showing the typical structure of the develop organ (Julia Victoria Rica 2008).There were multifocal necrosis with fibrin deposition and apoptotic cells in spleen (Kommers *et al.*, 2002). Marked degeneration of medullary region was seen in bursa (stevens *et al.*, 1976). The most remarkable histologic finding was observed in the brain .There may be non-suppurative encephalomyelitis ,neuronal necrosis, gliosis, perivascular cuffing, and endothelial hyperplasia in cerebellum, cerebrum others part of central nervous system (kuiken *et al.*, 1999; Okoye *et al.*, 2000). Additionally, hemorrhagic lesions of the digestive tract (Gohm *et al.*, 2000), particularly in the proventriculus (Jordan *et al.*, 2001).

## 2.2 NEWCASTLE DISEASE VIRUS

### 2.2.1 Etiology

The causative agent of ND is Newcastle disease virus (NDV) or avian paramyxovirus type-1.ND is one of the OIE list I diseases (OIE, 2000).

### 2.2.2 Classification

NDV, an avian paramyxovirus, is classified as the only member of the newly formed genus avulavirus belonging to the family paramyxoviridae within the order mononegavirales (De leeuw and Peters, 1999; Chang *et al.*, 2001; Mayo, 2002). Three virus families, Rhabdoviridae, Filoviridae and paramyxoviridae, form the order Mononegavirales. Paramyxoviridae family consists of two subfamilies, Pneumovirinae and Paramyxovirinae. The subfamily pneumovirinae consists of 2 genara: pneumovirus which includes respiratory syncytial virus and avian pneumovirus and other is

metapneumovirus which includes turkey rhinotracheitis virus. The subfamily paramyxovirinae consists of 6 genera. The genus morbillivirus includes measles, rinderpest and distemper virus genus respirovirus includes sendivirus and mammalian parainfluenza virus 1 and 3. The genus Rubulavirus includes mumps virus, simian parainfluenza virus 5.Genus henipavirus consists of hendravirus and Nipahvirus .The genus TPMV –like virus include Tupwawing. Genus Avulavirus include Newcastle disease virus or avian paramyxovirus type-1 (Alexander, 1998; Mayo, 2002).

**Recent taxonomy of Newcastle disease virus:**

Order-mononegavirales

Family-paramyxoviridae

Subfamily-paramyxovirinae

Genus-avulavirus

Species-Newcastle disease virus

### 2.2.3 Morphology of virus

Virions are enveleloped and this is formed from modified cell membrane as the virus is budded from the cell membrane as the virus is budded from the cell surface after capsid assembly in the cytoplasm (Melnick, 1982). Virions are generally pleomorphic, rounded and 100 to 500 nm in diameter, having helical capsid symmetry. A filamentous form 100 nm wide and variable in length, has been described but may be artifact (http://www.PMV-RH & H.htm).The virion surface is covered with 8 nm projections (so-called "herring bone" nucleocapsids) that may be released from disrupted particles (Alexander, 1997). Fusion protein and attachment protein (HN) appears as

spikes on the virion surface .Matrix proteins inside the envelope stabilize virus structure. The nucleocapsids core is composed of the genomic RNA,

### 2.2.4 Molecular biology

The genome of NDV is a single stranded non-segmented negative sense RNA consisting of 15, 186 Neucleotides (Krishnamutry and Samal, 1998; Phillips *et al.*, 1998; De Leeuw and Peeters, 1999). Non coding (extracistronic) region includes : A 3 inches leader sequence, 50 nucleotides in length, which nucleocapsid proteins, phosphoprotein and polymerase proteins .acts as a transcriptional promoter,, A 5 inches trailer sequence and inter genomic regions between each gene. Each gene contains transcription start/stop signals at the beginning and end, which are transcribed as part of gene. Gene sequence within the genome is covered across the family due to a phenomenon known as transcriptional polarity in which genes closest to the 3 inches end of genome are transcribed in greater abundance than those towards the 5 inches end. This mechanism acts as a form of transcriptional regulation. Plasma protein (p), Matrix(M) Fusion(F), Haemagglutinin (HN), Large polymerage (L)-5 (Millar and Emersion, 1988;Samson *et al.* 1991; Steward *et al.*, 1995). NDV produces two additional proteins, V and W, from P gene by alternative mRNAs that are generated by RNA editing (McGinnes *et al.* 1991; Steward *et al.* 1993; Hausmann *et al.* 1999; Jordan *et al.*; 2000).

In NDV, Insertion of two non template G residues gives rise to a V encoding mRNA. While insertion of two non –template G residue generates a W encoding mRNA. These V and W protein share their amino (N) terminal domains with the P protein and vary at their carboxy (C) termini. NDV V protein has a cysteine rich C terminal domain which blinds two atoms of Zn+2 (Steward *et al.*, 1995).Of the three NDV P gene products

the P protein ,together with L protein ,is known to form part of virus RNA polymerase complex (Lamb and Kolakofsky, 2001)

**Functions of genomic proteins are:**

**N**-The nucleocapsid protein associates with genomic RNA (one molecule per hexamer) and protects the RNA from nuclease digestion.

**P**-the phosphoprotein binds to the N and L protein and forms part of the RNA polymerase complex.

**M**-The matrix protein assembles between the envelope and the nucleocapsid core, it organizes and maintain virion structure.

**F**-the fusion protein projects from the envelope surface as a trimer and mediates cell entry by inducing fusion between the viral envelope and the cell membrane. One of the defining characteristics of members of the paramyxoviridae family is the requirement for a neutral pH for fusogenic activity (Morrison, 2003).

**HN**-The cell attachment proteins span the viral envelope and project from the surface as spike .they bind to sialic acid on the cell surface and facilitate cell entry. These proteins contains both haemagglutination and neuraminidase activity that cleaves sialic acid on the cell (Gotoh *et al.*, 1988;Takimoto *et al.*, 2002; Huang *et al.*, 2004).

**L**-the large protein is the catalytic sub-unit of RNA dependent RNA polymerase.

**V**-Vprotein is responsible for blocking the antiviral action of interferon (IFN). V protein is the additional virulence factor of NDV that affects the IFN and apoptosis responses of the infected host. V protein is a determinant

of host range restriction (Huang *et al.*, 2003; Park *et al.*, 2003a; Park *et al.*, 2003b).

### 2.2.5 Biological properties

#### 2.2.5.1 Haemagglutination activity

The ability of NDV to agglutinate red blood cells (RBCs) is due to binding of haemagglutinin neuraminidase (HN) protein to receptors on the surface of RBCs (Burnet, 1942).this property and the specific inhibition of agglutination by antisera (Burnet, 1942; Beach ,1948) are proven the powerful tools in the diagnosis of the disease. Chicken RBCs are usually used in haemagglutination tests, but NDV will cause agglutination of all amphibian, reptilian and avian cells (Lancaster, 1966) and human, mouse, guineapig, cattle, goat, sheep, swine and horse cells to some extent (Winslow *et al.*, 1950; Hanson *et al.*, 1967; Westbury, 1979; Yamada, 1981; Sueyoshi *et al*,; 2003) and this range differs between strains (Bell *et al.*, 1984).

#### 2.2.5.2 Neuraminidase activity

The enzyme neuraminidase is also a part of HN molecule. An obvious consequence of possession of this enzyme is the gradual elution of agglutinated RBCs (Ackerman, 1964). The rate of elution of chicken RBCs agglutinated by the virus has been used as a method of broadly grouping NDV isolates as rapid or slow eluters (Spalatin *et al.*, 1970). Rapid elution occurs in velogenic strain, whereas, lentogenic are slow eluter (Asahara, 1978; Kawamura *et al.*, 1987; Islam *et al.*, 1995). The elution of NDV from RBCs is promoted by high virus multiplicity, Ph between 6.8-7.7 and temperature of 37 C. Elution can occur when only one virus particle is attached per RBC provided the temperature is 37C (Segik and Levine, 1957).

### 2.2.5.3 Plaque formation

Plaque formation, size and morphology have been used to characterize virus (Hanson, 1975). Lentogenic strain do not form plaques in cell culture without the addition of diethylaminoethyl (DEAE) and Magnesium (Mg++) ions (Barahona and Hanson, 1968) and trypsin (Rott,1985) to the agar overlay. Plaques may be of two morphologic types, clear or red (Schloer and Hanson, 1968; Takehara *et al.*, 1987) and the size appears to be related to the virulence of the virus for chickens (Yoshimura, 1969; Reeve and poste, 1971; Cai-jiali and pan, 2000).

### 2.2.5.4 Resistance to agents

The infectivity of NDV may be destroyed by physical and chemical treatments such as heat, irradiation (including light and ultraviolet rays), oxidation process, pH effects and various chemical compounds .the rate at which infectivity is destroyed depends on the strain of virus, the length of time of exposure, the quantity of virus and the nature of suspending medium and interaction between treatments.

Kohn (1958) showed that when NDV was brought into contact with gizzard content at pH 2.6, its viability was considerably reduced .Doyle (1927) concluded that the effect of marked acidity and alkalinity on the NDV infectivity indicate greater resistance to the H ion than the OH ions. NDV are ether sensitive (Andrewes *et al.*, 1948). Harry and Stephen (1961) claimed that those agents that were susceptible to ether were also susceptible to chloroform while those that were resistant to ether were not affected by chloroform. The radiation inactivation of NDV infectivity at low temperature was considered to be due to nuclic acid (NA) degradation and at higher temperature to protein denaturation (Digioia *et al.*, 1970).

### 2.2.5.5 Thermostability

The thermostability of HA activity of NDV isolates varies (Hanson et al., 1949, Hanson and Spalatin ,1978) and has been used as a characterization test. This property has proven to be a useful tool in epizootiological studies (Hanson and Spalatin, 1978) and a rapid method for distinguishing between some avirulent and virulent viruses (Nitzschke and Schmittdid, 1963).

Some NDV isolates shows an exceptionally high thermostability at 56 degree C (Estola, 1974). The majority of NDV strains seem to lose their infectivity after 30 to 90 minutes at 56 degree C (Mcferran and Nelson, 1971; Hanson *et al.,* 1949).Bushnell and Erwin (1950) stated that the thermal death point of NDV was between 58 degree C and 64 degree C for a 30 minutes exposure.

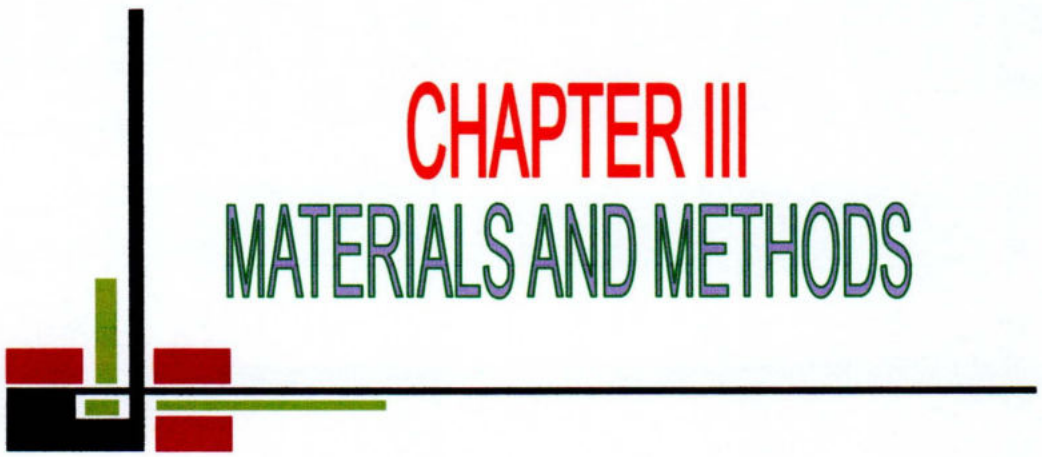
### 2.2.6 Antigenicity

Virus neutralization or agar-gel diffusion techniques have shown minor antigenic variation between different strain and isolates of NDV (Gomaz-Lillo *et al.* 1974; Scholoer *et al.* 1975; Pennington, 1978). NDV is shown to be neutralized by immune serum in an exponential manner that implies that only one antibody molecule is required for inactivation of an infectious particle. A very small fraction of neutralized particles can be reactivated upon dilution of the serum virus mixture (Rubin and Franklin, 1957).The basic mechanism of neutralization is to penetration in the host cell.

### 2.2.7 Molecular basis for pathogenicity

During the replication of NDV, it is necessary for the precursor glycoprotein F0 to be cleaved to F1 and F2 for the progeny virus particles to be infective (Rott and Klenk, 198B) This post trasnslatoinal cleavage is mediated by

host cell protease (Nagai *et al.*, 1976). If cleavage fails to take place, noninfectious virus particles are produced. F0 molecules of virulent viruses can be cleaved by a host protease or proteases found in a wide range of cells and tissues, but F0 molecules in virus of low virulence were restricted in their sensitivity and these viruses can grow only in certain host cell types. The amino acid sequence at Fusion (F) protein cleavage site has been postulated as a major determinant of NDV virulence (Alexander, 1997; Peeters *et at.,* 1999, Scanlon *et al.*, 1999; Terregino *et al.*, 2003). Cleavage at amino acid 117 produces disulfide-linked $F_2$ and $F_1$ polypeptides derived from the amino terminal and carboxyl terminal domains of F0 respectivery (Lamb anci Kolakofsky, 2001). The $F_1$ polypeptide has one and perhaps two fusion peptides (Peisajovich *et al.*, 2000; Peisajovich and Shai, 2002). Upon initiation of fusion, fusion peptides are thought to insert into target membranes, docking the protein to these membranes (Hernandez *et al.*, 1996; Eckert and Kim, 2oo1; Peisajovich and Shai, 2002). In case of velogenic strain, the amino acid sequence of the protease cleavage site of the fusion protein F0 is 112R-R-Q-R-R- F117 (Alexander, 1990; Liu *et al.*, 2002; Manin *et al.*, 2002). The activation of NDV requires not only cleavage of $F_0$ to $F_1$ and $F_2$ but also coexpression of homologus attachment protein, haemagglutinin- neuraminidase (HN) (Garten *et al.*, 1980; Kathryn and Trudy, 2003). Panda *et al.* (2004) shows that the efficiency of cleavage of F protein plays an important role if the NDV is delivered directly into brains of chicks, there could be other viral factors that probably affect peripheral replication, viremia or entry in CNS. Furthermore, V protein of NDV is able to mediate virus escape from interferon induced cellular antiviral mechanistn. V protein affects the host range of the virus via its species-specific IFN antagonist activity (Park *et al.*, 2003b).



# CHAPTER III
# MATERIALS AND METHODS

# CHAPTER III

# MATERIALS AND METHODS

The study was carried out in the Department of pathology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, for the pathological investigation of Ranikhet diseases in poultry.

## 3.1 EXPERIMENTAL CHICKENS

The chickens of different commercial poultry farms were considered as experimental chickens. Ranikhet outbreaks in the small scale commercial poultry farm were investigated at Dinajpur district of Bangladesh and the laboratory examinations were conducted in the Department of Pathology and Parasitology under the Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

A total of 35 farms were visited. 250 diseased and dead birds were examined out of which 135 birds were found to be positive form. The number of birds in the farms was variable ranging from 300 to 1400 and they were reared on litter. A detail flock history in relation to the incidence of disease including housing system, location of poultry farms, sources of birds, age and population of the birds per flock, rearing system, litter material, feeding and watering system, bio-security of the farms, previous history on Ranikhet outbreaks. The birds affected with Ranikhet were submitted to the Pathology laboratory for the diagnosis and treatment were the principal experimental chickens and some affected chickens were also collected physically.

## 3.2 RESEARCH AREA

Chickens (Sick and dead) were collected from different small scale commercial poultry farms at Dinajpur district and examined in the laboratory belonging to the Department of Pathology and Parasitology under Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

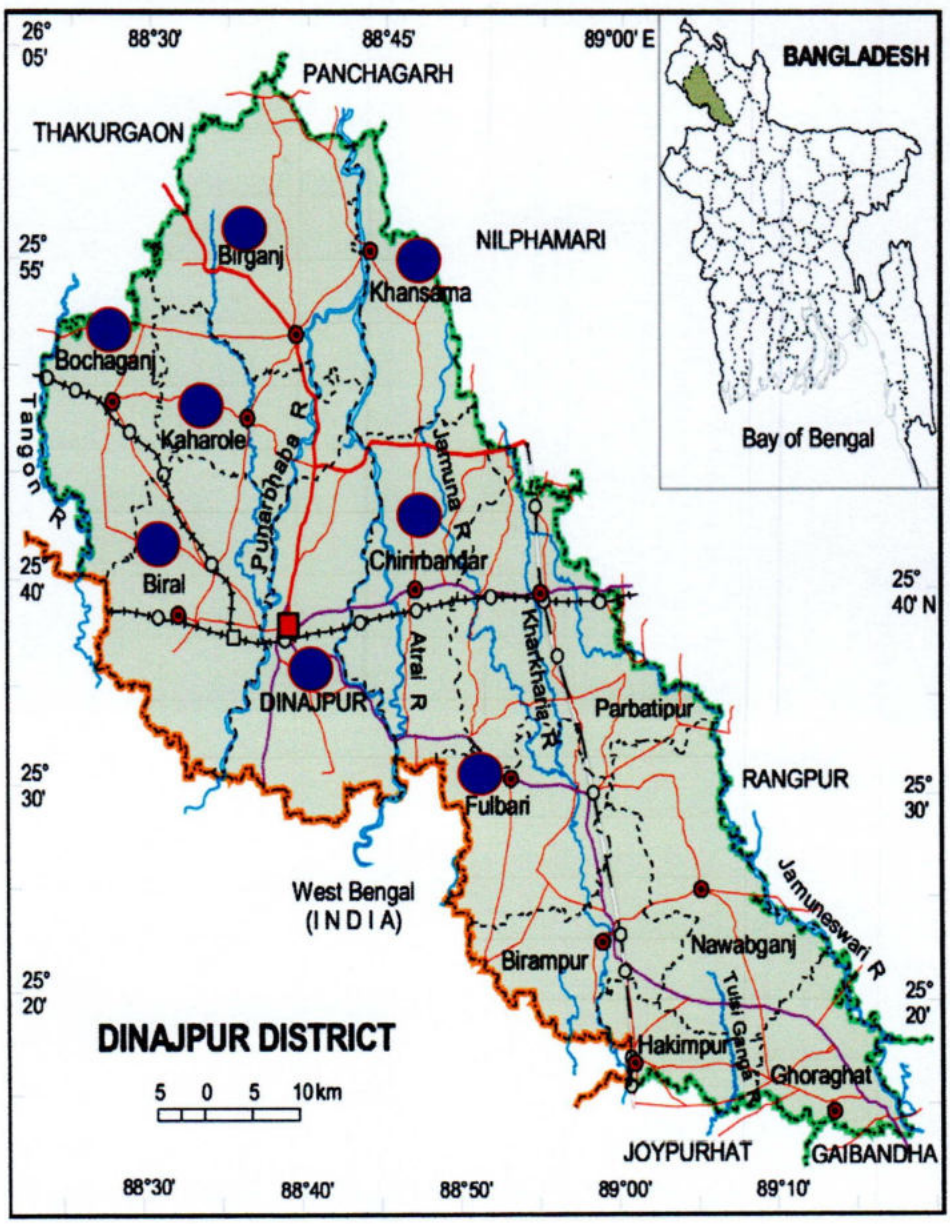



Fig. 1 Working areas are shown in the map by Special Circle

## 3.3 RESEARCH PERIOD

The duration of experiment was 6 months from July, 2012 to December, 2012.

## 3.4 SAMPLING OCCASION

There was no scheduled sampling occasion. Birds affected with Ranikhet were collected and examined when submitted to the laboratory only as well as the collection physically when informed.

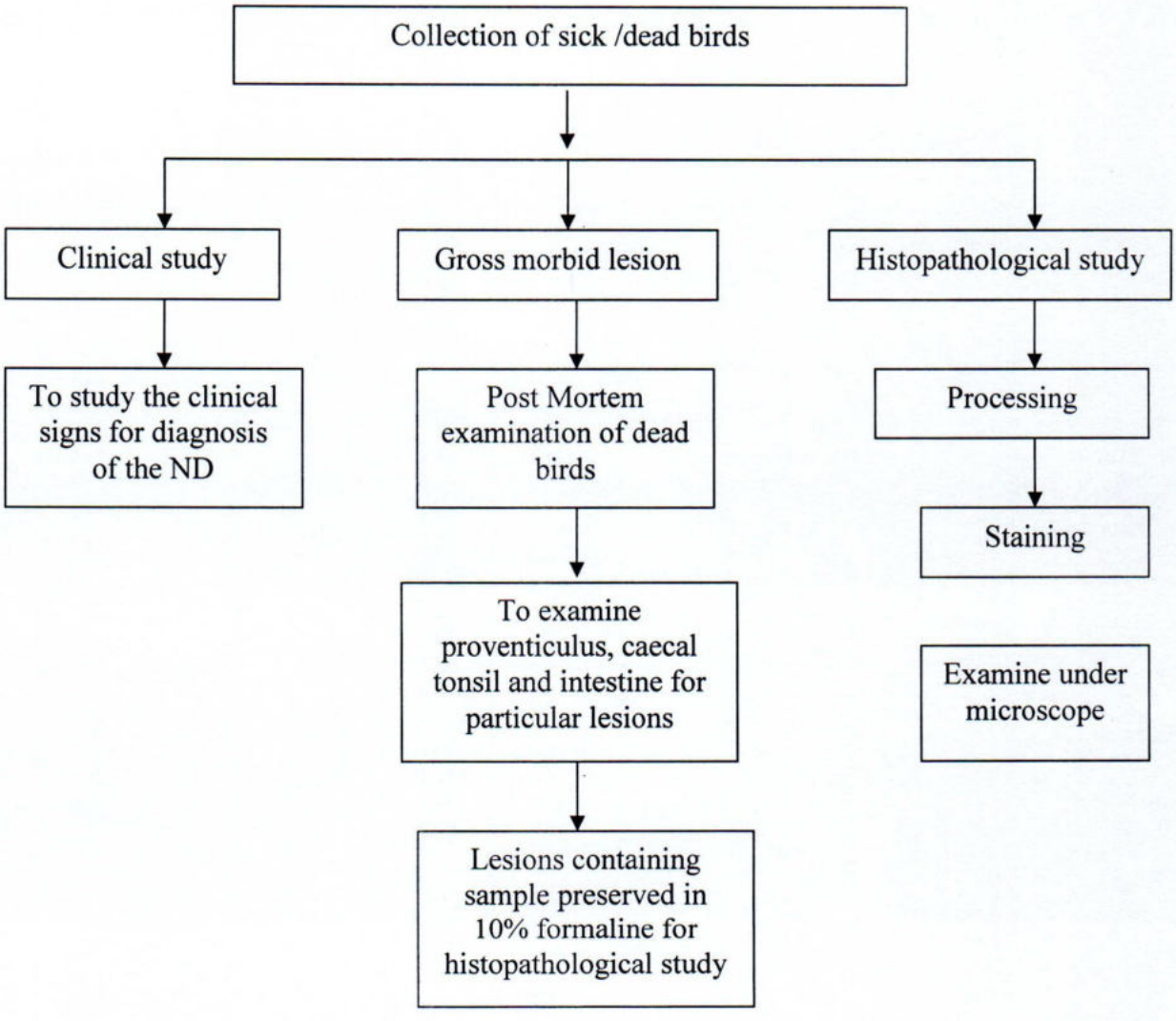
## 3.5 THE MAJOR WORKS OF THE PRESENT STUDY

Clinical Examination of affected birds.

Post mortem examination of dead birds to detect gross pathological changes.

Histopathological examination of proventiculus.

## 3.6 EXPERIMENTAL DESIGN



## 3.7 CLINICAL STUDY

The general health condition and age of the chicken were recorded. The chickens were observed to detect clinical signs. The clinical signs were observed from the visual examination. The clinical signs were recorded during the physical visit of the affected flocks and the farmer's complaints about the affected birds were also considered.

## 3.8. NECROPSY FINDINGS OF SUSPECTED CHICKENS

The necropsy was done on the selected chicken taken from different village, Dinajpur. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection. The samples were also collected in 10% neutral buffered formalin for the histopathological study.

### Equipment and appliances for necropsy

1. Bird
2. Scissors
3. Forceps
4. Gloves
5. Musk
6. Bone cutting saw
7. Scalpel
8. Chisel
9. 10% neutral buffered formalin

### Procedure

1. At first the chicken was wet in a detergent solution thoroughly to lessen the chances of feathers floating around the area while the examination.

2. The bird was laid on a pad of newspaper on post mortem table. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.
3. The bird was positioned in such way so that the legs and feet were facing the examiner. Then an incision was given on skin in between the thighs towards the back and through skinning was done to observe paleness condition of carcass for detection of anaemia. Body cavity of bird was opened.
4. Segments of the intestines, caecal tonsil, proventiculus were observed carefully for importants post mortem lesions. Then the parts opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal cotents.

## 3.9 CLEANING AND STERILIZATION OF REQUIRED GLASSWARE

Test tubes, glass tubes, glass slides, cover slips, beakers, pipettes, reagent bottles, glass bottle, spirit lamp, measuring cylinders etc. were used in this study. The conical flask, measuring cylinder, beakers, glass slides, cover slip, for slide preparation for histopathological study and staining of organisms after smear and pipettes, reagent bottle, glass tubes for different biochemical tests. New and previously used glassware were collected and dipped in 2% sodium hypochlorite solution and left there until cleaned. After overnight soaking in a household dishwashing detergent solution, the glassware were cleaned by brushing and washed thoroughly in running tap water and rinsed three times in distilled water. The cleaned glasswares were then dried on a bench at room temperature or in an oven at 50-70$^0$C.

**Gross lesion**

Gross morbid lesions of different organs were observed after necropsy examination of the birds.

## 3.10 HISTOPATHOLOGICAL EXAMINATION

During necropsy, various organs having gross lesions were collected, preserved at 10% formalin, processed. Formalin-fixed samples of the caecal tonsil, proventiculus, Intestine from the diseased and dead chicken were processed for paraffin embedding, sectioned and stained with haematoxylin and eosin according to standard method (Luna, 1968) for histopathological study. Details of tissue processing, sectioning and staining are given below.

### 3.10.1. Equipment and appliances

1. Sample( caecal tonsil, proventiculus, Intestine)
2. Formalin
3. Chloroform
4. Paraffin
5. Alcohol
6. Tape Water
7. Xylene
8. Hematoxylin and Eosin Stain
9. Distilled water
10. Microtome
11. Clean Slides
12. Cover slips
13. Mounting media (dpx)
14. Microscope

### 3.10.2 Processing of tissues and sectioning

1. The tissues were properly trimmed to obtain a good cross section of the tissue.
2. The tissues were washed under running tap water for overnight to remove the fixative.
3. The tissues were dehydrated in ascending grades of alcohol using 50%, 70%, 80%, 90% alcohol, and three changes in absolute alcohol, for 1hr in each.
4. The tissues were cleared in two changes in chloroform, 1.5hr in each.
5. The tissues were embedded in molten paraffin wax at $56^0$C for two changes, 1.5hr in each.
6. Paraffin blocks containing tissue pieces were made using templates and molten paraffin.
7. The tissues were sectioned with a microtome at 5 micrometer thickness, which were allowed to spread on warm water bath ($42^0$C) containing small amount of gelatin and taken on oil and grease -free glass slides. The slides were air dried and kept in cool place until staining.

### 3.10.3. Hematoxylin and Eosin Staining Procedure

Preparation of Harris' hematoxylin solution

| | |
|---|---|
| Hematoxylin crystals | 5.0g |
| Alcohol (100%) | 50.0 ml |
| Ammonium or potassium alum | 100 g |
| Distilled water | 1000.0 ml |
| Mercuric oxide (red) | 2.5 g |

Hemoatoxylin was dissolved in alcohol and alum in water by heat. The two solutions were thoroughly mixed and boiled as rapidly as possible. After removing from heat, mercuric oxide was added to the solution slowly. The solution was reheated to a simmer until it became dark purple, and then the vessel was removed from heat and immediately plunged into a basin of cold water until it became cool. 2-4ml glacial acetic acid was added per 100 ml of solution to increase the precision of the nuclear stain. Before use, the prepared solution was filtered.

**Preparation of eosin solution**

1% stock alcoholic eosin

| | |
|---|---|
| Eosin Y, water soluble | 1 g |
| Distilled water | 20 ml |
| 95% alcohol | 80 ml |

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

**Working eosin solution**

| | |
|---|---|
| Eosin stock solution | 1part |
| Alcohol, 80% | 3 parts |

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

**Staining protocol**

1. Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 mins in each),
2. Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 mins in each; 95% alcohol for 2 mins; 80% alcohol for 2 mins; 70% alcohol for 2 mins) and distilled water for 5 mins,
3. The tissues were stained with Harris' hematoxylin for 10 mins,
4. The sections were washed in running tap water for 10 mins,
5. Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol), 2-4 dips,
6. The tissue sections were then washed in tap water for 5 mins and dipped in ammonia water (2-4 times) until sections became bright blue,
7. The sections were stained with eosin for 1 min and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 mins in each),
8. The stained sections were then cleaned by 3 changes in xylene, 5 mins in each and finally the sections were mounted with cover slip using DPX,
9. The slide were dried at room temperature and examined under a low (10X) and high (40X) power objects.

# CHAPTER IV
# RESULTS

# CHAPTER IV

# RESULTS

Pathological investigation of Newcastle diseases encountered in small scale commercial poultry farms at Dinajpur district was studied and different clinical, parasitological, necropsy and microscopic conditions were recorded during the study period.

## 4.1 CLINICAL EXAMINATION

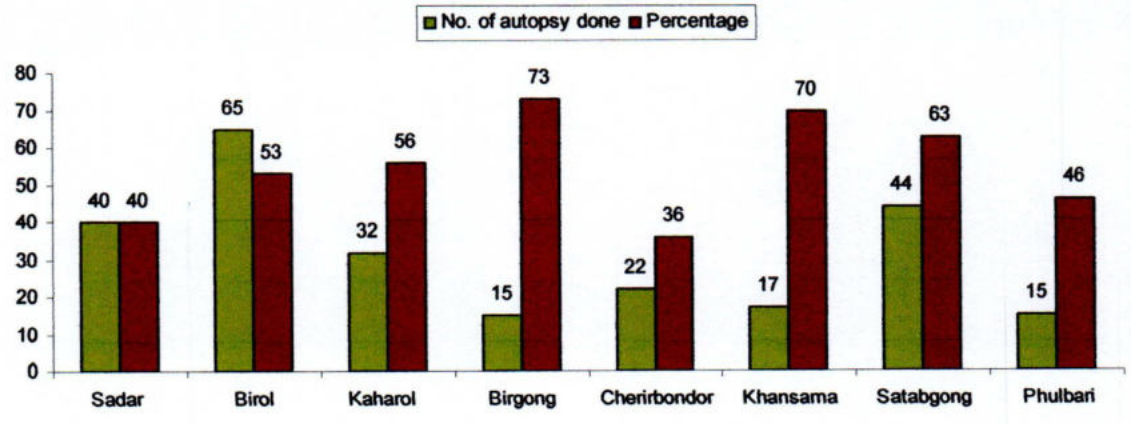
The clinical signs of the birds affected with NDV varied from farm to farm. The signs were sneezing, coughing, torticollis of the neck, and shaking of the head. Chicken may become marked depression, inactive and weak. Sometime complete inability to make sound. Greenish white Diarrhea may also occur. Nervous sign include paralysis of the neck and leg.

## 4.2 STATUS OF MORTALITY AND PREVALENCE OF THE DISEASE

The study revealed the following actual status of mortality and prevalence of Newcastle diseases virus (NDV) in broiler. Table-1 showed the prevalence of NDV at different region of Dinajpur district .A total of 250 birds were examined during the study period from which 135 birds were found infected with NDV. The Prevalence of NDV was 54.62%.where as Table-2 showed that the total mortality is higher in nonvaccinated than in vaccinated birds. The risk was 1.5 time higher in unvaccinated birds.

**Table 1: Prevalence of NDV at different commercial broiler farms in Dinajpur District**

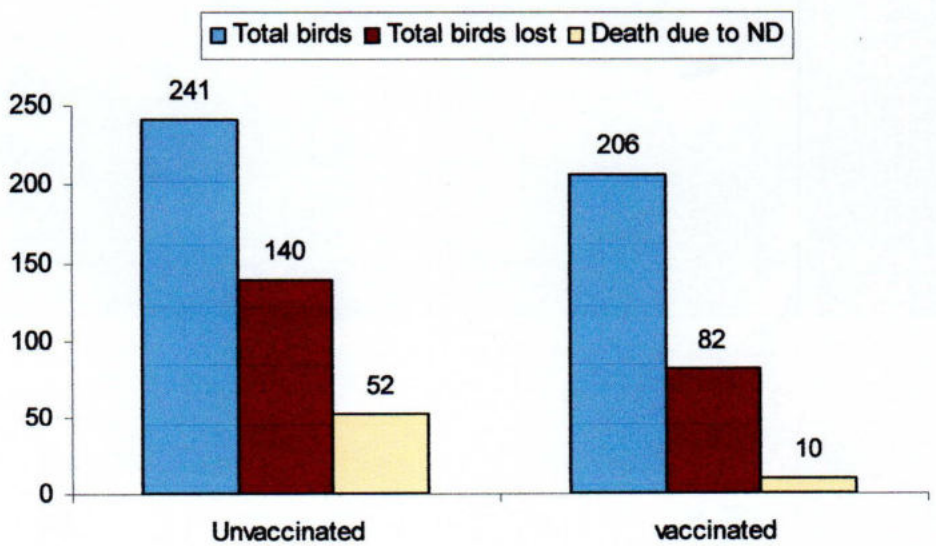
| Location of the farm (Upazilla) | No. of farm visited | No. of necropsy done | ND encountered | Percentage % |
|---|---|---|---|---|
| Sadar | 7 | 40 | 16 | 40 |
| Birol | 8 | 65 | 35 | 53 |
| Kaharol | 5 | 32 | 18 | 56 |
| Birgong | 2 | 15 | 11 | 73 |
| Cherirbondor | 3 | 22 | 8 | 36 |
| Khansama | 3 | 17 | 12 | 70 |
| Satabgong | 5 | 44 | 28 | 63 |
| Phulbari | 2 | 15 | 7 | 46 |
| Total | 35 | 250 | 135 | 54.62 |




Graph 1: Prevalence of NDV at different commercial broiler farms at Dinajpur district

**Table 2: Mortality in nonvaccinated and vaccinated flock**

| Vaccination status | Total birds | Total birds lost | Death due to ND |
|---|---|---|---|
| nonvaccinated | 241 | 140(58.1%) | 52(21.6%) |
| vaccinated | 206 | 82 (39.8%) | 10(4.9%) |
| Relative risk | | 1.5 | 4.4 |




Graph 2: Mortality rate of nonvaccinated and vaccinated birds of NDV affected

Fig. 2 Flocked affected with NDV

Fig: 3 NDV affected bird showing depression

Fig: 4 NDV affected birds showing torticolis

## 4.3 NECROPSY EXAMINATION

Gross pathological changes in different samples were nearly similar but varied in severity. These included slight to severe haemorrhages in the proventiculus (Fig. 6), haemorrhage in the internal wall of intestine and caecal tonsils (Fig. 7, 8 &9).

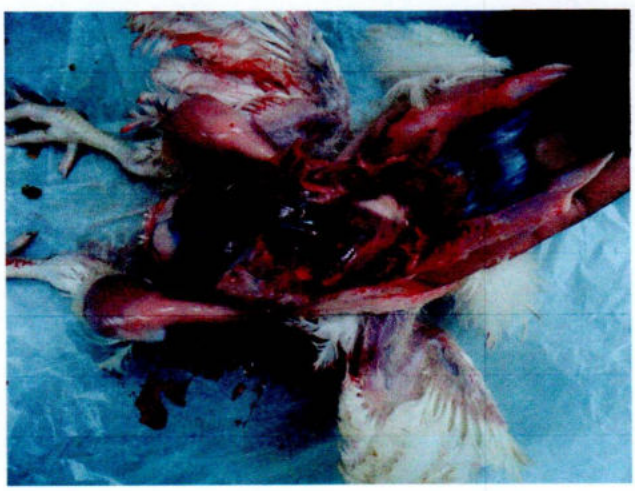


Fig: 5 NDV affected bird

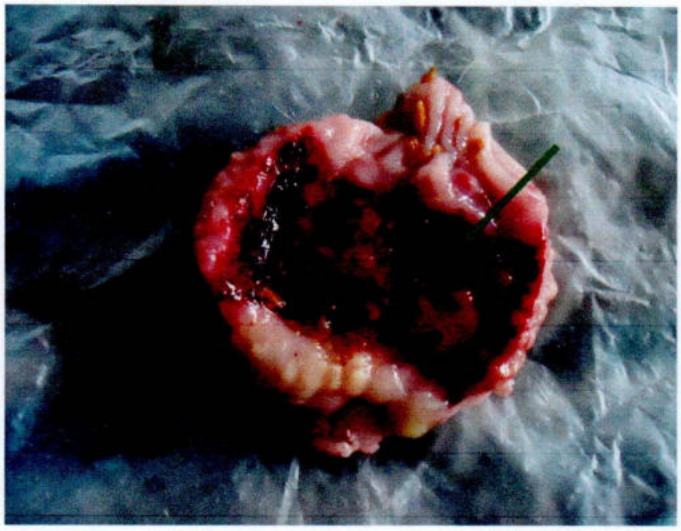


Fig: 6 Haemorrhages in the proventiculus

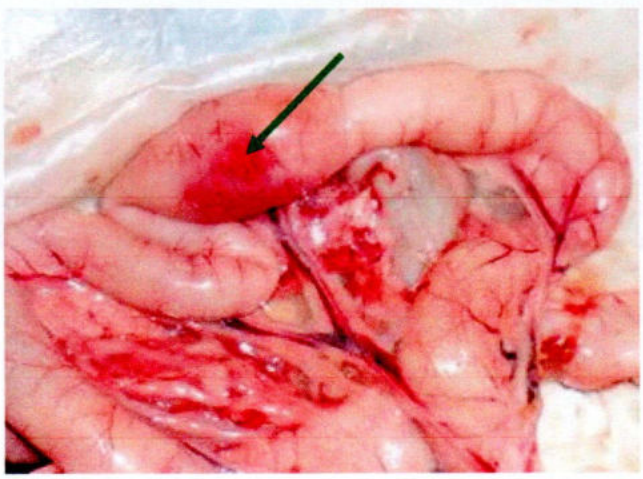


Fig: 7 Haemorrhages in the intestine

Fig: 8 Haemorrage in the internal wall of the intestine

Fig: 9 Haemorrhages in the caecal tonsil

## 4.4 HISTPATHOLOGICAL STUDY

Histopathological changes in proventiculus are distortion of normal architecture of tissue (Fig. 10). Necrosis and haemorrhages around the gland, Globular destruction of the proventiculus (Fig.11). Severe epithelial layer destruction in the proventiculus (Fig. 12).

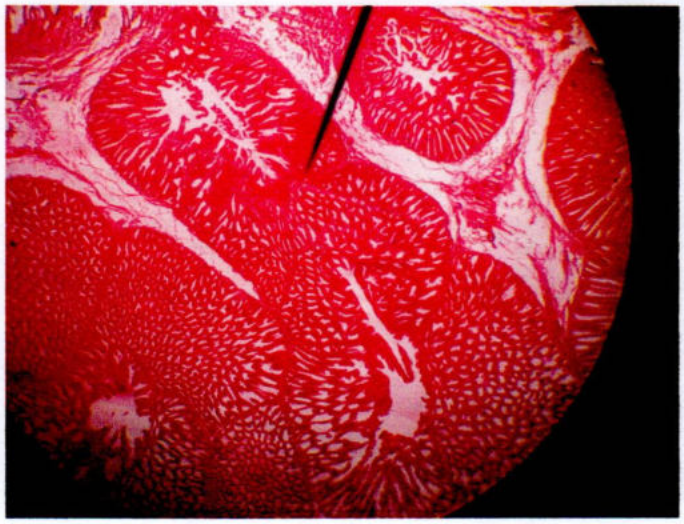


Fig: 10 Distortion of normal architecture of tissue

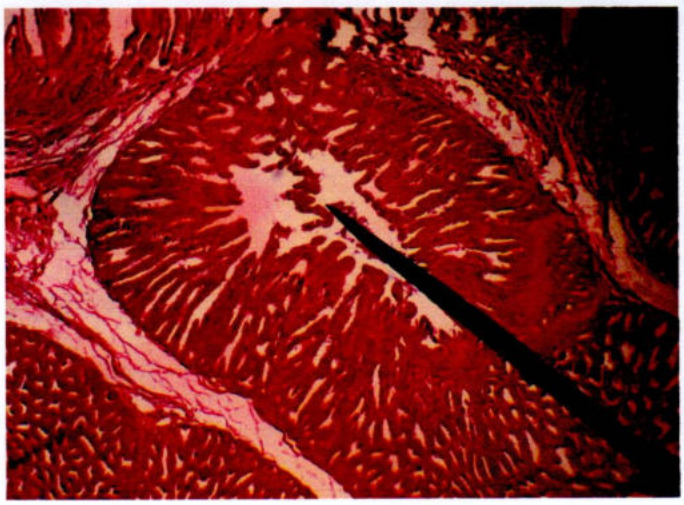


Fig: 11 Globular destruction in the Proventiculus section

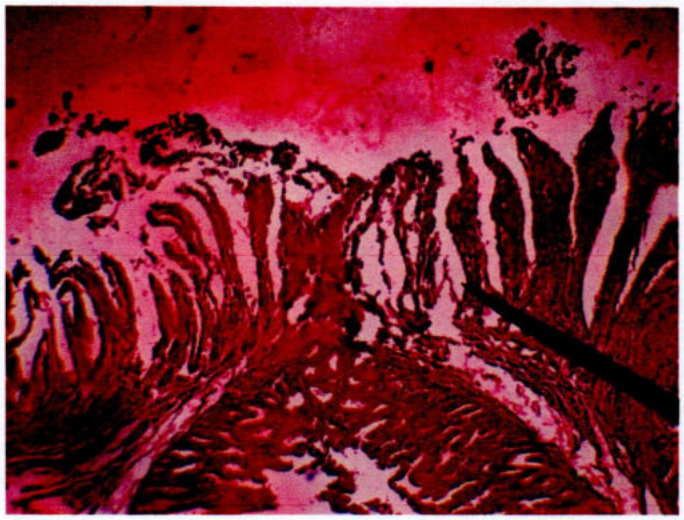


Fig: 12 Epithelial layer destruction in the proventiculus section

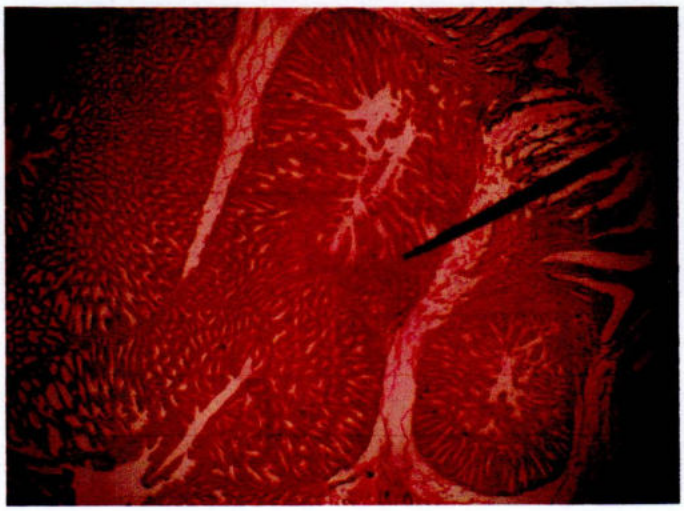


Fig: 13 Haemorrhage in the Proventiculus section

# CHAPTER V
# DISCUSSION

# CHAPTER V

# DISCUSSION

This study was undertaken to investigate the pathological condition of NDV at small scale commercial broiler farm in different upazilla of Dinajpur district from July to December, 2012.

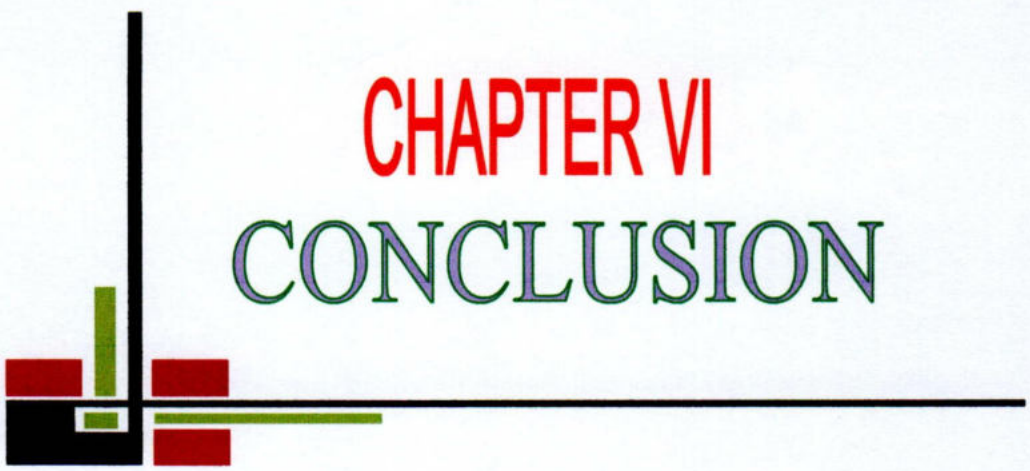
A total of 250 of the 135 affected broiler chickens were examined as NDV and observed clinical signs were sneezing, coughing, nasal discharge, laboured breathing, and torticolis which correspond with the findings of (Okoye *et al.* 2000). Greenish diarrhea which was also similar with the findings of (Alexander *et al.*, 1993).Nervous system is marked by paralysis of legs, neck and wing which correspond with the findings of (Ressang, *et al.* 1961).

(Table 1) Prevalence of NDV at different commercial broiler farms in Dinajpur District are showing- total 35 farms visited in different thana. Total 250 diseased and dead birds were examined out of which 135 birds were found to be positive for NDV. The Prevalence of NDV was (54.62%) which were similar findings reported by (A.D.EL.Yuguda *et al* 2007) stated that (46%)prevalence of Borno state in Nigeria, (A.E.Salihu *et al* 2012) stated that (54.67%) prevalence of Nasarawa state in Nigeria and not similar reported by (A.Idi Maikano *et al* 1999) reported by (28.3%) in Niger.

(Table 2) Out of 241 nonvaccinated birds 52 (21.6%) died due to ND, where as out of 206 vaccinated birds, 10 (4.9%) died of ND. The risk of dying from ND was 4.4 time higer in nonvaccinated birds. In vaccinated flock death from ND might be due to improper vaccination. The total mortality was higher in nonvaccinated than in vaccinated birds. Result is not agreement with (L. R. Barman *et al.*2010) stated that the risk was 1.5 time higher in nonvaccinated birds.

In this observation, the gross pathological lesions were Slight to severe haemorrhages in the proventiculus (Fig. 6), haemorrhage in caecal tonsils (Fig.9). These findings support earlier observation of (Mishra. *et al*, 2000; Okoye *et al.*, 2000) who reported that typical lesions are proventricular haemorrhage, most commonly seen in the surface near the junction with the ventriculus, and in the caecal tonsils . Haemorrhages in the internal wall of intestine (Fig.8) which supports the finding of (Orr and John, 1946; Jungherr, 2004; Kianizadeh *et al.*, 2002) who reported that Haemorrhagic lesions associated with necrosis are found in the intestinal wall.

Histopathological lesions in the proventiculus were distortion of normal architecture of tissue (Fig. 10). Globular destruction (Fig.11), Sever epithelial layer destruction (Fig.12), haemorrhages and congestion in the mucosa of proventriculus (Fig.13). Similar result were also reported by (Jordan, F., M. Patisson *et al* 2001), Julia Victoria Rodriguez barahonacosta Rica 2008).



# CHAPTER VI
# CONCLUSION

# CHAPTER VI

## CONCLUSION

Newcastle disease is consider to be one of the most important viral diseases in broiler though out the world and has a devasting effect on poultry production in most countries. The present study was conducted mainly to explore a pathological investigation of ND based on clinical, parasitological, gross and histopathological lesion. Total 35 farms were visited, 250 diseased and dead birds were examined out of which 135 birds were found to be positive for ND. In above the discussion mortality due to ND was higher in the nonvaccinated birds.

The clinical signs of the affected birds were recorded as sneezing, gasping, and coughing and sometime complete in ability to make sound. Broiler may become inactive, weak, and rough in appearance. Greenish Diarrhea may also occur. Nervous sign include clonic, spasm and paralysis of the legs.

The Investigation of Newcastle diseases grossly in the naturally infected birds were haemorrhages in the proventiculus, caecal tonsils, and intestine. Histopathological changes in proventiculus are congested blood vessels, haemorrhages, Globular destruction, & sever epithelial layer destruction.

On the basis of this study it is assumed that although ND is a serious problem at poultry industry in Bangladesh, it possible to control under routine preventive and control measure which is prime essential for substantial improvement in poultry production

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