PATHOLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE (IBD) IN SONALI CHICKEN AT DINAJPUR DISTRACT

A Thesis

By

SHADHANA RANI ROY
Registration No. 1705441
Semester: July-December, 2018
Session: 2017-2018

MASTER OF SCIENCE (M.S.)
IN
PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
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Dedicated to.....

My father who insisted me the value of my education, my mother whose unending love and sacrifices inspired and encouraged me ,my brother who support me to do anything and the Almighty God who blessed me with the ability and strength to accomplish it.

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The Author

ABSTRACT

The study was designed to investigate the prevalence and pathology of Infectious Bursal Disease (IBD) of Sonali chicken at different upazilas in Dinajpur district in a short six month duration starting from July to December 2018. Eight Sonali chickens farms with sum of 7750 birds of various age group from four different upazila like Sadar, Chirirbandar, Parbatipur, Birol, were suspected for Infectious Bursal Disease (IBD). On the basis of detail about farm history, clinical signs, and postmortem investigation of infected chicks, the prevalence of IBD was 8.95%, 36.5%, 49.16% and 35.45% in Sadar, Chirirbandar, Parbatipur and Birol of Dinajpur District respectively. On the basis of age group, the prevalence of IBD was 41.90%, 33.11%, 28.38% and 28.24% at the age of 3^{rd}, 4^{th}, 5^{th} and 6^{th} week of age (table 3), respectively. The prevalence of IBD in Sonali chickens was the highest 41.90% at the 3^{rd} week of age and the lowest 28.24% at 6^{th} week of age. No sonali chick was identified as positive for IBD in their first two weeks of age. The prevalence of vaccinated birds was 25.29% and non vaccinated birds were 49.16%. The necropsy findings of infected chick's revealed were hemorrhages in the breast muscle and thigh muscles. The main changes were enlarged, edematous and swollen bursa of Fabricius. Histopathological study revealed the findings are destruction of normal architecture and reducing size of the follicle**.** Thickening of the intermolecular space. Therefore, it was concluded that susceptibility of chicks to IBD is influenced by its age, ruffled feather, depression, whitish diarrhoea with haemorrhagic muscles and inflamed, edematous, hyperemic Bursa of Fabricious is attributable to Infectious Bursal Disease.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percentage
\	:	Per
BF	:	Bursa of Fabricious
CEF	:	Chicken embryo fiboblast
e.g.	:	Example
et al.	:	And his associates
Etc	:	Etcetera
F.F.Y.P	:	The Fifth Five –Year Plan
Fig.	:	Figure
Gm	:	Grams
H&E	:	Hematoxylin and Eosin
Hrs	:	Hours
HSTU	:	Hajee Mohammad Danesh Science and Technology University
IBD	:	Infectious Bursal Disease
IBDV	:	Infectious Bursal Disease virus
IPNV	:	Infectious Pancreatic Necrotic Virus
Kg	:	kilogram
Ib	:	pound
MDA	:	Maternally derived antibody
Min	:	Minutes
Ml	:	Milimeter
OIE	:	Office International des Epizooties
p.i	:	post infection
PBS	:	Phosphate buffered saline
VP	:	Virus protein
VV	:	Very virulent
vvIBDV	:	very virulent infectious bursal disease virus

CHAPTER I
INTRODUCTION

Bangladesh is an agricultural country. Bangladesh is also a highly populated country. Large amount of people depend on agriculture for their livelihood. Poultry sector is one of the branches of agriculture. Poultry production especially chickens and ducks has attained an important place in agricultural economy of Bangladesh both through contribution to GDP and employment especially in urban areas. About 80% of the total population of 160 million is living in the 68,000 villages of Bangladesh and almost each and every village home holds 6 to 7 chickens.

In Bangladesh, since the beginning of 21st century, the poultry industry has become an unparalleled platform for a quick profit, the generation of local employment, and the production of cheaper animal proteins.

Poultry meat and eggs available in Bangladesh are mostly originated from locally grown backyard poultry and also from small and large scale poultry enterprises. Meeting the domestic demand for meat and eggs through importation is very rare and sporadic (Anas, 2015).

The Bangladesh poultry industry primarily produces chicken although a few other species like duck, pigeon, quail, goose, turkey, and guinea fowl are available throughout the year. Chicken meat and eggs are, so far, the cheapest source of animal protein in Bangladesh and it is well accepted by all religious, economic, social, and demographic groups (Simon, 2009).

And the local chicken supply approximately 71% of the total meat (Paul and Islam, 2001). It is estimated that the share of poultry in the animal protein of human diet increased from 14% in 1977 to 23% in 1987 and in further estimated to 30% in 1995 (Alam, 1999).

Poultry farming is a versatile agro business all over the world. In Bangladesh, the poultry sector is also an integral part of the farming system. The number of poultry grew at an annual rate of 6.7 percent over the period 1990-97. About 50,000 poultry farms and 26,000 duck farms have already been established in private sector in addition to the government farms (F. F. Y. P., 1998).

The GDP contribution of this sub-sector has been a modest 2.6% annually in the 1990s (IMF 2005) which was lower than the previous estimates of 5% of total and 10% of agricultural GDP during the 1970s and 1980s (FAO 1990; Planning Commission 1990). Before industrialization, backyard poultry was the sole source of local, low-productive, and non-descript chickens in Bangladesh and it primarily met the demand of the producer's family consumption (Ahmed and Islam, 1985).

The major constraints in poultry farming are the outbreak of several devastating diseases causing economic loss and discouraging poultry rearing (Das *et al.,* 2005). IBD is economically important for the poultry industry in function of the immune depression that it causes (Moraes *et al.,* 2004). There are frequent occurrences of this disease, reported by the farm-owners, even when the flocks have been vaccinated against the disease (Bentue, 2004).

Among the various diseases, infectious bursal disease (IBD) popularly known as Gumboro disease is the number one killer disease of chickens. It is a major poultry pathogen in the poultry industry (Hein *et al.,* 2002). The virus has predilection for lymphoid tissue special target organ being the bursa, and also can be isolated from the thymus, spleen, and bone marrow. Besides the loss due to mortality and morbidity, immunosuppression is a very important problem associated with IBD infection (Saif, 1998). The primary target organ for IBDV is the bursa of Fabricius (Lukert and Saif, 1997). The IBD virus destroys lymphocytes and macrophages as a result cripples the immune system with marked immunosuppressive effect leading to vaccination failures and concurrent infections (FAO, 1991).

Infectious Bursal Disease (IBD) is caused by an acute, highly contagious *Birna virus* that results in mortality and immunosuppressant in young chickens. Be since its original isolation in Gumboro, Delaware, the disease has inflicted profound economic losses on the poultry industry worldwide. Infectious bursal disease virus (IBDV) and other *Birna viruses* are single-shelled, non-enveloped viruses that contain a bi-segmented, double stranded RNA genome (Muller, H., C. Scholtissek and H. Becht, 1979).

Although chickens are highly susceptible to IBD than poultry species such as turkeys and ducks show minimal or no susceptibility to the clinical disease under natural conditions. Serological evidence of infection has been established in turkeys, even though neither of the two IBDV serotypes has produced clinical disease in this species. In addition, IBDV has been isolated from clinically healthy ducks which were negative for IBD antibody. Thus, these

three poultry species appear to have different levels of susceptibility to IBDV infection, being highest in chickens (Kosters, J., H. Betch and R. Rudolph, 1972.)

The infectious bursal disease (IBD) is recognized as an important disease of young chickens worldwide. It causes unthrifitness, anorexia, ruffled feathers, diarrhoea and mortality in affected flocks. The infectious bursal disease virus (IBDV) infection of chickens less than three weeks of age causes immunosuppression with increased a susceptibility to other diseases and lack of humoral response to vaccinations (Hitchner, S.B., 1970). There are two distinct serotypes of IBDV: serotype1 and serotype 2. Serotype 1 is pathogenic to chicks and classified as classical, variant and very virulent (vv) IBDV while serotype 2 is not pathogenic to chicks.

The immunosuppression prevents the birds from optimally responding to vaccine (Sharma *et al.,* 1984) and ultimately leads to increase the incidence of numerous concurrent infections including Marek's disease (Sharma,1984), Newcastle disease (Faragher *et al.,* 1972), coccidiosis (Anderson *et al.,* 1977), infectious bronchitis (Pejkovski *et al.,* 1979), hemorrhagic-aplastic anemia and gangrenous dermatitis (Rosenberger *et al.,* 1975), infectious laryngotracheitis (Rosenberger and Gelb, 1978), inclusion body hepatitis (Bacon *et al*., 1986), reovirus (Montgomery and Maslin, 1991), chicken anemia agent, salmonellosis, colibacillosis *Mycoplasma synoviae* (Giambrone *et al.*, 1977b) and *Eimeriatenella* (Anderson *et al.*, 1977).

One of the significant components of the control of the disease is its vaccination which if improved may help in lowering the incidence of the disease in poultry (Zaheer *et al.,* 2003).

Importance of Sonali Chicken in Bangladesh

i. Sonali, with a phenotypic appearance similar to local chicken has higher market demand than exotic breed.

ii. As an important segment of livestock production, the Sonali chicken industry in Bangladesh is considered a great avenue for the economic growth and simultaneously creates numerous employment opportunities.

Considering the above facts, the present study was undertaken with the following Objectives:-

i. Investigate the prevalence and mortality rate in sonali chicken encountered at Dinajpur District.
ii. Study the clinical findings of Infectious Bursal Disease (IBD) in the affected flock.
iii. Study the prevalence of IBD in relation to age of birds.
iv. Study the gross and histopathological changes of different organs developed due to Infectious Bursal Disease.

CHAPTER II
REVIEW OF LITERATURE

In this part of the thesis an attempt is made to review available literature on the history, epidemiology, etiology, pathogenesis and pathology, clinical manifestations, and immunosuppressive effects against Infectious Bursal Disease.

2.1 History of Infectious bursal disease

Infectious bursal disease is a viral infection, affecting the immune system of poultry. The disease is highly contagious, affects young chickens, and is characterised by the destruction of the lymphoid organs, and in particular the bursa of Fabricius, where B lymphocytes mature and differentiate. The target cell of the virus is the B lymphocyte in an immature stage, and the infection, when not fatal, causes an immunosuppression, in most cases temporary, the degree of which is often difficult to determine.

Infectious bursal sickness was first perceived as an unmistakable clinical element in 1957 .It is also called ‘‘avian nephrosis’’ due to the tubular degenerative lesions. The first report of a specific disease affecting the bursa of Fabricius in chickens was made by Cosgrove in 1962. (Cosgrove A.S., 1962).

The etiological viral agent was isolated by Winterfield who differentiated the disease from nephrosis syndrome caused by certain variant strains of Infectious bronchitis viruses (Winterfield *et al.,* 1962).

Infectious bursal disease the first cases were observed in the area of Gumboro, in Delaware (United States of America [USA]), which is the origin of the name, although the terms 'IBD' or 'infectious bursitis' are more accurate descriptions. Between 1960 and 1964, the disease affected most regions of the USA (Lasher H.N. & Davis V.S.,1997) and reached Europe in the years 1962 to 1971 (Faragher J.T.,1972).

In Europe, the disease was first recognized in 1962 in Great Britain (Faragher, 1972).

Infectious bursal disease is currently an international problem: 95% of the 65 countries that responded to a survey conducted by the Office International des Epizooties (OIE) in 1995 declared cases of infection (Eterradossi N. (1995), including New Zealand which had been free of disease until 1993 (Jones B.A.H. (1986).

Following the initial outbreaks, the disease had been brought under control by extensive vaccination until the antigenic variant strains emerged in early 1980s in the USA (Snyder *et al.,* 1990).

Subsequent studies however, revealed that IBV immunized birds could still be infected with the "infectious bursal agent" (IBA) and developed changes in their cloacal bursas specific for the disease. Following successful isolation of IBA in embryonated chicken single eggs (Hitchner, S.B., 1970) proposed that the disease be termed "infectiousbursal disease" due to its pathognomonic bursal lesions. The disease has now spread throughout the world with the exception of New Zealand (Van der Sluis, 1994).

The acute disease first described in Europe at the end of the 1980s (Chettle *et al.,* 1989; Van den Berg *et al.,* 1991; Eterradossi *et al.,* 1992), then described in Japan as acute form in the early 1990s (Nunoya *et al.,* 1992; Lin *et al.,* 1993), and they rapidly spread all over the major parts of the world (Eterradossi, 1995).

From 1966 to 1974, the disease was identified in the Middle East, southernand western Africa, India, the Far East and Australia (Faragher J.T. (1972)., Firth G.A. (1974), Jones B.A.H. (1986)., Lasher Van den Berg T.P. (2000). H.N. & Shane S.M. (1994). Borredon C. & Bocquet P. (1972) and Van der Sluis W. (1999).

Infectious bursal disease is an acute, highly contagious lymphocytolytic viral infection of young chickens caused by a Birnavirus (Lukert and Saif, 1997 and Muller *et al.,* 2003).

Subsequent studies indicated that birds immune to infectious bronchitis virus (Gray virus) could still be infected with the Infectious bursal disease (IBD) virus and would develop changes in the cloacal bursa like IBD (Lukert *et al.,* 2003).

The first outbreaks of IBD occurred in Bangladesh at the end of 1992 (Islam *et al.,* 1994a and 1994b; Rahman *et al.,* 1996 and Chowdhury *et al.,* 1996) with high mortality in the poultry farms (Chowdhury *et al.,* 1996; Islam *et al.,* 1997 and Talha *et al.,* 2001). The virus has been isolated from the field outbreaks (Chowdhury *et al.,* 1996 and Islam *et al.,* 2001a) and the pathogenicity has been tested (Islam *et al.,* 1997). IBDV isolates from Bangladesh were also characterized at antigenic and molecular level and had been found to be antigenitically and genetically related to other very virulent strains isolated earlier in Europe, Asia and Africa (Islam, *et al.,* 2001a). The complete nucleotide sequence of both genome segments of a

vvIBDV from Bangladesh (BD-3/99) has established and full-length cDNA clones corresponding to the both segments have been established (Islam *et al.,* 2001b).

2.2 Epidemiology

2.2.1Incidence and distribution

Birds that are 3-6 weeks of age are the most susceptible to clinical disease. The IBDV is susceptible to mutation, highly resistant to heat and chemicals and can persist in faces, bedding, contaminated feed and water for up to four months in certain conditions. Mode of transmission is primarily through fecal oral route, with aerosol spread considered to be less important. There is no evidence that IBD can be transmitted in embryos or semen.

The viral incubation period is about 2-3 days and can be shed as soon as 24 hours following infection and can last up to two weeks. The disease is highly contagious, can spread through the movement of poultry products, equipment, feed bags, vehicles and people and to a lesser extent, through aerosols of dust. Transmission of IBD between wild birds and poultry is likely to be due to scavenging of dead chickens, ingestion of contaminated water, or exposure of respiratory or conjunctiva membranes to contaminated poultry dust (Woldemariam *et al*., 2007 and Okoyo *et al*.,2005).

Classical serotype 1 IBD infection in wild birds is believed to be subclinical. Recent research shows that wild birds play a role in the epidemiology of IBDV by acting as a reservoir for the virus. Classical serotype 1 IBDV strains are endemic throughout the world. Very virulent IBD is endemic in parts of southern Asia, Indonesian island region, South America, Middle East and Africa (Jackwood *et al.,* 2005).

Serotype 1 IBDV antibodies have been detected in Australian wild birds including carrion crows and rock pigeons which were found around barns and domestic chicken flocks. There is no data that suggest IBDV is transmitted by wild birds in Australia, however direct or indirect transmission of the virus between wild birds and domestic chickens probably may occur. It is strongly believed that the serotype IBDV 1 is highly host specific to chickens. However, IBDV has been isolated from a sparrow in China, which suggests that wild birds may have an important role in the natural history of IBDV. Reports have shown that serotype 2 of IBDV is more prevalent in many species of wild birds, with the natural host considered to be turkeys (Okoyo *et al*., 2005).

2.2.2 Susceptibility factors

The age of maximum susceptibility is between three to six weeks, corresponding to the period of maximum bursa development, during which the acute clinical signs are observed. Infections occurring prior to the age of three weeks are generally subclinical and immunosuppressive. Clinical cases may be observed up to the age of fifteen to twenty weeks (Ley *et al*.,1979 and J.O.A. *et al.,*1981). Light strains of laying stock are more susceptible to disease than the heavy broiler strains (Bumstead *et al* ., 1993; Hassan *et al*., 1996 and Van den Berg *et al.,* 1991).

2.2.3 Host

The natural hosts of IBDV are the domestic fowl including chickens and turkeys. Other wild bird like healthy ducks, guinea fowl, quail and pheasants have been found to be naturally infected by serotype 1 IBDV. There is no evidence that IBD virus can infect other animals, including humans (Sanchez *et al.,* 2005).

Only chickens *(Gallus gallus)* develop IBD after infection by serotype 1 viruses. Turkeys (*Meleagris gallopavo)* may be asymptomatic carriers of serotype 2 (Ismail *et al.,*1988; Jackwood *et al.,1982;* McFerran *et al.,* 1980), and at times, of serotype 1 viruses whose pathogenicity for turkeys is ill-defined (Owoade *et al., 1995;* Reddyaa *et al., 1991*). The Pekin duck *(Cairina moschata)* can also be an asymptomatic carrier of serotype 1 viruses (McFerran *et al.,* 1980).

Anti-IBDV antibodies have been detected in guinea-fowl *(Numida meleagris)* (1), common pheasants *(Phasianus colchicus)* (Louzis *et al., 1979*) and ostriches *(Struthio camelus)* (Cadman *et al.,* 1994) which have also been demonstrated to carry serotype 2 viruses (Guitte *et al.,* 1982). Neutralising or precipitating antibodies have been detected, *inter alia,* in various species of wild duck, goose, tern, puffin, crow and penguin, which may mean that wild birds act as reservoirs or vectors (Gardner *et al.,* 1997; Ogawa *et al.,* 1998 and Wilcox *et al.,* 1983).

2.2.4 Breeds Susceptibility

The population at risk includes broiler flocks and young pullets destined for breeder and commercial egg laying flocks. Lighter breeds (laying breeds) show severe reaction to IBDV infection than heavier broiler breeds (Lukert and Hitchner, 1984) and the highest susceptibility (about 80% mortality) was recorded in a Brown Leghorn line (Bumstead *et al.,* 1993). On the other hand, no difference found in the mortality between heavy and light

breeds in a survey of 700 outbreaks of the disease (Meroz, 1966). There is no report of IBD in the native breeds. Moreover, indigenous chickens also can be infected experimentally (Okoye *et al.,* 1999).

2.2.5 Morbidity and mortality

Infectious bursal disease is extremely contagious. In infected flocks, morbidity is high, with up to 100% serological conversion, after infection, whilst mortality is variable. Until 1987, the field strains isolated was of low virulence and caused only 1% to 2% of specific mortality. However, since 1987 an increase in specific mortality has been described in different parts of the world. In the USA, new strains responsible for up to 5% of specific mortality were described (Shakya *et al*., 1999). At the same time, in Europe and subsequently in Japan, high mortality rates of 5 0% to 60% in laying hens and 25% to 30% in broilers were observed. These hypervirulent field strains caused up to 100% mortality in specific-pathogen-free (SPF) chickens (Nunoya *et al*.,1992 and Van den Berg *et al*.,1991).

2.2.6 Seasons

IBD occurred round the year in Assam of India (Sami and Baruah, 1997), although IBD is more common during the winter months in Botswana (Binta *et al.,* 1995).

2.2.7 Transmission

Only horizontal transmission has been described, with healthy subjects being .infected by the oral or respiratory pathway. Infected subjects excrete the virus in faeces as early as 48 h after infection, and may transmit the disease by contact over a sixteen-day period (Vindevogel *et al*., 1976).

The possibility of persistent infection in recovered animals has not been researched. The disease is transmitted by direct contact with excreting subjects, or by indirect contact with any inanimate or animate (farm staff, animals) contaminated vectors. Some researchers have suggested that insects may also act as vectors (Howie *et al*., 1981).

The extreme resistance of the virus to the outside environment enhances the potential for indirect transmission. The virus can survive for four months in contaminated bedding and premises (Benton *et al., 1967)* and up to fifty-six days in lesser mealworms *(Alphitobius* sp.) taken from a contaminated building (McAlliste *et al*.,1995). In the absence of effective cleaning, disinfection and insect control, the resistance of the virus leads to perennial contamination of infected farm buildings.

Chickens infected with the IBD virus shed the virus in their feces. Feed, water and poultry house litter become contaminated. Other chickens in the house become infected by ingesting the virus (Saif, M.Y., 2003) the lesser mealworm has been shown to carry the virus. Because of the resistant nature of the IBD virus, it is easily transmitted mechanically among the farms by people, equipment and vehicles (Murphy *et al*.,1999).

2.2.8 Factors influencing the pathogenicity

Several viruses and host related factors can influence the pathogenicity of IBDV (Table 1).

Table 1: Factors influencing the pathogenicity of IBDV

Factors influencing the pathogenicity		**Reference (s)**
Virus factors	Genetic variation	Sharma *et al.*,1989; Nunoya *et al.*, 1992; Jing *et al.*, 1995 and Yamaguchi *et al.*,
	Virus antigen distribution in the nonbursal lymphoid organs	Tanimura *et al.*, 1995
Host factors	Species	Brown and Grieve, 1992
	Age	Winterfield and Hitchner, 1964
	Breeds	Lukert and Hitchner, 1984 and Bumstead *et al.*, 1993
	Serial passaging in cell culture	Yamaguchi *et al.*, 1996a
	Levels of MDA	lordanides *et al.*, 1991

2.3 Etiology

2.3.1 Classification of IBDV

Family: Birnaviridae

Genus: Birnavirus

Sub-genus: Avibirnavirus

Species: Infectious bursal disease virus

Infectious bursal disease virus (IBDV) is classified as a member of the *Birnaviridae* family The family includes 3 genera: *Aquabirnavirus* whose type species is infectious pancreatic necrosis virus (IPNV which infects fish, mollusks and crustaceans; *Avibirnavirus* whose type species is infectious bursal disease virus (IBDV), which infects birds; and *Entomobirnavirus*

whose type species is *Drosophila* X virus (DXV), which infects insects (Viruses in this family possess bi-segmented, double-stranded RNA (dsRNA) genomes, which are packaged into singleeggs shelled, non-enveloped virions. The capsid shell exhibits icosahedral symmetry composed of 32 capsomeres and a diameter ranging from 55 to 65 nm (Brown *et al.*, 1994).

2.3.2 Antigenic and pathotypic variation

The high mutation rate of the RNA polymerase of RNA viruses generates a genetic diversification that could lead to emergence in the field of viruses, with new properties allowing them to persist in immune populations. In the case of IBDV, these mutations lead to antigenic variation and modification in virulence *in vivo* and attenuation *in vitro*.

Antigenic variation

Two serotypes of IBDV are described and distinguished by cross-neutralization and cross-protec- tion tests. Antigenic variation among serotype 1 isolates of IBDV has been shown in the US since 1985. These antigenic variants were of different subtypes compared with classical strains, as determined by serum neutralization tests, and could be antigenically differentiated by the use of a selected panel of neutralizing monoclonal antibodies (Snyder *et al.*, 1992). Even though only one of these subtypes could be considered as truly variant based on cross-protection experiments (Jackwood & Saif, 1987), important economic losses have been sustained due to the emergence of these antigenic mutants. Neutralizing Mabs have been shown to bind to VP2, within a minimal region–called the variable domain–between amino acids 206 and 350, which is highly hydrophobic with a small hydrophilic region present at each terminus (Bayliss *et al.*, 1990). Sequencing of the VP2 gene of numerous different IBDV strains and selection of escape mutants have proven that this variable domain represents the molecular basis of antigenic variation (O" ppling *et al.*, 1991; Schnitzler *et al.*, 1993; van den Berg *et al.*, 1994a and Vakharia *et al.*, 1994b). Vaccination failures due to vvIBDVs have caused great concern for possible antigenic variation among the recent isolates. There is no evidence of antigenic variation in the very virulent strains as described in the US: they belong to classical serotype 1 (Van der Marel *et al.*, 1990; Van den Berg *et al*, 1991and Eterradossi *et al.*, 1992). Never the less, a modified epitope could be identified on all the vvIBDVs tested by Eterradossi *et al.* (1997b) by the use of a panel of neutralizing Mabs. This corresponded to a mutation of amino acid at position 222 (numbering following Bayliss *et al.*, 1990) that is located in the first hydrophilic peak, as demonstrated by the selection of an escape mutant. Anyway, no drift could be demonstrated by cross

neutralization tests (Eterradossi *et al.*, 1998). Other amino acid changes have been shown in the hydrophilic peaks of the variable domain in vvIBDVs but their antigenic relevance and epidemiological significance is questionable. For instance, in China, where poultry is one of the fundamental industries of animal production, there have been recent molecular indications for the emergence of variant very virulent strains (Cao *et al.*, 1998) but their biological and epidemiological relevance still needs to be established. In France, during their monitoring of the field, Eterradossi *et al.*, (1998) have also shown atypical antigen city in some vvIBDVs due to critical amino acid changes in the second hydrophilic peak, but these strains were not shown to replace the more typical prevalent vvIBDVs.

All these observations indicate that vvIBDVs are evolving but, in contrast to biological significance of several antigenic differences has to be demonstrated by cross-neutralization tests. Moreover, molecular investigations must be related to the field situation, with a good characterization of the circulating strains in terms of prevalence and virulence.

Pathotypic variation

In addition to antigenic differences in serotypes and subtypes, the viral strains can also be classified according to their virulence But there has been a great deal of confusion in these definitions. In particular, the term "very virulent" has been used to describe both European hypervirulent.

Strains and variant American strains that cause less than 5% mortality but are able to multiply to a higher degree in the bursa of Fabricius of vaccinated animals. In the absence of the identification of specific virulence determinants, the only valuable criteria for the classification of IBDV strains as "pathotypes" should refer to their virulence (mortality or lesions) in 3- to 6-week-old specific pathogen free birds and not to any antigenic specificity.

2.3.3 Evolution

The evolution of the virus since 1984 has been marked by two major events. The first was the discovery of an antigenic drift in serotype 1 viruses. Commencing in 1984, several strains of this serotype were isolated in the USA from broiler flocks that had been properly vaccinated (Rosenberger *et al.*, 1986). The new viruses did not cause the characteristic clinical signs of the infection, but had a major immunosuppressive potential. These strains were termed Variant' since they were capable of infecting chicks that possessed an antibody titre considered protective in normal circumstances. The variant viruses have since been found to carry modified neutralising epitopes, and several successive generations of these viruses,

which gradually have accumulated antigenic mutations, have been found in the USA. Thus, six sub-groups have been described among thirteen strains tested by serum neutralization (SN) (Jackwood *et al*., 1987). These results were confirmed using neutralizing monoclonal antibodies (Snyder *et al*., 1988 and Snyder *et al*., 1992). Nonetheless, only one of these sub-types was considered to be a 'true' variant in cross-protection tests (131). Vaccinal protection against the infections caused by these sub-groups has required the development of specific vaccines (Giambrone *et al*., 1990; Hassan *et al*., 1996; Ismail *et al*., 1991and Müller *et al*., 1992).

The second major epidemiological event was the emergence, in 1987, of 'hypervirulent' viruses (wIBDV) in Europe, particularly on farms that were well managed and on which all hygiene and sanitary control measures had been implemented (Chettle *et al*., 1989; Eterradossi *et al*., 1992; Stuart J.C. 1989; Tsukamoto *et al*., 1992; Van den Berg T.P., Gonze M. & Meulemans G. (1991). These viruses are significantly more pathogenic than the classical strains, and are also capable of infecting chicks with normally protective antibody titres (Van den Berg & Meulemans (1991). As no antigenic mutation characteristic of the wIBDV was detected, these viruses are generally considered to be pathotypic variants (Van den Berg Gonze & Meulemans 1991; Van der Marel *et al*., 1991). In the absence of specific virulence markers, the only valid criteria for classifying IBDV strains into 'pathotypes' is virulence (mortality, lesions) in SPF chickens. Moreover, increases in virulence are apparently unrelated to antigenic variation, and research is currently underway to determine virulence markers.

2.4 Immunosuppression

The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds (Faragher *et al*., 1974). In addition to the impact on production affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (Giambrone *et al*., 1976).

The most severe and longest-lasting immunosuppression occurs when day-old chicks are infected by IBDV (Allan *et al*., 1972; Faragher *et al*., 1974; Sharma *et al*., 1989 and Sharma *et al*., 1994). In field conditions, this rarely occurs since chickens tend to become infected at approximately two to three weeks, when maternal antibodies decline. Evidence suggests that the virus has an immunosuppressive effect at least up to the age of six weeks (Giambrone, 1979); Lucio *et al*., 1980 and Wyeth, 1975).

Immunosuppression is most often demonstrated using experimental models based on the measurement of humoral responses induced by different antigens such as *Brucella abortus* (Hopkins *et al*., 1979), sheep red blood cells, or Newcastle disease vaccines (Allan *et al*., 1972; Faragher *et al.*,1974 and Giambrone *et al.*, 1976).

The best assessment is clearly the measurement of vaccinal protection against a challenge infection by the Newcastle disease virus, as described in the OIE *Manual of Standards for Diagnostic Tests and Vaccines* (Office International des Epizooties (OIE); 2000) since this constitutes a measurement of both humoral and cellular immunity. Unfortunately, these techniques are time-consuming, tedious, costly, and require the use of animals. Thus, they are usually confined to IBD vaccine registration procedures.

2.5.1 Incubation Period, Clinical Signs

IBDV has a short incubation period of 2 to 3 days and the infection generally lasts 5 to 7 days. One of the earliest signs of IBDV infection is the tendency for birds to engage in vent picking. Clinical signs are described as acute onset of depression, trembling, white and watery diarrhoea, anorexia, prostration, ruffled feathers, vent feathers soiled with urates and hemorrhages in pectoral and thigh muscles. In severe cases, birds become dehydrated and in the terminal stages subnormal 3 to 6 weeks of age are most susceptible to the clinical form of IBD, which causes impaired growth, immune suppression and mortality. Clinical signs are mainly characteristic of IBDV serotype I classic strains (Giambrone *et al.*, 1977).

Mortality commences on the third day of infection, reaches a peak by day four, then drops rapidly, and the surviving chickens recover a state of apparent health after five to seven days. Disease severity depends on the age and breed sensitivity of the infected birds, the virulence of the strain, and the degree of passive immunity. Initial infection on a given farm is generally very acute, with very high mortality rates if a very virulent strain is involved. If the virus persists on the farm and is transmitted to successive flocks, the clinical forms of the disease appear earlier and are gradually replaced by subclinical forms. Nonetheless, acute episodes may still occur. Moreover, a primary infection may also be in apparent when the viral strain is of low pathogenicity or if maternal antibodies are present. The clinical signs of IBD vary considerably from one farm, region, country or even continent to another. Schematically, the global situation can be divided into three principal clinical forms, as follows:

- The classical form, as described since the early 1960s, is caused by the classical virulent strains of IBDV. Specific mortality is relatively low, and the disease is most often subclinical, occurring after a decline in the level of passive antibodies (Faragher, J.T. 1972).
- The immunosuppressive form, principally described in the USA, is caused by low-pathogenicity strains of IBDV, as well as by variant strains, such as the Delaware variant E or GLS strains, which partially resist neutralisation by antibodies against the so-called 'classical' viruses (Jackwood *et al.,*1987 and Snyder D.B. 1990).
- The acute form, first described in Europe, and then in Asia, is caused by 'hypervirulent' strains of IBDV, and is characterised by an acute progressive clinical disease, leading to high mortality rates on affected farms (Chettle *et al.,* 1989, Stuart, J.C. 1989 and Van den Berg *et al.,* 1991).

In fully susceptible flocks, mortality associated with infection due to classic strain may range from 1-60%, with high morbidity of up to 100%. A variant IBDV strains do not produce overt clinical signs, but cause immunosuppression and may cause mortality due to secondary opportunistic infections in immuno compromised birds. In contrast, vvIBDV strains cause mortality of 50-60% in laying hens, 25-30% in broilers and 90-100% in susceptible leghorns. Susceptible chickens younger than three weeks of age may not exhibit clinical signs, but develop subclinical infections. This results in a decreased humoral antibody response due to B lymphocyte depletion in the cloacal bursa and a severe and prolonged immunosuppression. The most significant economic losses result from subclinical infections. This form of IBD infection greatly enhances the chicken's susceptibility to sequelae such as gangrenous dermatitis, chicken anemia virus, inclusion body hepatitis, respiratory postdiseases and bacterial infections (Lukert *et al*., 1984).

2.5.2 Subclinical and Clinical IBD

Infectious bursal disease follows one of two courses, depending on the age at which chickens are infected. The subclinical form of the disease occurs in chickens less than 3 weeks of age. Chickens present no clinical signs of disease, but experience permanent and severe in imrnuno-suppression.

The reason young chickens exhibit no clinical signs of disease are not known. However, immune-suppression occurs due to damage to the bursa of Fabricius (Jordan *et al*., 2002).

The majority of field infections are subclinical and this form is the more economically important form of the disease. Broiler integrations commonly have farms described as problem farms. Broilers grow on these farms typically have poor body weights and feed conversions, high mortality, excessive reactions to respiratory vaccines and high rates of condemnation at processing. In man cases, investigations have shown that these farms are heavily contaminated with the IBD virus. The poor performance of the broilers is due to factors relating to immune-suppression caused by subclinical IBD.

The clinical form of IBD usually occurs in chickens from 3 to 6 weeks of age. The clinical disease has a sudden onset and the mortality rate in the flock increases rapidly Clinical signs of disease include dehydration, trembling, ruffled feathers, vent pecking and depression. Affected chicken experiences a transient immune-suppression. On necropsy, the principle lesions are found in the BF (Saif *et al*., 2003).

2.6 Pathogenesis

To understand how the IBD virus adversely affects the chicken's immune system, relevant factors above early development immune system have to be understood. During embryonic development and through approximately 10 weeks of age, immune system become antibody-producing cells (Hitchner, S.B., 1970).

Pathogenesis can be defined as the method used by the virus to cause injury to the host with mortality, disease or immunosuppression as a consequence. These injuries can be evaluated at different levels: the host, the organ and the cell, and are exacerbated in the acute forms of the disease.

If the IBD virus damages the BF in young chickens, the bursa of Fabricus will not be capable of programming sufficient numbers of lymphocytes. Thus, the chickens will experience reduced immune system capabilities and immunosuppression (Pattison *et al*., 1975).

The earlier the damage to the BF occurs; the lesser lymphocytes with antibody-producing capability will be programmed. Therefore, any IBD virus control program should attempt to protect the BF as early as possible. In practical terms, if the BF can be protected against disease until at least 3 weeks of age with a chance for, an adequate number of lymphocytes to be programmed and the immune-suppressive effects of an IBD outbreak will be minimal (Müller *et al.*, 1987).

The selected host of the virus is young chickens where a clinical disease occurs, while in older birds the infection is essentially subclinical. Susceptibility of different breeds has been described with higher mortality rates in light than in heavier breeds (Bumstead *et al.*, 1993 and Nielsen *et al.*, 1998). Inoculation of IBDV in other avian species fails to induce disease (McFerran, 1993).

The target organ of IBDV is the bursa of Fabricius at its maximum development, which is a specific source for B lymphocytes in avian species. Bursectomy can prevent illness in chicks infected with virulent virus (Hi number of susceptible cells present in the bursa of Fabricius; therefore, the highest age susceptibility is between 3 and 6 weeks, when the bursa of Fabricius is at its maximum development. This age susceptibility is broader in the case of vvIBDV strains (vanden Berg *et al.*, 1991and Nunoya *et al.*, 1992).

After oral infection or inhalation, the virus replicates primarily in the lymphocytes and macrophages of the gut-associated tissues. Then virus travels to the bursa via the blood stream, where replication will occur. By 13 h post-inoculatio (p.i.), most follicles are positive for virus and by 16 h p.i., a second and pronounced viraemia occurs with secondary replication in other organs leading to disease and death (M¨uller *et al.*, 1979). Similar kinetics is observed for vvIBDVs but replication at each step is amplified.

Actively dividing, surface immunoglobulin Raga *et al.*, 1994). The severity of the disease is directly related to the M-bearing B cells are lysed by infection (Hirai 1994), but cells of the monocyte–macrophage lineage can be infected in a persistent and productive manner, and play a crucial role in dissemination of the virus (Burkhardt & Muller, 1987; Inoue *et al.*, 1992 and Van den Berg *et al.*, 1994b) and in the onset of the disease (Sharma & Lee, 1983; Kim *et al.*, 1998 and Lam, 1998). Indeed, the exact cause of clinical disease and death is still unclear but does not seem to be related only to the severity of the lesions and the bursal damage. Indeed, after infection, some birds with few bursal lesions can be found dead, while others can survive despite extensive bursal damage. Moreover, mortality rates are often variable and the establishment of median lethal dose for standardization has always been hazardous. In addition, the narrow age range for susceptibility to clinical disease has not yet been clearly explained. Prostration (with ruffled feathers, diarrhoea and inappetence) preceding death is very similar to what is observed in acute coccidiosis, and is reminiscent of a septic shock syndrome.

The macrophage could play a specific role in this pathology by an exacerbated release of cytokines such as tumor necrosis factor or interleukin 6 (Kim *et al.*, 1998). However, an intermediate role of TH cells in this pathophysio-logical mechanism should also be considered (Tanimura & Sharma, 1997 and Vervelde & Davison, 1997). As chicken macrophages are known to be activated by interferon (Dijkmans *et al.*, 1990), this role could occur through an increased secretion of interferon as has been described *in vitro* after infection of chicken embryo cultures or *in vivo* in chicken (Gelb *et al.*,1979a, b).

Depletion of lymphoid cells in the bursa of Fabricius after IBDV infection is due to both necrosis and apoptosis. Apoptosis, or programmed cell death, is a process where, in response to specific stimuli, cells die in a controlled, programmed manner. Many different cell species can undergo apoptosis but immature B and T cells are particularly susceptible to apoptotic cell death.

2.7 Pathology

Although the other lymphoid organs are affected (Sharma *et al* 1993; Tanimura *et al.*,1995 and Tanimura *et al.,* 1997), the principal target of the virus is the bursa of Fabricius (Kaufer *et al.,* 1980), which is the reservoir of B lymphocytes in birds. Indeed, the target cell is the B lymphocyte in active division, for which the infection is cytolytic (Burkhardt *et al.,* 1987). Cell sorting studies have demonstrated that the B lymphocyte is susceptible in the immature stage, during which immunoglobulin M is carried on the surface of the lymphocyte (Hirai *et al.,* 1981and Nakai *et al.*,1981). This accounts for the paradoxical immune response to IBDV, in which immunosuppression co-exists with high anti-IBDV antibody titres. The mature and competent lymphocytes will expand as a result of stimulation by the virus whereas the immature lymphocytes will be destroyed.

2.7.1 Gross Lesion

Gross lesions observed in birds that are common to IBDV infection include dehydration hemorrhage in breast and leg musculature, darkened discoloration of the pectoral muscles, occasional hemorrhages in the leg, thigh and pectoral muscles, increased mucus in the intestine and renal changes.

The gross appearance of the kidneys may appear normal in birds that are necropsied during the course of infection. In birds that die or are in advanced stages of the disease, kidneys frequently show swelling and pallor with accumulation of urates in the tubules and ureters.

The bursa of Fabricius is the predominant lymphoid organ affected by IBDV. Infections with classic strains of IBDV cause inflammation and hypertrophy of the bursa as early as day 3 post-infection. By day 4, the bursa is double its original size and weight due to edema and hyperemia. By day 5, the bursa returns to its normal weight, but continues to atrophy until reaching one-third or less of its original weight following day 8 post infection. In contrast, variant strains of IBDV typically cause a rapid atrophy, mucosal edema and firmness of the bursa in the absence of inflammation. Only one variant isolate has been reported to cause bursal inflammation by day 2 or 3 post-infection, a gelatinous yellowish transudate covers the serosa surface of the bursa and longitudinal striations become visible. The bursa's normal white color shifts to cream and then, in some cases, gray during and following the period of atrophy. In addition, necrotic foci and petechial or ecchymotic hemorrhages on the mucosal surface may be observed in infected bursa (Weiss *et al*., 1994).

Moderate to severe splenomegaly with small gray foci uniformly distributed on the surface has been reported. Occasionally, petechial hemorrhages have been in the mucosa at the junction of the proventriculus and gizzard. Compared to moderately pathogenic IBDV strains, vvIBDV strains induce similar bursal lesions, but cause more severe damages to the cecal tonsils, thymus, spleen and bone marrow (Ashraf *et al.,* 2006).

2.7.2 Histopathology

IBDV infections produce microscopic lesions primarily in the lymphoid tissues i.e. cloacal bursa, spleen, thymus, cecal tonsils and Hardariangland. Degeneration and necrosis of B lymphocytes in the medullary region of the bursal follicles is apparent within one day of exposure. Depleted lymphocytes are quickly replaced by heterophils, pyknotic debris and hyperplasic reticulo-endothelial (RE) cells. By 3 or 4 post-infection, IBDV-associated lesions are visible within all bursal follicles. (Cheville, 1967).

At this time, infections with classic IBDV strains have caused an inflammatory response marked by severe edema, heterophil infiltration and hyperemia in the bursa. Inflammation diminishes by day 4 post-infection (PI) and as necrotic debris is cleared by phagocytosis cystic cavities develop in the medullary areas of the, lymphoid follicles. Necrosis and infilteration of heterophils and plasma cells occur within the follicle, as well as, the inter follicular connective tissue. In addition, a fibroplasia the inter follicular connective tissue may appear and the surface epithelium of the bursa becomes involuted and abnormal (Peters, 1967).

Proliferation of the bursal epithelial layer generates a glandular structure of columnar epithelial cells that contains globules of mucin. During this stage of the infection, scattered foci of repopulating lymphocytes were observed; however, these did not develop into healthy follicles Microscopic lesions caused by variant strains are characterized by extensive follicular lymphoid depletion and rapid atrophy of the cloacal bursa in the absence of an inflammatory response (Campbel *et al.,* 1986).

Macroscopic lesions are observed principally in the bursa which presents all stages of inflammation following acute infection (McFerran J.B. (1993) and Vindevogel, *et al.,* 1974) Autopsies performed on birds that died during the acute phase (three to four days following infection) reveal hypertrophic, hyperaemic and oedematous bursas. The most severe cases are characterised by a major infection of the mucous membrane and a serous transudate, giving the bursal surface a yellowish colour. This appearance is often accompanied by petechiae haemorrhages. By the fifth day, the bursa reverts to normal size and by the eighth day becomes atrophied to less than a third of the normal size.

The affected animals are severely dehydrated, and many birds have hypertrophic and whitish kidneys containing deposits of urate crystals and cell debris. Haemorrhages in the pectoral muscles and thighs are frequently observed, probably due to a coagulation disorder (Skeeles *et al.,*1980). Certain variants from the USA are reported to cause rapid atrophy of the bursa without a previous inflammatory phase (Lukert *et al.,* 1997).

Moreover, in the acute form of the disease caused by hypervirulent strains, macroscopic lesions may also be observed in other lymphoidorgans (thymus, spleen, caecal tonsils, Harderian glands, Peyer's patches and bone marrow) (Hiraga *et al* 1994; Inoue M *et al.,* 1994; Inoue M *et al.,*1999 and Tsukamoto *et al.,* 1995) have developed a system for evaluating microscopic lesions of the affected organs, with a score ranging from one to five according to severity (Henry *et al.,* 1980). The B lymphocytes are destroyed in the follicles of the bursa as well as in the germinal centres and the perivascular cuff of the spleen. The bursa is infiltrated by heterophils and undergoes hyperplasia of the reticulo-endothelial cells and of the interfollicular tissue. As the disease evolves, the surface epithelium disappears and cystic cavities develop in the follicles. Severe panleukopenia is also observed.

2.8 Potential risk of spreading infectious bursal disease virus through trade

Vertical transmission of the disease has not been reported. Horizontal transmission due to external contamination of egg shells has not been documented (but fertile eggs to be

incubated can be disinfected by fumigation). As a result, the most likely sources of contamination during commercial trade of poultry products are live animals and poultry meat. Infectious bursal disease is an OIE List B disease, and countries importing live poultry may refer to Chapter 3.6.1 of the *International Animal Health Code* (Office International des Epizooties (OIE) (1999).

The IBD-free status of imported live animals can only be established by a negative serological test, repeated after aquarantine sufficiently long to allow for the eventuality of seroconversion (at least three weeks).

Although imported meat has not been demonstrated to be responsible for the spread of IBDV, this remains a theoretical possibility. Contaminated meat may be produced, either by the slaughter of viraemic asymptomatic chickens (Vindevogel, *et al*., 1976 and Winter field *et al.,* 1972). or by the slaughter of convalescent chickens which, ten to sixteen days after infection, are no longer symptomatic, but continue to carry pathogenic virus in the digestive tract, and thus may constitute a viral source of cross-contamination along the slaughter line. The resistance of infectivity of IBDV to temperatures below freezing (at least three years at -20°C) (Cho Y *et al*., 1969) and to heat (Alexander *et al*., 1998 and Benton *et al*.,1967) is another factor in the spread of IBD through trade in poultry meat derivatives.

Aside from these theoretical possibilities, it should be noted that current scientific data are in many respects insufficient to quantify precisely the risk under discussion. In particular, more precise data are required on the prevalence, the tropism of the different strains (in particular for muscle tissue), the risk of the spread of an imported virus to an IBD-free population, and the preferred technique(s) for detecting IBDV in meat.

2.9 Distribution and persistence of the virus

A kinetic study using immunofluorescence (Müller *et al*., 1979) has shown that, 4 h after oral inoculation, the virus is found in the lymphoid tissues associated with the digestive tract, where the first cycle of viral replication occurs. The virus subsequently enters the general circulation via the hepatic portal vein. A phase of primary viraemia ensues, during which the virus reaches the bursa, 11 h after infection, and a major secondary replication cycle occurs. A phase of secondary viraemia then occurs, and the other lymphoid organs become massively infected.

2.10 Resistance to disinfectants

The virus is sensitive to sodium hydroxide (it is totally inactivated when pH exceeds 12), but it is not affected at pH 2 (Benton *et al*., 1967) The iodinated and chlorinated derivatives, as well as the aldehydes (formaldehyde, glutaraldehyde) are also active (Landgraf *et al.,* 1967; Meulemans *et al*., 1982 and Shirai *et al*.,1994).

2.11Public health issues

No evidence exists of transmission of IBDV to humans (Pedersen *et al*., 1990) the disease thus has no direct impact on public health.

2.12 Economic Significance of IBD

It has been described throughout the world and its socioeconomic significances recognized worldwide. The most economic significances of this disease are; higher mortality especially during initial outbreak, immune suppression, especially during initial outbreak, immune suppression, susceptibility and vaccination failure (Muller *et al.,* 2003).

The economic impact of IBD is difficult to assess due to the multi-factorial nature of the losses involved. In addition to direct losses related to specific mortality (which in turn depends on the dose and virulence of the strain, the age and breed of the animals and the presence or absence of passive immunity), indirect losses also occur, due to acquired immunodeficiency or potential interactions between IBDV and other viruses, bacteria or parasites. Further losses may occur as a result of growth retardation or the rejection of carcasses showing signs of haemorrhages.

CHAPTER III

MATERIALS AND METHODS

The present studies were conducted during the period of July to December 2018 at the Pathology laboratory of the Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The detailed outline about the materials and methods used are given below.

3.1 Materials

3.1.1 Samples

Sources of the population in this study were different Sonali farms raised commercially by farmers from different Upazila of Dinajpur District. From the flocks suspected with infectious bursal disease, all the dead as well as sick birds were collected for furthers examination. The organs or tissue like liver, bursa of Fabricius, breast and thigh muscles, kidney were submitted to the Laboratory of the Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur for the final diagnosis.

3.1.2 Instrument and appliances

Equipment and appliances for histopathology:

- Samples (Bursa of Fabricious)
- 10% neutral buffered formalin
- Chloroform
- Paraffin
- Alcohol
- Tape water
- Xylene
- Hematoxylin and Eosin stain
- Distilled water
- Clean slides
- Cover slips
- Mounting media (DPX)
- Microscope

Equipment and appliances for necropsy:

- Birds (Liver, Bursa of Fabricius, Breast and Thigh muscle)

- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- Knife
- A pair of shears,
- 10% neutral buffered formalin

3.1.3 Chemical and reagents used

10% neutral buffered formalin, Xylene, Hematoxylin and Eosin stain. PBS, Distilled water etc were used for necropsy and histopathology of collected samples.

3.1.3.1 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	1 part
Alcohol, 80%	3 parts

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

3.1.3.2 Preparation of harris' hematoxylin solution

Hematoxylin crystals	5.0 g
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. 2-4 ml 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.

3.1.4 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^{0}C.

3.2 Methods

3.2.1 Experimental layout

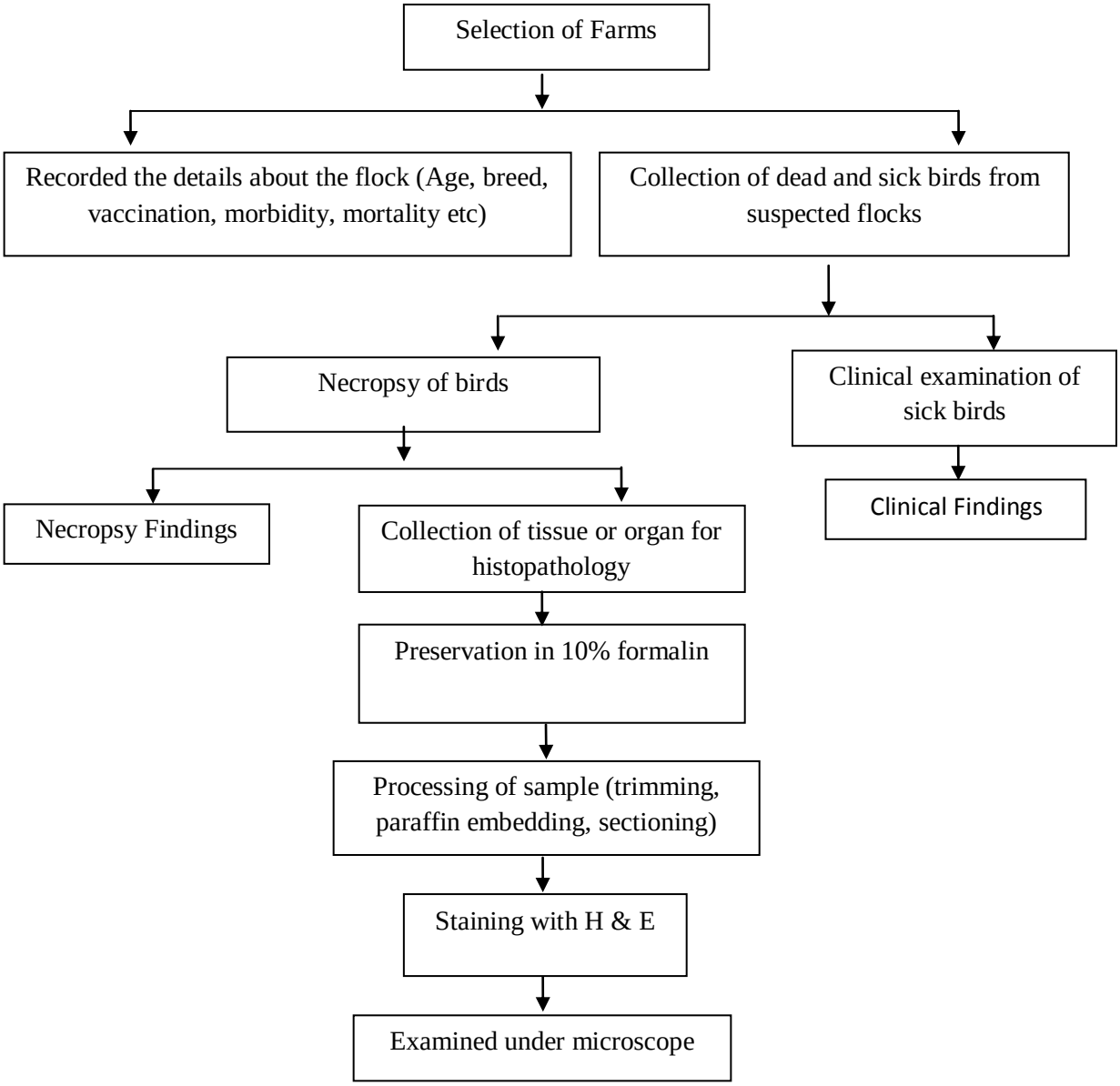

Figure 1: Schematic illustration of the experimental layout

3.2.2 Clinical examination

The general health condition and age of the chicken were recorded. The clinical signs were observed from the visual examination. The clinical signs were recorded during the physical visit to the affected flocks. Farmer's complaints about the affected birds were considered in some cases.

3.2.3 Sample collection and examination

In this study, a total of 7750 birds of various age group from four different upazila (Sadar, chirirbandar, parbotipur and birol) were suspected for the disease and considered as experimental birds. From those farms all dead as well as live sick chickens were collected with detailed particular of the outbreaks of IBD including farm location, history, age, breed, total number of birds and affected birds in farm, intervals between the batches, vaccine schedule, daily mortality and total mortality and clinical signs of affected birds were also recorded. In each case sampling was done following standard sampling methods and send to the laboratory. Different organ like liver, bursa of Fabricious, breast and thigh muscle, kidney were collected during necropsy for further this study. All the diagnostic works were carried under the Laboratory of Department of Pathology & Parasitology, Hajee Mohammad Danesh Science and Technology University (HSTU). Clinical diagnosis and in some cases necropsy examinations were carried out at the place of sampling where as histopathology of all samples were done in the laboratory.

3.2.4 Necropsy examination of suspected birds

- The necropsy was done on the selected birds taken from suspected flocks. At necropsy, gross changes were observed and recorded carefully by systemic dissection. The lesion containing tissues and organs were also collected and preserved in 10% neutral buffered formalin for the histopathology. The routine necropsy examination was carried out as follows-
- At first the bird was laid on its back and each leg, in turn drawn outward away from the body while the skin was incised between the leg and abdomen on each side.
- Then the both legs were then grasped firmly in the area of the femur and bent forward, downward, and outward, until the heads of both femurs were broken free of the acetabular attachment so that both legs lied flat on the table.

- The skin was cut between the two previous incisions at a point midway between keel and vent.
- The cut edge was then forcibly reflected forward, cutting was necessary until the entire ventral aspect of the body including the neck was exposed.
- For exposing of the viscera, knife was used to cut through the abdominal wall transversely midway between the keel and vent, then through the breast muscle on each side.
- Positioning shears were used to cut the rib cage, the coracoid and clavicle on both sides.
- This was done carefully without severing the large blood vessels and through examination of the organs was done.
- The bursa of Fabricius was located by opening the cloaca, laid on its distal side and was examined.

3.2.5 Histopathological study

During necropsy, Bursa of Fabricius was collected, preserved in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed for paraffin embedding, sectioned and stained with hematoxylin and eosin according to standard method (Luna, 1968). Details of tissue processing, sectioning and staining are given below.

3.2.5.1 Processing of tissues and sectioning

- The tissues were properly trimmed into a thin section to obtain a good cross section of the tissue.
- The tissues were washed under running tap water for overnight to remove the fixative.
- The tissues were dehydrated in ascending grades of alcohol to prevent shrinkage of cells using 50%, 70%, 80%, 90% alcohol, and three changes in absolute alcohol, for 1hr in each.
- The tissues were cleaned in two changes in chloroform to remove alcohol, 1.5hr in each.

- The tissues were embedded in molted paraffin wax at 56-60^{0}C for two changes, 1.5hr in each.

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

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

3.2.5.2 Routine Hematoxylin and Eosin staining procedure

The sectioned tissues were stained as described below:

- Deparaffinization of the sectioned tissues was done by 3 changes in xylene (3 minutes in each).
- Rehydration of the sectioned tissues was done through descending grades of alcohol (3 changes in absolute alcohol, 3 minutes in each; 95% alcohol for 2 minutes; 80% alcohol for 2 minutes; 70% alcohol for 2 minutes) and distilled water for 5 minutes.
- The tissues were stained with Harris' hematoxylin for 10 minutes.
- The sections were washed in running tap water for 10-15 minutes.
- Then the staining was differentiated in acid alcohol (1part HCl and 99 parts 70% alcohol), 2-4 dips.
- The tissue sections were then washed in tap water for 5 minutes and dipped in ammonia water (2-4 times) until sections became bright blue.
- The sections were stained with eosin for 1 minute and then differentiated and dehydrated in alcohol (95% alcohol, 3 changes, 2-4 dips in each; absolute alcohol 3 changes, 2-3 minutes in each),
- The stained sections were then cleaned by 3 changes in xylene, 5 minutes in each and finally the sections were mounted with cover slip using DPX.
- The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

3.3 Statistical analysis

All collected data were analysed by SPSS version 22 using Chi-squre test

3.3.1 Determination of mortality rate

Mortality rate is a measure of the number of deaths due to a specific cause in a given population. In this study the mortality rate was calculated by the following statistical formula-

$$Mortality\ rate\ (\%) = \frac{Deaths\ occurrring\ during\ a\ given\ time\ period}{Birds\ Population\ during\ the\ same\ time\ period} \times 100$$

3.3.2 Determination of prevalence

Prevalence of a disease is the proportion in a given population which have a particular disease at a specified point in time, or over a specified period of time. In this study the Prevalence was calculated by the following statistical formula-

$$Prevalence\ (\%) = \frac{IBD\ infected\ birds\ during\ specified\ time\ period}{Birds\ Population\ during\ the\ same\ time\ period} \times 100$$

CHAPTER IV

RESULTS

A total of 7750 sonali chicks from four different upazila like Sadar, chirirbandar, parbatipur and Birol of Dinajpur District were considered as the study population for this research work. The dead and sick birds were collected randomly and subjected to pathology laboratory of Hajee Mohammad Danesh Science and Technology University (HSTU) to determine the status of mortality, prevalence, gross and histopathological lesion of IBD in sonali chicken of Dinajpur district. The results of different clinical and pathological examination are as follows:

4.1 Results of clinical examination

4.1.1 Clinical signs

The clinical signs of the birds affected with IBDV varied from farm to farm and age to age. The signs were clinically characterized as marked depression (Fig 3), anorexia, ruffled feathers, whitish or watery diarrhea (Fig 4), vent picking, reluctant to move, huddling together and severe prostration and death.

4.1.2 Status of mortality and prevalence of the disease

The study revealed the following actual status of mortality and prevalence of infectious bursal disease (IBD) in sonali chicks. Table-2 showed the mortality and prevalence of IBD at different region of Dinajpur District where as Table-3 showed the prevalence of IBD at different age group. Table-4 showed prevalence of IBD in Sonali chicks on the basis of vaccination status

A total of 7750 birds were examined during the study period from which 2390 birds (30.83) are found infected with IBD. The mortality rate is 42.80%. No case was found in first two weeks of age.

Significant variation found between vaccinated (25.29%) and non-vaccinated (49.16%) chicken.

Table-2: Prevalence and mortality rate of IBD in Sonali chicks at different Upazila of Dinajpur

Area	Total	Positive cases	Prevalence (%)	P-value	Died	Mortality (%)	P value
Sadar	2400	215	8.95		42	19.53	
Chirirbandar	3000	1095	36.5		493	45.02	
Parbatipur	1800	885	49.16	0.00***	433	48.92	0.00***
Birol	550	195	35.45		55	28.20	
	7750	2390	30.83		1023	42.80	

*** Highly significant (P>0.01)

Table-3: Prevalence of IBD in Sonali chicks at different age groups

Age (Week)	Total Bird	Positive	Prevalence %	P value
3rd	704	295	41.90	
4th	2114	700	33.11	0.012**
5th	1409	400	28.38	
6th	3523	995	28.24	

** means significant (P>0.05)

Table-4: Prevalence of IBD in Sonali chicks on the basis of vaccination status

Vaccination Status	Total	Positive	Prevalence %	P value
Vaccinated	5950	1505	25.29	0.001***
Non vaccinated	1800	885	49.16	

*** means highly significant (P>0.01)

4.2 Results of necropsy examination

For the conformation of infectious bursal disease the pathological lesions of different parts of the body were examined mainly on bursa of Fabricious and thigh muscle. During necropsy examination the most frequent gross lesions of IBD were haemorrhages in the breast muscle and thigh muscles (Fig 5 and 6). The main changes, enlarged and edematous, swollen bursa of Fabricious (Fig 7) were found in primary stage. The bursal folds become edematous, haemorrhagic and abnormally thick with accumulation of exudates. The junction of proventiculus and gizzard showed haemorrhagic lesions in some cases and kidneys were swollen.

4.3 Results of histopathological examination

Section of the bursa of Fabricious showed destruction of normal architecture and reducing size of the follicle (Fig 8). Thickening of the interfollicular space (Fig 9).

4.4 Results on photo focus

Fig 2: Birds affected with IBD

Fig 3: Depression of chicks

Fig 4: IBD affected birds excreted white color feces

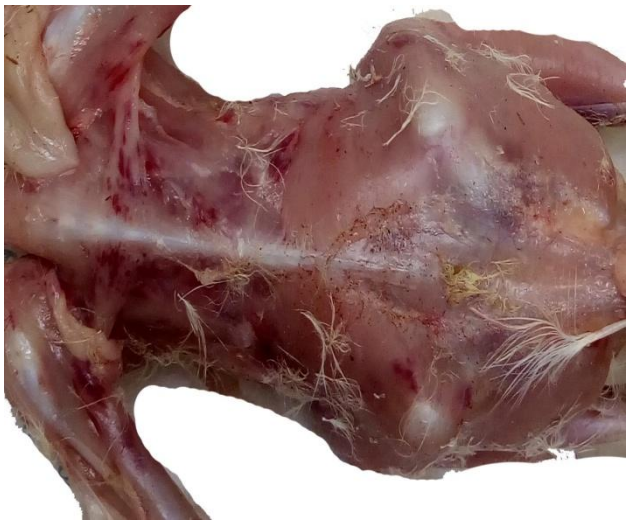

Fig 5: Hemorrhage in breast muscle

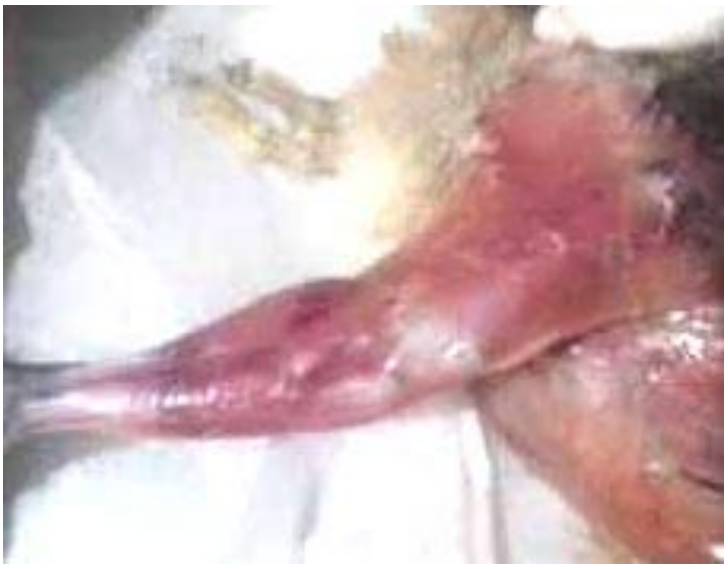

Fig 6: Hemorrhage in thigh muscles

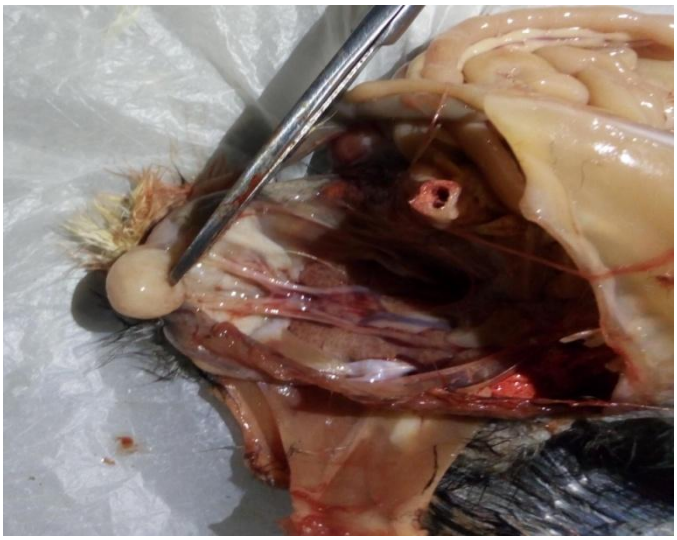

Fig 7: Showing swollen bursa of Fabricious

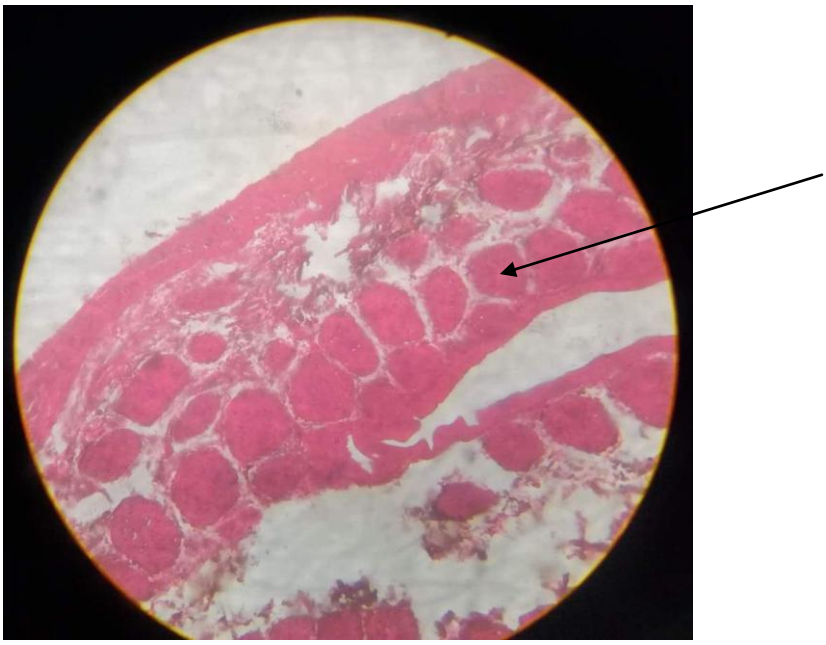

Fig 8: Destruction of normal architecture, Reducing size of the follicle

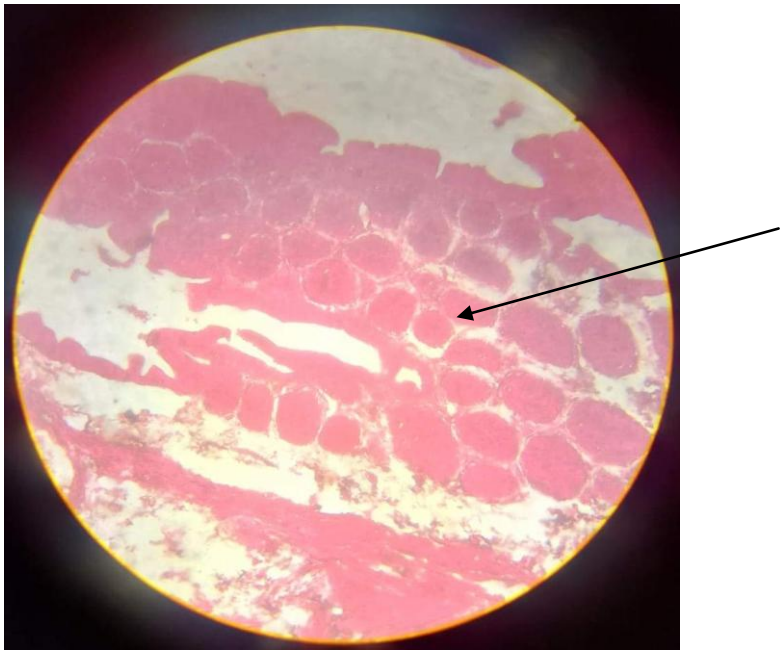

Fig 9: Thickening of the interfollicular space

CHAPTER V

DISCUSSION

The present investigation was carried out to determine the actual status of mortality, morbidity, prevalence, and clinico-pathological features of Infectious Bursal Disease (IBD) of Sonali chickens. In Dinajpur district from July to December 2018. In this study the diagnosis of IBD was made on the basis of the farm history and gross pathological lesions and histopathology.

A total of 7750 of the of the 2390 affected Sonali chickens werw diagnosed as IBD (Table 2) and observed clinical signs were morbidity, high mortality, watery or whitish diarrhoea , vent picking , unsteady gait, ruffled feathers and sudden death which correspond with the findings of Lukert and Saif (2003).

The present study showed that overall prevalence of IBD in Sonali chicken were 8.95%, 36.5%, 49.16%, 35.45% respectively in Sadar, Chirirbandar, Parbatipur and Birol of dinajpur District respectively (Table2).The highest prevalence was found in Parbatipur and lowest was found in Sadar upazila. The highest prevalence was in Parbatipur due to those birds were non vaccinated and lowest was in Sadar due those bids were vaccinated and age 5^{th} weeks.

On the basis of age group, the prevalence of IBD were 41.90%, 33.11%, 28.38%, 28.24% at the age of 3^{rd} , 4^{th} , 5^{th} and 6^{th} week of age (table 3) respectively. The prevalence of IBD in Sonali chickens was the highest 41.90% at the 3^{rd} week of age and the lowest 28.24% at 6^{th} week of age. At 3^{rd} week of age was more susceptible due to decrease maternal immunity and increase pressure on bursa during rapid body growth. While no case was found in first two weeks of age and the sonali chickens of 3^{rd} weeks of old were highly susceptible to IBD. Rajaonarison *et al*., (2006) who observed the highest prevalence of IBD in sonali during the 3^{rd} to 5^{th} week of age.

The highest mortality 48.92 % was found in parbatipur Upazila due to those birds were non vaccinated and lowest mortality 19.53% was sadar (table 2) which support the findings of Mohanty *et al*., (1971). The variation of prevalence of gumboro disease in sonali chickens of the present study area from another area may be due to managemental variation such as vaccination, feed intake, biosecurity, season, and region of the study area.

The prevalence of vaccinated birds was 25.29% and non vaccinated birds was 49.16%. (Table 4).

The diagnosis of the disease was based on history, clinical signs and gross lesions as well as histopathological alterations.

The general health status and age of the chickens were recorded. The chickens were observed for detection of clinical signs. The clinical signs were observed by visual examination. The clinical signs were recorded during the visit of the infected flocks and the farmer's complaints about the affected birds were also considered.

In this observation, the gross pathological lesions were haemorrhages in the breast muscle and thigh muscles (Fig 4 and 5). The main changes, enlarged edematous and swollen bursa of Fabricious (Fig 6) were found in primary stage. These findings support with the earlier observation of Paul (2004); Richard and Miles (2004) and Rajaonarison *et al.,* (2006) who reported the gross pathological lesions were dehydrated and darkened carcass, hemorrhages on pectoral, leg and thigh muscles.

The postmortem changes of all the cases were performed immediately after collection of the dead birds. At necropsy, gross changes were observed and recorded very carefully. The representative tissue samples containing lesions were fixed in 10% buffered neutral formalin.

During necropsy, different organs having gross lesions were collected, preserved at 10% buffered neutral formalin, processed, sectioned and stained for histopathological examination following a standard procedure.

Histopathological study revealed the findings as showed destruction of normal architecture andreducing size of the follicle (Fig 7). Thickening of the intermolecular space (Fig 8).

CHAPTER VI

SUMMARY AND CONCLUSION

It is summarized that condition showing marked depression, unsteady gait, ruffled feathers, whitish diarrhea, atrophy of bursa of Fabricious and sudden death is attributable to Infectious Bursal Disease virus (IBDV). At necropsy, haemorrhages were found in the breast and thigh muscles. Enlarge, edematous swollen bursa of Fabricious were found in primary stage. In histopathological study, showed destruction of normal architecture and reducing size of the follicle was found. Thickening of the interfollicular space was found. The prevalence was very high at the age of 3rd week but low in 6th week of age in chick. The occurrence of IBD outbreaks in sonali chickens farms as observed in this study indicates not only due to lack of immunization plan but also poor management system such as vaccination, feed intake, biosecurity and regional variation etc., resulting heavy economic loss. Scheduled vaccination along with good management practices are the basic tools to control of Infectious Bursal Disease (IBD) in the study area.

- In the context of this study, it may be concluded that Infecious Bursal Disease could be pathologically characterized and identified by necropsy and histopathological examination.
- Prevalence was higher in Parbatipur 49.16% and lower in Sadar 8.95%
- Age wise prevalence was higher in 3 weeks (41.90%) and lower in 6 weeks (28.24%)
- Significant variation found between vaccinated (25.29%) and non-vaccinated (49.16%) chickens.

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APPENDIX

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

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.