PREVALENCE AND PATHOLOGY OF NEWCASTLE DISEASE IN BROILER AT BOCHAGANJ UPAZILA OF DINAJPUR DISTRICTS

A Thesis

By

PABITRA KUMAR

Registration No. 1305079
Semester: January- June, 2014
Session: 2013-2014

Master of Science (M.S.)
in
Pathology

Department of Pathology and Parasitology

Hajee Mohammad Danesh Science and Technology University
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ABSTRACT

The study was schemed to investigate the prevalence, pathology, mortality and clinical features of Newcastle disease in the small scale commercial broiler farms at Bochaganj upazila of Dinajpur district during the period from January to June, 2014. A total 1950 birds (from 5 farms), among which 160 diseased and dead birds were randomly selected, out of which 99 (5.35%) birds were found to be positive for Newcastle disease. Thorough clinical and necropsy examination was done and the characteristics clinical signs and gross lesions were recorded. Different organs mainly proventiculus, caecal tonsil and intestine were collected, preserved and processed for histopathological examination. The clinical signs of the affected birds were sneezing, coughing, nasal discharge, laboured breathing and torticolis, Broilers were inactive, weak and rough in appearance, greenish watery diarrhoea occur severely. Nervous signs includes clonic spasm and paralysis of the legs and wings. In this observation, the gross pathological lesions were slight to severe haemorrhages in caecal tonsils, typical lesions were proventricular haemorrhage, most commonly seen in the surface near the junction with the proventriculus. Haemorrhages were found in the internal wall of intestine, liver and lungs during post-mortem examination. Histopathological lesions in the proventiculus were distortion of normal architecture of tissue, globular destruction, severe epithelial layer destruction, haemorrhages and congestion in the mucosa of proventriculus. The prevalence of Newcastle disease in Bochaganj upazila was 5.35%. Mortality of Newcastle disease in non-vaccinated and vaccinated broiler flock was 20.76% and 4.6%, respectively. The study indicates that birds of this areas were at high riskof NDV. Further studies like serological, epidemiological and clinical examination can be done regarding sex, age and climate to find out the real scenario of ND in the poultry sector at Bochaganj.

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LIST OF ABBREVIATIONS AND SYMBOLS

C	-	Celcious
DW	-	Distilled water
et al.	-	And his associates
Etc.	-	Etcetera
F	-	Fusion protein
Fig.	-	Figure
G	-	Gram
H and E	-	Hematoxylin and Eosin
HN	-	Haemagglutinin neuraminidase
IFN	-	Interferon
IM	-	Intramuscular
IN	-	Intranasal
hr	-	Hour
HSTU	-	Hajee Mohammed Danesh Science and Technology University
MAB	-	Monoclonal Antibody
M	-	Matrix protein
mg	-	Milligram
Min	-	Minute
ml	-	Milli Liter
MS	-	Master of Science

N	-	Normal
NP	-	Nucleoprotein
ND	-	Newcastle disease
NDV	-	Newcastle disease virus
NVNDV	-	Neurotropic Velogenic Newcastle disease virus
nm	-	Nanometer
No.	-	Number
P	-	Phosphoprotein
PBS	-	Phosphate buffer solution
PM	-	Postmortem
PPD	-	Purified protein derivative
RBC	-	Red blood cell
RFLP	-	Restriction fragment length polymorphism
RNA	-	Ribonucleic acid
SEM	-	Standard Error Mean
SPSS	-	Standard package for Social Science
Sq	-	Square
VVNDV	-	Viscerotropic Velogenic Newcastle disease virus
%	-	Percent
µg	-	Micro gram

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 poultry rearing. The vast majority of the world's hungry people live in developing countries, where 13.5 percent of the population is undernourished (WFP, 2015). 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. The industry in Bangladesh is expanding rapidly. A parent population of 5 million produces 52 million day old chicks a year and the annual growth rate is 15 to 20%. Broiler meat production is currently at 2.2 million tones a year, with 300 million eggs produced. The country has a population of 14 million people who need a daily protein requirement of 70 to 100 grams a day. Currently, the average poultry meat consumption is just one kilogram a person a year, while just 28 eggs per person are eaten each year, (Peter, 2014). 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 Avulavirus, a newly formed genus under Paramyxoviridae (De Leeuw and Peters, 1999; Chang *et al*, 2001; Mayo, 2002). Outbreaks of 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. 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 of 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). It was a very common disease in the project area in semi-intensive system of rearing. Many studies have been done regarding this disease in large scale commercial farms. So far no pathological investigation has been done to identify the common abnormalities of Newcastle disease (ND) in small scale commercial broiler farms. The owner of the small scale farm faces many problems and loses their birds due to Newcastle disease infection that cause major economic loss.

The present research work on the pathological investigation of Newcastle disease in broiler was undertaken with the following objectives

1. To study the gross and histopathological lesions of Newcastle disease in various organs
2. To determine the prevalence of Newcastle disease in broiler
3. To know the mortality rate of Newcastle disease in nonvaccinated and vaccinated broiler

CHAPTER II

REVIEW 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

Newcastle disease (ND) also known as Ranikhet disease (RD) is a highly contagious viral disease that attacks many species of domestic and wild birds (Al-Garib *et al.,* 2003).

In Africa and Asia ND is a major constraint against the development of both industrial and village poultry production (Alders *et al.,* 2001). NDV infections of poultry range from latent to rapidly fatal depending upon the pathotype of virus involved (Alexander, 2003).

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 is most important. Avian Newcastle diseases is 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 envelope single standard RNA virus. The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability (Alexander, 2000).

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 pneumo-encephalitis.

The causative agent of the disease in Newcastle upon type 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; and 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; andHerczeg *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

NDV 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). Six breeds (mallard, Gaoyou, Shaoxing, Jinding, Shanma and Pekin ducks) were infected intramuscularly (IM) with JSD-0812 strain at the dose of 5×108 ELD50. Susceptibility to NDV infection among breeds varied, per morbidity and mortality. Mallard ducks were the most susceptible, and Pekin ducks the most resistant. Fifteen-, 30-, 45-, 60-, and 110-day-old Gaoyou ducks were infected with JSD-0812 strain at the dose of 5×108 ELD50 either IM or intranasally(I/N) BMC Veterinary Research (2014).

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; and 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);and 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; and 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 (Yuguda, A. D. E. L. *et al.,* 2007). In Nigeria of Nasarawa state prevalence was (54. 67%) (Salihu, A. E. *et al.,* 2012). Mortality was higher in nonvaccinated than in vaccinated birds. The risk was 1. 5 time higher in nonvaccinated birds (Barman, L. R. *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 a.,*, 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).

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. Egg production falls dramatically but diarrhoea is usually absent. 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 egg 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.

2.1.4 Pathogenesis and Pathology

Newcastle disease virus (NDV) or avian paramyxovirus 1 (APMV-1) is a non-segmented, single-stranded, negative-sense RNA virus in the family Paramyxoviridae (de Leeuw and Peeters, 1999, Krishnamurthy and Samal, 1998 and Phillips *et al*., 1998). The RNA genome consists of 15, 186 bases and contains six genes encoding the six structural proteins in order from 3' to 5': nucleoprotein (NP)–phosphoprotein (P)–matrix (M)–fusion (F)–hemagglutinin–neuraminidase (HN)–large protein (L) (Chambers *et al.,* 1986 and Wilde *et al*., 1986). In addition, transcriptional editing of the P gene mRNA results in two non-structural proteins, V and a potential W (Peeters *et al.,* 2004 and Steward *et al*., 1993).

The disease resulting from an NDV infection of birds varies from mild to severe with high mortality depending on virulence of the infecting strain and host susceptibility (Alexander, 1995 and Alexander, 2001, 2003). Because NDV strains are of a single serotype, virulence differentiation among those strains must be determined by standard pathotyping assays. The results of those tests which utilize inoculation of embryonated chicken eggs and live chickens are the basis for classifying NDV as velogenic (highly virulent), mesogenic (moderately virulent), or lentogenic (low virulent) (Alexander, 1998). Further division of the velogenic pathotype into viscerotropic velogenic (VVNDV) and neurotropic velogenic (NVNDV) pathotypes, those strains that cause an acute lethal disease with frequent visceral hemorrhage or an acute and often lethal disease with neurological and respiratory signs, respectively, is accomplished by intracloacal inoculation of chickens (Alexander, 1998 and Alexander, 2003). Pathogenesis studies to assess virus distribution in tissues and resultant lesions from an NDV infection have been completed by inoculation of chickens with a lower virus dose by a natural route in contrast to the inoculation of a high virus dose by a systemic route in pathotyping tests (Brown *et al*., 1999). Prior pathogenesis studies demonstrated that viruses of both velogenic pathotypes produce severe clinical disease and infect multiple tissues. Gross and histologic lesions that are the result of those infections are usually more extensive and severe with VVNDV than with NVNDV (Brown et al., 1999). No overt clinical signs were usually observed with infections from either mesogenic or lentogenic NDV. However, mesogenic isolates do cause some gross and histologic lesions that are considerably less extensive than those caused by a velogenic virus infection (Brown *et al*., 1999 and Kommers *et al*., 2003). Minimal lesions, if present, occurred in birds with lentogenic infections, affecting mostly the respiratory tract (Hamid *et al*., 1990). In lentogenic NDV infections, viral replication is detected primarily at the inoculation sites (Kommers *et al*., 2003) but minimal replication can also be present in cardiac myofibers (Brown *et al*., 1999).

The marked strain-dependent difference in tropism and virulence observed with NDV are hypothesized to depend upon the presence of cellular proteases required for the activation of the viral fusion glycoprotein precursor (Alexander, 2001, Gotoh *et al*., 1992, Nagai, 1995 and Nagai and Klenk, 1977). Recent studies utilizing viruses containing mutations generated by reverse genetics have supported the importance of the amino acid sequence at the F cleavage site for NDV virulence (de Leeuw *et al.,* 2003, Panda *et al.,* 2004b, and Peeters *et al.,* 1999 and Römer-Oberdörfer *et al.,* 2003) and viral distribution in embryos (Al-Garib *et al.,* 2003). However, some investigators have suggested involvement of other factors (de Leeuw *et al.,* 2003 and Panda *et al.,* 2004b). The loss of glycosylation sites from the HN protein altered

NDV pathogenicity (Panda *et al.,* 2004a), and HN chimeras generated from low virulent or virulent viruses either increased or decreased viral pathogenicity depending on the virulence of the virus that was the origin of the HN gene (Huang *et al.,* 2004).

Evidence for a P gene product, the V protein, contribution to NDV virulence was demonstrated in chickens (Huang *et al.,* 2003), in embryonating chicken eggs (Mebatsion *et al.,* 2001 and Park *et al.,* 2003a), and during in vitro cell culture (Huang *et al.,* 2003, Park *et al.,* 2003a and Park *et al.,* 2003b). These prior studies with infectious clones demonstrated the potential role of the F, HN, and P genes in NDV virulence, but the dissemination of these infectious clones and induction of pathological changes was not reported for infected mature chickens. Therefore, the purpose of this study was to extend the understanding of the role of the F, HN, and P genes in the pathogenesis of NDV by comparing the results of a clinicopathologic assessment in chickens infected via a natural route with selected wild-type NDV, their infectious clones, and those clones with various gene changes or mutations. The virulence of those viruses was also determined by standard pathogenicity assays (Alexander, 1998).

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; and 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; and 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).

Lesions in the respiratory tract may consist of mucosal haemorrhages and marked congestion of trachea (Mc Ferran and Mc Cracken, 1988). Air sacculitis and thickening of air sacs with 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).

Respiratory tract lesions include: loss of cilia of the epithelia, congestion, and oedema of themucosa with dense mononuclear cells infiltration (Saif, *et al.,* 2005).

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). Focal vacuolation and destruction of lymphocytes may be seen in the cortical area and germinal centres of the spleen and thymus. Marked degeneration of lymphocytes in the medulla of cloacal bursa often observed. In the intestinal tract, haemorrhages and necrosis of mucosal lymphoid tissue are seen withinfections of virulent strains of ND virus (Saif *et al*., 2005).

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; and 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;

and 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; and 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; and 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; and 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; and Beach, 1948) are proven the powerful tools in the diagnosis of the disease. Chicken RBCs are usually used in haemagglutination test, 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; and 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; and 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; and 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; and 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.

Saif and colleagues (2005), pointed out that for all practical purposes, isolates of ND virus can be considered to represent a single antigenically homogeneous group.

Monoclonal antibody (MAB) technology provides a new approach to antigenic differentiation of ND virus strains and isolates.

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, l98B). 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; and 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, 2001; 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- F1l7 (Alexander, 1990; Liu *et al.,* 2002; and 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.,* l980; 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).

2.2.8 Diagnosis of Newcastle disease:

At present, definitive diagnosis of ND is by isolation of the ND virus (Saif, *et al,* 2005). Other reliable methods of diagnosis include; direct detection of viral antigens by immunohistologic techniques, which offer a rapid method for the specific demonstration of the presence of the virusor viral antigen in organs or tissues.

Immunohistochemical staining on formalin-fixed, paraffin embedded sections has been revolutionized in 1991 by the discovery of heat-mediated retrieval (Antigen Retrieval, AR) of immunoreactivity (Shin *et al.,* 1991).

This method is now widely used and applies to the detection of the overwhelming majority of antigens, with few exceptions for which enzymatic retrieval is required (www. immunohistochemistry. html). Nobuko and colleagues in (2007) compared reverse transcription- polymerase chain reaction (RT-PCR) from formalin fixed paraffin embedded tissues to the immunohistochemistry and insitu hybridization assays for detection of ND virus and found that PCR is more effective diagnostic test than others.

Serological tests for ND include, single radial immunodiffusion test, agar gel precipitation and enzyme-linked immunosorbent assays (ELISA) which are semi automated techniques and have become popular as part of flock screening procedures (Synder, *et al.,* 1984). Good correlation has been reported between ELISA and HI test (Cvelic-Cabrilo *et al.,* 1992).

The hemagglutination (HA) and HI tests are not greatly affected by minor changes in the methodology, although 9Brugh, *et al.,* 1978) stressed the critical nature of the antigen and antiserum incubation period in test standardization.

2.2.9 Economic and Public Health Significance

The global economic impact of velogenic ND is enormous and it certainly surpassed any other poultry disease (Saif, *et al.,* 2005). In many developing countries, velogenic ND is endemic and represents an important limiting factor in the development of commercial poultry production and the establishment of trade links. The constant loses from ND severely affect the quality and quantity of food for people on marginal diets (Saif, *et al.,* 2005). Newcastle disease virus is a human pathogen and clinically presents as eye infection, seen asexcessive lacrymation, reddened eyes, oedema of eyelids, conjunctivitis and sub-conjunctivalhaemorrhages (Chang, 1981). Human infections with ND virus have usually resulted from direct contact with the virus, such asfrom splashing infective allantoic fluid into the eye in laboratory accidents, rubbing the eye with contaminated hands, handling infected birds or their carcasses.

CHAPTER III

MATERIALS AND METHODS

The study was carried out in the laboratory of the Department of pathology and parasitology, 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 broiler poultry farms were considered as experimental chickens. Ranikhet outbreaks in the small scale commercial poultry farm were investigated at Bochaganj upazila in 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 1950 birds were observed (from 5 farms) 160 diseased and dead birds were examined out of which 99 birds were found to be positive form. The number of birds in the farms was variable ranging from 350 to 800 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 were recorded. 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 of Bochaganj upazila and examined in the laboratory belonging the Department of Pathology and Parasitology under the Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

3.3 RESEARCH PERIOD

The duration of experiment was 6 months from January to June, 2014.

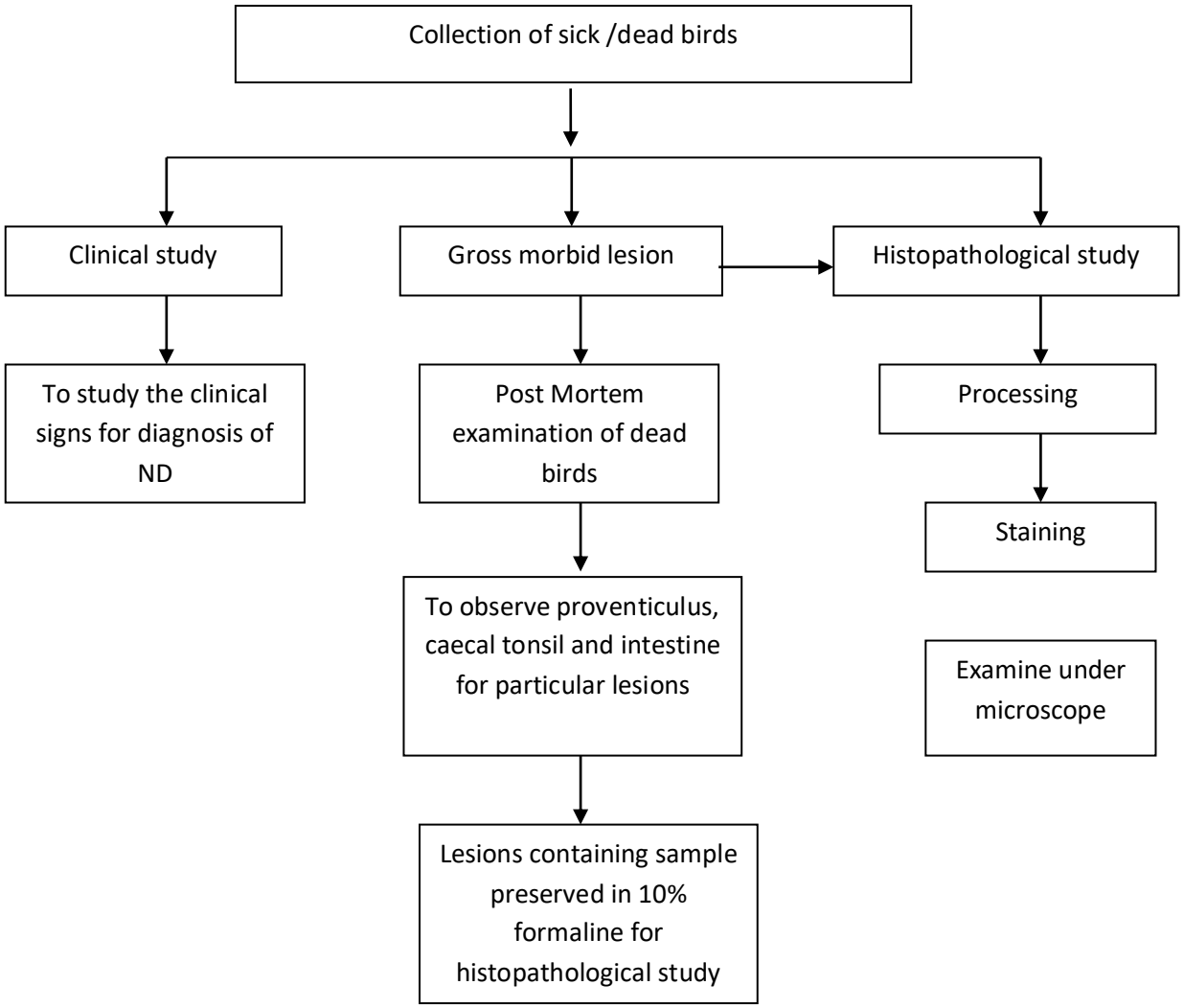
3.4 SAMPLING OCCASION

Birds affected with Ranikhet were collected and examined when submitted to the laboratory as well as collected 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 FLOWCHART/LAYOUT



3.7 CLINICAL STUDY

The general health condition and age of the chicken were recorded. The chickens were observed to detect clinical signs by usual inspection. 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 collected from different farms of Bochaganj. At necropsy, gross tissue changes were observed and recorded carefully by systemic dissection. The samples were also preserved in 10% neutral buffered formalin for the histopathological study.

Equipment and appliances for necropsy

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

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 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 anemia. Body cavity of bird was opened.

4. Segments of the intestines, caecal tonsil and proventiculus were observed carefully for important 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 and 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 and trimmed at 10% formalin. Formalin-fixed samples of the caecal tonsil, proventiculus and 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 56^0C 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 (42^0C) 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, H& E) power objects.

CHAPTER IV

RESULTS

Pathological investigation of Newcastle disease encountered in small scale commercial broiler farms at Bochaganj upazila in Dinajpur district was studied and different clinical, 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 showed marked depression, inactive and weak. Sometime complete inability to make sound. Greenish watery diarrhoea occured. Nervous sign include paralysis of the neck and legs.

4.2 STATUS OF PREVALENCE AND MORTALITY OF THE DISEASE

The study revealed the following status of mortality and prevalence of Newcastle diseases virus (NDV) in broiler. Table-1 showed the prevalence of ND at different region of Bochaganj upazila of Dinajpur district. A total of 1950, among which 160 birds were selected during the study period from which 99 birds were found infected with ND. The prevalence of ND was 5.35%. Mortality rate of vaccinated broiler was 4.65% and in non-vaccinated broiler was 20.76% showed in Table-2. The total mortality rate was 4. 5 times higher in non-vaccinated than in vaccinated birds. Table:3 showed the percentages varriation of most common gross lesions that were found in different organs during postmortem examination.

Table 1: Prevalence of ND at different commercial broiler farms at Bochaganj Upazila

Farms	No. of birds	No. of infected birds	ND encountered	Prevalence of ND(%)	Level of significance
F-1	250	27	12	4. 80	-

F-2	500	38	22	4. 40	-
F-3	450	30	24	5. 33	-
F-4	200	20	15	7. 50	*
F-5	550	45	26	4. 73	-
Mean±SEM				5. 35±0. 56	

* = Significant at 5% level of significance

SEM=Standard Error of Mean

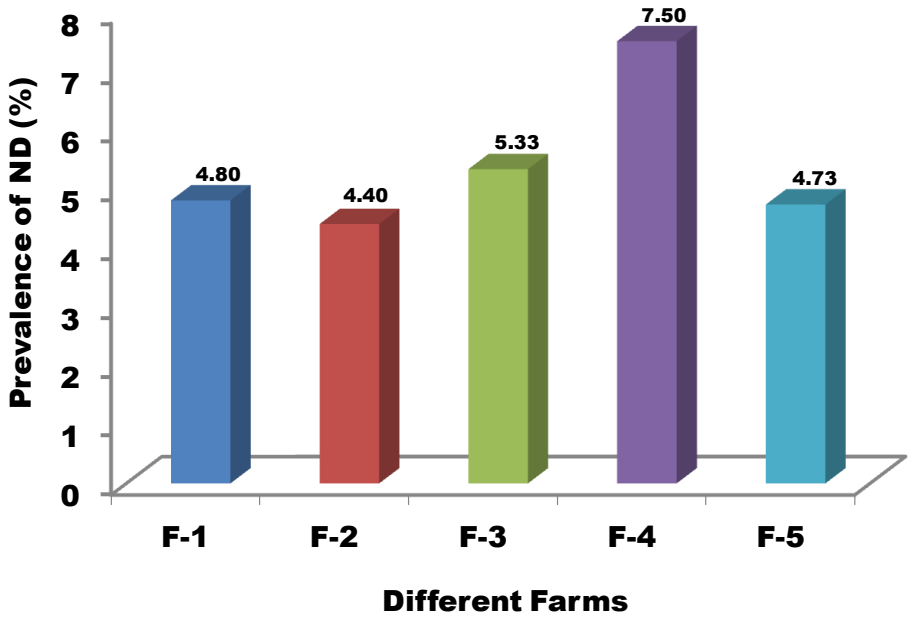


Fig 1: Prevalence of ND in different farms

Table 2: Mortality rate in nonvaccinated and vaccinated flock

Vaccination status	Total birds	Death due to ND	Mortality rate of ND (%)
nonvaccinated	130	27	20. 76%
vaccinated	130	6	4. 6%
Relative risk			4. 5

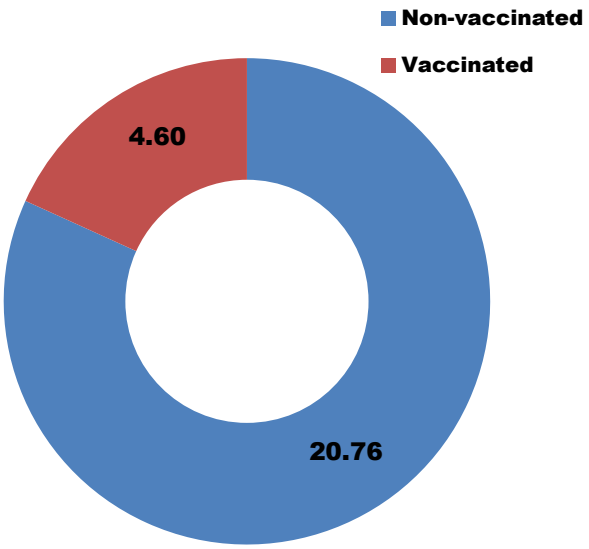


Fig 2: Mortality rate of ND in nonvaccinated and vaccinated birds

Table 3: Common gross lesions observed in the Newcastle Diseases affected broiler during necropsy

SL. No.	Gross Lesions	No. of birds exhibit the lesions out of 25 birds	Percentages(%)
1	Enlarged haemorrhagic or congested caecal tonsils	21	84
2	Haemorrhage in the mucosa of proventicuous	14	56
3	Haemorrhages, congestion and edema in the lungs	19	76
4	Haemorrhages, congestion in tracheal mucosa	7	28
5	Hepatic necrosis and haemorrhage	18	72
6	Haemorrhages in the intestinal mucosa	12	48

Fig 3: Flock affected with NDV

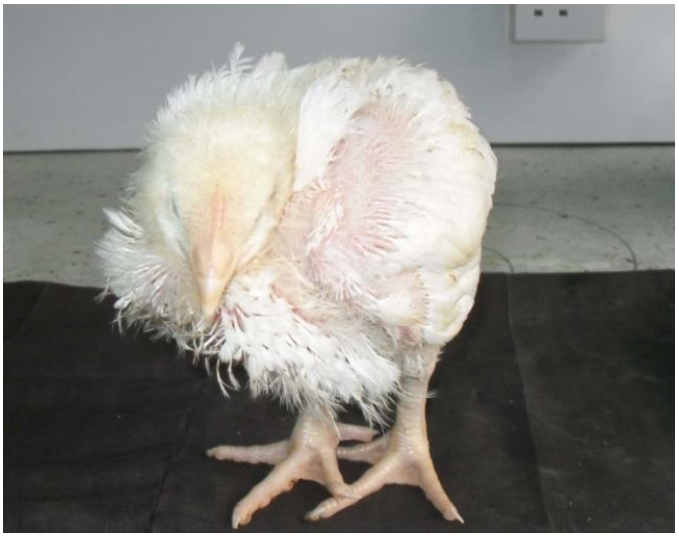


Fig 4: NDV affected bird showing depression

Fig5: NDV affected bird 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:7), haemorrhage in the internal wall of intestine (Fig: 8) and haemorrhage in caecal tonsils (Fig: 9).

Fig 6: NDV affected bird after dissection

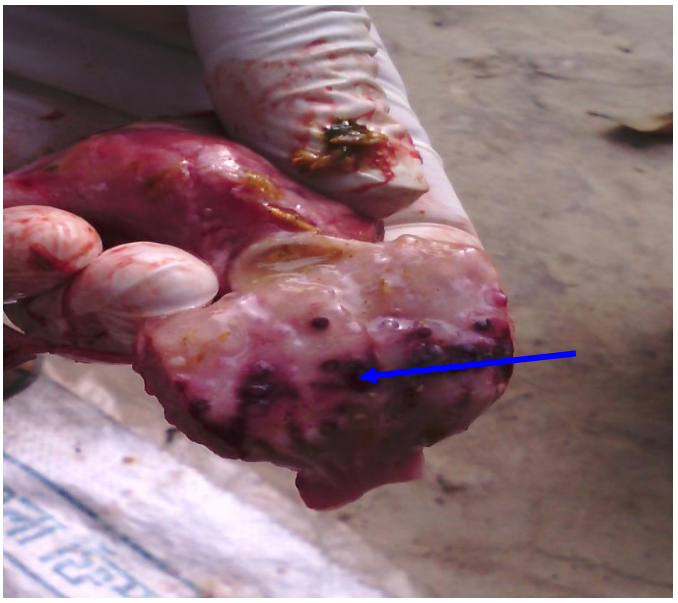


Fig 7: Haemorrhages in the proventiculus

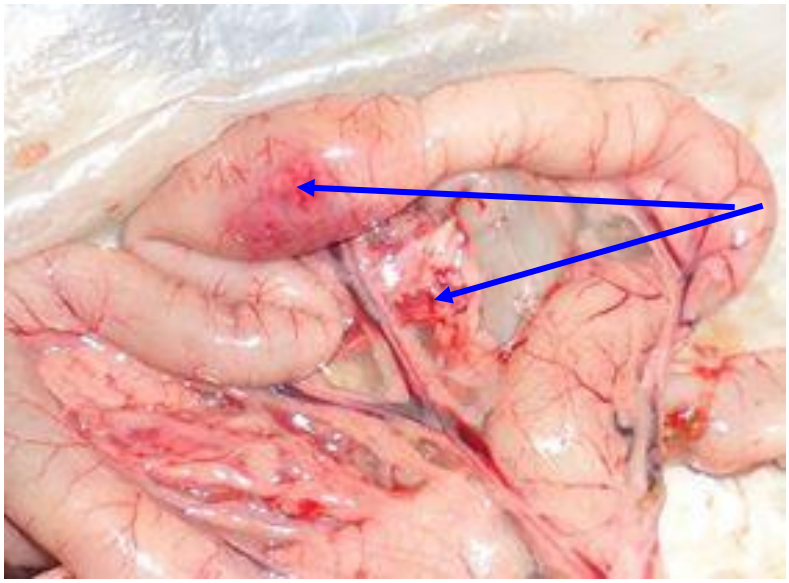


Fig 8: Haemorrhages in the intestine

Fig 9:Haemorrhages in the caecal tonsil

4.4 HISTPATHOLOGICAL STUDY

Histopathological changes in proventiculus are distortion of normal architecture of tissue (Fig:11). Necrosis and haemorrhages around the gland, globular destruction of the proventiculus (Fig:12). Severe epithelial layer destruction in the proventiculus (Fig: 13). Haemorrhage in the proventiculur section(Fig:14).

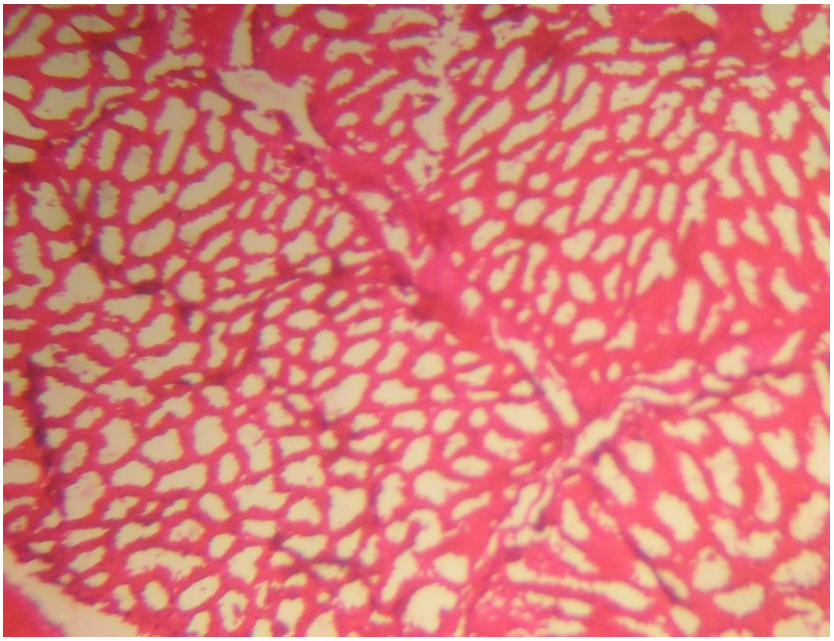


Fig 10: Normal architecture of proventiculur tissue (40X, H& E)

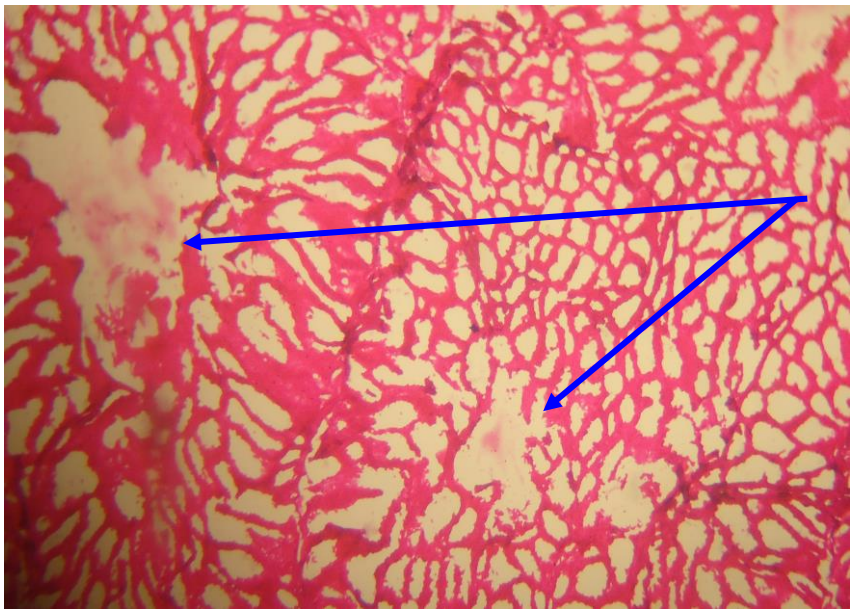


Fig 11: Distortion of normal architecture of tissue (40X, H& E)

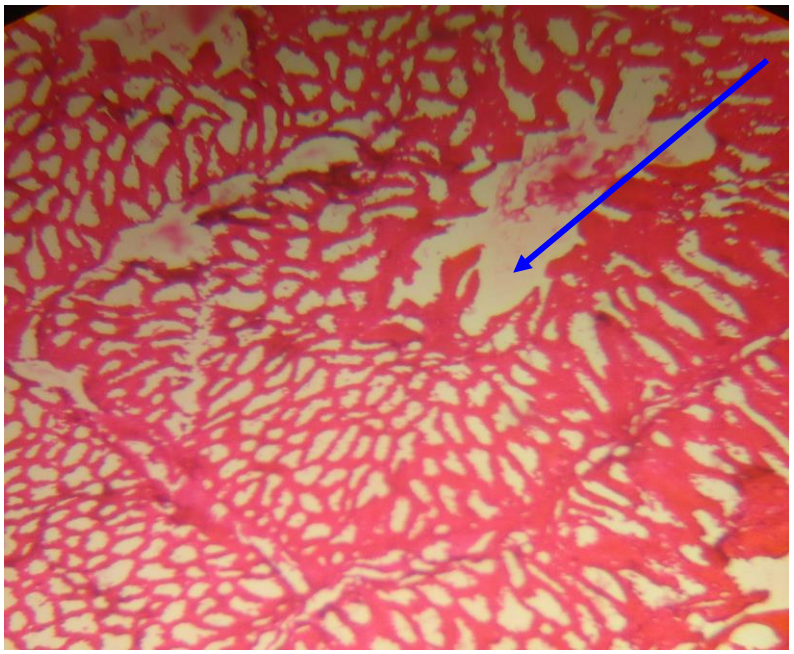


Fig 12: Globular destruction in the Proventiculus section (40X, H& E)

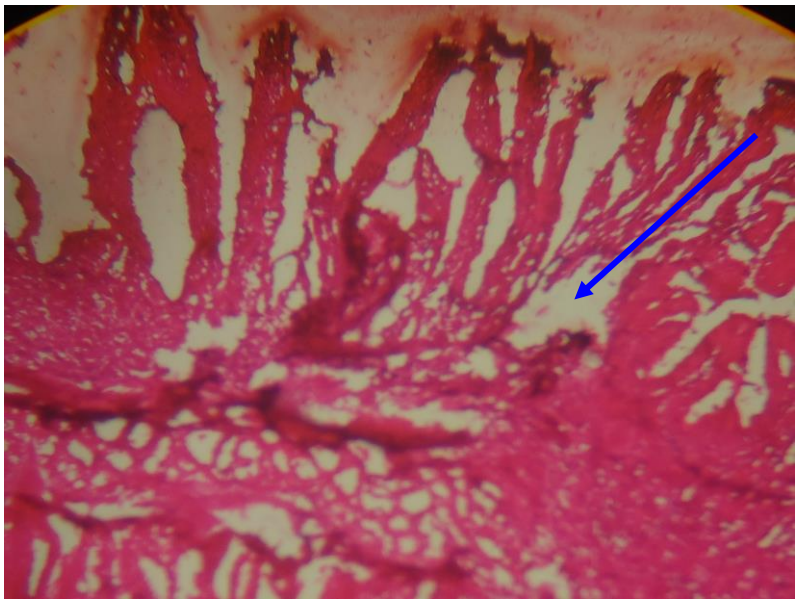


Fig 13: Epithelial layer destruction in the proventiculus section(40X, H& E)

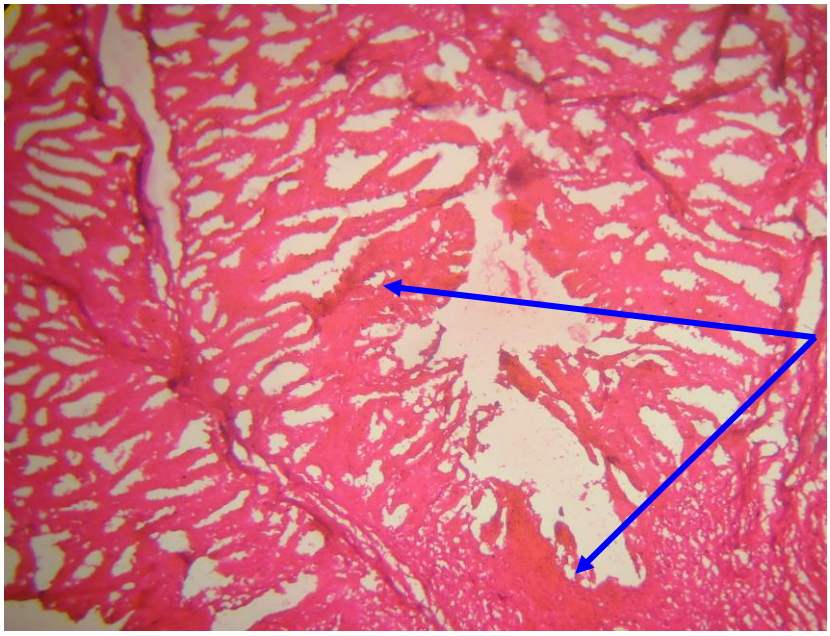


Fig 14: Haemorrhage in the Proventiculus section(40X, H& E)

CHAPTER V

DISCUSSION

This study was undertaken to investigate the pathological condition of NDV at small scale commercial broiler farm at Bochaganj upazilla during the period from January to June, 2014.

A total 1950 birds, among which number of infected birds was 160, out of which 99 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 diarrhoea which was also similar with the findings of Alexander *et al.,* (1993). Nervous system was marked by paralysis of legs, neck and wing which correspond with the findings of Ressang, *et al*. (1961).

Prevalence of NDV at different small scale commercial broiler farms in Bochaganj upazila are showing in table-1 where total 5 farms visited in this upazila. Total 160 diseased and dead birds were examined out of which 99 birds were found to be positive for NDV. The prevalence of ND at Bochaganj was 5.35% which were dissimilar with findings report by (Yuguda, A. D. EL. *et al.,* (2007) stated that (46%) prevalence of Borno state in Nigeria, (Salihu, A. E. *et al.,* (2012) stated that (54.67%). 7.5% prevalence shown in the journal of biological science (04/2002). (Njagi, LW. *et al.,* (2010) stated 17.8% prevalence in hot dry zone and 9.9% in cool wet zone. The variation of prevalence may be due to variation in geographical location, seasonal variation, species variation, managemental error and so on.

Out of 130 non-vaccinated birds, 27 died due to ND and mortality rate was (20.76%), where as out of 130 vaccinated birds, 6 died of ND and 4.6% mortality rate showed in table-2. The relative risk of NDV was 4.5 times higher in nonvaccinated birds than vaccinated one. In vaccinated flock death from ND might be due to vaccination failure (wrong time vaccination, wrong route, use of denaturated vaccine etc.), improper management. The mortality was 4.5 times higher in nonvaccinated birds than in vaccinated birds. Result was not agreed with Barman, L. R *et al.,* (2010) stated that the risk was 1.5 time higher in nonvaccinated birds.

Percentages varriation of most common gross lesions that were found in different organs during postmortem examination showed in table-3.

In this observation, the gross pathological lesions were slight to severe haemorrhages in the proventiculus (Fig.7), haemorrhage in caecal tonsils (Fig.9). These findings support with the earlier observation of Mishra. *et al.*, (2000); and 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 intestine (Fig:8) which supports with the finding of others (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: 11), globular destruction (Fig: 12), sever epithelial layer destruction (Fig: 13), haemorrhages and congestion in the mucosa of proventriculus (Fig: 14ss). Similar result were also reported by (Jordan, F. , M. Patisson *et al* (2001), Julia Victoria Rodriguez barahonacosta Rica (2008) were severe haemorrhages and congestion in the mucosa of proventriculus and globular destruction.

CHAPTER VI

CONCLUSION

Newcastle disease is consider to be one of the most important viral diseases in broiler throughout the world and has a devastating effect on poultry production in most countries. The present study was conducted mainly to explore a pathological investigation of ND based on clinical, gross and histopathological lesion. Total 5 farms were visited, A total of 1950 birds, among which 160 diseased and dead birds were selected out of which 99 birds were found to be positive for ND. The prevalence of ND at Bochaganj upazila was 5.35%. In above discussion mortality due to ND was 20.76% in the non-vaccinated birds and 4.6% in vaccinated birds remarks that mortality rate in the non-vaccinated birds was 4.5 times higher than vaccinated birds.

The clinical signs of the affected birds were recorded as sneezing, gasping, coughing and sometime complete inability to make sound. Broiler were totaly inactive, weak and rough in appearance. Greenish diarrhoea occured. Nervous sign includes clonic spasm and paralysis of the legs.

The investigation of Newcastle disease grossly in the naturally infected birds were haemorrhages in the proventiculus, caecal tonsils and intestine. Histopathological changes in proventiculus were congested blood vessels, haemorrhages, globular destruction and severe epithelial layer destruction.

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

REFERENCES

Ackerman, W. W. (1964): Cell surface phenomena of Newcastle disease virus. In: R. P. Hanson (Ed.). Newcastle disease virus an evolving pathogen. University of Wisconsin press Medison, wl. pp 153-166.

Alexander D. J. (1988a): Historical aspects of disease. Kluwer Academic publishers, Boston, Mass. pp. 1-10.

Alexander, D. J.; Alexande Y. M.; Saif, H. J.; Barnes, I. R.; Glisson, A. M.; Fadly, J. R.; McDougald, D. E.; Swayne (Eds.), (2003): Newcastle disease, other avian paramyxoviruses, and pneumovirus infections Disease of Poultry (11th ed.), Iowa State University Press Ames (2003), pp. 63–8 Italian Veterinary Journal. 18:75-79.

Alexander, D.J. and Parsons, G. (1986): Pathogenicity for chickens of avian paramyxovirus type 1 isolates obtained from pigeons in Great Britain during 1983-1 985. Avian Pathology. 15: 487-49.

Alexander, D. J. (1988b): Newcastle Disease: Methods of Spread. In DJ Alexander (Ed.). Newcastle Disease. Kluwer Acadernic Publishers, Boston, MA. pp. 256-272.

Alexander, D. J. (1995):Newcastle disease in countries of the European Union Avian Pathol. , 24 (1995), pp. 3–10

Alexander, D. J. (2000): Newcastle disease and other avian paramyxoviruses. Review of Science and Technology. 443-462.

Alexander, D. J. (2001): Gordon Memorial Lecture. Newcastle disease. British Poultry Science. 42: 522.

Alexander, D. J. and Allano W. H. (1974): Newcastle disease virus patho types. Avian pathology. 3:269-278.

Alexander, D. J. Parsons, G. and Marshall, R. (l984): Infection of fowls with Newcastle disease virus by food contaminated with pigeon faeces. Veterinary Record 115: 60l-602.

Alexander, D. J. Swayne, D. E. Glisson, J. R. Jackwood M. W, Pearson J. E, Reed W. M. (Eds.), (1998): Newcastle disease virus and other avian paramyxoviruses in a Laboratory

Manual for the Isolation and Identification of Avian Pathogens, American Association of Avian Pathologists, Kennett Square (4th ed) (1998), pp. 156–163

Alexander, D.J. (1997): Newcastle disease and avian paramyxovirus infections. In: B. W. Calnek, H. J. Bernes, Beard C. W, Beard, L. R. McDougald (ed), Disease of Poultry, 10th (ed). Lowa State University Press, Ames, pp. 541-569.

Alexander, D.J. (1993): Newcastle Disease, Other Avian Paramyxoviruses, and Pneumovirus Infections. 63 - 80. *In:* Saif YM Diseases of Poultry*, Volume* 111993. Iowa State Press, Ames, Iowa. Pp. 505-535.

Alexandero D. I. (1985): Avian pararnyxovinrs typc 1 (NDV) infcction in pigeons and poultry. Proceedtng of Annual West Poultry Conference 34:131-134.

Al-Garib Al-Garib, S. O.; Gielkens, A. L. J.; Gruys, E.; Peeters, B. P. H.; Koch,G. (2003): Tissue tropism in the chicken embryo of non-virulent and virulent Newcastle diseases strain that express green fluorescence protein Avian Pathol. , 32 (2003), pp. 591–596

Al-Garib, S. O.; Gielkens, A. L. J.; Gryus, E. and Koch, G. (2003): Review of Newcastle disease virus with particular references to immunity and vaccination, World's poultry Science Journal. 59: 185-200.

Ali, M. I. (1994): Current status of Veterinary Biologies production in Bangladesh and their quality control. Proceding of the BSVER symposium held on July28, 1994 at Nipsom Auditorium, Mohakhali, and Dhaka, Bangladesh.

Asahara (1973): Study on the virulence of Newcastle disease virus. Kitasato Archives of Experimental Medicine. 51:15-29.

Ballagi-Pordany, A. ; Wehmann, E.; Herczeg, J.; Belak, S. and Lomniczi, B. (1996) : Identification and grouping of Newcastle disease virus strains by restriction site analysis of a region from the F gene. Archives of Virology. l41: 243-261.

Banerjee, M.; Reed, W. M.; Fitzgerald, S. D.; and Panigrahy, B. (1994) : Neurotropic velogenic ND in Cormorants in Michigan: Pathology and virus characterization. Avian Disease. 38: 873-878.

Barahona, H. H. and Hanson, R. P. (1968) : Plaque enhancement Newcastle disease virus (lentogenic strains) by magnesium diethylaminoethyl dextran. Avian Disease. 12:15l-158.

Beach, J. R. (1943): Avian Pneumoencephalitis. North American veterinary Journal. 24:288-292.

Beach, J. R. (1942) : avian pneumoencephalitis proceding of animal Meeting in US Livestock Sanitary Association. 203-223.

Beach, J. R. (1948) : the application of haemagglutination inhibition test in the diagnosis of avian pneumoencephalitis (Newcastle disease). Journal of American Veterinary Medical Association. l12: 85.

Beard, C. W. and Hanson, R. P.; Hofstad, M. S. ; Barnes, H. J.; Calnek, B. W.; Reid, W. M.; Yoder, H. W. (1984): Newcastre Disease of poultry, 8th ed. Iowa State University press, Ames, IA, pp 452-470.

Bell, J. G.; Berrada, J.; Wyffels, R.; and Houadfi, M. E..I. (1984: Isolation and biological properties of some Moroccan strains of newcastle disease virus. Avian Disease. 28: 3l9-322.

Box, P. G. ; Helliwel, B. I.; and Halliwell, P. H.; (1970) : Newcastle disease in turkeys. Veterinary Record. 86: 524-527.

Brandly, C. A.; (1950): Newcastre disease. American Journal of Medical Association. 116: 139.

Brown, C.; King, D. J.; Seal, B. S.; (1999): Pathogenesis of Newcastle disease in chickens experimentally infected with viruses of different virulence Vet. Pathol., 36 (1999), pp. 125–132

Brugh, M., Beard, C. W. ; and Wilkes. W. J. (1978) : The influence of test conditions on Newcastle disease hemagglutination- inhibition titers. Avian Dis 22: pp 320-328.

Burnet, F. M. (1942): The affinity of Newcastle disease virus to the influenza virus group. Australian Journal of Experimental Biology and Medical Science. 20: 81-88.

Burnet, F. M. (1943): Newcastle disease: Human infection with virus of Newcastle disease of fowls. Medical Journal of Australia. 2: 131.

Bushnell, L. D.; and Erwin, L. F.; (1950): Studies on Newcastle disease IV. Thermostability of Newcastle disease virus and its antibody kam Academy of Science Institute. 53: 68.

Cai, Jiali and Pan, G. Q. (2000): Isolation identification and immunological study of a highly virulence strain of Newcastle disease virus, Journal of southwest Agricultural University. 21: 74-7 6.

Chambers, P.; Millar, N. S.; Bingham, R. W. (1986): Emmerson Molecular cloning of complementary DNA to Newcastle disease virus, and nucleotide sequence analysis of the junction between the genes encoding the hemagglutinin–neuraminidase and the large proteinJ. Gen. Virol. 67: 475–486

Chang, P. C. ; Hsieh, M. L. ; Shien, J. H. ; Graham, D. A.; Lee, M. S. and Shieh, H. K. (2001): Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks. Journal of general Virology. 82: 2157 -2168.

Chang, P. W. (1981): Newcatle disease. In D. W. Beran CRC Hand book series in zooonosis volume II. CRC press: Baton Raton, pp. 261- 274

Chauhan, H. V. S. and Roy, S. (1996) : Poultry Diseases, Diagnosis and Treatment. 2nd ed. New Age International (P) Limited Publishers, New Delhi. pp. 58-64.

Cooper, H. (1931) : Ranikhet disease: a new disease of fowl in lndia due to a filter passing virus. lndian Journal of Veterinary Science and Animal husbandry. 1: 107.

Corpus, Z. C.; and shortridge, K. F. (1982): Newcastle disease in Philippines. Newcastle disease and its control in Southeast Asia. 61-66.

Csatary L. K. ; Csatary, E. and Moss R. W. (2000) : Scientific interest in Newcastle disease virus is reviving. Journal of National cancer Institute. 92: 493 -494.

Cvelic- Cabrilo, Mazija, V. H. ; Bidin, Z. and Ragland, W. L. (1992): Correlation of hemagglutination inhibition and enzyme linked immunosorbent assays for antibodies to Newcastle disease virus. Avian pathol 21: 509 – 512.

Cynthia M, Kahn and Scott Line (2005): The Merck veterinary manual, ninth edition, pp 2255–2256.

De Leeuw O. S,; Hartog, L.; Koch, G.; Peeters, B. P. H. (2003): Effect of fusion protein cleavage site mutations on virulence of Newcastle disease virus: non-virulent cleavage site mutants revert to virulent to virulence after one passage in chicken brain J. Gen. Virol. , 84 : 475–484

De Leeuw O. S. ; Koch, G. ; Hartog, L.; Ravenshorst, N.; Peeters, B. P. H. (2005) :Virulence of Newcastle disease virus is determined by the cleavage site of the fusion protein and by both the stem region and globular head of the haemagglutinin–neuraminidase proteinJ. Gen. Virol. , 86 : 1759–1769

De Leeuw, O. and Peeters, B, (1999): Cornplete nucleotide sequence of Newcastle disease virus: evidence for the existence of a new genus within the subfamily Paramyxovirinae. Journal of General Virology. 80:131-136

Dekock, G. (1954): Studies on the histological and pathogenesis of ND of fowls in South Africa with spccial reference to the lymphoid tissue Onderstepoort Journal of Veterinary research. 26: 599-620.

Digioia, G. A. Licciardello, J. J.; Nickerson, J. J. R. and Goldblith, S. A. (1970): Thermal inactivation of Newcastle disease Virus. Applied Microbiology. 9: 451-454.

Doyle, T. M. (1927): A hitherto unrecorded disease of fowls due to a filter passing virus. journal of comparative pathology and theraputics 40: 144-169.

Duque, S. B and Estupinan, A. J. (1976): Pathogenicity or Newcastle diseasaes in parrots. Revista instituto Colombiano, Agro Pecuario, 11:163-171.

Eckert, D. M. ; and Kim, P. S. (2001) : Mechanisms of viral mernbrane fusion and its inhibition, Annual Review biochemistry. 70: 777 -810

Edwards, J. T. (1928) : A new fowl disease. Annual Report of Institute of Veterinary Research, Mukteswar. pp. 15-15.

Eisa, M. and Omer, B. A. (1984) : A natnral outbreak of Newcastle disease in pigeons in the Sudan. Veterinary Record. ll4: 297.

Emmerson, P. T. (1999): Newcastle disease virus (Pararnyxoviridae); Virology and Microbiology; Acadernic Press. University of Newcastle upon tyne, U. K. 236-256

Estola, T. (1974): Isolation of a Finish New castle disease virus with an exceptionally high thermostability. Avian Disease. l8: 274-277.

Ezeokoli, C. D., Umoh, J. U. , Adesiyan A. A. and Abu, P. (1984): Prevalence of NDV virus antibodies local and exotic chicken under different management system in Nizeria, Bulletin of animal health and production in Africa 32 : 253-257.

Fan, G; Zhu, W.; Wang, Y.; Jiang, Y. S. and Du, Y. Z. F. L. (1999): Isolation and identification of highly virulent Newcastle disease virus. Chinese Journal of Veterinary Science. 19: ll4-117.

Garten, W.; Burke, W.; Nagai, Y.; Rott, R. and Klenk, H. D. (1980): Mutational changes of the protease susceptibility of glycoprotein of Newcastle disease virus: Effects on Pathogenicity. Journal of general virology. 50: 135-147.

Glickman, R. L.; Syddall, R. J.; Iorio, R. M.; Sheehan, J. P.; Bratt, M. A. (1988) : Quantitative basic residue requirements in the cleavage-activation site of the fusion glycoprotein as a determinant of virulence for Newcastle disease virusJ. Virol. , 62 (1988), pp. 354–356

Gomez-Lillo, M.; Bankowski, R. A.; and Wiggins, A. D. (1974): Antigenic relationships among viscerotropic velogenic and domestic strains of Newcastle disease virus. American Journal of' Veterinary research 35:2 471-475

Gotoh, Ohnishi, Y.; Inocencio,N. M.; Esaki, E.; Nakayama, K.; Barr, P. J.; Thomas, G. and Nagai, Y. (1992): Mammalian subtilisin related proteinases in cleavage activation of the paramyxovirus fusion glycoprotein: superiority of furin/PACE to PC2 or PC1/PC3J. Virol. , 66 : 6391–639

Gotoh, B.; Sakaguchi, T; Nishikawa, K; Inocencio, N. M.; Hamaguchi, M; Toyoda, T. and Nagai, Y. (l999): Structural features unique to each of the three antigenic sites on the hernagglutinin-neuraminidase protein of Newcastle disease virus. Virology. 163: 174-182.

Gravel and Morrison, Gravel, K. A.; Morrison, T. G. (2003): Interacting domains of the HN and F proteins of Newcastle disease virus J. Virol., 77 (2003), pp. 11040–110.

Hamid, H.; Champbell,R. S. F. ; Lamichhane, C. (1990): The pathology of infection of chickens with the lentogenic V4 strain of Newcastle disease virus Avian Pathol., 19 : 687–696

Hanson, R, P. ; Hitchner, S. B.; Domermuth, C. H.; Purchase, H.G.; and Williarns, J. E. (1975): Isolation and Identification of Avian Pathogens. American association of Avian Pathologist, Kennett Square, PA. pp 160-173.

Hanson, R. P. and Spalatin, J. (1978): Thermostability of hemagglutinin of Newcastle disease virus as a strain marker in epizootic studies. Avian Disease. 22: 659-665.

Hanson, R. P. (1956) : An inffacerebral inoculation test for determining the safety of NCD vaccines. American Journal of Veterinary Research. 17: 16.

Hanson, R. P. ; Spalatin, J. and Estupinatn, J. (1967) : Identification of lgntogenic strains of Newcastle disease virus. Avian Disease. 1l- 49.

Hanson, R. P. ; Upton, E; Brandly, C. A. and Winslow, N. S. (1949) : Heat stability of haemagglutinin of various strains of Newcastle disease virus. Proceeding of Society for Experimental Biology in NewYork. 70: 283-287.

Harry, A. F. and Stephen S. W. (1961): Sensitivity of various viruses to chloroform.

Hausman, S; Garcin, D.; Delenda, C. and Korakofsky, D. (1999): The versatility of paramyxovirus RNA polymerase stuttering, Journal of Virology. 73: 5568- 557 6.

Herczeg, J.; Wehmann E.; Bragg, R. R.; Travassos Dias, P. M.; hadjiev, G.; werner O. and Lomniczi, B. (1999): Two novel genetic groups (Viib and Viii) responsible for recent Newcastle disease outbreaks in Southern Africa. One (viib) of which reached Southern Europe Archives of Virology 144: 2087 -2099.

Hernandez, L, D. ; Hoffman, L. R. ; Wolfsberg, T. G. and White, J. M. (1996): Virus. cell and cell-cell fusion. Annual Review o. f Cell Biology. 12:627 -661.

Huang, Z.; Krishnamur Chy, S.; Panda, A. and Samal, S. K. (2003): Newcastle Disease Virus V Protein Is Associated with viral Pathogenesis and Functions as an Alpha interferon Antagonist. Journal virology 77 : 867 6 -8 6 8 5 .

Huang, Z; Panda, A.; Elankumaran, S.; Govindarajan, D.; Rochemann D. D. and Samal, S. K. (2004): The Hemagglutinin-Neuraminidase Protein of Newcastle Disease Virus Determines Tropism and Virulence Journal of Virology. 78: 417 6-4184.

Huchzermeyer, F. W. (1993): Why is velogenic Newcastle disease endemic in some countries and not in others? Zimbabwe Veterinary Journal. 24: 111- 113.

Islam, M. A.; Ito, T. and Kida. H. (1995) : comparison of the haemagglutinin pattern and pathotypic activities of some Japanese isolates of Newcastle disease virus with reference strains. Bangladesh Veterinary Journal. 29 : 1 -8.

Johnson, E. P.; Doll, E. R. and Boney, W. A. (1953): Newcastle disease of fowl. virginia polytechnic Institute, Blacksbug, virginia; Agar. Expt. Station. Bull. p. 964.

Jordan, F., M. Patisson, D. Alexender, Faragher, U. T. (2001): Poultry Diseases. 5th Ed. Saunders, Hong Kong.

Jordan, I. K. ; Sutter, IV B. A. ; and McClure, M. A. (2000) : Molecular evolution of the Paramyxoviridae and Rhabdoviridae rnultiple-protein encoding P gene. Molecular Biology. l7: 75-86.

Journal of biological science (04/2002) : prevalence of poultry disease in Bangladesh DOI:10. 3923/jbs. 2002. 212. 213

Julia Victoria Rodriguez barahonacosta Rica (2008) : Lectin and immuno histochemical investigations on cellular alterations in chicken embryos following inoculation with Newcastle diseases virus of different virulence at the University of Veterinary Medicine, Hannover . p. 93.

Jungherr, E. L. and Hanson, R. P. (2004): Pathogenecity of NDV for the chicken.). Newcastle Disease virus. An evolving Pathogens. university, of Wisconsin, Medison. p p. 257 - 272.

Jungherr, E. L. and Terell, N. (1946) : Observation on the spread of ND. Proceeding in Annual Meeting in US Livestock Sanitary Association 50:158-171

Kaleta, E. F. and Baldauf, C. (1988) : Newcastle disease in free-living and pet birds. In: D. J. Alexander (ed.), Newcastle Disease, Kluwaer Academic Publishers, Boston/ Dordrecht/London. Pp. 197 -246.

Kawamura, M.; Nerome, K; Kodama, H.; Izawa, H. and Mikami, T. (1987) : Serological and pathological studies of ND virus isolated from cased binds from South East Asia. Avian Disease. 3l: 564-589.

Kianizadeh, M. I Aini, L. and Ghoami, G. R. (2002) : A corrrparatrvc study on histopathologic effects of Iranian Newcastle disease virus isolates. Archieves of Razi Institute. 54:17 - 29 .

King, D. J. (1993): Newcastle disease virus passage in MDBK cells as an aid in detection of a virulent subpopulation Avian Dis. , 37 (1993), pp. 961–969

Kingston, D. J. and Dharsana, R. (1977): Mortality in crabs in the presence of Newcastle disease virus. philippines Journal of Veterinary record and Medicine. 18: 125-130.

Kohn, A. (1958): Quantitative aspects of alimentary infection by Newcastle disease virus. Poultry Science. 37: 792-796.

Kommers, G. D.; King, D. J. ; Seal, B. S. and Brown , C. C. (2003): Pathogenesis of chicken-passaged Newcastle disease viruses isolated from chickens and wild and exotic birds Avian Dis. , 47 : 319–329

Kommers, G. D. ; King, D. J. ; seal, B. S. ; carmichael, K. p. and Brown, C. C. (2002) : Pathogenesis of six pigeon-origin isolates of Newcastle disease virus for domestic chickens. Veterinary Pathology. 39: 353- 362

Kommers, Kommers, G. D. ; King, D. J. ; Seal, B. S. And Brown, C. C. (2001): Virulence of pigeon-origin Newcastle disease virus isolates for domestic chickens Avian Dis. , 45: 906–921

Koncicki, A. and Rotkiewicz, T. (1988): Course of Newcastle diseases virus infection in turkey. zeszyty NeuKowe Akadermii. Rolniczey., we wroclamiu. Weterynaria. 45: 119-l2l.

Kraneveld, F. D. (1926) : A poultry disease in the Dutch East Indies. NedIndisch BI Diegeneeskd. 38: 448-450,

Krishnamurthy, S.; Huang, Z. H. And Samal, S. K. (2000) : Recovery of a virulent strain of Newcastle disease virus from cloned cDNA: expression of a foreign gene results in growth retardation and attenuation Virol., 278 : 168–182.

Krishnamurty, S. and Samal, S. K. (1998): Nucleotide sequence of trailer, nucleocapsid protein gene and intergenic regions of Newcastle disease virus strain Beaudette C and completion of the entire genome sequence. Journal of General Virology. 79: 2419-2424.

Kuiken, T.; Wobeser, G.; Leighton, F. A.; Haines, D. M.; Chelack, B.; Bogdan, J.; Hassard, L. ; Heckert, R. A. and Riva, J. (1999): Pathology of Newcastle disease in double-crested carmorants frorn Saskatchewan, with comparison of diagnostic methods, Journal of Wildlife Diseases. 35: 8-23.

Barman1, L. R.; M. F. Flensburg and Islam, M. R. (2010): A controlled study to assess the effects of vaccination against Newcastle disease in village chickens Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh . 27(2): 56 – 61

Lafia, Akwange and keffi metropolis, nasarawa state Nigeria, International journal of Agricultural science, Vol. 2. No. 2. pp-109-112.

Lamb, R. A. and Kolakofsky, D. (2001): Paramyxoviridae: the viruses and their replication. In: D. M. Knipe and P. M. Howley (ed.), Fields virology, 3rd ed. , vol. l. Lippincott Williarns & Wilkins, Philadelphia, Pa. pp. 1305-1340.

Lancaster, J, E. and Alexander, D. I. (1975): Newcastle disease: virus and spread. Monograph No. 11, Canadian Department of Agriculture, Ottawa. Pp.356-365

Liu, H.; Wang, Y.; Zhu, G.; Yan, W. and Tian, H. (2002): The isolation molecular identification of a genotype VII Newcastle disease virus (NDV) isolate. Chinese, Journal of Veterinary Science. 22: 216-218

Lomniczi, B. ; Wehmann, E. ; Herczeg, J. I Ballagi-pordany, A.; Kaletfl, E. F.; Werner, O. L. Meulemans, G. Jorgenser, p. H.; Mante, A. P.; Gielkens, A. L.: capua, I. and Damoser, J. (1998): Newcastle disease outbreaks in recent years in Western Europe were caused by an old (vi) and a novel genotypc (vii). Archivcs of virology143: 49-64.

Lorence, R. M. ; Roberts, M. S. ; Groene, W. S. and Rabin, H. (2001): Replication-competent, oncolytic Newcastle disease virus for cancer therapy. In: Driever pH, Rabkin SD (eds): Replication-competent viruses for cancer Therapy. Monographs in virology,doerr HW, Karger: 22:160-182.

Luna, L. G. (1963): Manual of Histopathologic Staining Methods of the armed forces institute of pathology 3rd Edn. McGrow-Hill Book Company, London. 21: 325-376

Manin, T. B. ; sticherbakova, L. O. ; Bochkov, Y. A.; Elnikov, V. V.; Pehlkina, I. P.; starov, S. K. and Drygin, V. V. (2002): Characteristics of field isolates of Newcastle disease virus isolated in the corurse outbreak. voprosy virusologii. 47: 4l -43

Mayo, M. A. (2002): A summary of taxonomic changes recently approved by ICTV. Archives of virology. 147:1655-1656.

McFerran, J. B. and McCracken, R. M. (1988): Newcastle Disease. Kluwer Acadernic Publishers. Noston. M. A. pp. 16l-183.

McFerran, J. B. and Nelson, R. (1971): Some properties of an avirulent Newcastle disease virus. Archive von gesamte Virus forschung 34: 64-74.

McGinnes, L. I. McQuain, C. and Morrison, T. (1988): The P protein and the nonstructural 38K and 29K proteins of Newcastle disease virus are derived fiom the same open reading frame. Virology. 164:256-264.

Mebatsion, T.; Verstegen, S.; De Vaan, L T.; Romer-Oberdorfer, A. And Schrier, C. C. (2001): A recombinant Newcastle disease virus with low-level V protein expression is immunogenic and lacks pathogenicity for chicken embryos J. Virol., 75: 420–428

Melnick, J. L. (1982), Taxonomy and nomenclature of Viruses. Progress on Medical Virology. 28:208-221.

Millar, N. S. and Emmerson, P. T. (1988): Molecular cloning and nucleotide sequencing of Newcastle disease virus. In: D. J. Alexandar (Ed.). Newcastle Disease. Kluwer Academic Publisher, Boston, MA, pp. 79- 97.

Mishra, S.; Kataria, J. M.; Sah, R. L.; Verma, K. C. and Mishra, J. P. (2000) : Pathogenesis of Newcastle disease virus isolates in pigeon. Indian Journal of Animal science. 70: 1125-1126.

Morrison, T. G. (2003) : Structure and function of a paramyxovirus fusion protein. Biochemical et Biophysica Acta. 6l4:73-84.

Nagai, Y. and Klenk, H. -D. (1977) : Activation of precursors to both glycoproteins of Newcastle disease virus by proteolytic cleavage Virol. , 77 (1977), pp. 125–134

Nagai, Y. (1995) : Virus activation by host proteinases. A pivotal role in the spread of infection, tissue tropism and pathogenicity Microbiol. Immunol. , 39 (1995), pp. 1–9

Nagai, Y.; Kelnk, H. D. and Rott, R. (1976): protelytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology. 722 494-508.

Nitzschke, F. and Schmittdid (1963): Thermoresistance of the hemaglutinin as acharacteristics features of strains of Newcastle diseases virus. J. Zbl. Vet. Med. Ser. Bio. pp. 121-126.

Njagi, L. W., Nyaga P. N., Mbuthia, P. G. ,Bebora L. C. ,Michieka J. N., Kibe, J. K., Minga UM (2010): "Prevalence of Newcastle disease virus in village indigenous chickens in varied agro ecological zones in Kenya. 576-598

Nobuko, W., Daniel J. King, Bruce S. Seal and Corrie C. Brown1 (2007): Detection of newcastle disease virus RNA by reverse transcription-polymerase chain reaction using formalin-fixed, paraffin-embedded tissue and comparison with immunohistochemistry and in situ hybridization. Correspondence: 1 Corresponding Author: Corrie C. Brown, Department of Pathology College of Veterinary Medicine, University of Georgia, 501 DW Brooks Drive, Athens, GA 30602-7388

Office, International Des Epizootics (OIE). (2000): Newcastle disease Manual of Standards for Diagnosis Test and Vaccine. 4th Edn . OIE, Paris. pp. 189-207.

Okoye, J. O. A. ; Agu, A. O. ; Chineme, C. N. and Echeonwu, G. O. N. (2000): Pathologic characterization in chickens of a velogenic Newcastle disease virus isolated from Guinea fowl. Revue-d'Elevage-et-de- Medicine-Veterinaire-des-pays-tropicaux, 53: 325-330.

Orr, W. and John, K. T. (1946): A Malaysian virus disease of fowls. Veterinary Record. 58: 117-119.

Panda, A. Panda, S. Elankumaran, S. Krishnamurthy, Z. and Huang, S. K. (2004a) Samal Loss of N-linked glycosylation from the hemagglutinin–neuraminidase protein alters virulence of Newcastle disease virus J. Virol. , 78 (2004), pp. 4965–4975

Panda, A.; Huan E, Z.; Elankumaran, S.; Rockemann, D. D. and Samal, S. K. (2004): Role of Fusion Protein Cleavage Site in the Virulence of Newcastle Disease virus. Microbial pathogenesis, 36: l-10.

Park' M. S.; shaw, M. L.; Munoz-Jordan, J.; Cros, J. F.; Nakaya, 'l'; Bouvier, N.; Palese, P.; Garcia-sastre, A. ancl Basler, C. F. (2003a): Newcastle disease virus (NDV)-based assay demonstrates interferon-antagonist activity for the NDV V protein and the Nipah virus v, w, and c proteins. Journal of virology. 77: l50l-1511.

Park, Man-seong; Garcia-Sastre, A.; Cros, J. Ii. ; Basler, C. T. and Palese, P. (2003b): Newcastle Disease virus v protein is a Determinant of Host Range Restriction. Journal of Virology. 77: 9522- 9532.

Peeters, P,;Verbruggen, F. Nelissen, O. de Leeuw,(2004): The P gene of Newcastle disease virus does not encode an accessory X protein J. Gen. Virol. , 85 : 2375–2378

Peeters, B. P. H. ; de Leeuw, o. S. ; Koch, G. and Gielkens, A. L. (1999) : Rescue of Newcastle disease virus from cloned cDNA: evidence that cleavability of the fusion protein is a major determinant for virulence, Journal of Virology. 73: 5001-5009.

Peisajovich, S. G. , and Shai, Y. (2002): New insights into the mechanism of virus-induced membrane fusion. Trends in Biochemical Science. 27: 183-190.

Peisajovich, s. G. ; Samuel, o. and shai, Y. (2000): Paramyxovirus F1 protein has two fusion peptides: implications for the mechanisrn of membrane fusion. Journal of Molecular biology 296: 1353- I 365

Pennington, T. H. (1978) : Antigenic difference between strains of NDV. Archives of virology. 56: 345-351.

Peter H, (2014): Poultry production in Bangladesh. WORLD POULTRY - Elsevier Volume 17(7) : 01.

Phillips, R. J. ; Samson, A. C. R. and Emmerson, P. T. (1998): Nucleotide sequence of Newcastle disease virus and assembly of the complete genomic sequence: agreement with the "rule of six. " Archives of virology. 143: 1993-2002.

Raszewaska, H. (1964): Occurrence of the La Sota strain NDV in the reproductive tract of laying hens. Bulletin for Veterinary Institute, Pulawy, 8:130-136,

Reeve, P. and Poste, G. (1971) : Studies on the cytopathologenicity of Newcastle disease virus: Relationship between virulence, polykaryocytosis and plaque size. Journal of General Virology. 1l: 17 -24.

Ressang, A. A. (1961) : Newcastle disease in Indonesia. Part-II. Its symptomatology, gross and microscopic anatomy. Communications in Veterinary. (Boge Indonesia) 5: 16-37.

Römer-Oberdörfer A.; O. Werner, J. Veits, T. Mebatsion, T. C. Mettenleiter, (2003) : Contribution of the length of the HN protein and the sequence of the F protein cleavage site to Newcastle disease virus pathogenicity J. Gen. Virol. , 84 : 3121–3129

Rott, R. (1985) : In vitro Differenzierung von pathologenen und apathogenen aviaren Influenzaviren. Ber. Munch. Tieraerztl Wochenschr. 98: 37 -39.

Rott, R. and Klenh, H. D. (1988): Molecular basis of infectivity and Pathogenicity of Newcastle Disease. Kluwer Academic Publishers. Boston, M. A. pp. 98-112.

Rubin, H. and Franklin, R. M. (1967) : On the mechanisrn of NDV neutralization by immune serum. Virology, 3: 84.

Saha, S.; Islam, M. A.; Rahman, M. M. and Alam, K. H. T. (1998): Efficacy of an inactivated Newcastle disease virus vaccine prepared frorn a local isolate. Bangladesh Veterinary Journal. 32:57 -62.

Saif Y. M, Barnes H. J, Glisson J. R, Fadly A. M, McDougald L. R and Swayne D. E. (2005): Diseases of poultry, 11th ed. , pp 66 – 78.

Sakaguchi, T. Sakaguchi, T.; Toyoda, B.; Gotoh, N. M.; Inocencio, K.; Kuma, T. and Miyata, Y. (1989): Nagai Newcastle disease virus evolution. I. Multiple lineages defined by sequence variability of the hemagglutinin–neuraminidase gene Virol. , 169 (1989), pp. 260–272

Samson, A. C.; Levesley, I. and Russell, P. H. (1991): The polypeptide synthesized in Newcastle disease virus-infected cells possesses properties predicted for the hypothesized protein. Journal of General Virology. 72: 1709-1713.

Seal B. S,; King, D. J. and Bennett, J. D. (1995): Characterization of Newcastle disease virus isolated by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis J. Clin. Microbiol. , 33 : 2624–2630

Talha, A. F. S. M: Hossain, M. M.; Chowdhury, E. H.; Bari, A. S. M. Islam, M. R. and Das, P. M. (2001): Poultry diseases occurring in Mymensing. District of Bangladesh. The Bangladesh Veterinarian 18, 20-23. chickens. Chinese journal of Veterinary Medicine, Zhorgguo Shouy, Zazhi. 10:6-8.

Vindevogel, H. and Duchatel, J. P. (1988): Panzootic Newcastle diseases in pigeons. in: Alexander (Ed). Newcastle Diseases Kluwer Academic Publishers, Boston, M. A. P. P 184-196.

Wan, S. X. ; Gao,Q. Y. and Shaho, Z. H. (1984): Pathomorphological model of Experimental infection with the Beijing strain of Newcastle diseases virus in chickens. Chainese journal of veterinary medicine, Zhorgguo Shouy, Zazhi. 10:6-8

World Food Programme (WFP) (2015). Hunger Statistics. Available at http://www. wfp. org/hunger/stats

Youguda, A. D. E. L. ; Ngulde, I. S. ; Abubakar, M. B and Baba, S. S. (2007): 'village chicken health, management and production indices in selected villages of borno state', Nigeria family poultry journal.