PATHOLOGICAL INVESTIGATION OF INFECTIOUS BURSAL DISEASE (IBD) IN SONALI CHICKEN AT CAIBANDHA DISTRICT

A THESIS

BY

ABDUR ROUF
Registration No.: 1305082
Semester: January-June, 2014
Session: 2013-2014

MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

Department of Pathology and Parasitology
Hajee Mohammad Danesh Science and Technology University
Dinajpur-5200

June, 2014

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June, 2014

ABSTRACT

The study was designed to investigate prevalence and pathology of Infectious Bursal Disease (IBD) of sonali chicken at different upazila in Gaibandha Distict in a short six month duration starting from Janury to June2014. Eight sonali chicken farms with sum of 3230 birds of various age group from four different upazila like Sadar, Palashbari, Suddulapur and Gobindogonj 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 10%, 10.95%, 7.89% and 12% in Sadar, Palashbari, Suddulapur and Gobindogonj upazila, respectively with an overall prevalence 10.21% at Gaibandha district. The prevalence of IBD in sonali chickens was the highest (11.98%) at 4^{th} week of age and the lowest (7.88%) at 6^{th} week of age. No sonali chick was identified as positive for IBD in their first two weeks of age. The highest mortality was observed at Gobindogonj upazila (5%) and the lowest (3.80%) at Palashbari upazila, with total mortality rate 4.19%. The necropsy findings of infected chicks revealed haemorrhages on thigh and brest muscles; enlarged, edematous, hyperemic and haemorrhagic Bursa of Fabricious followed by atrophy. In some cases kidneys were found swollen. Severe lymphoid depletion and reactive cells infiltration in the interfollicular space were found in histopathological studies by using H & E stain. Therefore, it was concluded that susceptibility of chicks to IBD is influenced by its age. Ruffled feather, depression, whitish diarrhoea with haemorrhagic muscles and inflammed, edematous, hyperemic Bursa of Fabricious is attributable to Infectious Bursal Disease (IBD).

CONTENTS

CONTENTS (CONTINUED)

CONTENTS (CONTINUED)

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF ABBREVIATIONS AND SYMBOLS

%	:	Percentage
/	:	Per
µg	:	Microgram
µl	:	Micro liter
^{0}C	:	Degree Celsius
BF	:	Bursa of Fabricius
CEF	:	Chicken embryo fibroblast
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
lb	:	Pound
Ltd	:	Limited
MDA	:	Maternally derived antibody
min	:	Minutes
ml	:	Mililiter
mm	:	Milimeter
OIE	:	Office International des Epizooties
p.i.	:	Post inoculation or post infection
PBS	:	Phosphate buffered saline
RNA	:	Ribonuclic acid

Sec	:	Second
SL.	:	Serial
SPF	:	Specific pathogen free
spp.	:	Species
Sq.	:	Square
VP	:	Virus protein
vv	:	Very virulent
vvlBDV	:	very virulent infectious bursal disease virus
SEM		Standard Error Mean

CHAPTER I

INTRODUCTION

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INTRODUCTION

The economy of Bangladesh is mainly based on Agriculture. Our agriculture primarily depends on Livestock. Livestock is considered as the backbone of agriculture (Ahmed, 2000). The contribution of the livestock sub-sector to GDP at constant prices was 1.84% in the fiscal year 2012-13 (BER, 2014). There are 78171 Lac registered poultry farms in 64 districts of Bangladesh till February, 2014. From poultry, Bangladesh gets 67452.80 Lac egg annually (BER, 2014). Meat and eggs are two major sources of animal protein. Bangladesh is one of the developing countries facing acute shortage of animal protein. The poultry meat alone contributes a substantial 37% of the total meat production in Bangladesh (Begum *et al.* 2011). Protein is the most important constituent of human's food. Poultry meat and eggs provide approximately 38% total animal protein in the country (FAO, 1999). 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, 1997) and the local chicken supply approximately 71% of the total meat (Paul and Islam, 2001).

Poultry can be an important tool to fight poverty not only for this group of people but also for the distressed women as poultry requires minimum land, short capital and skill. 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 major constraints in poultry farming are the outbreak of several devastating diseases causing economic loss and discouraging poultry rearing (Das *et al.*, 2005). 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).

In Bangladesh, the first outbreak of IBD occurred at the end of 1992 (Islam *et al,* 1994; Chowdhury *et al,* 1996; Rahman *et al,* 1996) and has become a major problem in the poultry industry, causing up to 80% mortality in the field outbreaks (Battacharjee *et al.,* 1996; Chowdhury *et al.,* 1996; Islam *et al.,* 1997).

Infectious Bursal Disease (IBD) or Gumboro disease is an acute, highly contagious viral disease of growing chickens specially chickens of 3-6 wks of age. It is caused by a double stranded, bi segmented RNA virus belonging to the genus Birnavirus (Murphy *et al.* 1995), sub-genus Avibirnavirus (Pringle, 1998), family Birnaviridae (Dobos *et al.,* 1979; Brown, 1986). There are two distinct serotypes of IBDV: serotypel 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.

One of the earliest signs of infection in a flock is picking of their own vent. Other signs included depression, anorexia, soiled vent feathers, whitish watery diarrhoea, ruffled feathers, trembling, severe prostration and finally death (Saif, 1998; DiFabio *et al,* 1999). The disease is characterized mainly by severe damage of the Bursa of Fabricius (BF) followed by immunosuppression (Cheville, 1967; Fadley *et al.,* 1976; Rosenberger and Gelb, 1978; Saif, 1994; Lukert and Saif, 1997). There are frequent occurrences of this disease, reported by the farm-owners, even when the flocks have been vaccinated against the disease (Bentue, 2004). IBD is economically important for the poultry industry in function of the immune depression that it causes (Moraes *et al.,* 2004).

The primary target organ for IBDV is the Bursa of Fabricius (Lukert and Saif, 1997). IBDV affects the actively dividing B-lymphocytes bearing cell surface IgM (Hirai and Calnek, 1979; Miiller, 1986), developing the severe

morphological alteration of Bursa of Fabricius (Lukert and Saif, 1997) and producing a profound immunosuppression (Ivan *et al.*, 2001).

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 *Eimeria tenella* (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).

Many researchers reported the prevalence and incidence of Infectious Bursal Disease in different regions of Bangladesh. In greater Mymensingh district the incidence was 21.1% (Das *et al.*, 2005) whereas the prevalence of IBD in Rajshahi region was found 12.23% (Hossain *et al.*, 2010). However there is no such study in Gaibandha district which is one of the major belts in Bangladesh.

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

i. To investigate the prevalence and mortality rate of disease in sonali chicken encountered at Gaibandha district
ii. To study the clinical findings of Infectious Bursal Disease (IBD) in the affected flock
iii. To known the prevalence of IBD in relation to age of birds
iv. To study the gross and histopathological changes of different organs developed due to Infectious Bursal Disease

CHAPTER II

REVIEW OF LITERATURE

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 IBDV

Infectious Bursal disease (IBD) was first recognized as a distinct clinical entity in 1957. A. S. Cosgrove initially described the malady as "avian nephrosis" on account of the tubular degenerative lesions. The popular name of the disease is Gumboro disease; as the initial outbreaks occurred in an area known as Gumboro in Southern Delware, USA (Cosgrove, 1962). Subsequently, the term infectious Bursal was proposed by Hitchner because it produces specific pathognomic lesions in the Bursa of Fabricius (Hitchner, 1970). 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). The disease has now spread throughout the world with the exception of New Zealand (Van der Sluis, 1994). 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).

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

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

Prior to 1987 the European strains of IBDV were of low pathogenicity, causing less than 1% mortality (Cavanagh, 1992). In 1987, the picture changed, a very

virulent (vv) pathotype of IBDV emerged, which caused an acute disease with very high mortality (Van den Berg *et al.,* 1991) in Belgium and Netherland.

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).

The first outbreaks of IBD occurred in Bangladesh at the end of 1992 (Islam *et al.,* 1994a and 1994b; Rahman *et al.,* 1996; Chowdhury *et al.,* 1996) with high mortality in the poultry farms (Chowdhury *et al.,* 1996; Islam *et al.,* 1997;Talha *et al.,* 2001). The virus has been isolated from the field outbreaks (Chowdhury *et al.,* 1996; Islam *et al.,* 2001a) and their 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).

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).

2.2 EPIDEMIOLOGY

2.2.1 Geographical distribution of IBDV

Infectious Bursal Disease is currently an international problem. IBDV is worldwide distributed, occurring in all major poultry producing areas (Eterradossi, 1995; Lukert and Saif, 1997; Van den Berg *et al.,* 2000; Wit and William Baxendale, 2004). A survey is conducted by the Office International des Epizooties (OIE, 1995) during the 63rd General Session in 1995 declared cases of infection and the disease is present in more than 95% of the Member Countries

(Eterradossi, 1995). In Ethiopia, there is no recorded occurrence of IBD case (OIE, 2003). Australia, Newzealand, Canada and the US are also so far unaffected (Snyder, 1990; Sapats and Ignjatovic, 2000). Variant IBD viruses were first reported in the Delmarva Peninsula region of the eastern United States in 1984. Variant strains are the predominant viruses in the United States (Lukert and Saif 2003). Australia has remained free of vvIBDV mainly due to geographical isolation and strict quarantine barriers, but a disease outbreak during which IBD virus was isolated occurred in 1999 (Ignjatovic *et al.*, 2004).

2.2.2 Susceptible Hosts

The natural hosts of IBDV are domestic fowls (Helmboldt and Garner, 1964). Natural infections of turkeys and ducks have also been recorded (Page *et al.*, 1978; McNulty *et al.,* 1979; McFerran *et al.,* 1980). IBDV infections of turkeys are subclinical in 3-6 weeks old poults, producing microscopic lesion in the Bursa (Giambrone *et al.*, 1978). IBD virus has been isolated from a goose in China (Wang *et al.,* 2007). The couternix quail is not infected with a chicken strain of IBDV (Weisman and Hitchner, 1978). Experimental inoculation of pheasants, partridges, guinea fowls and quails showed no signs of disease (Van den Berg *et al.,* 2001).

Antibodies against IBDV have been detected in various wild birds like penguins (Gardner *et al.,* 1997), commercially raised ostrich (Ley *et al.,* 2000), wild ducks, crows, goose (Hollmen *et al.,* 2000), which may mean that wild birds may act as targets or reservoirs (Hollmen, *et al.,* 2000).

2.2.3 Breeds Susceptibility

The population at risk includes broiler flocks, sonali chicken 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).

2.2.4 Susceptible Age

The time when chickens are the most susceptible to clinical infection of IBD is between 3 and 6 weeks, when the Bursa of Fabricius is at its maximum rate of development (Cosgrove, 1962; Winterfield and Hitchner, 1964; Hanson, 1967; Ley *et al.,* 1983; Lukert and Saif, 1997; Rajaonarison *et al.,* 2006; Khan *et al.,* 2009). But the disease has also been reported to occur in birds between 9 days to 20 weeks of age (Lukert and Saif, 1997; Chauhan and Roy, 1996). In chicken the disease has also been reported to occur upto 20 weeks of age (Okoye and Uzoukwn, 1981). Sub-clinical infection has been reported to occur in chicks less than three weeks of age (Allan *et al.,* 1972; Ley *et al.,* 1979; Savova and Liupkel, 2002; Butcher, 2003; Richard and Miles, 2004) and even in newly hatched chicks (Fadley and Nazerian, 1983). Clinical disease also occurred in chicken upto 18 weeks of age (Ley *et al.,* 1979 and 1983).

2.2.5 Mode of Transmission

Because of high contagious nature, IBDV spreads rapidly by direct contact, fomites, contaminated feed, water, litter and equipments (Benton *et al.,* 1967a; Sun Ming *et al.*, 2001). Natural infection is usually via the oral route, but the upper respiratory tract and conjunctiva (eye) probably also play a role. Chicken usually become infected through ingestion of contaminated faeces or other organic materials (Breytenbach, 2003). After infection infected chickens excrete virus in their dropping and can transmit the disease for at least 14 days (Vindevogel *et al.,* 1976; Baxendale, 2002) but not exceeding 16 days (Winterfield *et al.,* 1972). Indirect transmission of virus most probably occurs on fomites (clothing and litter) or through airborne, virus laden feathers and poultry

house dust (Benton *et al.,* 1967a). Virus can remain viable for up to 60 days in poultry house litter (Vindevogel *et al.*, 1976). Insect means lesser mealworm and mosquito may be involved in the spread of the disease (Snedeker *et al.*, 1967; Howie and Thorson, 1981). IBD virus has recently been isolated from a sparrow in China, suggesting that wild birds could act as mechanical carriers (Wang *et al.,* 2007). According to another report, houses that contained infected birds were infective for innate birds after 54 and 122 days (Benton *et al.,* 1967a). The virus is not egg transmitted but can survive on the eggshell surface. IBDV is a very stable virus and can therefore persist in poultry houses after thorough cleaning and disinfection (Lukert & Hitchner, 1984).

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 Morbidity and mortality rates

The main features of the disease are the sudden and high morbidity rate, spiking death curve and rapid flock recovery (Lukert and Hitchner, 1984).

IBD occurred first among birds aged 19-20 days then spread to birds aged 29-33 days with a higher mortality (9.22%) in the later group. Immunization of subsequent group batches at 14 days of age with a drinking water vaccine overcomes the problem (Barnes *et al.,* 1982). The disease spread rapidly but mortality rate is low (3.5%) (Okoye and Uzoukwn, 1981).

Morbidity is usually 100% but mortality varies depending on the virus strains (Saif, 1998). Morbidity could be 100% (Islam *et al.,* 2008) and mortality could reach up to 80% in field outbreaks (Chowdhury *et al.,* 1996; Islam *et al.,* 1997; Hoque *et al.,* 2001; Islam *et al.,* 2008).

Mortality due to IBD on various farms ranged from 1 to 40% in broilers (Saif and Abdel-Alim, 2000; Kurade *et al.*, 2000; Islam and Samad, 2004; Rajaonarison *et al.*, 2006; Uddin *et al.*, 2011).

The morbidity of the IBD following infection with classical strains may be may be higher than 80% while mortality may be as low as 5 to 12% (Mohanty *et al.*, 1971) or may reach up to 50% in layer pullets and 25% in broilers (Lukert and Hitchner, 1984).

Flock mortality ranges from 20-30% and 60-100% with classic virulent strains and vvIBDVs, respectively (Cao *et al.*, 1998). On the other hand, the attenuated strains do not cause disease in chicken (Xue and Lim, 2001).

However infection with the newly emerged very virulent strain of IBDV may cause up to 100% morbidity and over 70% mortality (Brown *et al.*, 1994). The strains of very virulent IBDV may cause mortality up to 90% (Chettle *et al.*, 1989); whereas mortality can reach up to 100% with the infection of this isolates (Van den Berg *et al.*, 1991). Experimentally, infection to SPF chickens with vvIBDV causes 90-100% mortality (Chettle *et al.*, 1989; Van den Berg *et al.*, 1991; Wenky *et al.*, 1994). The genetically engineered tissue culture adapted vvIBDV did not show any mortality in SPF chickens (Van Loon *et al.*, 2001).

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; Yamaguchi *et al.*, 1996b; van Loon *et al.*, 2001; Hoque *et al.*, 2001
	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; Bumstead *et al.*, 1993
	Serial passaging in cell culture	Yamaguchi *et al.*, 1996a; Hassan *et*
	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

The etiological agent of the disease is Infectious Bursal Disease virus (IBDV) belonging to the family Birnaviridae of the genus Avibirnavirus. The genus name Birnavirus was proposed to describe viruses with two segments of double stranded RNA. Other viruses included in this group are Infectious Pancreatic Necrotic Virus (IPNV) of fish, Tellina virus, oyster virus, blotched snakehead virus (BSVN) (Da Costa *et al.,* 2003) and crab virus of bivalve mollusks belonging to Aquabirnavirus while Drosophila X virus belongs to genus Entomobirnavirus. All of these contain two segments of double stranded RNA surrounded by a single protein capsid of icosahedral symmetry (Dobos *et al.,* 1979).

2.3.2 Morphology of the virus

IBDV is a small, non-enveloped virus with icosahedral symmetry (Hirai and Shimakura, 1974). IBDV particles have a diameter of 55-60 nm (Hirai and Shimakura, 1974) and posses a bisegmented, double-stranded RNA genome (Dobos *et al.,* 1979; Muller *et al.,* 1979a; Muller and Becht, 1982). The molecular weight of the virus ranged from 2.2 to 2.5 X 106 Daltons with the buyoant density of 1.34 g/ml (Hirai and Shimakura, 1974; Dobos *et al.*, 1979; Jackwood *et al.*, 1982).

The virus consists of four structural proteins, VP1 to VP4 (Dobos *et al.,* 1979) and the molecular weight of VP1, VP2, VP3 and VP4 polypeptides is 11000, 50000, 35000 and 25000 Daltons, respectively. The capsid proteins (VP2 and VP3) arranged in the capsid, a single capsid shell composed of 32 capsomeres arranged in a 5:3:2 symmetry (Hirai and Shimakura, 1974).

The three dimensional structure of IBDV virion has been determined by electron cryomicroscopy. The outer and inner surfaces of the capsid are made of trimeric subunits (Bottcher *et al.,* 1997). Capsid is 9 nm thick and non-spherical in shape since the subunits close to the 5 fold symmetry axes are at a larger radius than those close to 2-3 fold axes. The VP2 forms the external trimeric subunits and protrude out of the shell forming a honeycomb surface. The VP3 forms the inner Y- shaped trimers that are packed closely to form a continuous shell and are connected to VP1. VP4 formed the rim around each 5 fold axis on the inner surface of the capsid (Bottcher *et al.,* 1997). This model suggests 780 copies of VP2, 600 copies of VP3, 60 copies of VP4 and is in accordance with the observed composition of 51% VP2, 40% of VP3, 6% VP 4 and 3% VP1 (Dobos *et al.,* 1979).

2.3.3 Serotypes and pathotypes of IBDV

There are two distinct serotypes of IBDV: serotype 1 and serotype 2 (Lukert *et al.*, 1979; McFerran *et al.,* 1980; Jackwood *et al.,* 1982). Serotype 1 is isolated from both chickens and turkeys while serotype 2 is isolated mainly from turkeys (Jackwood *et al.*, 1982) and also from chickens (Ismail *et al.,* 1988). Serotype 1 IBD virus has been isolated from the faeces of clinically healthy adult ducks, but the significance of the isolation is uncertain (Wang *et al.,* 2007). Serotype1 viruses differ significantly in their pathogenicity and antigenicity (Winterfield and Thacker, 1978; McFerran *et al.*, 1980; Rosenberger and Cloud, 1986; Jackwood and Saif, 1987), whereas, serotype 2 is apathogenic to chickens (Brown and - Grieve, 1992; Ashraf, 2005). Antibody has been detected but no clinical disease has been reported in chickens or turkeys as a result of infection with IBD virus

serotype 2 (Lukert and Saif, 2003). Serotype 1 viruses can be further categorized on the basis of their pathogenicity: Classical strains, variants strains and very virulent strains (Lim *et al.,* 1999; van den Berg *et al.,* 2000; Lukert and Saif, 2003) depending on their pathogenicity and/or antigenicity (Jackwood and Saif, 1987; Lasher and Shane, 1994).

Classical IBDV has traditionally affected poultry worldwide since the first reported incident from Gumboro. Classical strains of IBD virus vary in pathogenicity (Ignjatovic *et al.,* 2004). Classical strains cause Bursal inflammation and severe lymphoid necrosis in infected chicken, resulting in immunodeficiency and moderate mortality from 20 –30% in specific pathogen free (SPF) chicken (Lim *et al.,* 1999).

Variant strains appeared in the US in 1983. These strains were antigenically different from classic strains and caused a rapid and severe Bursal atrophy (Vakharia *et al.,* 1994) and in contrast to classical strains produced no clinical signs of illness. Antigenic variants have been recognized by their ability to escape cross-neutralization by antiserum against the classical strains (Lim *et al.,* 1999).

Attenuated strains have been generated by adapting the classical and variants strains to chicken embryo fibroblasts (CEF) or other cell lines (Lim *et al.,* 1999). Since they are not pathogenic they have been used as live vaccines.

Emergence of the very virulent strains during the 1980's in Europe, Japan and China resulted in dramatic losses to the poultry industry. Very virulent strains have been characterized by severe clinical signs and high mortality ranging from 60-100%. Very virulent strains can break through the immunity provided by the maternal antibodies. The vvIBDV produce similar signs as of the classical strains and the same incubation period of 4 days but the acute phase is more severe and more generalized in the affected flocks (Van den Berg, 2000).

Recently, emerged very virulent pathotypes of IBDV are closely related to classical serotype 1 strain of IBDV (Van der Marel *et al.,* 1991; Van den Berg *et*

al., 1991; Tsukamoto *et al.*, 1995b; Abdel-Alim and Saif, 2001), but molecularly distinct from classical strains (Brown *et al.,* 1994). Molecular and antigenic characterization of Bangladeshi isolates of IBDV demonstrates their similarities with recent European, Asian and African vvIBDV strains (Islam *et al.*, 2001a).

Serotype 1 also includes many attenuated vaccine strains with different degrees of residual pathogenicity. They are designated as mild, intermediate and intermediate plus strains. A serotype 2 strain causes neither mortality nor bursal lesions in SPF birds. Serotype 1 vaccine causes no mortality but possess residual pathogenicity with bursal lesions varying from mild to moderate or even severe. Virulent serotype 1 field strains induce both mortality and Bursal lesions.

2.3.4 Physico-chemical properties

The virus is non-enveloped and highly resistant to physical conditions and chemical agents. Due to the stability and hardiness of the virus, it persists in poultry premises even after thorough cleaning and disinfection. IBDV is resistant to a temperature of 56°C for 5 hours (Benton *et al.,* 1967b), at 60°C for 90 minutes, at room temperature 25°C for 21 days (Cho and Edgar, 1969), viable for up to 60 days in poultry house litter (Vindevogel *et al.*, 1976) and outside the host for at least four months (Baxendale, 2002). The hardiness of the virus makes it difficult to eradicate it from poultry houses after outbreaks of IBD (Alexander *et al.,* 1998).The virus is inhibited by formalin and wescodyne but not by chloroform, phenol, ether, thimerosal and hyamine 2389 treatments (Benton *et al.,* 1967b). There is a marked reduction in the virus infectivity when exposed to 0.5% formalin for 6 hours (Lukert and Hitchner, 1984). The virus was inactivated by exposure for 1 hour to 1% formalin, 1% cresol and 1% phenol (Cho and Edgar, 1969). The virus could survive outside the host for at least four months (Allan *et al.,* 1982). A solution of 2% chloroform, formalin at suitable temperature, gluteraldehyde and a complex disinfectant containing formaldehyde, gluteraldehyde and alkyldimethyl benzylammonium are suitable disinfectants effective against IBDV (Van der Sluis, 1994).

2.4 CLINICAL SIGNS

Severity of the signs depends on the age and breed of the chickens, the virulence of the strain, and the degree of passive immunity (Van den Berg *et al.,* 1991a). The clinical signs of IBD also vary considerably from one farm, region, country or even continent to another. The clinical signs of the affected birds were more or less similar to the signs generally developed due to the infection with vvIBDV (Islam *et al.,* 2008). The exact cause of clinical symptoms and death is still unclear, but the signs do not seem to be related only to the severity of the lesions and the Bursal damage (Van den Berg, 2000).

The incubation period (time between infection and the appearance of clinical disease) of IBDV in chickens is very short and clinical signs of the disease are seen in 2-3 days (Cho and Edgar, 1972; Hirai *et al.,* 1974; Lukert and Hitchner, 1984; Saif, 1998).

The disease is characterized clinically by marked depression, prostration, ruffled feathers, whitish or watery diarrhoea, vent picking, inappetance or anorexia, dehydration, emaciation, progressive weakness, reluctant to move, soiled-vent feathers significantly elevated body temperature at 48 hours of infection but dropped below normal later, lateral recumbence before death and coma. Similar observations were also obtained from many literatures (Cosgrove, 1962; Snedeker *et al., 1967;* Cho and Edgar, 1972; Wyeth, 1980; Nunoya *et al.,* 1992; Islam *et al.,* 1997; Van den Berg, 2000; Rodriguez-chavez *et al.,* 2000; Butcher and Miles, 2001; Hafez *et al.,* 2003; Paul, 2004; Islam and Samad, 2004; Okoyo and Uzoukwu, 2005; Islam *et al.,* 2008; Hossain *et al.,* 2010).

The virus causes immunosuppression in young chickens whereas clinical signs and death may be evident in older chickens at a time when the BF is more developed (Lukert and Saif, 1991). Chickens infected with IBDV when older than 12 weeks do not show clinical signs (Becht, 1980).

2.5 PATHOGENESIS

Pathogenesis is defined as the method used by the virus to cause injury to the host with mortality, disease or immuno-suppression as a consequence (Van den Berg *et al.,* 2000). IBDV usually infects young chickens between 3-6 weeks of age (Asraf, 2005) and causes a clinical disease, while sub-clinically infecting older birds.

IBDV first infect the lymphocytes and macrophages of the gut-associated tissues (duodenum, jejunum, caeca) (Muller *et al.,* 1979b; Weis and Kaufer-Weis, 1994). These organs are considered as the organs of primary replication or organs of primary affinity. The virus containing cells or virus particles reach the BF, the target organ of IBDV (Kaufer and Weis, 1976), producing transient viremia (Winterfield *et al.,* 1972; Weis and Kaufer-Weis, 1994) and by way a considerable part of them are phagocytized by kupffer cells of liver, but the virus materials are not trapped in the liver (Weis and Kaufer-Weis, 1994). Presumably the virus is first taken up by the follicle-associated epithelium (Bursal tufts) and then reaches the medulla of the follicles (Kaufer and Weis, 1976). The failure of the electron microscope to demonstrate adsorption and uptake of the virions is due to the fact that the follicle-associated epithelium normally contains numerous vacuoles, filled with electron-densed granular material, making it almost impossible to identify phagocytized virus particles (Kaufer and Weis, 1976).

After entering into the follicles, the virus infect and replicate within the B lymphocytes (Nakai and Hirai, 1981; Muller,1986) and then a second and pronounced viremia occur with secondary replication in other organs leading to the development of the clinical signs and sometimes death (Weis and Kaufer-Weis, 1994; Van den Berg, 2000).

Virus is spread in various organs, but due to the absence of a sufficient number of susceptible cells, virus multiplication is moderate and can be kept in check by the host defense mechanism. With the occurrence of circulating specific antibodies the virus can be rapidly eliminated. The availability of a large number of highly

susceptible cells is a crucial point in the pathogenesis of IBD (Weis and Kaufer-Weis, 1994).

2.6 PATHOLOGY

2.6.1. Affected Organs

The principal target organ for pathogenic IBDV is the Bursa of Fabricius (BF) (Cheville, 1967; Hirai and Calnek, 1979; Kaufer and Weis, 1980; Lukert and Saif, 1991; Tanimura *et al.,* 1995; Elankumaran *et al.,* 2001). The BF reaches the maximum development between 3-6 weeks of age and at this time chickens are most susceptible to the disease. But other lymphoid organs such as spleen (Rinaldi *et al.,* 1965; Cho and Edgar, 1972; Tanimura *et al.,* 1995; Islam *et al.,* 1997; Hoque *et al.,* 2001; Rudd *et al.,* 2001), thymus (Islam *et al.,* 1997; Hoque *et al.,* 2001; Rudd *et al.,* 2001; Okoye and Uzoukwu, 2001), caecal tonsils (Islam *et al.,* 1997; Elankumaran *et al.,* 2001) and other non lymphoid organs like kidneys (Cosgrove, 1962; Van der Sluis, 1994), liver (Chowdhury *et al.,* 1996; Islam *et al.,* 1997) are also affected.

2.6.2 Gross pathology

2.6.2.1 Bursa of Fabricius

The pathognomonic lesions of IBD are found in Bursa and is characterized by oedematous (Chowdhury *et al.,* 1996; Butcher and Miles, 2001; Singh *et al.,* 2002; Hafez *et al.,* 2003; Islam *et al.,* 2008; Goud, *et al.*, 2009; Hossain *et al.,* 2010; Uddin *et al.*, 2011), swollen (Mohanty *et al.*, 1971; Nunoya *et al.,* 1992; Chowdhury *et al.,* 1996; Saif and Abdel- Alim, 2000; Singh *et al.,* 2002; Islam *et al.,* 2008; Rahman *et al.,* 2010; Hossain *et al.,* 2010), haemorrhagic bursa (Van der Sluis, 1994; Chowdhury *et al.,* 1996; Haque *et al.,* 2001; Singh *et al.,* 2002; Islam *et al.,* 2008; Goud, *et al.*, 2009; Uddin, *et al.,* 2011), changes in shape and colour- yellow, red, black (Rajaonarison *et al.,* 2006; Paul, 2004; Richard and Miles, 2004), formation of gelatinous film around the bursa (Butchner and Miles,

2001; Hafez *et al.,* 2003; Paul, 2004; Richard and Miles, 2004; Rajaonarison *et al.,* 2006), cheesy mass within the bursal lumen (Chowdhury *et al.,* 1996; Islam *et al.,* 2008) and finally, atrophy of the bursa (Mohanty *et al.*, 1971; Jhala *et al.,* 1990; Chowdhury *et al.,* 1996; Rodriguez-chavez *et al.,* 2000; Islam *et al.,* 2008; Uddin *et al.,* 2011).

2.6.2.2 Spleen

Spleen becomes swollen (Chowdhury, *et al.,* 1996; Helmboldt and Garner, 1964; Rinaldi *et al.,* 1965) or may become atrophied (Chowdhury *et al.,* 1996; Cho and Edgar, 1972), sometimes mottling and paler than normal in appearance (Chowdhury *et al.,* 1996). Hemorrhages are common (Cho and Edgar, 1972; Hoque *et al.,* 2001) and small gray and whitish foci may be present (Rinaldi *et al.,* 1965; Cullen and Wyeth, 1978; Ley *et al.,* 1979), hypertrophy of the spleen (Craig *et al.,* 1979).

2.6.2.3 Kidneys

The kidneys become swollen (Ley *et al.,*1979; Van der Sluis, 1994; Chowdhury, *et al.,*1996 ; Van den Berg, 2000; Rajaonarison *et al.,* 2006; Hossain *et al.,* 2010; Uddin *et al.,* 2011), paler than normal (Chowdhury *et al.,*1996), mottled (Ley *et al.*, 1979). Inflammatory swelling of the ureters is caused by retention of urine and hydronephrosis (Weis and Kaufer-Weis, 1994). Kidneys with pronounced tubules (Barron, 1966), ureters filled with urates (Cosgrove, 1962), nephrotic lesions or congestion (Mohanty *et al.,* 1971; Dongaonkar *et al.,* 1979) are also reported.

2.6.2.4 Caecal tonsil

Haemorrhages (Chowdhury, *et al.,* 1996) and partially damaged caecal tonsils are found in some cases (Islam *et al.,* 1997).

2.6.2.5 Thymus

Necrosis (Chowdhury *et al.,* 1996), haemorrhages (Hoque *et al.,* 2001) and opaque boiled meat appearance with a thickened, gelatinous connective tissue capsule and hyperemia on the surface (Cosgrove, 1962; Dongaonkar *et al.,* 1979) are found.

2.6.2.6 Liver

Congestion (Chowdhury *et al.,* 1996; Islam *et al.,* 1997), paler than normal in appearance (Chowdhury *et al.,* 1996) and occasionally with focal necrosis (Nunoya *et al.*, 1992; Islam *et al.,* 1997), swollen and streak appearance (Hanson, 1967; Rajaonarison *et al.,* 2006), hypertrophy of the liver (Cho and Edgar, 1972) are reported.

2.6.2.7 Other features

On post mortem examination of chickens which died in outbreaks of IBD, the carcass is characterized as well developed and good bodily condition but with dehydration of the subcautenous tissue and muscles (Cosgrove, 1962; Hanson, 1967; Chowdhury *et al.*, 1996; Rudd`*et al.,* 2001; Islam *et al.,* 2008; Hossain *et al.,* 2010) and darkened carcass (Chowdhury *et al.,* 1996; Paul, 2004; Okoye and Uzoukwu, 2005; Rajaonarison *et al.,* 2006). Varying degrees of haemorrhages are found in the leg, thigh and/or breast muscles (Cosgrove, 1962; Schat *et al.,* 1981; Lukert and Hitchner, 1984; Chowdhury *et al.,* 1996; Hoque *et al.,* 2001; Hafez *et al.,* 2003; Anku, 2003; Islam *et al.,* 2008, Hossain *et al.,* 2010; Uddin, M. B *et al.,* 2011), haemorrhages also found at the junction between the gizzard and proventriculus (Hanson, 1967; Cullen and Wyeth, 1978; Van der Sluis, 1994; Chowdhury *et al.,* 1996; Islam *et al.,* 1997; Hoque *et al.,* 2001), skeletal muscles are darkly discoloured (Nunoya *et al.,* 1992).

2.6.3 Histopathology

2.6.3.1 Bursa of Fabricius

IBD viruses cause Bursal changes including lymphocytic depletion of varying degrees from the follicles (Islam *et al.*, 1997; Rodriguez-chavez *et al.*, 2000; Van Loon *et al.,* 2001; Rautenschlein *et al.,* 2001; Rudd *et al.,* 2001; Hoque *et al.,* 2001; Franciosini and Coletti, 2001; Islam *et al.,* 2008), interfollicular oedema (Czifra and Jonson, 1999; Hoque *et al.,* 2001; Franciosini and Coletti, 2001; Flensburg and Ersboil, 2000; Islam *et al.,* 2008), heterophilic infiltration in the interfollicular space (Mohanty *et al.,* 1971;Tanimura *et al.,* 1995; Ignjatovic and Sapats, 2002) and also in the follicles (Hoque *et al.,* 2001), formation of purple coloured necrotic cellular mass within the follicles (Tanimura *et al.,* 1995; Islam *et al.,* 1997), fibroplasia surrounding the follicles (Hoque *et al.,* 2001; Rodriguez-chavez *et al.,* 2000; Mahajan et al., 2002; Hemalatha et al., 2009), haemorrhages and congestion in the Bursa, necrosis of lymphocytes with pyknotic and karyorrhectic nuclei (Islam *et al.,* 1997; Del Bono *et al.,* 1968; Flensburg and Ersboil, 2000; Mahajan *et al.,* 2002) in the follicles, formation of cystic spaces within the follicles (Hoque *et al.,* 2001; Franciosini and Coletti, 2001; Islam *et al.,* 2008) as well as in the Bursal epithelium, thickness and oedematous serosa and finally follicular atrophy (Del Bono *et al.,* 1968; Franciosini and Coletti, 2001) have been reported. Infiltration of macrophages in the follicles (Tanimura *et al.,* 1995) and varying degree of follicular regeneration were also recorded.

2.6.3.2 Spleen

Histopathological appearance of the spleen of the IBDV infected birds are characterized as lymphocytic depletion with marked haemorrhages (Chowdhury *et al.,* 1996; Islam *et al.*, 1997; Del Bono *et al.,* 1968), thickening of the arterial wall with fibrinoid degeneration (Chowdhury *et al.,* 1996; Helmboldt and Garner, 1964), lymphoid necrosis (Cheville, 1967; Del Bono *et al.,* 1968; Cho and Edgar, 1972), eosinophilic tissue debris containing karyorrhectic nuclei of necrotic

lymphocytes (Henry *et al.* 1980; Islam *et al.,* 1997), hyaline degeneration of the arterioles (Dongaonkar *et al.,* 1979), pronounced heterophilic infiltration in the sinusoids as well as in the germinal centres, round aggregations of eosinophilic materials surrounding the germinal centres (Henry *et al.,* 1980) and splenic hyperplasia of the white pulp with cell death (Cho and Edgar, 1972; Rautenschlein *et al.,* 2001). The devoid of lymphocytic elements of the spleen are replaced by macrophages and heterophils (Nunoya *et al.,* 1992).

2.6.3.3 Kidneys

Degeneration (Cosgrove, 1962; Chowdhury *et al.*, 1996), dissociation or sloughing of (Henry *et al.,* 1980; Chowdhury *et al.,* 1996) and coagulation necrosis (Chowdhury *et al.,* 1996) of the tubular epithelium; heterophilic infiltration but a few mononuclear leukocytes and some eosinophilic materials and cellular debris in the tubules (Cheville, 1967), interstitial haemorrhage (Barron, 1966), glomerular nephrosis (Mandelli *et al.,* 1966), a large oedematous space between many tubules and collecting ducts (Henry *et al.,* 1980) are found in the kidneys of IBDV infected birds.

2.6.3.4 Caecal tonsils

Severe haemorrhages (Islam *et al.,* 1997), varying degrees of lymphocytic depletion (Helmboldt and Garner, 1964; Nunoya *et al.,* 1992; Tanimura *et al.,* 1995; Chowdhury *et al.,* 1996; Islam *et al.,* 1997), macrophage and heterophilic infiltration (Nunoya *et al.,* 1992; Tanimura *et al.,* 1995), hyperemia and reticular cells proliferation (Dongaonkar *et al.,* 1979) are found in the caecal tonsil of IBDV infected birds. The devoid of lymphocytic elements of the caecal tonsils are replaced by macrophages and heterophils (Nunoya *et al.,* 1992).

2.6.3.5 Thymus

Moderate to severe lymphocytic depletion (Cheville, 1967; Cho and Edgar, 1972; Chowdhury *et al.,* 1996; Islam *et al.,* 1997) with presence of tissue debris and

interlobular oedema (Nunoya *et al.,* 1992; Islam *et al.,* 1997), hyperemia and reticular cells proliferation (Dongaonkar *et al.,* 1979), presence of empty spaces in the cortex, heterophilic infiltration especially in the medulla, numerous round aggregations of cell debris and karyorrhectic nuclei in the cortex and medulla (Henry *et al.,* 1980) of thymus are found in Gumboro disease affected birds.

2.6.3.6 Liver

Congestion in the central vein (Chowdhury *et al.,* 1996), fatty changes, necrosis of hypatocytes (Nunoya *et al.,* 1992; Chowdhury *et al.,* 1996; Otaki, 1993; Cho and Edgar, 1972), heterophilic infiltration and edema (Cho and Edgar, 1972) and dilatation of the sinusoids of the liver (Nunoya *et al.,* 1992) are reported. No detectable histological changes found in the liver (Ley *et al.,* 1979; Dongaonkar *et al.,* 1979; Henry *et al.,* 1980; Schat *et al.,* 1981).

2.6.3.7 Other features

Extensive haemorrhagic lesions found in the intestinal tract, thigh, pectoral muscle and petechial haemorrhage found in myocardium (Schat *et al.,* 1981). Reduced number of haemopoietic cells and a greater decrease in myelocyte numbers in the extra-sinusoidal spaces, erythrocytes in the sinusoidal spaces (Nunoya *et al.*, 1992; Tanimura *et al.,* 1995); congestion, haemorrhages and alveolar emphysema in the lungs (Islam *et al.,* 1997) are reported.

2.7 CLINICO-PATHOLOGICAL OBSERVATIONS

Blood calcium level is significantly lower than normal (Cosgrove, 1962) in IBDV infected birds. Marked increase in serum gamma globulin (van der Sluis, 1994), markedly increased lactic dehydrogenase (Kumar and Rao, 1991; Nunoya *et al.,* 1992; van der Sluis, 1994), decreased alkaline phosphatase (Nunoya *et al.,* 1992), raised chlolesterol, creatine (Kumar and Rao, 1991), creating phosphokinase, glutamic oxaloacetate transaminase level (Nunoya *et al.,* 1992), decreased serum levels of glucose, uric acid and urea (Kumar and Rao, 1991), decreased total

cholesterol and phospholipid (Nuroya *et al.,* 1992), but no significant changes in the serum electrolytes levels (Cosgrove, 1962) are reported.

Panleukopenia (van der Sluis, 1994), lymphopenia (Cosgrove, 1962; Asdrubali and Mughetti, 1972), leukocytosis with heterophilia (Chineme, 1977; Kumar and Rao, 1991), eosinopenia, monocytosis, basophilic, decreased haemoglobin and PCV values (Kumar and Rao, 1991), prolonged clotting time (Chineme, 1977; Kumar and Rao, 1991), prolonged prothrombin time (Kumar and Rao, 1991) are also the haematological pictures in the IBDV infected birds.

2.8 EFFECTS OF IMMUNOSUPPRESSION

Immunosuppression caused by IBDV has a significant economic impact due to widespread nature of the disease in commercial chickens. Reduction in the number of B cells in the BF due to viral infection is the major cause of immunosuppression.

IBDV drew the attention of avian virologists mostly because of its severe immunosuppressive effects (Allan *et al.,* 1972). Actively dividing B-lymphocytes bearing cell surface IgM (Hirai and Calnek, 1979; Miiller, 1986) are the target cells of IBDV. Alteration of immunoglobulin production (Ivanyi and Morris, 1976) and significant depression of serum IgM level (Hirai *et al.,* 1979) were observed after infection, regardless the time of infection.

IBDV alters hosts immunological capacity, affecting humoral or cellular immune responses or both by destruction of the lymphoid elements of the Bursa of Fabricius and sometimes of spleen, thymus and caecal tonsils (Hirai *et al.,* 1974 and 1979). The localization of viral replication and the immunosuppressive effect of IBDV on the humoral immune response may differ between strains (Rosales *et al.,* 1989a, b, c; Mazariegos *et al.,* 1990; Tsukamoto *et al.,* 1995b; Abdel-Alim and Saif, 2001). Selective stimulation of the proliferative B cells committed to anti-IBDV antibody production seems to occur (Lukert and Saif, 2003).

IBDV multiplies in the lymphocytes, macrophages, heterophils and reticular epithelial cells of the bursa (Mandell *et al.,* 1972; Kaufer and Weiss, 1980). IBDV does not multiply in T lymphocytes or in peripheral B lymphocytes (Cursiefen, 1980). Depression of the humoral antibody response in IBDV infected chickens (Allan *et al.,* 1972; Faragher *et al.,* 1974 and 1979) and the suppression of cell mediated immune response, as determined by lymphocyte transformation assay (Sivanandan and Maheswaran, 1981) have already been documented. IBDV affects the Harderian gland influencing the local immune system (Dohms *et al.,* 1981; Rosenberger, 1994) but IBDV infection leads to the accumulation of T cells in the Bursa, concurrently to B cell depletion (Kim *et al.,* 2000). Thus, IBDV infection causes immunosuppression and the immunosuppression ultimately leads to increase the incidence of many diseases (Table-2).

Table 2: Concurrent infections occurring during the course of IBD

Causal agent	Disease or concurrent infection	Reference(s)
Bacteria	*E. coli* infection or colisepticemia	Wyeth, 1975; Ahmed *et al.*, 1993; Singh *et al.*, 1994; Binta *et al.*, 1995; Igbokwe *et al.*, 1996
	Salmonellosis	Wyeth 1975; Binta *et al.*, 1995
	Infectious coryza	Ahmed *et al.*, 1993
	Hemophilus gallinarum infection	van der Sluis, 1994
	Staphylococcus aureus infection	Binta, *et al.*, 1995
	Gangrenous dermatitis	Rosenberger *et al.*, 1975
Virus	Newcastle disease	Faragher *et al.*, 1974; Yachida *et al.*, 1975; Binta *et al.*, 1995
	Infectious laryngotracheitis	Rosenberger *and* Gelb, 1978
	Infectious bronchitis	Giambrone *et al.*, 1977
	Marek's disease	Cho, 1970
	Inclusion body hepatitis	LiWeijen and Cho, 1980
	Chicken infectious anaemia	Clould *et al.*, 1992a and 1992b
Protozoa	Coccidiosis	Anderson *et al.*, 1977; Ahmed *et al.*, 1993; Singh *et al.*, 1994; Chowdhury *et al.*, 1996
Fungus	Aspergillosis	Chowdhury *et al.*, 1996
	Aflatoxicosis	Chang and Hamilton, 1982; Somvanshi *et al.*, 1992
Mycoplasa	*Mycoplasma synoviae* infection or mycoplasmosis	Gimabrone *et al.*, 1977; Binta *et al.*, 1995
Other	Haemorrhagic aplastic anaemia	Rosenberger and Gelb, 1978

2.9 ECONOMIC IMPACT

IBD is a serious menace in the development of poultry enterprise and has resulted in major worldwide economic losses (Chettle *et al.*, 1989; Berg *et al.*, 1991). Immunosuppression induced by IBDV is the primary cause of economic loss associated with the virus (Khatri *et al.,* 2005). 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.

The IBDV being a non-zoonotic pathogen is not regarded as a human food safety issue, nevertheless movement of birds with IBDV infections is a cause for concern because of the possible introduction of new antigenic and pathogenic strains into a geographic area can have a negative economic impact on the chickens grown in that region (Jackwood and Sommer-Wagner, 2010).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The present studies were conducted during the period of January to June, 2014 in 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 chicken farms raised commercially by farmers from different upazila at Gaibandha 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 necropsy

- Birds (Liver, Bursa of Fabricius, Breast and Thigh muscle)
- Scissors
- Forceps
- Gloves
- Musk
- Scalpel
- Knife
- A pair of shears,
- 10% neutral buffered formalin

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

3.1.3 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.

3.1.4 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.

3.1.4.1 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.

3.1.4.2 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.

3.2 METHODS

3.2.1 EXPERIMENTAL LAYOUT

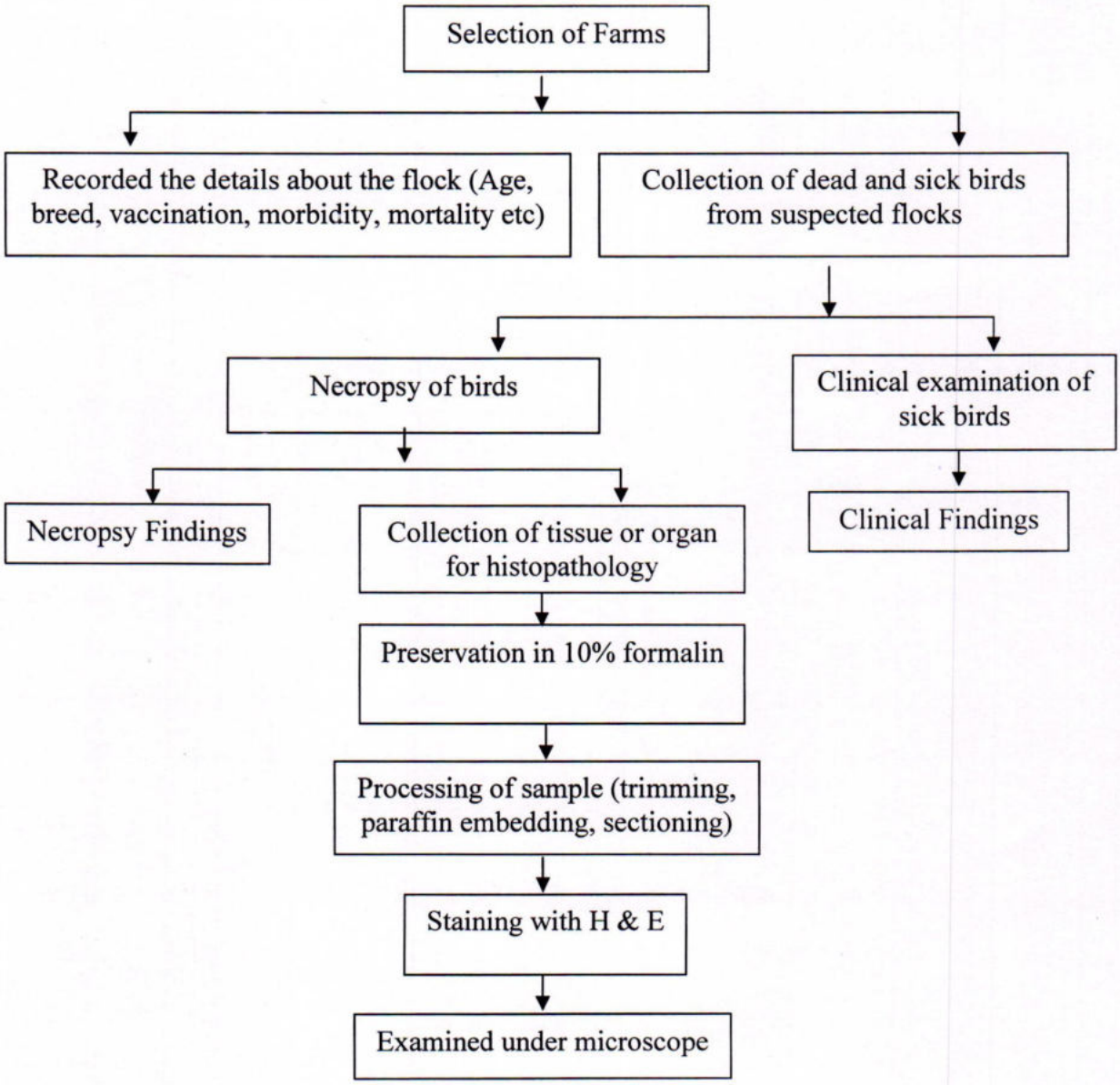


Figure 1: Schematic illustration of the experimental layout

3.2.2 SAMPLE COLLECTION AND EXAMINATION

In this study, a total of 3230 birds of various age groups from four different upazila (Sadar, Palashbari, Saddullapur and Gobindogonj) 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 study. All the diagnostic works were carried under the Laboratory of Department of Pathology and 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.

3.2.3 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.

3.2.4 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 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 °C) 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 METHODS

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-

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

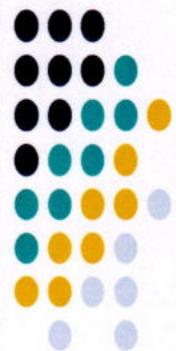
3.3.2 DETERMINATION OF PREVALEANCE

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-

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

CHAPTER IV

RESULTS



CHAPTER IV

RESULTS

A total of 3230 sonali chicken from four different upazila like Sadar, Palashbari, Saddulapur and Gobindogonj of Gaibandha 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 of Gaibandha 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.

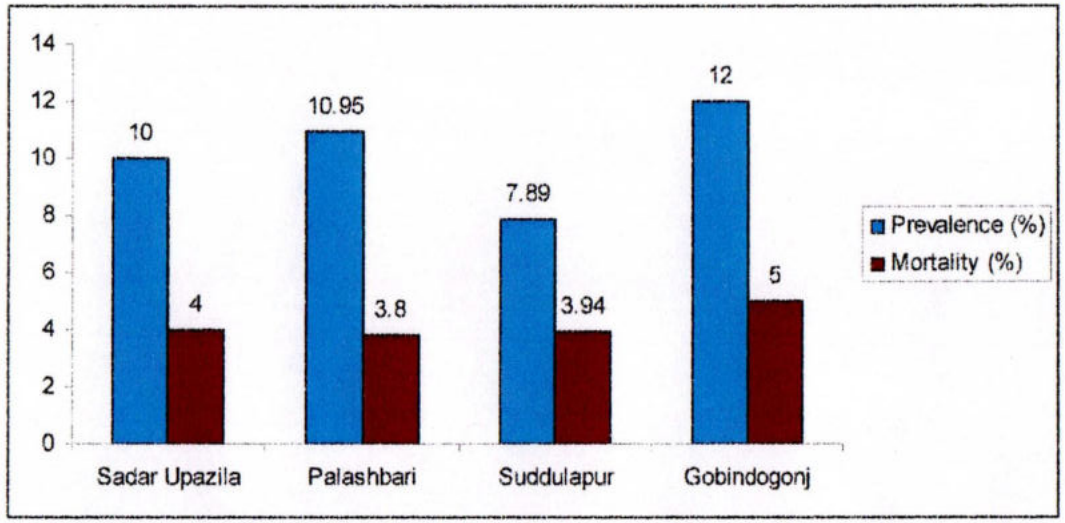
4.1.2 STATUS OF MORTALITY AND PREVALENCE OF THE DISEASE

The study revealed the following actual status of mortality and prevalence of infectious Bursal disease (IBD) in sonali chicken Table-3 showed the mortality and prevalence of IBD at different region of Gaibandha district where as Table-4 showed the prevalence of IBD at different age group. A total of 3230 birds were examined during the study period from which 327 birds (10.21%) are found infected with IBD. The mortality rate is 4.19% No case was found in first two weeks of age.

Table-3 Prevalence and mortality rate of IBD in Sonali chicken at different Upazila of Gaibandha

Name of Upazila	No. of Farm Visited	No. of Birds observed	No. of infected birds	No. of Dead Birds	Mortality (%)	Prevalence (%)
Sadar Upazila	3	1250	125	50	4	10
Palashbari	1	420	46	16	3.80	10.95
Suddulapur	2	760	60	30	3.94	7.89
Gobindogonj	2	800	96	40	5	12
Mean ± SEM					**4.19±0.27**	**10.21±0.87**

*SEM: Standard Error Mean

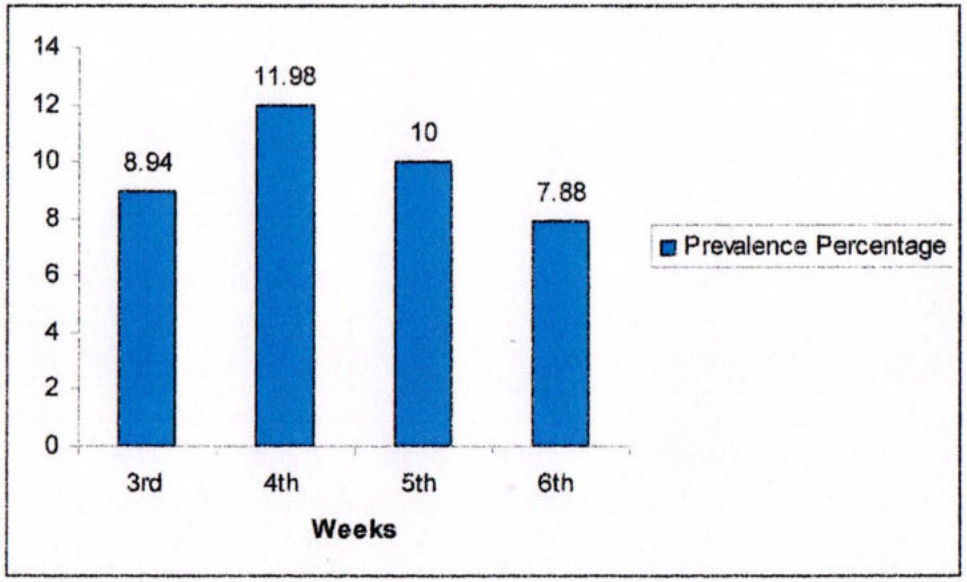


Graph 1. Prevalence and mortality rate of IBD in Sonali chicken at different Upazila in Gaibandha

Table-4 Prevalence of IBD in Sonali chicken at different age group

Age of Birds(Weeks)	No. of Birds observed	No. of infected birds	Prevalence (%)
3rd	850	76	8.94
4th	1160	139	11.98
5th	700	70	10
6th	520	41	7.88
Mean ± SEM			**9.70±0.87**

*SEM: Standard Error Mean



Graph 2. Prevalence of IBD in Sonali chicken at different age group at different Upazila in Gaibandha

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 5and 6). The main changes, enlarged and haemorrhagic Bursa of Fabricious (Fig 7) were found in primary stage. A cut surface of Bursa of Fabricius showing haemorrhage (Fig 8). Haemorrhage in the internal wall of Bursa (Fig 9). In some cases kidneys were swollen.

4.3 RESULTS OF HISTOPATHOLOGICAL EXAMINATION

Section of the Bursa of Fabricious showed loss of normal corticomedullary architecture of Bursa and in most follicles severe lymphoid depletion was observed (Fig 10 and 11). Reactive cells infiltration by heterophils and macrophages in the interfollicular space (Fig 12).

4.4 RESULTS ON PHOTOS

Figure 2: Birds affected with IBD

Figure 3: Birds showing depression

Figure 4: IBD affected birds excreted white colour faec

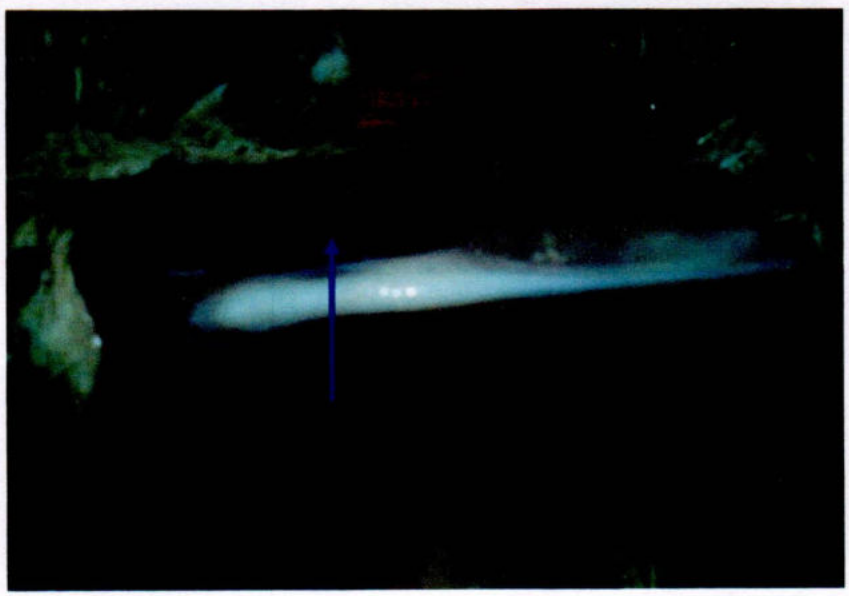


Figure 5: Haemorrhage in the breast muscle

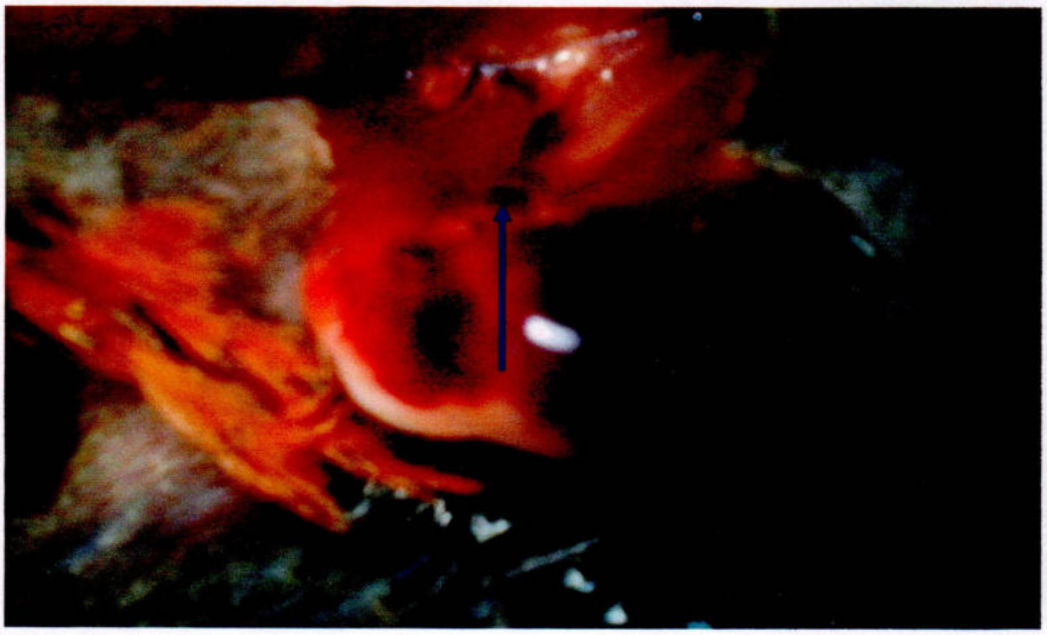
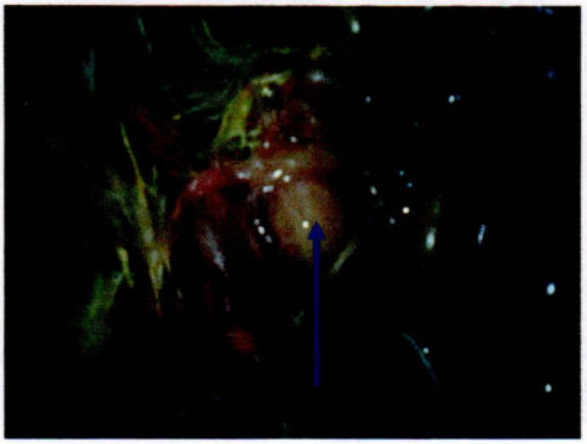


Figure 6: Haemorrhage in the thigh muscles



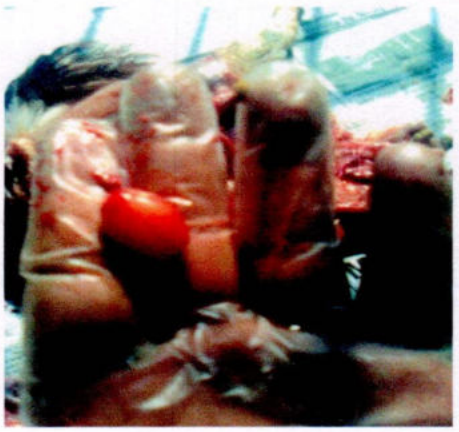


Figure 7: Showing Swollen and haemorrhagic Bursa

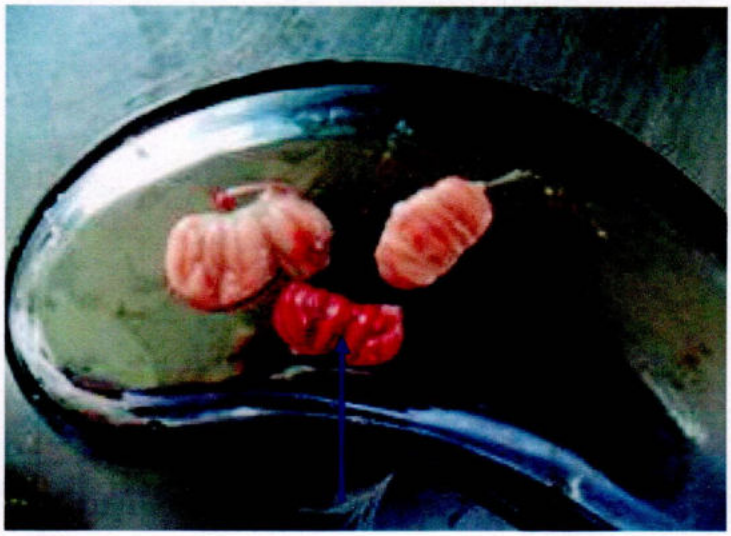


Figure 8: A cut surface of Bursa of Fabricius showing haemorrhage

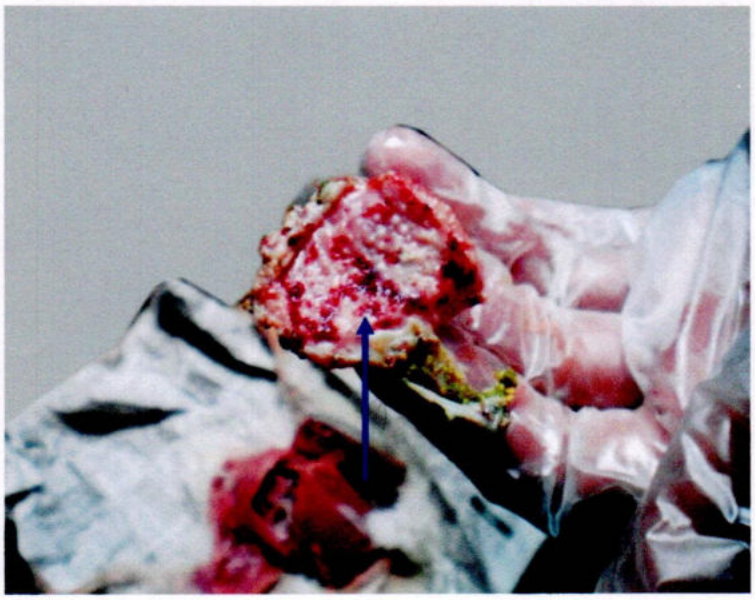


Figure 9: Haemorrhage in the internal wall of Bursa

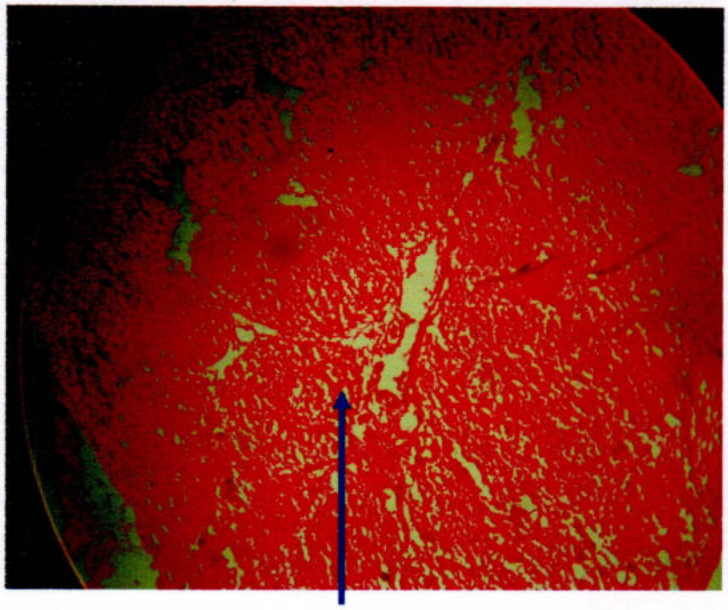


Figure 10: Few Lymphoid depletion in Bursal Follicles

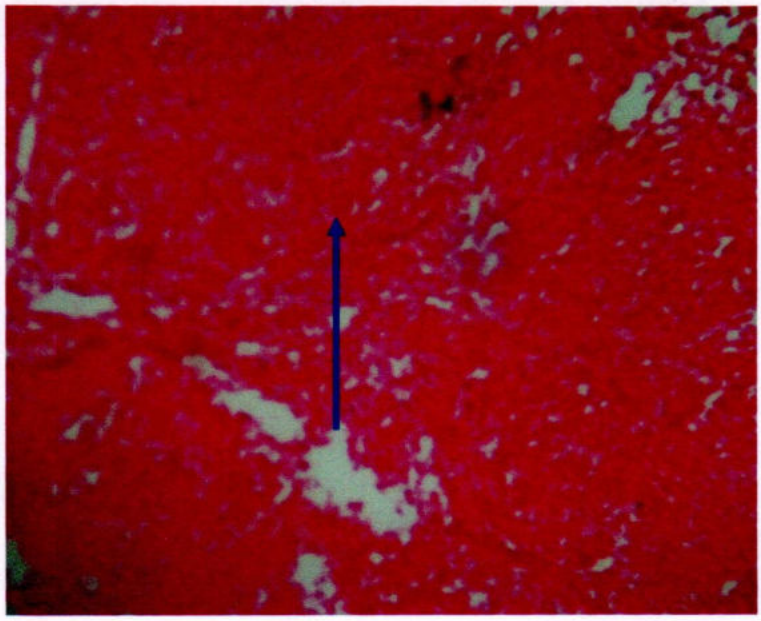


Figure 11: Severe lymphoid depletion in Bursal Foilicles

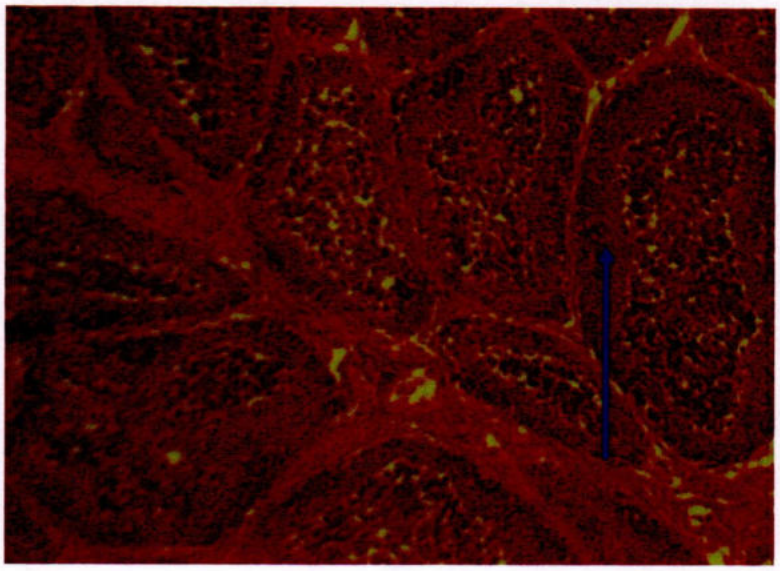


Figure 12: Reactive cells infiltration by heterophils and macrophages in the interfollicular space

CHAPTER V

DISCUSSION

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 chicken in Gaibandha district from January to June, 2014. In this study the diagnosis of IBD was made on the basis of the farm history and gross pathological lesions as had been diagnosed by Sharoon (2002).

A total of 3230 of the 327 affected sonali chicks were diagnosed as IBD (Table 3) 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); Islam and Samad (2004).

The present study showed that overall prevalence of IBD in Sonali chicken was 10%, 10.95%, 7.89% and 12% prevalence in Sadar, Palashbari, Suddulapur and Gobindogonj upazila of Gaibandha district respectively (Table 3). The highest prevalence was found in Gobindogon and lowest was found in Suddulapur upazila (Table 3). The prevalence variation in different upozila of Gaibandha district due to poor management system such as vaccination, feed intake, biosecurity and regional variation. These results support to the reports of Hossain *et al.*, (2010) who reported 12.23% prevalence of IBD in sonali chicken at Rajshahi district. These results agree with the reports of some others. Khan *et al.,* (2009) stated that 7.75% prevalence in Peshawar. Mbuko *et al.*, (2010) found overall prevalence (7.26%) in Nigeria.

On the basis of age group, the prevalence of IBD was 8.94%, 11.98%, 10% and 7.88% at the age of 3rd, 4th, 5th and 6th week of age (Table 4) respectively. The prevalence of IBD in sonali chickens was the highest (11.98%) at 4th week of age and the lowest (7.88%) at 6th week of age. At 4th week of age is 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

chicken of four weeks of old were highly susceptible to IBD. Similar reports have been described by Hirai *et al.*, (1972), Khan *et al.,* (2009) who reported that susceptibility of chickens to IBD is influenced by their age reaching a peak at 4 weeks of age. Rajaonarison *et al.* (2006) who observed the highest prevalence of IBD in sonali during the 3rd to 5th week of age.

The highest mortality 5% was found in Gobindogonj upazila and 3.80% was found in Palashbari upazila (Table 3) which support the finding of Mohanty *et al.,* (1971) and Islam and Samad (2004). The variation of prevalence of gumboro disease in sonali chicken of the study area from another area may be due to managemental variation such as vaccination, feed intake, biosecurity, season and region of the study area.

In this observation, the gross pathological lesions were hemorrhages in the breast (Fig 5) and thigh muscles (Fig 6); enlarged, edematous, hyperemic and haemorrhagic Bursa of Fabricious (Fig 7). A cut surface of Bursa of Fabricius showing haemorrhage (Fig 8). Haemorrhage in the internal wall of bursa (Fig 9).In some cases kidneys were found swollen. These findings support with the earlier observation of Paul (2004); Richard and Miles (2004) and Rajaonarison *et al.*, (2006) who reported necropsy the gross pathological lesions were dehydrated and darkened carcass, hemorrhages on pectoral, leg and thigh muscles.

Histopathological study revealed the finding as severe lymphoid depletion (Fig 10 & Fig 11), reactive cells infiltration by heterophils and macrophages in the interfollicular space (Fig 12). These lesions were in agreement with those described by Hoque *et al.,* (2001).

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSIONS

It is summarized that conditions showing marked depression, unsteady gait, ruffled feathers, whitish diarrhoea, atrophy of Bursa of Fabricius and sudden death is attributable to Infectious Bursal Disease virus (IBDV). At necropsy, haemorrhages were found in the breast and thigh muscles. Enlarged and haemorrhagic Bursa of Fabricious were found in primary stage. The Bursal folds become haemorrhagic. In histopathological study, severe lymphoid depletion was observed. Reactive cells infiltration by heterophils and macrophages in the interfollicular space. The prevalence is very high at the age of 4^{th} but low in 6^{th} age of chick. The occurrence of IBD outbreaks in sonali chicken 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-

i. Infectious Bursal Disease could be pathologically characterized and identified by necropsy and histopathological examination
ii. Average prevalence and mortality of IBD at Gaibandha district is 10.21% and 4.19% respectively
iii. The bird at the age of 4^{th} weeks revealed highest prevalence
iv. Ruffled feathers, whitish diarrhoea, vent picking, atrophy of Bursa of Fabricious and sudden death is attributable to Infectious Bursal Disease (IBD)

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APPENDICES

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.