CLINICO PATHOLOGICAL INVESTIGATION OF NEWCASTLE DISEASE IN BROILER CHICKEN AT SADAR UPAZILA OF DINAJPUR

A Thesis
By

SABUJ CHANDRA ROY

REGISTRATION NO. 1705443

SESSION: 2017-2018

SEMESTER: JULY-DECEMBER, 2018

MASTER OF SCIENCE
IN
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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
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DINAJPUR

December, 2018

Dedicated
To My
Beloved Parents

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ABSTRACT

This study was carried out to investigate the prevalence and pathological lesions of Newcastle disease in different small scale commercial broiler farm at Dinajpur sadar upazila from July to December 2018. Newcastle disease in commercial broiler farms causes huge economic loss through a relatively high mortality and loss of production. In the present study, a total of 1600 birds from 5 farms were observed. Infected birds on the basis of clinical signs, post mortem and histopathological lesions. The highest prevalence found in F1 farm (10%) and lowest prevalence found in F5 farm (5.33%) which was not significant ($p<0.05$). Highest mortality was found in non vaccinated birds (20.69%) and lowest in vaccinated birds (6.67%) which was statistically significant ($p<0.05$). The overall prevalence of Newcastle diseases at different farms of Dinajpur sadar upazila were not significantly varied. Mortality rate in non vaccinated birds was 3.10% times higher than vaccinated birds which was statistically highly significant ($p<0.05$). The most frequent clinical signs were sneezing, gasping, coughing. Broiler were totally inactive, weak and rough in appearance. Greenish diarrhoea was also found occurred. Nervous sign include clonic, spasm and paralysis of the legs. NDV infected birds showed 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.

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LIST OF ABBREVIATIONS

%	= Percentage
°C	= Degree Centigrade
APMV-1	= Avian Paramyxovirus 1
BLRI	= Bangladesh Livestock Research Institute
CNS	= Central Nervous System
ELISA	= Enzyme- Linked Immunosorbent Assays
et al.	= And others
F	= Fusion
FAO	= Food and Agriculture Organization
H	= Haemagglutinin
HN	= Haemagglutinin Neuraminidase
HSTU	= Hajee Mohammed Danesh Science and Technology University
L	= Large Protein
M	= Matrix
MAB	= Monoclonal Antibody
ND	= Newcastle disease
NDV	= Newcastle disease virus
NP	= Nucleoprotein
NS	= Not Significant
NVNDV	= Neurotropic Velogenic Newcastle Disease Virus
P	= Phosphoprotein
RBCs	= Red Blood Cells
RD	= Ranikhet disease
RT-PCR	= Reverse Transcription- Polymerase Chain Reaction
Sp.	= Species
SPSS	= Statistical Package for Social Science
VVNDV	= Velogenic Viscerotropic Newcastle Disease Virus

CHAPTER I

INTRODUCTION

Bangladesh is one of the key players in the south Asian livestock industry, especially in poultry industry (Ladda Mongkolchaivivat, 2011). The livestock sector is the integral part of the agro-based economy. Chicken meat is relatively cheap and affordable source of animal protein (Yami and Dessie, 1997). Poultry industries play an important role in poverty alleviation and economic development of Bangladesh. Poultry meat contributes approximately 37% of total animal protein supplied in the country (Rahman and Rahman, 1998). According to WHO-FAO joint survey, meat consumption^{-1} head in Bangladesh is 15.23 kg^{-1} year and poultry contributes 35.25% of total meat supply (Akbar *et al.,* 2013). Traditionally in Bangladesh, poultry rearing is one of the most important sources of income for rural women especially for landless and marginal farmers (Paul *et al.,* 1990). The poultry sector employs about 5 million people and has experienced a long-term growth rate of about 4.50%, which is highest in the economy (BLRI Reort, 2009). It is an important component of farming system and plays a significant role to 80% rural people of Bangladesh.

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. Newcastle disease is caused by Avulavirus, a newly formed genus under Paramyxoviridae (Peters *et al.,* 1999; Chang *et al*, 2001; Mayo, 2002). Outbreaks Newcastle disease have a tremendous impact of backyard chickens in developing countries, where these birds are a significant source of protein and tills disease is endemic. In developed countries, where the more virulent forms of the virus have been eradicated, cause significant economic losses during outbreaks. The affected broiler showed varied types of symptoms. These included difficult breathing, coughing, and loss of appetite. Paralysis of the leg and/or wings along with torticollis and incoordination of movement was also notice greenish diarrhoea was a common feature. Postmortem

examination revealed petechial hemorrhages in the proventriculus, hemorrhage in larynx, trachea, heart and stomach (Jungherr, 1964; Alexander and Allan, 1974; Wan *et al*., 1984). Strains NDV are present in most countries. In many countries there is a wide spectrum of strains from non-pathogenic to highly virulent. In 2002 outbreaks occurred in Australia and later on in Japan. ND is endemic in our country (Saha *et al*., 1998). The virus mainly infects birds through their respiratory and gastrointestinal tract (Alexander, 1988). Embryos can be infected if their egg 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 farm. The owner of the small scale farm faces many problems and loses their birds due to Newcastle disease infection that cause major economic loss.

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

1. To identify the clinical features of Newcastle disease in broiler
2. To study the gross and cytopathological lesions of Newcastle diseases in various organs
3. To determine the prevalence of Newcastle disease in broiler
4. To know the mortality rate of Newcastle disease in non-vaccinated and vaccinated broiler

CHAPTER II

R EVIEW OF LITERATURE

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

2.1 Newcastle Disease

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 highly contagious viral diseases of chickens. Usually the diseases are manifested as a respiratory problem and may cause high mortality rate in young flock. An envelop single standard R Thedisease 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 pneumoencephalitis.

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

2.1.2 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).

2.1.3 Epidemiology

2.1.3.1 Distribution

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

Three panzootics of ND have occurred since the disease was first recognized (Alexander, 1988a; Alexander; 1997). By restriction site mapping and sequence analysis of the F gene, Newcastle disease virus (NDV) strains were divided into eight genotypes (Ballagi-pordany *et al*., 1996; Lomniczi *et al*., 1998; Herczeg *et al*., 1999). Among these, at least three genotypes (ii, iii, and iv) were involved in first panzootic, genotypes v and vi were considered to be responsible for the second and third panzootics. In addition, it was indicated that the severe outbreaks in weatern Europe (Lomniczi *et al*., 1998), South

Africa and southern Europe (Herczeg *et al*., 1999) and Taiwan (Yang *et al*., 1999) in the 1990s were caused by prevalent genotypes vii. Genotype I consists of the virulent strains of NDV, whereas genotype viii appears to be endemic to South Africa during the past few decades (Herczeg *et al*., 1999).

2.1.3.2 Host and age susceptibility

ND occurs in domestic fowl, turkey, pheasants, pigeons, quail, and guinea fowl. Ducks and geese are susceptible but severe disease is rare (OIE, 2000). Some wild birds like crows, sparrows, jungle fowls, kites, and vultures can suffer and spread the disease to poultry farms (Chauhan and Roy, 1996). Six breeds (mallard, Gaoyou, Shaoxing, Jinding, Shanma, and Pekin ducks) were infected intramuscularly (IM) with JSD0812 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 JSD0812 strain at the dose of 5×108 ELD50 either IM orintranasally (IN) 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.

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).

2.1.3.3 Transmission

The virus mainly infects birds through their respiratory and gastrointestinal tract (Alexander, 1988b). Embryos can be infected if their shells are contaminated with virus i.e. virus can penetrate the shell after laying (Williams and Dillard, 1968). Vertical transmission can occur, but is rare with velogenic strains because viremic hens usually stop laying. Infected embryos have been reported during naturally occurring infections of

laying hens with virulent strains (Lancaster and Alexander, 1975; Beard and Hanson, 1984), but this generally results in the death of the infected embryo during incubation. Lentogenic and apathogenic NDV might be egg transmitted via the vitelline membrane. This route of transmission is thought to occur regularly following vaccination with live lentogenic strains (Hitchner B1) (Raszewaska, 1964).

To demonstrate the presence of lentogenic virus in chick embryos and young progeny, including day-old chicks, of a vaccinated laying flock (Pospisil *et al*. 1991). 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.

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 (Alexander *et al.,* 1984). The virus may be spread by the wind or insects it can also settle on equipment and on peoples shoes or clothing and spread to birds (Lancaster, 1966; Alexander, 1988b).

2.1.3.4 Morbidity and mortality

Newcastle disease virus reacts with avian hosts in various ways .when non –immune domestic chickens encountered highly pathogenic strain of NDV, The common sequel is an acute disease with mortality close to 100% (Fan *et al*., 1999; Spradbrow,2004).there are several reports on morbidity and mortality due to ND in various countries .in Philippines morbidity and mortality rate in chickens were 2.05% and 1.55% ,respectively ,in 12 regions of islands (Corpuz and Shortridge 1982), in Sudan 100% morbidity and 80% mortality in pigeon were recorded (Eisa and Omer , 1984). In Faisalabad, Pakistan, mortality ranged from 2-50% in different vaccinated chicken flocks (Siddique *et al*., 1986).In A.P. of India mortality was 6.31% (Srinivas *et al.*,1983) and in northern India morbidity reached up to 100% and mortality up to 60% in pigeon (Manager *et al.*, 1988). Alexander (1997) reviewed the morbidity and mortality due to ND in chickens and reported that morbidity may reach up to 100% and mortality up to 50% in adult birds and 90% in young chickens. In Bangladesh, ND accounted for 10.24% mortality of total submission of samples for diagnosis during period from July 1998 to October 1999 (Thalha *et al*., 2001). The Prevalence of NDV was (54.62%) of Borno state in Nigeria (Yuguda *et al.,* 2007). In Nigeria of Nasarawa state prevalence was (54.67%) (Salihu *et al.,* 2012).

Mortality was higher in nonvaccinated than in vaccinated birds. The risk was 1.5 time higher in nonvaccinated birds (Barman *et al*., 2010).

2.1.4 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 in apparent and the affected birds may have no evidence of illness. Some flocks have only mild respiratory infection indicated by "cold" like signs over a period of a few days (Johnson *et al*., 1953).

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

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

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

Clinical sign in different form

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 (Kahn *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.

2.1.5 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,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.

2.1.5.1 Gross lesions

Depending on the strain of virus and how it reacts, post mortem findings are variable. Affected birds typically have haemorrhage in larynx, trachea, and heart and stomach (Jungherr, 2004; Alexander and Allan, 1974; Wan *et al.,* 1984, Koncicki and Rotkieicz, 1988). Although the disease does not have lesions pathognomonic to it, typical lesions are proventricular haemorrhage, most commonly seen in the surface near the junction with the ventriculus, and in the caecal tonsils (Mishra *et al.*, 2000; Okoye *et al*., 2000). Haemorrhagic lesions associated with necrosis are found in the intestinal wall, specially in the posterior half of the duodenum, in the jejunum forming button ulcers (Orr and John 1946; Jungherr, 2004; Kianizadeh *et al.,* 2002). The presence of haemorrhagic lesion in the intestine of infected chickens has been used to distinguish velogenic viscerotropic ND virus from non-velogenic ND virus.

Birds with CNS signs may have no gross lesion or only hyperemia of the brain. Air sacculitis may be present even after infection with relatively mild strains, and thickening of the air sacs with catarrhal or caeseous discharge and congestion of lung is often observed (Koncicki and Rotkiewicz, 1988).

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.

2.1.5.2 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-supportive 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).

In the intestinal tract, haemorrhages and necrosis of mucosal lymphoid tissue are seen with infections of virulent strains of ND virus (Saif *et al*., 2005).

2.2 Newcastle Disease Virus

2.2.1 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 (Peters *et al.*, 1999; Chang *et al*., 2001; Mayo, 2002). Three virus families, Rhabdoviridae, Filoviridae and paramyxoviridae, form the order Mononegavirales. Paramyxoviridae family consists of two subfamilies, Pneumovirinae and Paramyxovirinae. The subfamily pneumovirinae consists of 2 genara: pneumovirus which includes respiratory syncytial virus and avian pneumovirus and other is metapneumovirus which includes turkey rhinotracheitis virus. The subfamily paramyxovirinae consists of 6 genera. The genus morbillivirus includes measles, rinderpest and distemper virus genus respirovirus includes sendivirus and mammalian parainfluenza virus 1 and 3. The genus Rubulavirus includes mumps virus, simian parainfluenza virus 5.Genus henipavirus consists of hendravirus and Nipahvirus .The genus TPMV –like virus include Tupwawing. Genus Avulavirus include Newcastle disease virus or avian paramyxovirus type-1 (Alexander, 1998; Mayo, 2002).

Recent taxonomy of Newcastle disease virus:

Order-Mononegavirales

Family-Paramyxoviridae

Subfamily-Paramyxovirinae

Genus-Avulavirus

Species-Newcastle disease virus

2.2.2 Morphology of virus

Virions are enveloped 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,

2.2.3 Molecular biology

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

In NDV, Insertion of two non template G residues gives rise to a V encoding mRNA. While insertion of two non –template G residue generates a W encoding mRNA. These V and W protein share their amino (N) terminal domains with the P protein and vary at their carboxy (C) termini. NDV V protein has a cysteine rich C terminal domain which blinds two atoms of Zn+2 (Steward *et al*., 1995). Of the three NDV P gene products the P protein, together with L protein is known to form part of virus RNA polymerase complex (Lamb and Kolakofsky, 2001)

2.2.4 Biological properties

2.2.4.1 Haemagglutination activity

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

2.2.4.2 Neuraminidase activity

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

2.2.4.3 Plaque formation

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

2.2.4.4 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).

2.2.4.5 Thermostability

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

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

2.2.5 Antigenicity

Virus neutralization or agar-gel diffusion techniques have shown minor antigenic variation between different strain and isolates of NDV (Gomaz-Lillo *et al*.,, 1974; Scholoer *et al*., 1975; Pennington, 1978). NDV is shown to be neutralized by immune

serum in an exponential manner that implies that only one antibody molecule is required for inactivation of an infectious particle. A very small fraction of neutralized particles can be reactivated upon dilution of the serum virus mixture (Rubin and Franklin, 1957). The basic mechanism of neutralization is to penetration in the host cell.

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.

2.2.6 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 al.,* 1999, Scanlon *et al*., 1999; Terregino *et al*., 2003). Cleavage at amino acid 117 produces disulfide-linked F_2 and F_1 polypeptides derived from the amino terminal and carboxyl terminal domains of F0 respectivery (Lamb anci Kolakofsky, 2001). The F_1 polypeptide has one and perhaps two fusion peptides (Peisajovich *et al*., 2000; Peisajovich and Shai, 2002). Upon initiation of fusion, fusion peptides are thought to insert into target membranes, docking the protein to these membranes (Hernandez *et al*., 1996; Eckert and Kim, 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; 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).

2.2.7 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 *et al*. (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 Brugh, *et al.* (1978) stressed the critical nature of the antigen and antiserum incubation period in test standardization.

2.2.8 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-conjunctival haemorrhages (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 Department of Pathology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, for the pathological investigation of Ranikhet diseases in poultry.

3.1 Experimental Chickens

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

A total of 5 farms were visited where 1600 total birds were examined. Out of which 138 birds were found to be positive. The number of birds in the farms was variable ranging from 300 to 500 and they were reared on litter. A detail flock history in relation to the incidence of disease including housing system, location of poultry farms, sources of birds, age and population of the birds per flock, rearing system, litter material, feeding and watering system, bio-security of the farms, previous history on Ranikhet outbreaks. The birds affected with Ranikhet were submitted to the Pathology laboratory for the diagnosis and treatment were the principal experimental chickens and some affected chickens were also collected physically.

3.2 Research Area

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

3.3 Research Period

The duration of experiment was 6 months from July to December, 2018.

3.4 Sampling Occasion

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

3.5 The Major Works of the Present Study

Clinical Examination of affected birds.

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

Histopathological examination of proventiculus.

Cytopathological examination of intestine.

3.6 Experimental Flowchart/Layout

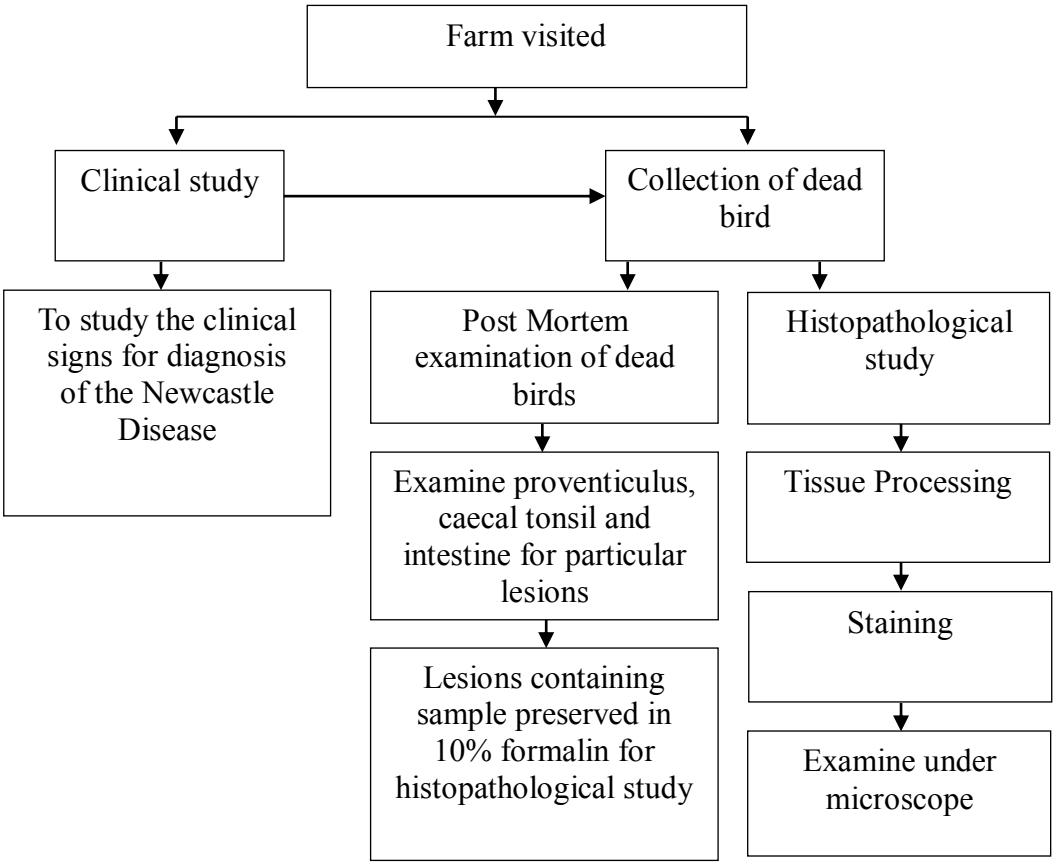

Figure 1: Experimental Flowchart/Layout

3.7 Clinical Study

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

3.8. Necropsy Findings of Suspected Chickens

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

Equipment and appliances for necropsy

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

Procedure

1. At first the chicken was wet in a detergent solution thoroughly to lessen the chances of feathers floating around the area while the examination.
2. The bird was laid on a pad of newspaper on post mortem table. The paper served to absorb most blood and fluid, and provided a convenient wrapper for the carcass after examination.

3. The bird was positioned in such way so that the legs and feet were facing the examiner. Then an incision was given on skin in between the thighs towards the back and through skinning was done to observe paleness condition of carcass for detection of anaemia. Body cavity of bird was opened.

4. Segments of the intestines, caecal tonsil, proventiculus were observed carefully for importants post mortem lesions. Then the parts opened longitudinally by knife or scissors to observe the colour, consistency and appearance of intestinal contents.

3.9 Cleaning and Sterilization of Required Glassware

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

Gross lesion

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

3.10 Histopathological Examination

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

3.10.1 Equipment and appliances

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

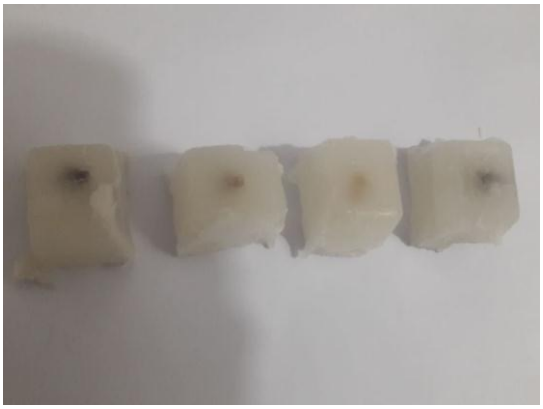

Figure 2: Block

Figure 3: Chemical Used for Histopathological Examination

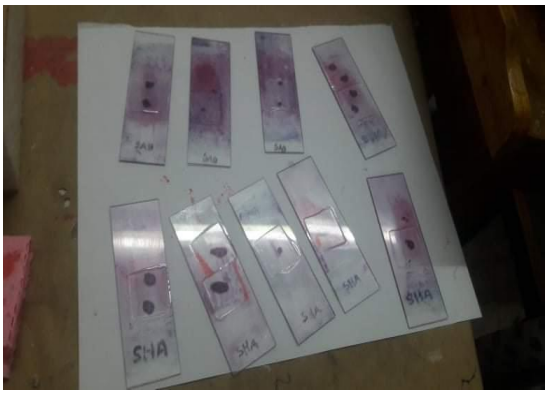

Figure 4: Slides

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°C) containing small amount of gelatin and taken on oil and grease -free glass slides. The slides were air dried and kept in cool place until staining.

3.10.3 Hematoxylin and Eosin Staining Procedure

Preparation of Harris‟ hematoxylin solution

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

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

Preparation of eosin solution

1% stock alcoholic eosin

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

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

Working eosin solution

Eosin stock solution 1part

Alcohol, 80% 3 parts

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

Staining protocol

1. Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 mins in each),
2. Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 mins in each; 95% alcohol for 2 mins; 80% alcohol for 2 mins; 70% alcohol for 2 mins) and distilled water for 5 mins,
3. The tissues were stained with Harris hematoxylin for 10 mins,
4. The sections were washed in running tap water for 10 mins,
5. Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol), 2-4 dips,
6. The tissue sections were then washed in tap water for 5 mins and dipped in ammonia water (2-4 times) until sections became bright blue,
7. The sections were stained with eosin for 1 min and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 mins in each),

8. The stained sections were then cleaned by 3 changes in xylene, 5 mins in each and finally the sections were mounted with cover slip using DPX,

9. The slide were dried at room temperature and examined under a low (10X) and high (40X) power objects.

3.11 Statistical Analysis

The data were recorded and analyzed statistically by using statistical software 'SPSS' (version 20). Chi–Square Test were performed and the results were expressed in percentage with P–value and significance was determined when $P<0.05$. The mean intensity was calculated and analyzed by F-variance test.

CHAPTER IV

RESULTS

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

4.1 Clinical Examination

The clinical signs of the birds affected with ND 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 occurred. Nervous sign include paralysis of the neck and legs.

4.2 Status of Mortality and Prevalence of the Disease

The study revealed the status of mortality and prevalence of Newcastle diseases virus (NDV) in broiler. Table 1 showed the prevalence of ND at different region of Dinajpur Sadar Upazilla. Among 1600, birds 138 were found infected with ND. The Prevalence of ND was 7.96% where as Table 2 showed that the total mortality rate was 3.10 times higher in non-vaccinated than in vaccinated birds. Table 3 showed the percentages variation of most common gross lesions that are found in different organs during postmortem examination.

Table 1: Prevalence of ND at different commercial broiler farms at Sadar Upazila in Dinajpur

Name of farm visited	Total number of birds	No. of ND infected birds	Prevalence of ND	P value
F-1	350	35	10	0.274 (NS)
F-2	200	12	6	
F-3	400	38	9.5	
F-4	500	45	9	
F-5	150	8	5.33	

NS=Not significant (P <0.05)

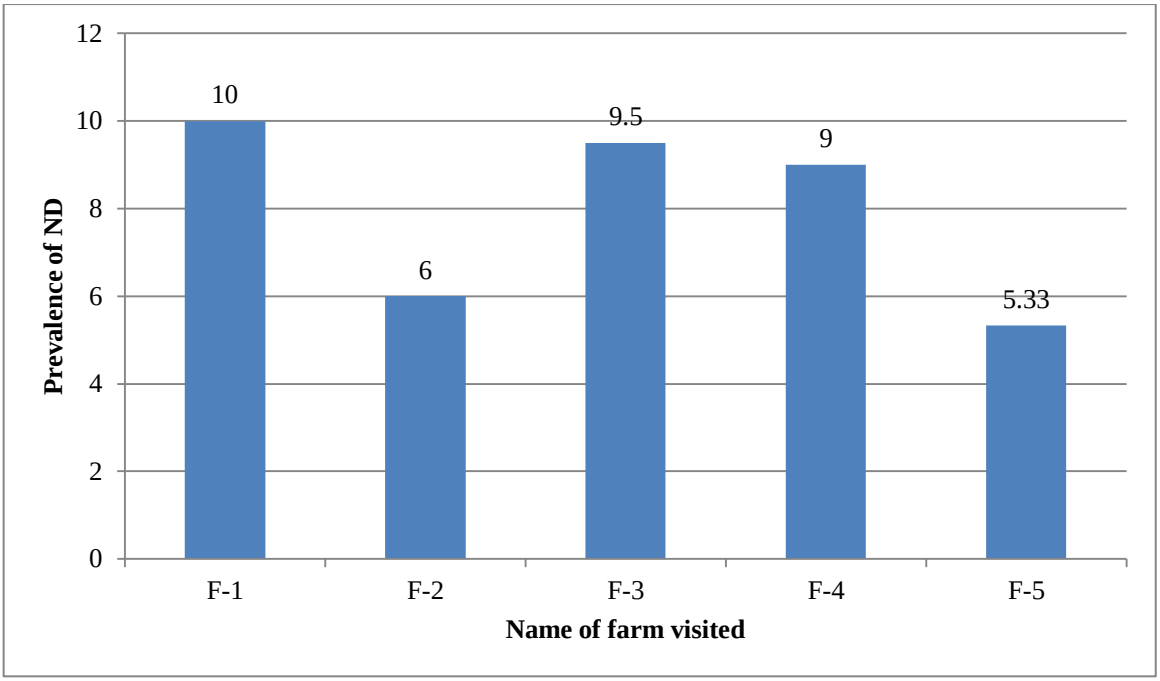

Figure 5: Prevalence of ND at different commercial broiler farms at Sadar Upazila in Dinajpur

Table 2: Mortality rate in non-vaccinated and vaccinated flock

Vaccination status	Total birds	Infected birds	Death due to ND	Mortality rate of ND (%)	P value
Non vaccinated	800	145	30	20.69	0.001*
Vaccinated	800	120	8	6.67	
Relative risk	-	-	-	3.10	

***significant at 5% level of significance (P <0.05)**

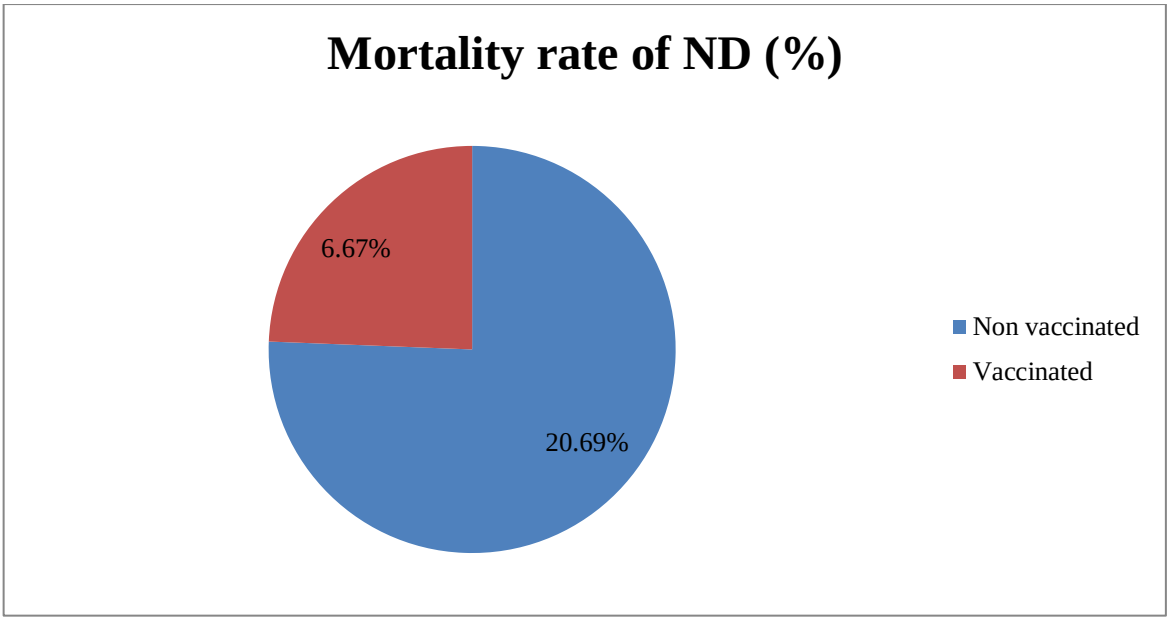

Figure 6: Mortality rate in non-vaccinated and vaccinated flock

Table 3: Common gross lesion seen in the ND disease cases Diagnosed from July to December 2018 during necropsy

SL. No.	Lesion seen in ND cases	No of cases with lesions out of 25 cases diagnosis as ND	Percentage occurrence of such lesions (%)
1	Enlarged haemorrhagic congested caecal tonsils	21	84
2	Haemorrhage in the mucosa of proventiculus	14	56
3	Haemorrhage, congestion and odema in the lungs	19	76
4	Haemorrhages, congestion in tracheal mucosa	14	56
5	Hepatic necrosis and haemorrhage	18	72
6	Haemorrhage in the intestinal mucosa	12	48

4.3 Necropsy Examination

Gross pathological changes in different samples were nearly similar but varied in severity. These included grasping cauging wheezing and paralysis (Figure 7), NDV affected birds showing torticolis (Figure 8), Haemorrhages in the proventiculus (Figure 9), Button shape ulcer in the intestine (Figure 10) and Haemorrhages in the caecal tonsil (Figure 11).

Figure 7: Grasping, cauging, wheezing and paralysis

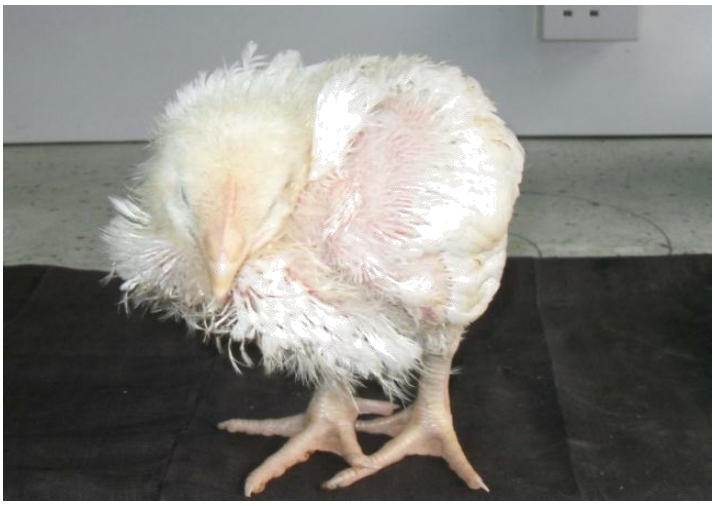

Figure 8: NDV affected bird showing torticolis

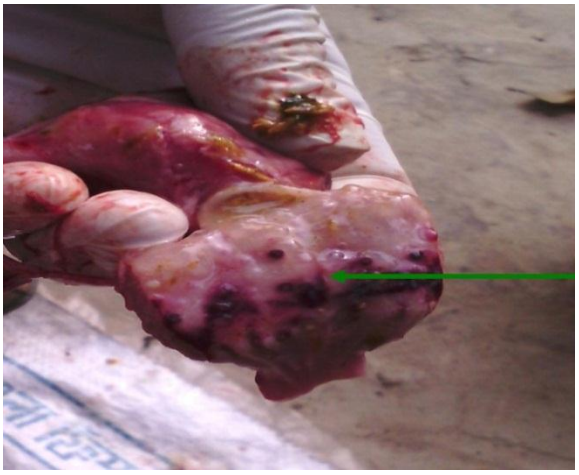

Figure 9: Haemorrhages in the proventiculus

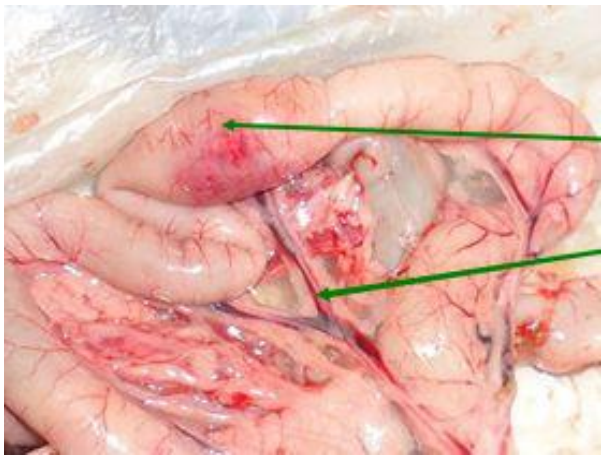

Figure 10: Button shape ulcer in the intestine

Figure 11: Haemorrhages in the caecal tonsil

4.4 Histpathological Study

Haemorrhages in the proventiculus (Figure 12) and globular destruction in the proventiculus section (Figure 13).

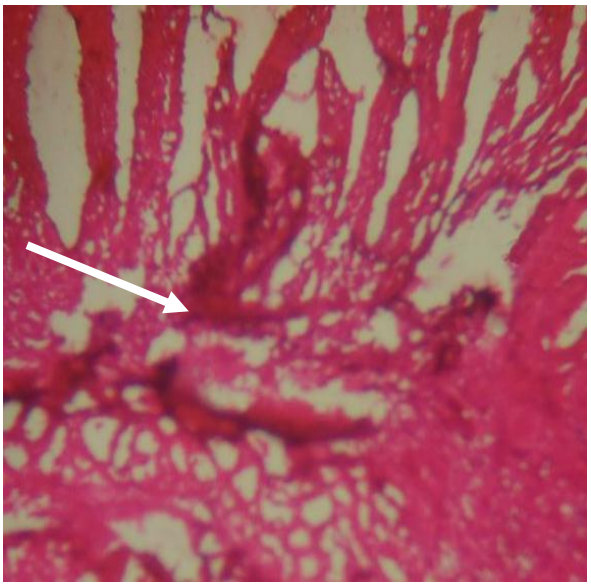

Figure 12: Haemorrhages in the proventiculus

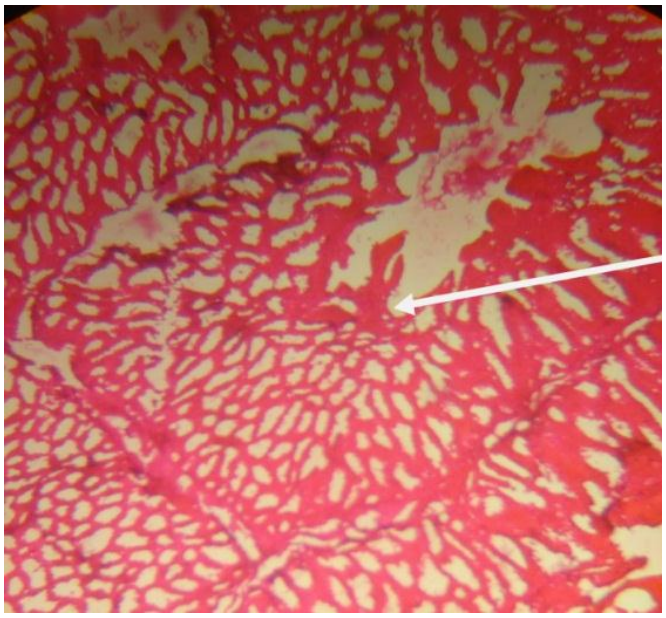

Figure 13: Globular destruction in the Proventiculus section

CHAPTER V

DISCUSSION

This study was undertaken to investigate the pathological condition of NDV at small scale commercial broiler farm at Dinajpur Sadar Upazilla from July to December, 2018.

A total 1600 birds, among which number of infected birds was 138. 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 were marked by paralysis of legs, neck and wing which correspond with the findings of (Ressang, *et al*., 1961).

Table 1 showed the prevalence of NDV at different small scale commercial broiler farms in Dinajpur Sadar Upazilla are showing- total 5 farms visited in this upazila. 138 birds were found to be positive for NDV. The prevalence of ND was 7.96% which were not similar findings reported by (Yuguda *et al.,* 2007) stated that (46%) prevalence of Borno state in Nigeria, (Salihu *et al.,* 2012) stated that (54.67%) prevalence of Nasarawa state in Nigeria. In my recent study percentages variation of prevalence from previous study due to geographical location, seasonal variation, species variation, managemental error, methods of examination and so on.

Table 2 showed the out of 800 non-vaccinated birds, from145 infected birds 3 died due to ND and mortality rate was (20.69%), whereas out of 120 vaccinated birds, 8 died of ND and gave 6.67% mortality rate. The risk of dying from ND was 3.10 times higher in non-vaccinated birds than vaccinated one. In vaccinated flock death from ND might be due to vaccination failure, improper management. The total mortality was 3.10 times higher in non-vaccinated birds than in vaccinated birds. Result is not agreement with (Barman *et al*. 2010) stated that the risk was 1.5 time higher in non-vaccinated birds.

Table 3 showed the percentages variation of most common gross lesions that are found in different organs during postmortem examination.

In this observation, the gross pathological lesions were slight to severe haemorrhages in the proventiculus (Fig. 9), haemorrhage in caecal tonsils (Fig. 11). These findings support earlier observation of Mishra *et al.* (2000); Okoye *et al*. (2000) 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. 10) which supports the findings of Orr and John, 1946; Jungherr, 2004; Kianizadeh *et al*. (2002) reported that Haemorrhagic lesions associated with necrosis are found in the intestinal wall.

Haemorrhages in the proventiculus (Figure 12) and globular destruction in the proventiculus section (Figure 13). Similar result were also reported by (Jordan *et al.* (2001), Julia Victoria Rodriguez barahonacosta Rica (2008).

CHAPTER VI

CONCLUSION

Newcastle disease is consider to be one of the most important viral diseases in broiler throughout the world. It has devastating effect on poultry production in most countries. The present study was conducted mainly to explore pathological investigation of ND based on clinical, gross and histopathological lesion. Total 5 farms were visited. A total of 1600 birds from 5 different farms were observed, 138 birds were found to be positive for ND in which the mortality due to ND was 20.69% in the non-vaccinated birds and 6.67% in vaccinated birds remarks that mortality rate in the non-vaccinated birds was 3.10% 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 totally inactive, weak and rough in appearance. Greenish diarrhoea occurred. Nervous sign include clonic, spasm and paralysis of the legs.

The infected birds showed haemorrhages in the proventiculus, caecal tonsils and intestine. Histopathological changes in proventiculus were congested blood vessels, haemorrhage, globular destruction & sever epithelial layer destruction.

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

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