PATHOLOGY OF BURSA OF FABRICIUS OF THE BIRDS VACCINATED WITH COMMERCIAL GUMBORO VACCINES

A THESIS

BY

MOST. REBA BEGUM

SEMESTER: MARCH - AUGUST, 2010
REGISTRATION NO.: 0905083
SESSION: 2009-2010

MASTER OF SCIENCE (M. S.)

IN

PATHOLOGY

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

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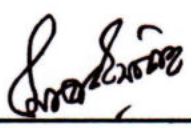

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DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
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DEDICATED

TO
MY

BELOVED HUSBAND AND SONS

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ABSTRACT

Pathology of bursa of Fabricius of the birds vaccinated with commercial Gumboro vaccine was studied. The course of the study was six months from December/2009 to May/2010. A total of 20 flocks were recorded and these flocks were previously vaccinated with commercially available Gumboro vaccines. The vaccination schedule and the instruction of the vaccination were strictly followed as per producers. The samples of the bursae were collected from the birds submitted to the Pathology laboratory during necropsy as well as during the physical visit of the farms. The clinical signs including morbidity and mortality rate, gross morbid lesions, histopathological lesions including bursal lesion scores were also determined. An apparently normal flock was included to compare the results. The morbidity rate and mortality rate was around 100% and 7-40%, respectively. Gross morbid lesions and histopathological features of the bursa of Fabricius were as typical as the naturally Gumboro affected flock. Maximal bursal lesion score was found in these affected flocks.

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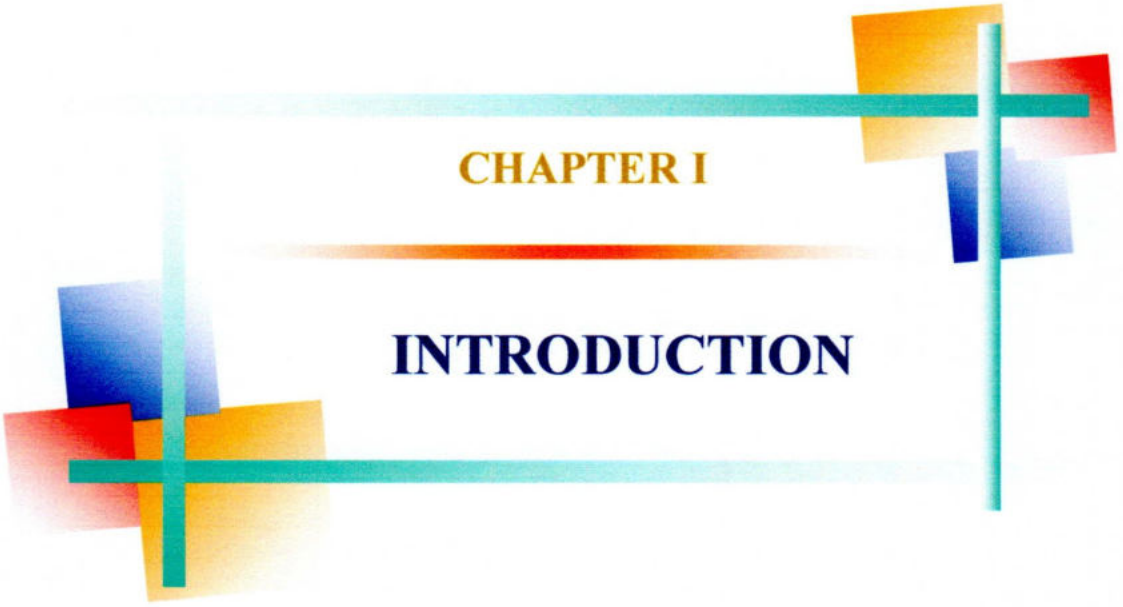
ABBREVIATION AND SYMBOLS

α	:	Alpha
γ	:	Gamma
µg	:	Microgram
µl	:	Microlitre
@	:	At the rate of
AGPT	:	Agar gel precipitation test
Ala	:	Alanine
B/BW	:	Bursa/body weight
HEPES	:	Hydroxy Ethyl Piperazine Ethene Sulfonic acid
BF	:	Bursa of Fabricius
CAM	:	Chorio-allantoic membrane
cDNA	:	Complementary deoxy ribonucleic acid
CEF	:	Chicken embryo fibroblast
cm	:	Centimeter
CPE	:	Cytopathic effect
D	:	Day
d.p.i.	:	Days post inoculation or days post infection
ds	:	Double-stranded
ELISA	:	Enzyme -linked immunosorbent assay
g	:	Gram
Glu	:	Glutamine
His		Histidine
IBD	:	Infectious bursal disease
IBDV	:	Infectious bursal disease virus
IFN	:	Interferon
lbs	:	Pounds
Ltd	:	Limited
MDA	:	Maternally derived antibody
mg	:	Milligram
ml	:	Milliliter
rim	:	Nanometer
°C	:	Degree centigrade
ORF	:	Open reading frame
p.i.	:	Post inoculation or post infection

PBS	:	Phosphate buffered solution
RNA	:	Ribonucleic acid
S/BW	:	Spleen/body weight
SPF	:	Specific pathogen free
Sq.	:	Square
TCID50	:	50% tissue culture infective dose
Thr	:	Threonine
TNF	:	Tumor necrosis factor
VNT	:	Virus neutralization test
VP	:	Virus protein
vv	:	Very virulent
vvIBDV	:	very virulent infectious bursal disease virus
W/V	:	Weight/volume
et al	:	And his associates
etc.	:	Etectera
Fig	:	Figure
H & E	:	Hematoxylin and Eosin
Min	:	Minute
hr	:	Hour
MS	:	Master of Science
No.	:	Number
PBS	:	Phosphate buffered saline
Sec	:	Second
UV	:	Ultraviolet



CHAPTER I

INTRODUCTION

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INTRODUCTION

Infectious bursal disease is an acute contagious viral disease of young chickens caused by an RNA-virus Birnavirus (Murphy *et al.* 1995) which is classified as a member of the genus avibirnavirus (Pringle, 1998) of the Family Birnaviridae (Dobos *et al.*, 1979; Brown, 1986).

The disease was first reported in USA in 1962 by Cosgrove and was referred to as "avian nephrosis" because of the extensive kidney damage in birds that succumbed to the infection (Cosgrove, 1962); and subsequently in other countries including Bangladesh (Rahman, 1994; Chowdhury *et al.*, 1996)

Although, IBD is primarily a disease of young (between 3 and 6 weeks) and unimmunized chickens, reports of the disease in vaccinated chicken flocks aged 16-20 weeks have been reported (Okoye and Uzoukwu, 1981; Durojaye *et al.*, 1984; Awolaja and Adene,1995).

Infectious bursal disease (IBD) or Gumboro disease is an acute, highly contagious viral disease of young chickens characterized mainly by severe lesions in the bursa of Fabricius (BF) followed by immunosuppression (Rosenberger and Gelb, 1978; Saif, 1994; Lukert and Saif, 1997). Infectious bursal disease virus (IBDV), the oetiological agent of Gumboro disease has been widely studied mainly for two reasons:
Firstly, the highly contagious virus can cause severe economic losses in poultry industries resulting from both the high mortality in the acute course of the disease and the consequences of B cell-dependent immunodeficiency (Muller *et al.*, 1992; Lasher and Shane, 1994; Lukert and Saif, 1997; Nagarajan and Kibenge, 1997; van den Berg, 2000).

Secondly, the pathological mechanism of IBDV is yet difficult to explain and interesting since only one organ system, the bursa of Fabricius, is almost exclusively involved (Hirai and Calnek, 1979; Kaufer and Weiss, 1980).

The effects of IBDV in chickens have been extensively reviewed (Lukert and Saif, 1997, van den Berg, 2000). The severity of these effects varies with the virulence of the field virus, age of the birds, and the maternally derived antibodies (MDA) (Lucio and Hitchner, 1979).

There are two distinct serotypes of IBDV: serotype 1 and serotype 2. Both serotypes can infect chickens and turkeys, but clinical disease is recognized only in chickens (Jackwood and Saif, 1987; Lana *et al.* 1992; Hassan and Saif, 1996; Yamaguchi *et al.* 1996a). Serotype 1 has three pathotypes: classical virulent, very virulent and antigenic variant. Very severe clinical outbreaks with high mortality rates caused by very virulent IBDV (vvIBDV) have been reported in Europe (van den Berg *et al.*, 1991; van den Berg, 2000), Africa (Zierenberg *et al.*, 2000), South America (Di Fabio *et* al.,1999), Asia (Nunoya *et al.*, 1992; Chen *et al.*, 1998; To *et al.*, 1999) including Bangladesh (Rahman,1994; Chowdhury *et al.*, 1996; Islam *et al.*, 1997) Bangladeshi strains of IBDV have been found to be antigenically and genetically similar to other very virulent strains (Islam *et al.*, 2001a; Hoque *et al.*, 2001). IBDV is now the major killer disease in the poultry farms of Bangladesh.

IBDV is exclusively a lymphotrophic virus targeting and destroying the growing B lymphocytes bearing cell-surface IgM (Hirai and Calnek, 1979; Nakai and Hirai, 1981), developing the severe morphological alteration of BF (Winterfield and Hitchner, 1962; Lukert and Saif, 1997), and producing a profound immunosuppression (Ivan *et al.*, 2001). The immunosuppression prevents the birds from optimally responding to vaccine (Winterfield and Thacker, 1978; Sharma *et al.*, 1984), and ultimately leads to increase in the incidence of numerous concurrent bacterial (Wyeth, 1975), viral

(Giambrone *et al.*, 1977; Rosenberger and Gelb, 1978), protozoal (Anderson *et al.*, 1977) and fungal (Chowdhury *et al.*, 1996) infections as well as microbial toxicosis (Somvanshi and Mohanty, 1993).

There is no alternative of vaccination in the prevention of IBD, although the clinical outbreaks are also reported in vaccinated flocks (Chettle *et al.*, 1989; van den Berg *et al.*, 1991; Eterradossi *et al.*, 1992; Muhammad et *al.*, 1996; Hafez *et al.*, 2002). Neutralization of vaccine virus by the neutral antibodies is considered to be one of factors causing vaccination failure. To overcome this problem stronger vaccine with higher residual pathogenicity has been developed to withstand maternal antibodies (Kouwenhoven and van den Bos, 1994). The antigenic variation among viruses also may causes vaccination failure, mainly when antigenic structures among field and vaccine strains no longer coincide (Jackwood and Saif, 1987: Cao *et al.*, 1998; van den Berg, 2000).
IBDV is highly infectious and very resistant to inactivation. There is none alternative without vaccination to prevent IBD or Gumboro disease (Lukert and Saif, 1997), but the outbreaks in the vaccinated flocks are also reported (Muhammad *et al.*, 1996; Hafez *et al.*, 2002). In order to control IBD with live vaccines, it is critical to vaccinate commercial have the ability to overcome the maternal antibody at a certain level. Vaccination during low maternal antibody titre shows better immune response than high maternal antibody titre (Giasuddin *et al.*, 2003). But it is very much difficult to field based determine when maternal antibodies in chicks will decline to levels that vaccine can overcome as well as the optimum time of vaccination.

The apparent inability to control IBDV infections through vaccination sometimes may be due to improper administration of vaccine virus, antigenic differences among the viruses (Rosenberger *et al.*, 1987), insufficient potency of the live attenuated vaccine virus (Ismail and Saif, 1991), interference between the residual maternally derived antibodies and the vaccine virus (Eterradossi, 2001). The

vaccine prepared from classical strain did not give protection against variant IBDV strains (Snyder, 1990). Again, the immunogenicity of the virus may differ between strain to strain (Rosales *et al.*, 1989a, b, c; Abdel-Alim and Saif, 2001). This study was conducted for the field based evaluation of the pathogenicity of the viruses responsible for the outbreak in the vaccinated flocks and to investigate the probable causes of vaccination failure.

OBJECTIVES

- To study the morbidity and mortality rate of IBD in vaccinated flock.
- To identify the causes of outbreak of Gumboro disease in vaccinated flocks.

CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Available literature for the determination of Pathology of bursa of Fabricius of the birds vaccinated with commercial Gumboro vaccines in commercial chicken is reviewed in this part of the thesis after a brief overview on the history, epidemiology, oetiology, pathogenesis and pathology, clinical manifestations, immunosuppressive effects, and immunization strategies against IBD.

2.1. HISTORY OF IBD AND IBDV

The syndrome which emerged in 1957 (Cover, 1960) was formally documented by Cosgrove (1962) in broiler flocks located near the town of Gumboro, Delware, USA, while gave the common eponym of the malady as `Gumboro disease'. Originally the condition was referred to as 'avian nephrosis' or 'nephritis-nephrosis syndrome of chickens' because of prominent kidney lesions (Cosgrove, 1962). Subsequently, the disease was called infectious bursal disease (IBD) because of the consistent involvement of the bursa of Fabricius (Hitchner, 1970). 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).

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).

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 (Bhattacharjee *et al.*, 1996; 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 genom segments of a vvIBDV from Bangladesh (BD-3/99) has been established and full-length cDNA clones corresponding to the both segments have been established (Islam *et al.*, 2001 b).

2.2. EPIDEMIOLOGY

2.2.1. Geographical distribution and prevalence of IBD

IBDV is worldwide distributed (Eterradossi, 1995; Lukert and Saif, 1997). Australia, Newzealand, Canada and the US are so far unaffected (Snyder, 1990; Proffitt *et al.*, 1999; Sapats and Ignjatovic, 2000). Australia has remained free of vvIBDV mainly due to geographical isolation and strict quarantine barriers.

2.2.2. Host ranges

Domestic fowls are the natural host of IBDV (Helmboldt and Garner, 1964). Natural infection of turkeys and ducks have also been recorded (Page *et al.*, 1978; McNulty *et al.*,1979; McFerran *et al.*, 1980; Johnson *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).The couternix quail is not infected with a chicken strain of IBDV (Weisman and Hitchner, 1978).

Antibodies against IBDV have been detected in various wild birds like penguines (Gardner *et al.*, 1997), commercially raised ostrich (Ley *et al.*, 2000), wild ducks, crow,

goose (Wilcox *et al.*, 1983; Hollmen *et al.*, 2000), which may mean that wild birds may act as targets or reservoirs (Wilcox *et al.*, 1983; Gardner, *et al.*, 1997; Ogawa *et al.*, 1997a; Hollmen, *et al.*, 2000).

2.2.3. Breeds susceptibility

Lighter breeds show severe reaction to IBDV infection than heavier ones (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, Meroz (1966) found no difference in the mortality between heavy and light breeds in a survey of 700 outbreaks of the disease.

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

Chickens of 3-6 weeks of age are more commonly affected (Ley *et al.*, 1983). Sub-clinical infection has been reported to occur in chicks less than 2 weeks of age (Allan *et al.*, 1972; Ley *et al.*, 1979) and even in newly hatched chicks (Fadley and Nazerian, 1983). Clinical disease also occurred in chickens up to 18 weeks of age (Ley *et al.*, 1979 and 1983).

2.2.5. Sources and transmission of infection

Infected chickens shed IBDV one day after infection and can transmit the disease for at least 14 days (Vindevogel *et al.*, 1976) 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). According to another report, houses that contained infected birds were infective for innate birds after 54 and 122 days (Benton *et al.*, 1967a). No egg transmission of IBDV has yet been reported.

2.2.7. 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).

2.2.8. Morbidity and mortality rates

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

Morbidity could be 100% and mortality could reach up to 80% in field outbreaks (Chowdhury *et al.*, 1996; Islam et *al.*, 1997; Hoque *et al.*, 2001). Experimentally, infection to SPF chickens with vvlBDV causes 90-100% mortality (Chettle *et al.*, 1989; van den Berg *et al.*, 1991; Wenky *et al.*, 1994). The genetically engineered tissue culture adapted vvlBDV did not show any mortality in SPF chickens (van Loon *et al.*, 2001).

2.2.9. Factors influencing the pathogenicity

Several virus 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	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 al.*, 1996
	Levels of MDA	lordanides *et al.*, 1991

2.3. OETIOLOGY

2.3.1. Classification of IBDV

Family: Birnaviridae

Genus: Birnavirus

Sub-genus: Avibirnavirus

Species: Infectious bursal disease virus

2.3.2. 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 turkyes (Jackwood *et al.*, 1980 and also from chickens (Ismail *et al.*, 1988). Serotype 1 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). Serotype 1 field viruses are further categorized as classical virulent, antigenic variant and very virulent depending on their pathogenicity and/or antigenicity (Jackwood and Saif, 1987; Lasher and Shane, 1994). Recently, emerged very virulent pathotypes of IBDV are closely related to classical serotype 1 strain of IBDV (Box, 1991; 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 demonstrate their similarities with recent European, Aisan and African vvlBDV 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. Gradations in the pathogenicity of different serotypes and pathotypes are shown below (Fig. 1)

2.3.3. Morphology and physico-chemical properties

IBDV is a small, non enveloped virus with icosahedral symmetry (Hirai and Shimakura, 1974). IBDV particles have a diameter of 55-60nm (Hirai and Shimakura, 1974; Nick *et al.*, 1976) and posses a bisegmented double-stranded RNA genome (Dobos *et al.*, 1979; Muller *et al.*, 1979a; Muller and Becht, 1982; Kibenge *et al.*, 1988). The virus has a buoyant density of about 1.32 g/ml and the molecular weight of RNA is close to 2×10^6 dalton (Nick *et al.*, 1976).The virus consists of four structural proteins, VP1 through VP4 (Nick *et al.*, 1976; Dobos *et al.*, 1979) and the molecular weight of VP1, VP2, VP3 and VP4 polypeptides is 11000, 50000, 35000 and 25000 daltons, respectively (Nick *et al.*, 1976). The capsid proteins (VP2 and VP3) are arranged in the capsid of 32 capsomeres (Hirai and Shimakura, 1974).

IBDV is resistant to a temperature of 56°C for 5 hours (Benton *et al.*, 1967b). The virus is inhibited by formalin and wescodyne but not by chloroform, phenol, either, thimerosal and thymine 2389 (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 becomes inactivated when exposed to 1% formalin, 1% creasol and 1% phenol for one hour (Cho and Edgar, 1969). Chloramine (0.5%) killed the virus after 10 minutes (Landgraf *et al.*, 1967). 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, giuteraldehyde and a complex disinfectant containing formaldehyde, gluteraldehyde and alkyldimethyl benzylammonium are suitable disinfectants effective against IBDV (van der Sluis, 1994).

2.4. CLINICAL MANIFESTATIONS

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). 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 of IBD is 2-3 days (Cho and Edgar, 1972; Hirai *et al.,* 1974). During the acute phase of IBDV infection, the symptoms are similar to that observed in a septic shock like syndrome (Stocquardt *et al.,* 2001) or very similar to what is observed in acute coccidiosis. It has been shown that ChIFN (Yun *et al.,* 2000; Rothwell *et al.,* 2000) and TNF (Zhang *et al.,* 1995) might play an important role in the onset of the clinical signs. The disease is characterized clinically by marked depression, prostration, ruffled feathers, whitish or watery diarrhoea, inappetance or anorexia, dehydration, emaciation, progressive weakness, reluctance to move, vent picking, 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; Islam *et al,* 1997; Thangavelu *et al,* 1998; van den Berg, 2000). Morbidity could be 100% and mortality could reach upto 80% in field outbreaks (Chowdhury *et al,* 1996; Islam *et al.,* 1997; Hoque *et al,* 2001). Experimentally, infection to SPF chickens with vvIBDV causes 90-100% mortality (Chettle *et al,* 1989; van den Berg *et al.;* 1991). The wild-type vvIBDV strain and a virus generated by reverse genetics technology showed 100% morbidity but a tissue culture adapted vvIBDV strain did not show any clinical manifestation in SPF birds (van Loon *et al.,* 2001).

2.5. PATHOGNESIS AND/OR IMMUNOPATHOGENESIS OF IBD

2.5.1. Apoptosis

Apoptosis has been shown to be one of the major mechanisms by which IBDV causes lesions (Eterradossi, 2001). Some IBDV strains induce apoptosis of bursal lymphocytes (Vasconcelos and Lam, 1995), but this was not confirmed with another IBDV strains (Hill and Sharma, 1999). Apoptosis has also been demonstrated in peripheral blood lymphocytes (Vasconcelos and Lam, 1995) and chickens embryo fibroblasts (Tham and

Moon, 1996) when infected *in vitro* with IBDV. Both IBDV positive and IBDV negative cells of bursa of Fabricius (Tanimura and Sharma, 1998; Nieper *et al.,* 1999), and antigen negative cells of thymus (Tanimura and Sharma, 1998) are died by apoptosis in IBDV infected chickens. IBDV probably induces apoptosis indirectly in nonbursal organs (Eterradossi, 2001). IBDV induced protein VP5 plays the crucial role in the pathogenesis of IBD (Yao *et al.,* 1998) and the degree of intensity of apoptotic death is mediated by this protein (Yao *et al.,* 1998; Raue *et al.,* 2000). During the replication of IBDV in growing B lymphocytes the viral proteins induce apoptosis, resulting in a rapid depletion of B lymphocytes (Vasconcelos and Lam, 1995; Jungmann *et al.,* 2001).

A population of proliferating lymphoblasts, representing about 20% of the total population of the bursa lymphocytes was identified as target cells (Muller, 1986). These observations are in accordance with the presence of IBDV specific antigens in avian cells (Cursiefen 1980; Lange 1985; Muller, 1986; Burkhardt and Muller, 1987).

2.5.2. Role of T cells in the pathogenesis

IBDV infection leads to the dramatic accumulation of T cells (Tanimura and Sharma, 1997; Kim *et al.,* 1999; Kim *et al.,* 2008; Sharma *et al.,* 2001) around the site of virus replication, concurrently to B cells depletion in the bursa (Kim *et al.,* 2000), but IBDV does not multiply within the T lymphocytes (Cursiefen, 1980).

CD4+ and CD8+ cells are present in the bursa in similar proportion in the early infection, but later, mainly the CD8+ cells remain (Sharma *et al.,* 2000). Early after IBDV infection the role of bursal T cells are as follows:

- Expression of high levels of MHC class II and 11-2 receptors
- Proliferation when stimulated *in vitro* with IBDV antigens but have a reduced response to T cell mitogens such as ConA (Sharma *et al.,* 2000).
- Inhibition of the mitogenic response of normal splenocytes by a soluble fact produced by themselves (Sharma *et al., 2001)* or CD4+ or CD8+ cells (Kim and Sharma, 2000).

In late stage of IBDV infection, bursal T cells play an important role in the recovery (Kim *et al.*, 2000).

The possible role of IBDV on antigen presenting cells or impairment of T cells function need to be further investigated. Indeed, the effect of IBDV infection on cell mediated immunity is still not fully understood (Eterradossi, 2001). IBDV modulates T cells function (Sharma *et al.*, 2001; Stocquart *et al.*, 2001).

Experimentally induced T cell immunodefiency modulate the IBDV pathogenesis as follows (Kim *et al.*, 2000; Rautenschlein *et al.*, 2001; Sharma *et al.*, 2001):

- The viral antigen load in the BF becomes significantly higher.
- The severity of local inflammatory response in the bursa is increased.
- The incidence of apoptotic bursal cells is increased.
- The follicular recovery becomes significantly faster.

2.5.3. Role of chemokines in the pathogenesis

There are various chemical mediators such as IFNγ (Kim *et al., 2000),* TNFα (Klasing and Peng, 1990; Kim *et al.*, 1998), nitric oxide (NO) (Green *et al.*, 1982; Kim *et al.*, 1998), interleukins (Kim *et al.*, 1998) that are produced by the biological interaction between IBDV and host cells. The acute IBDV infection induce the development of a septic shock like syndrome as in acute coccidiosis where IFNγ (Yun *et al.*, 2000; Rothwell *et al.*, 2000) and TNLFα (Zhang *et al.*, 1995) might play an important role in the onset of the clinical signs and be involved in the susceptibility to infection. Nitric oxide (NO), TNFα may promote the cellular destruction (Kim *et al.*, 1998) and ChIFNα is able to activate macrophages (Digby and Lowenthal, 1995; Karaca *et al.*, 1996). Excessive or insufficient production of cytokine may contribute significantly to the pathophysiology of the disease (Koghut, 2000).

2.5.4. Role of immune complexes in the pathogenesis

Previously the disease was recognized as avian nephrosis as because of its prominent kidney lesions (Cosgrove, 1962). Lodging of immune complexes in the glomeruli of IBDV infected chicks reveals its important role in the pathogenesis of IBDV infection in chickens (Ley and Yamamoto, 1979).

2.5.5 Role of bursal secretory dendritic cells (BSDC) in the pathogenesis

Principally, the BSDC plays the role in the transportation of IBDV to the different organs (Olah *et al.*, 2001).

2.5.6. General cyclic sequence of IBD

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 viraemia (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 viraemia

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. Organs affected

Bursa of Fabricius is the principal target organ of IBDV (Cheville, 1967; Hirai and Calnek, 1979; Kaufer and Weis,1980; Lukert and Saif, 1991; Tsukamoto *et al.*, 1995b; Tanimura *et al.*, 1995; Elankumaran *et al.*, 2001) 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 swollen (Mohanty *et al.*,1971; Chowdhury *et al.*, 1996), oedematous (Chowdhury *et al.*,1996; Czifra and Jonson, 1999), hemorrhagic (van der Sluis,1994; Chowdhury *et al.*,1996; Haque *et al.*,2001) bursa , cheesy mass within the bursal lumen (Chowdhury *et al.*,1996) and finally, atrophy of the bursa (Jhala *et al*,.1990; Chowdhury *et al.*, 1996). The bursa/ body weight ratios are lower than normal (Rosales *et al.*, 1989c; Thangavelu *et al.*, 1998).

The degree of virulence is assessed by the measurement of bursa/ body weight indices and bursal damage (Mazariegos *et al.*, 1990). Chickens vaccinated with intermediate strain exhibit low B/BW indices (Mazariegos *et al.*, 1990). Chickens inoculated with bursa derived and tissue culture attenuated classical or variant serotypes have significantly smaller bursa and larger spleen than the uninoculated control (Hassan *et al.*, 1996).

2.6.2.2. Spleen

Spleen becomes swollen (Chowdhury, *et al.*, 1996), enlarged (Rinaldi *et al.*, 1965) or may become atrophied (Chowdhury *et al.*, 1996), sometimes mottling and paler than normal in appearance (Chowdhury *et al.*, 1996). Haemorrhages are common (Cho and Edgar, 1972; Hoque *et al.*, 2001) and small gray and whitish foci may be present (Rinaldi *et al.*, 1965; Ley *et al.*, 1979).

2.6.3.3. Caecal tonsil

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

2.6.2.4. 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.

2.6.2.5. Kidneys

The kidneys become swollen (Ley *et al.*,1979; van der Sluis, 1994; Chowdhury, *et al.*,1996 ; van den Berg, 2000), 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,

ureters filled with urates (Cosgrove, 1962), hyperemia, subcapsular haemorrhages and pronounced hydronephrosis (Somvanshi *et al.* 1992) are also reported.

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) are also reported.

Others

The carcass is grossly characterized as good bodily condition (Cosgrove, 1962), dehydration of the fascia and musculature (Gosgrove, 1962; Chowdhury *et al.*, 1996; Rudd *et al.*, 2001) and emaciation (Chowdhury *et al.*, 1996). Echymotic hemorrhages are found in the thigh and/or breast muscles (Cosgrove, 1962; Schat *et al.*, 1981; Lukert and Hitchner, 1984, Chowdhury *et al.*, 1996; Hoque *et al.*, 2001), skeletal muscles are darkly discoloured (Nunoya *et al.*, 1992) and hemorrhages also found at the junction between the gizzard and proventriculus (van der Sluis, 1994; Chowdhury *et al.*, 1996; Islam *et al.*, 1997; Thangavelu *et al.*, 1998; Hoque *et al.*, 2001).

2.6.3. Histopathology

2.6.3.1. Bursa of Fabricius

Varying degrees of lymphocytic depletion from the follicles (Islam *et al*, 1997; van Loon *et al.*, 2001; Rautenschlein *et al.*, 2001; Rudd *et al.*, 2001; Hoque *et al.*, 2001; Franciosini and Coletti, 2001), interfollicular oedema (Czifra and Jonson, 1999; Hoque *et al.*, 2001; Franciosini and Coletti, 2001), heterophilic infiltration in the interfollicular space (Tanimura *et al.*, 1995) 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), formation of

cystic spaces within the fillicles (Hoque *et al.*, 2001; Franciosini and Coletti, 2001) as well as in the bursal epithelium, haemorrhages and congestion in the bursa, thickness and oedematous serosa and finally follicular atrophy (Franciosini and Coletti, 2001) have been reported. Infiltration of macrophages in the follicles (Tanimura *et al.*, 1995) necrosis of lymphocytes with pyknotic and karyorrhectic nuclei (Islam *et al.*, 1997) in the follicles and varying degree of follicular regeneration were also recorded.The pathogenicity and the degree of lesions varies according to the strain involved (Cheville, 1967; Ley *et al.*, 1983; Rosales *et al.*, 1989a; Sharma *et al.*, 1989; Nunoya *et al.*, 1992).

Depending on the residual virulence of the attenuated virus, some vaccine strains can also cause bursal damage (Mazariegos *et al.*, 1990) and induce immunosuppression (Muskett *et al.*, 1979; Edward *et al.*, 1982; Reece *et al.*, 1982). Highest bursal lesions score occur in chickens vaccinated with intermediate strain, followed by mildly attenuated strain (Mazariogos *et al.*, 1990; Tsukamoto *et al.*, 1995a). The intermediate strain caused extensive bursal damage but follicular repopulation was detected, whereas, there was absence of repopulation in chickens inoculated with virulent strain (Rautenschlein *et al.*, 2001).

The intermediate vaccine strain of IBDV caused lymphocytic depletion (Mazariegos *et al*, 1990; Franciosini and Coletti, 2001), acute necrosis (Mazariegos *et al.*, 1990; Tsukamoto *et al.*, 1995a; Franciosini and Coletti, 2001; Rautenschlein *et al.*, 2001), follicular atrophy (Mazariegos *et al*, 1990; Franciosini and Coletti, 2001), inflammation (Mazariegos *et al.*, 1990) and bursal damage (Muskett *et al.*, 1979; Tsukamoto *et al.*, 1995a; Rautenschlein *et al.*, 2001), cyst formation (Tsukamoto *et al*, 1995a; Rautenschlein *et al.*, 2001; Franciosini and Coletti, 2001), and increase of interstitial connective tissue (Franciosini and Coletti, 2001).

2.6.3.2. Spleen

Histopathological appearance of the spleen of the IBDV infected brids are characterized as lymphocytic depletion with marked haemorrhages (Chowdhury *et al.*, 1996; Islam *et al.*, 1997), thickening of the arterial wall with fibrinoid degeneration (Chowdhury *et al.*, 1996), 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), periarteriolar lymphoid and periellipsoid lymphoid sheaths (Tanimura *et al.*, 1995) and splenic hyperplasia of the white pulp with cell death (Rautenschlein *et al.*, 2001).

2.6.3.3. Caecal tonsils

Varying degrees of lymphocytic depletion (Nunoya *et al.*, 1992; Tanimura *et al.*, 1995; Chowdhury *et al.*, 1996; Islam *et al.*, 1997), associated with severe haemorrhages (Islam *et al.*, 1997), macrophage and heterophilic infiltration (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 lymphocytic elements of the caecal tonsils are replaced by macrophages and heterophils (Nunoya *et al.*, 1992).

2.6.3.4. 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.

2.6.3.5. 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; a large oedematous space between many tubules and collecting ducts (Henry *et al.*, 1980) are found in the kidneys of IBDV infected birds.

2.6.3.6. Liver

Congestion in the central vein (Chowdhury *et al.*, 1996), fatty changes, necrosis of hypatocytes (Nunoya *et al.*, 1992; Chowdhury *et al.*, 1996) and dilatation of the sinusoids of the liver (Nunoya *et al*, 1992) are reported.

Others

Reduced number of haemopoietic cells and a greater decrease in myelocyte numbers in the extra-sinusoidal spaces, erythrocytes in the sinusoidal spaces (Tanimura *et al.*, 1995); congestion, hemorrhages and alveolar emphysema in the lungs (Islam *et al.*, 1997) are reported.

2.7. IMMUNOSUPPRESSIVE EFFECTS

IBDV drew the attention of avian virologists mostly because of its severe immunosuppressive effects (Allan *et al.*, 1972). Actively dividing (Lasher and Shane, 1994; Lukert and Saif, 1997; Nagarajan and Kibenge, 1997) or growing (Lukert and Saif, 1997) or differentiating (Hirai, 1979) or IgM bearing (Hirai and Calnek, 1979; Rodenberg *et al.*, 1994) B lymphocytes 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; 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; Thangavelu *et al.,* 1998; Abdel-Alim and Saif, 2001).

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). So, IBDV infection causes immunosuppression and the immunosuppression ultimately leads to increase the incidence of many diseases

2.8. IMMUNIZATION STRATEGIES AGAINST IBDV

IBD can be controlled by vaccination (Hitchner, 1971; Rosales *et al.*, 1989b; Ismail and Saif, 1991; Lukert and Saif, 1997), but the outbreaks in the vaccinated flocks are also reported (van den Berg *et al.*, 1991; Eterradossi *et al.*, 1992; Muhammad *et al.*, 1996; Hafez *et al.*, 2002). The apparent inability to control IBDV infections through vaccination sometimes may be due to improper administration of vaccine virus, antigenic differences among the viruses (Rosenberger *et al.*, 1987; Snyder, 1990; Jackwood and Jackwood, 1997), insufficient potency of the live-attenuated vaccine virus (Ismail and Saif, 1991), interference between the residual maternally derived antibodies and the vaccine virus (Wyeth and Cullen, 1978; Lukert and Saif, 1997; Eterradossi, 2001).

The vaccine prepared from classical strain did not give protection against variant IBDV strains (Snyder, 1990). Again, the immunogenicity of the virus my differ between strain to strain (Rosales *et al.*, 1989a, b, c; Mazariegos *et al.*, 1990; Tsukamoto *et al.*, 1995a; Thangavelu *et al.*, 1998; Abdel-Alim and Saif, 2001).

The invasive vaccine strains are able to break through higher maternal antibody levels (Kouwenhoven and van den Bos, 1994). Therefore, the chicks could be immunized at an earlier age despite the presence of MDA (Kouwenhoven and van den Bos, 1994). Moreover, the better protection with more virulent strains of IBDV is due to more antigenic stimulation based on higher and longer replication in lymphoid tissues (Rautenschlein *et al.*, 2001).

There is no evidence of antigenic variation between classical and vvIBDV strains: and they belong to classical serotype 1 (van der Marel *et al.*, 1991; van den Berg *et al.*, 1991; Eterradossi *et al.*, 1992). No vaccine based on vvIBDV is yet commercially available, although the research work on the development of a vaccine with vvIBDV is still going on (van Loon *et al.*, 2001; Abdel- Alim and Saif, 2001). Recently, vvIBDV strains have been adopted to grow in CEF cell culture by genetic engineering (Lim *et al.*, 1999; Islam *et al.*, 2001b; van Loon *et al.*, 2001 and 2002) and residual pathogenicity of one of these has been tested in SPF chickens (van Loon *et al.*, 2001).The inactivated vaccine

made from the vvIBDV provided full protection against challenge with classical virulent strain as indicated by the low bursal body weight ratio (Abdel-Alim and Saif, 2001).

Table 3: General characteristics of live and inactivated vaccines for poultry

Live vaccines	Inactivated vaccines
Smaller quantity of antigen. Vaccination response relies on multiplication within the bird	Large amount of antigen. No multiplication after administration
Easily killed by chemicals and heat	Easier to store
Relatively inexpensive, easy to administer, and can be mass administered: drinking water, spray	Expensive to produce and to apply, since almost always individually administered
Adjuvanting live vaccines is not common	Adjuvanting killed vaccines is frequently necessary
Susceptible to existing antibody present in birds (e.g. maternal immunity)	More capable of eliciting an immune response in the face of existing antibody
In immune birds, booster vaccination is ineffective	In immune birds, additional immune response is frequently seen
Local immunity stimulated (i.e. trachea or gut)	Local immunity may be restimulated if used as a booster but secondary response is poor or absent
Danger of vaccine contamination (e.g. EDS)	No danger of vaccine contamination
Tissue reactions (commonly referred to as a 'vaccine reaction') are possible and frequently visible in a variety of tissues	No microbe replication; therefore, no tissue reaction outside that which is adjuvant dependent
Relatively limited combinations, due to interference of multiple microbes given at the same time (e.g. IB, ND and LT)	Combinations are less likely to interfere
Rapid onset of immunity	Generally slower onset of immunity

IB: infectious bronchitis, LT: laryngotracheitis, ND: Newcastle disease, EDS: egg drop syndrome

Table 4: Factors which interfere with vaccine efficacy in poultry

Type of factors	Impact on vaccine efficacy
Factors associated with the vaccine itself	
Virus serotype	Many infectious agents (e.g. infectious bronchitis virus) have different serotypes, and vaccine antigens do not provide protection against all field strains
Level of protection	Field strain of very high virulence, and/or highly attenuated vaccine strains
Factors associated with vaccine administration	
Handling	Certain live vaccines (e.g. live cell-mediated Marek's disease vaccines) are easily killed if mishandled
Diluent used	Viable vaccines administered in drinking water are destroyed if water sanitisers are not removed
Route	Vaccines administered by injection fail if vaccinators do not deliver the vaccine to the appropriate vaccination site
	Mass vaccination (drinking water and aerosol) tends towards lower uniformity than individual administration
Associations	Administration of certain combinations of live virus vaccines affects the single virus response if they have the same target tissues
Factors associated with the bird/flock	
Maternal immunity	In presence of high levels of maternal antibodies, live vaccines administered during the first two weeks of life may be neutralised
Immunosuppression	Stress, certain infectious agents (e.g. infectious bursal disease, infectious anaemia and Marek's disease in chickens, haemorrhagic enteritis in turkeys), mycotoxins (in particular

	aflatoxins) impair immune response
Sanitary status	The birds are already infected (incubation period) with the pathogen against which the vaccination is directed
Genetic factors	Different vaccine responses with respect to species or commercial hybrids
Management conditions	
Hygienic practices	Without clean-out and disinfection over successive flocks, the challenge dose might be too high or infection might occur too soon

2.9. FACTORS WHICH INTERFERE WITH IMMUNIZATION OF COMMERCIAL POULTRY FARM

Factors which interfere with immunization of commercial poultry can be divided into three main groups (PaulMcMullin 1985). They are:

- Factors associated with the vaccine itself,
- Those of vaccine administration, and
- Those which are endogenous to the bird.

2.9.1. The vaccine itself

All of The factors associated with the vaccine itself tend to be closely inter-related. A deficiency in one can be partially compensated by another. A vaccine of moderate-to-poor titre may give satisfactory results if very carefully applied, while it may be a disaster if poorly applied.

2.9.1.1. Vaccine Quality

Vaccine quality is sometime blamed when antibody titers are insufficient or disease breaks out in a flock. However, evidence shows that in the great majority of cases, vaccines are of excellent quality and are not responsible for the failure. To allay

concerns about vaccine quality, purchase only from reputable pharmaceutical companies whose products are manufactured under stringent quality control practices.

2.9.1.2. Vaccine Modifications

Commercial poultry companies may try to reduce costs by eliminating vaccines or administering partial doses. The decision to vaccinate is based on a risk analysis assessment. If the disease is not present, do not vaccinate. If it is a risk, the vaccine must be administered according to the recommendations of the manufacturer. When partial doses are given, birds will not get enough vaccine to properly stimulate their immune system.

2.9.1.3. Titre and Stability

It is self-evident that the live-virus vaccines must have an adequate titre and this titre must have sufficient stability so that under normal conditions it can cause an infection of appropriate intensity. Stability of live-virus vaccines is affected by the success of lyophilization and the temperature under which it is stored. Periods of validity must me strictly followed, or the vaccine re-titrated.

2.9.1.4. Inactivation and Adjuvant

These factors have similar importance for inactivated vaccines as do liophilization and titre for live vaccine. Type and quality of emulsion can influence the serological response to oil-adjuvant vaccines.

2.9.2. Administration of the Vaccine to the Bird

2.9.2.1. Administration and Handling of the Vaccine

A well designed vaccination program will not be effective if the vaccine is damaged by improper handling prior to administration. Live vaccines can be inactivated when

exposed to adverse conditions. Once a vaccine is reconstituted, the "time clock is ticking" for it to be used.

2.9.2.2. Vaccine Administration Deficiencies

Improper vaccine administration of the vaccine is the most common cause of vaccine failure in poultry. Prior to application of the vaccine, the details of the whole process must be well planned. This includes ensuring that the crew is trained in handling and applying the vaccine. The results of proper vaccination will be improved disease control and performance of the poultry. As one poultry grower commented, "Vaccines are no good if they do not get into the chicken."

2.9.2.3. Time of administration

Chickens may also already be incubating the disease at the time of vaccination. Despite proper administration, the birds become diseased because time is needed for antibody production to reach protective levels. Following first exposure to a live virus vaccine, antibody type G is detected approximately four to five days following exposure. Additional days are required for titers to reach protective levels.

2.9.2.4. Uniformity

In ultimate analysis it is essential that the antigen present in the vaccine is uniformly distributed within the flock. The use of "mass vaccination" (drinking water and aerosol) tends towards less uniformity in application than individual application, and need considerable operator care in order to control this tendency.

2.9.2.5. Association

Adiministration of certain combinations of live virus vaccines may affect the response to each virus, especially when they contain viruses which have the same target tissues (Beard & Brugh, 1975). It should be remembered, however, that in industrial poultry production the aim is maximum productivity and not necessarily maximum

protection against a given virus. Compromises must be reached. There is a number of live-virus combinations commonly used in practice. A combination Marek's disease/Fowl pox vaccination at one day of age has been shown to provide acceptable protection against both diseases (Heredia, 1977).

2.9.2.6. Vaccination Program

Each region typically has its own specific diseases. Thus it is not wise to try to develop a "one size fits all" or international vaccination program. In areas with a high density of poultry production, small flocks in close proximity to commercial flocks, or where farms have poor biosecurity and management practices, more comprehensive and intensive vaccination programs may be necessary.

When administering vaccines to flocks, records must include details on vaccine type, lot number and expiration date. In addition, details on chicken ages, route of administration and person administering the vaccine could be valuable when investigations are conducted at a later time. Without detailed records, it is often not possible to determine that a vaccination problem has occurred.

2.9.2.7. Diluent

The diluent used for live virus vaccines is very important to ensure that an adequate titre of virus actually reaches the birds. The classical problem of administering live-virus vaccine in chlorinated drinking water is well known, but less extreme.

2.9.3. Birds (endogenous factors)

The importance of adequate priming by prior exposure to the agent has been discussed above. It could be added that repeated exposure over too short a period may not be advantageous. Common practice dictates that the same vaccine should not be re-applied to a flock within 14 days.

2.9.3.1. Passive protection

Circulating antibody may effect the response to vaccination, even independently from the previous factor, i.e. when it is not produced by the bird itself. This may come about in two ways. Hyperimmune antiserum may be injected to provide passive protection but this is rarely used in commercial poultry today. The commonest source of passive protection is that transmitted from the breeder bird to her chick via the yolk. The baby chick has circulating antibodies in similar concentrations to those found in the breeder at 1-3 days of age. They fall to undetectable titres by 14 - 30 days (depending on the method of detection used).

With infectious bursal disease, on the other hand, maternal antibody plays an important part in avoiding infection and the adverse effects of field virus, but unfortunately it also prevents immunization with live-virus vaccines (Muskett *et al* 1979).

2.9.3.2. Immunosuppression

Stress of any sort is well known to reduce disease resistance and can also be expected to affect response to vaccination. Exceptionally poor environmental conditions could contribute to vaccination failure under some circumstances.The three infectious agents most associated with the immunological system and most capable of producing immunosuppression are infectious bursal (Gumboro) disease virus, Chick Anaemia Virus and Marek's disease virus. The latter can produce serious immunosuppression when it infects susceptible chicks early in life, but its other effects make it fairly easy to recognize. It has been well documented that early infection with infectious bursal disease virus in susceptible chicks can produce long-lasting immunosuppression against many diseases.

2.9.3.3. Maternal Antibodies

The immune status of the breeder flock can have an affect on the success of progeny vaccination. If the breeder flock has high levels of circulating antibodies which pass to the progeny through the egg, they may interfere with the replication of live vaccine viruses as they would for field challenge viruses. This will decrease the immune response to the vaccine because it is not stimulating the immune system as long and to as great an extent. For example, if a chick comes from a breeder hen with high levels of antibody against Infectious Bursal Disease (IBD), the chick will typically have high levels of maternal antibodies for several weeks. If vaccination is attempted in the presence of these antibodies, some of the vaccine virus will be neutralized and a decreased response to the vaccine results. On the other hand, delaying vaccination until maternal antibodies have been catabolized may leave the birds susceptible to field challenge.

2.9.4. Management Practices

Poor management practices in poultry flocks may contribute to vaccine failures. If infectious disease agents are allowed to build up in successive flocks without prior decontamination, it is possible that the challenge dose of a particular infectious agent will be large enough so that a normally effective vaccination program will be overwhelmed. In the long run, vaccines cannot replace a good management program.

Table 5: Vaccine delivery systems commonly used in the poultry industry: main advantages and disadvantages

Type of operation	Vaccination route	Diseases	Type of vaccine	Advantages	Disadvantages
Hatchery	In ovo	MD, IBD	Live and live cells mediated vaccines	Early protection; both the innate and adaptive immune responses are stimulated, 20,000-30,000 eggs per hour	Expensive equipment; training needed; poor early liveability due to possible fungal or bacterial contamination through the open hole in the egg
	Spray	IB, ND, coccidiosis	Live vaccines	Minimize handling, good mucosal immunity, inexpensive	Possible respiratory reaction (very small particle) particle size depends on relative humidity, temperature and hygiene
	S/c or i/m	MD	Live cell-mediated vaccines	Absence of respiratory reaction, uniform level of immunity, 1600 -2000 chicks per hour	Regular equipment sanitisation required; possible localised tissue damage; birds are stressed
On-farm	Drinking water	IBD, IB, ND	Most common route for live vaccines	Labour -saving, easy administration in drinking water	Improper/unequal distribution; inconsistency and variability of water quality, inactivation by impurities or residues; birds are stressed by water starvation
	Spray	IBD , IB, ILT ND	Live vaccines	Good mucosal immunity, mass application, minimize bird stress, inexpensive	Possible inconsistencies of vaccine dosages, possible respiratory reactions (in relation to particles size) need to target tissues that stimulate immunity.
	Intraocular/ , nasal drop	ILT, ND IBD.	Live vaccines	Effective and accurate vaccination type for live vaccines, uniform humoral and local immunity	Labour-intensive (individual handling); need to verify vaccine coverage

Table cond.					
	Wing web	Fowl pox, avian encephalomyelitis, fowl cholera	Live vaccines	May result in 95%-100% protection	Labour-intensive (individual handling); need to verify the 'vaccine take'; possible contamination at the injection site
	S/c or i/m	AI, MD, ND, salmonellosis	Most common route for inactivated vaccines	Use of inactivated vaccines (no spread of virus, no risk of residual virulence, stable). Uniform levels of immunity, low level of adverse of reactions	Labour-intensive (individual handling), possible localised tissue damage; use of inactivated vaccine (high costs), regular equipment sanitisation required

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. EXPERIMENTAL CHICKENS

The chickens of different small scale broiler farms were considered as experimental chickens. Gumboro outbreaks in the vaccinated flocks and the probable causes of vaccination failure were investigated at Dinajpur district of Bangladesh and the laboratory examinations were conducted at the Department of Pathology and Parasitology under the Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

A total 20 outbreaks in the vaccinated flocks were recorded during the course of the observation (Table 6).The number of birds in the farms was variable ranging from 250 to 1250 and they were rared on litter. The age and population of the birds per flock, biosecurity of the farms, previous history on Gumboro outbreaks, intervals between the batches, rearing of one more batches in the same farm at the same time, etc. were also recorded. An apparently normal flock was also included in this experiment for the comparison. The birds affected with IBD submitted to the Pathology laboratory for the diagnosis and treatment were the principal experimental chickens and some affected chickens were also collected physically.

3.2. RESEARCH AREA

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

Table 6: Grouping of different flocks and their relative clinical history

Experimental flocks	No. of incidences	Age of birds when reported (Day)	Morbidity (%)	Total No. of birds reared	No. of birds died	Mortality rate (%)	Birds examined at necropsy	Bursae examined at histopathology
Flock 1	4	17	Around 100	300	21	7	3-5 birds/ flock	3-5/ flock
Flock 2	7	19	Around 100	250	20	8	As above	As above
Flock 3	5	20	Around 100	400	28	7	As above	As above
Flock 4	3	25	Around 100	500	100	20	As above	As above
Flock 5	4	30	Around 100	350	35	10	As above	As above
Flock 6	6	32	Around 100	750	100	13	As above	As above
Flock 7	5	28	Around 100	1000	250	25	As above	As above
Flock 8	7	17	Around 100	500	50	10	As above	As above
Flock 9	4	29	Around 100	1200	360	30	As above	As above
Flock 10	6	18	Around 100	600	50	9	As above	As above
Flock 11	8	22	Around 100	900	150	17	As above	As above
Flock 12	11	27	Around 100	300	27	9	As above	As above
Flock 13	10	25	Around 100	250	25	10	As above	As above
Flock 14	4	29	Around 100	1100	350	32	As above	As above

Table contd.								
Flock 15	3	19	Around 100	500	150	30	As above	As above
Flock 16	9	31	Around 100	250	75	30	As above	As above
Flock 17	6	20	Around 100	750	200	27	As above	As above
Flock 18	7	21	Around 100	1250	500	40	As above	As above
Flock 19	10	26	Around 100	800	220	28	As above	As above
Flock 20	5	23	Around 100	500	150	30	As above	As above
Flock 21(Apparently normal)	0	0	0	0	0	0	As above	As above

3.3. RESEARCH PERIOD

The duration of experiment was 6 months from December, 2009 to May, 2010.

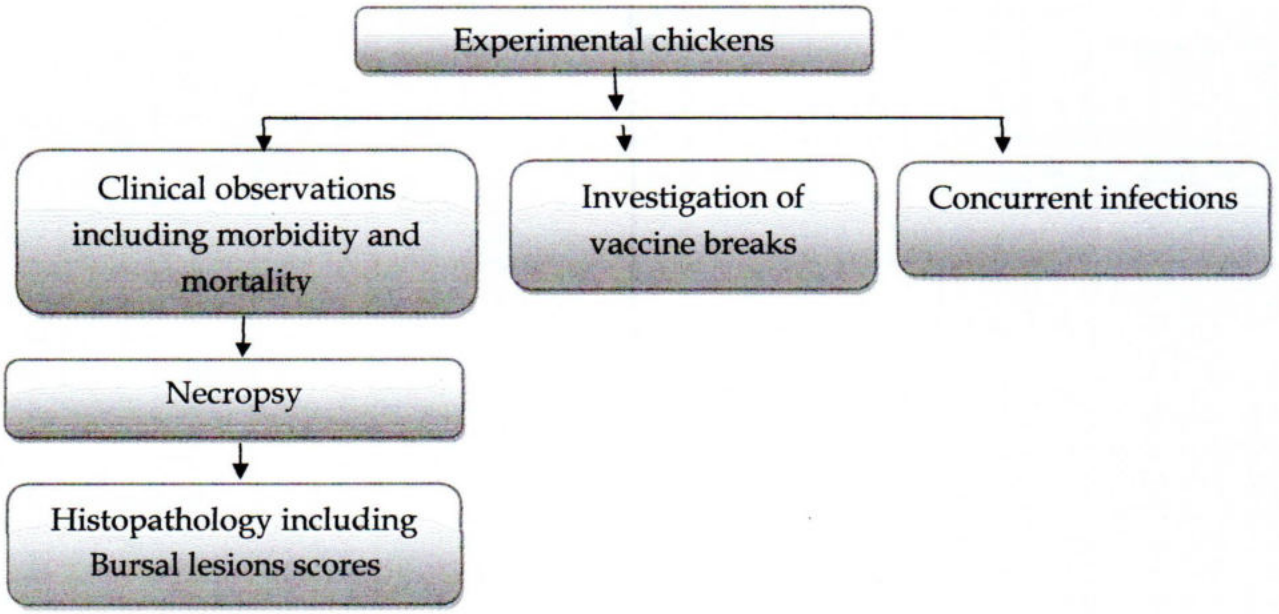
3.4. MANAGEMENT OF CHICKENS

The management systems of chickens of different farm were different. It was varied from farmer's experience, personal knowledge, efficiency and capacity.

3.5. SAMPLING OCCASION

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

3.6. EXPERIMENTAL DESIGN



3.7. CLINICAL OBSERVATION

The clinical signs were recorded during the physical visit of the affected flocks and the farmer's complaints about the affected birds were also considered. One apparently normal flock was also included in this study for the comparisons.

3.8. NECROPSY

Necropsy of birds obtained from different small scale broiler farms was done following a standard procedure (Charlton, 2000).

3.9. HISTOPATHOLOGY

During necropsy, various organs having gross lesions were collected, preserved at 10% formalin, processed, impregnated with paraffin, sectioned and stained with H&E for histopathological studies following a standard procedure (Luna, 1968).

3.9.1. Collection of bursa

Bursa of Fabricius was collected from each bird following standard procedures of necropsy (Charlton, 2000).

3.9.2. Preservation and processing of bursa of Fabricius

- Preservation of collected samples in 10% formalin solution for at least 3 days
- Trimming of preserved samples at suitable sizes
- Watering for overnight to remove formalin
- Dehydration in an ascending grades of alcohol
    - 50% alcohol: 1hr
    - 70% alcohol: 1hr
    - 80% alcohol: 1hr
    - 95% alcohol: 1hr
    - 100% alcohol: 3 changes and 1 hr for each change
- Chloroform treatment: 2 changes and 1.5 hrs for each change

- Impregnation by paraffinization at melting point (56^{0}C): 2 changes and 1.5 hrs for each change
- Blocking of the cooked tissue samples
- Sectioning at 5-7 µm in thickness, placing on water bath, taking on a glass slide and air dry

Routine haematoxylin and eosin (H & E) staining procedures

Preparation of Ehrlich's Haematoxylin solution

Chemicals	Amount
Haematoxylin crystals	4.0 g
Alcohol, 95%	200.0 ml
Potassium or ammonium alum	6.0 g
Distilled water	200.0 ml
Glycerine	200.0 ml
Glacial acetic acid	20.0 ml

Preparation of Eosin stock solution

Chemicals	Amount
Eosin Y, water soluble	1.0 g
Distilled water	20.0 ml
Alcohol, 95%	80.0 ml

Preparation of eosin working solution

Chemicals	Amount
Eosin stock solution	1 part
Alcohol, 80%	3 part

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

3.9.3. Protocol of H & E staining

- Xylene treatment: 3 changes and 3 minutes for each change
- Rehydration in descending grades of alcohol
  - 100% alcohol: 2 minutes
  - 95% alcohol: 2 minutes
  - 80% alcohol: 2 minutes
  - 70% alcohol: 2 minutes
  - Distilled water: 10 minutes
- Haematoxylin: 10-15 minutes
- Distilled water: 15 minutes
- Bluing in lithium carbonate: Few dips
- Eosin: 30 minutes
- Dehydration in ascending grades of alcohol
  - 80% alcohol: Few dips
  - 95% alcohol: Few dips
  - 100% alcohol: Few dips
- Xylene treatment: 3 changes and 3 minutes for each changes
- Mounting with Canada Balsam
- Examined under microscope using both low and high power objectives

3.10. INVESTIGATION OF VACCINE BREAKS

Investigation of probable vaccine breaks was done taking relevant flock history and record book. Conformation was ensured by farmer's complaints, clinical observations, necropsy examination and histopathology of the bursae of the affected birds.

3.11. CONCURRENT INFECTION

Different concurrent infections were also recorded. Conformation was ensured by clinical observations and necropsy examination.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4.1. PROBABLE CAUSES OF VACCINE BREAKS

Investigation of probable vaccine breaks was done taking relevant flock history and record book. Conformation was ensured by farmer's complaints, clinical observations, necropsy examination and histopathology of the bursae of the affected birds. The probable causes of vaccine breaks was enlisted bellow –

Table 7: Suspected factors of vaccination failure with their tentative interpretations

Suspected factors causing vaccination failure	Tentative interpretations on vaccination failure	No. of incidences
Previous history of Gumboro outbreaks	Virus loads in the farms and the birds of the newly batch (es) became exposed	4
Vaccination at early age (Between 7 to 10 days of age limit)	Inactivation of vaccine viruses by maternally derived antibodies	2
Vaccination beyond the optimal age limit	Vaccination after exposure	1
Intervals between the succeeding batches not more than 10-20 days	The shaded virus could be viable and the birds of the succeeding batch (es) might be infected	3
Single dosing without boosting	Insufficient immune response	4
Cold chain break during transportation, preservation and processing	Inactivation of the vaccine viruses	2
Completion of dosing taking prolonged time	The vaccine viruses might be inactivated and subsequent infection	1
Lower dosing	Insufficient immune responses	1
Rearing of one more batches of different ages in the same farms	Continuous exposure by different sources of IBDV	2

	infection	
Vaccination and disinfection simultaneously as spray or in drinking water	Inactivation of the vaccine viruses	2
Vaccination at stressful condition	Inadequate immune response	1
Vaccination through inappropriate drinking water	Inactivation of the vaccine viruses	2

4.2. MORBIDITY AND MORTALITY RATE

The morbidity rate of the affected flocks was around 100% and the mortality rate was variable ranging from 7- 40%. Highest mortality rate was recorded in flock 18.

4.3. CLINICAL MANIFESTATIONS

The clinical signs of the affected birds of the vaccinated flocks varied from farm to farm. The signs were clinically characterized as -

- Anorexia
- High fever
- Ruffled feather
- Reluctant to move
- Variable degrees of whitish /watery diarrhea
- Trembling
- Huddling together
- Severe prostration and death

4.4. NECROPSY, HISTOPATHOLOGY AND BURSAL LESION SCORE

The characteristic gross morbid lesions, the histopathological features of the bursae including the bursal lesion scores of the relevant flocks were mentioned in bellow -

Table 8: Gross and histopathological lesions with bursal lesion scores of the different flocks

Experimental flocks	Gross morbid lesions	Histopathological lesions	Bursal lesion scores
Flock 1	• Swollen,edematous bursa • Hemorrhage in bursa • Atrophy of bursa • Echymotic hemorrhage in thigh and / or breast muscle • Hemorrhage also found at the junction between the gizzard and proventiculus • Discoloured skeletal muscle	• Moderate to severe lymphoid depletion • Focal necrosis • Follicular atrophy and interfollicular edema • Cystic formation	2, 4, 3
Flock 2	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Infolding of epithelium • Cystic formation	4, 4,4
Flock 3	• As above	As above	4,4,4
Flock 4	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy • Cystic formation	4,4,4
Flock 5	• As above	• Interfollicular edema • Infolding of epithelium • As above	4,4,4
Flock 6	• Swollen, edematous bursa • Atrophy of bursa • Echymotic hemorrhage in thigh and / or breast muscle • Hemorrhage also found at the junction between the gizzard and proventiculus • Discoloured skeletal muscle	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Infolding of epithelium • Cystic formation	3,4,4

Table contd.			
Flock 7	• Swollen,edematous bursa • Hemorrhage in bursa • Atrophy of bursa • Echymotic hemorrhage in thigh and / or breast muscle • Hemorrhage also found at the junction between the gizzard and proventiculus	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation • Infiltration of reactive cells	4,4,4
Flock 8	• As above	• As above	4,4,4
Flock 9	• As above	• As above	4,4,4
Flock 10	• As above	• Severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4
Flock 11	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4
Flock 12	• As above	• As above	4,4,4
Flock 13	• As above	• As above	4,4,4
Flock 14	• As above	• As above	4,4,4
Flock 15	• As above	• As above	4,4,4
Flock 16	• Swollen,edematous bursa • Atrophy of bursa • Echymotic hemorrhage in thigh and / or breast muscle • Hemorrhage also found at the junction between the gizzard and proventiculus	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4

Table contd.			
Flock 17	• Swollen,edematous bursa • Hemorrhage in bursa • Atrophy of bursa • Echymotic hemorrhage in thigh and / or breast muscle • Hemorrhage also found at the junction between the gizzard and proventiculus • Discoloured skeletal muscle	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4
Flock 18	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,3,4
Flock 19	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4
Flock 20	• As above	• Moderate to severe lymphoid depletion • Follicular atrophy and interfollicular edema • Cystic formation	4,4,4
Flock 21 (Apparently normal)	0	0	0, 0, 0

CHAPTER V

DISCUSSION

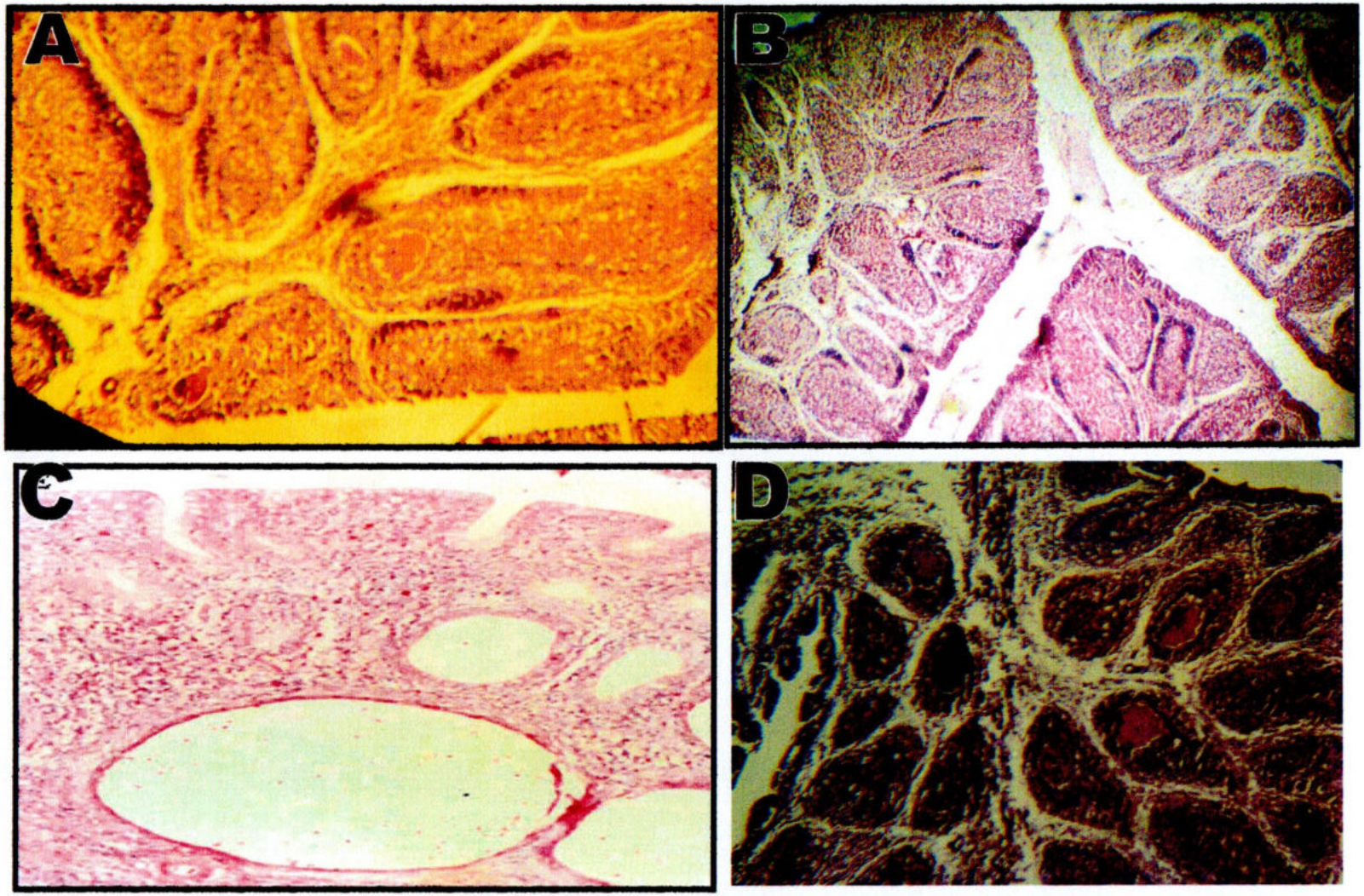


Figure 2: A: Cyst formation within the follicle B: Atrophy of the follicle C: Infolding of epithelium, cyst formation within the follicle D: Atrophy of the follicle, cyst formation within the follicle

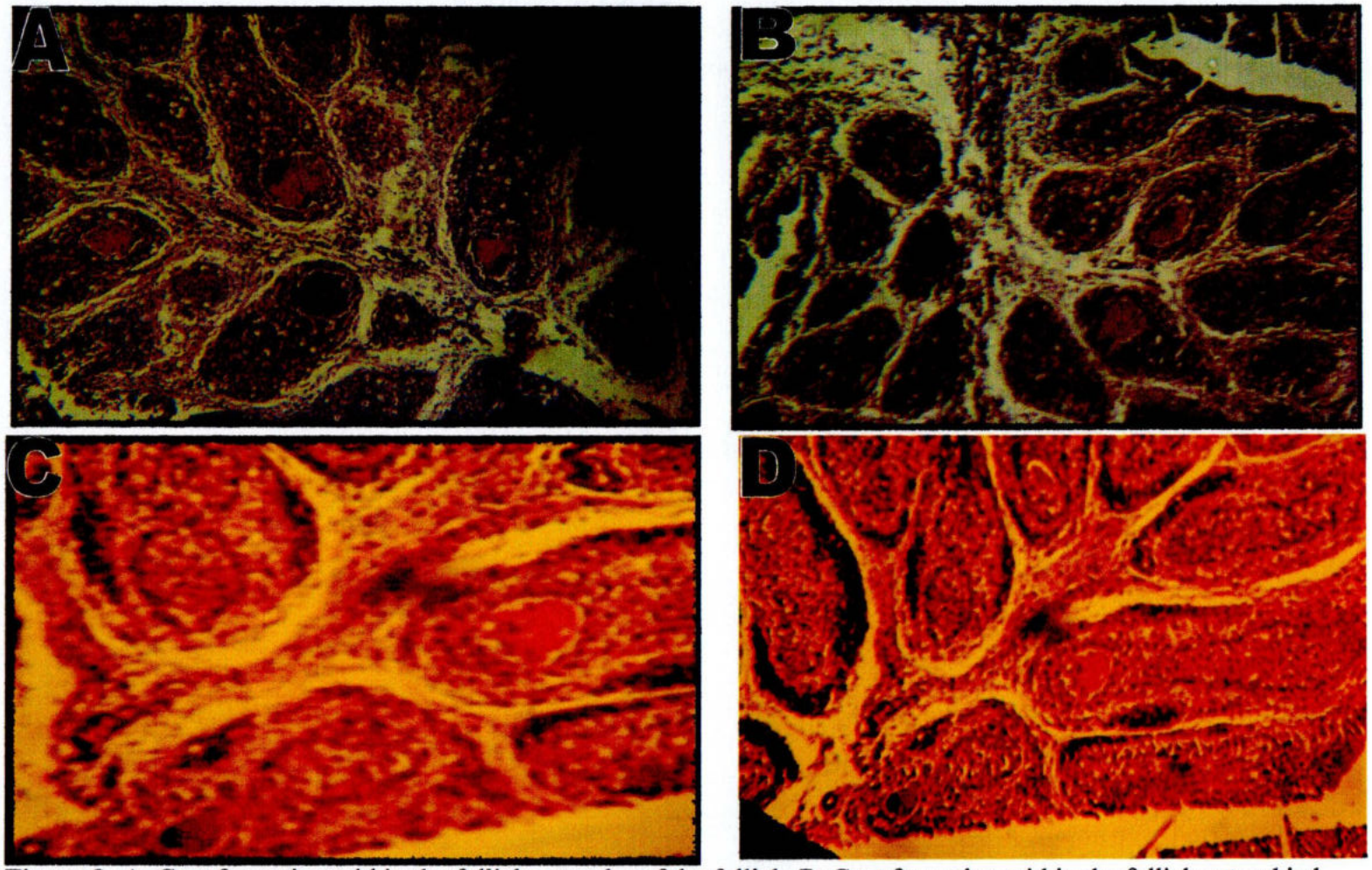


Figure 3: A: Cyst formation within the follicle, atrophy of the follicle B: Cyst formation within the follicle, atrophied follicle,densed interfollicular space C: Cyst formation within the follicle D: Cyst formation within the follicle

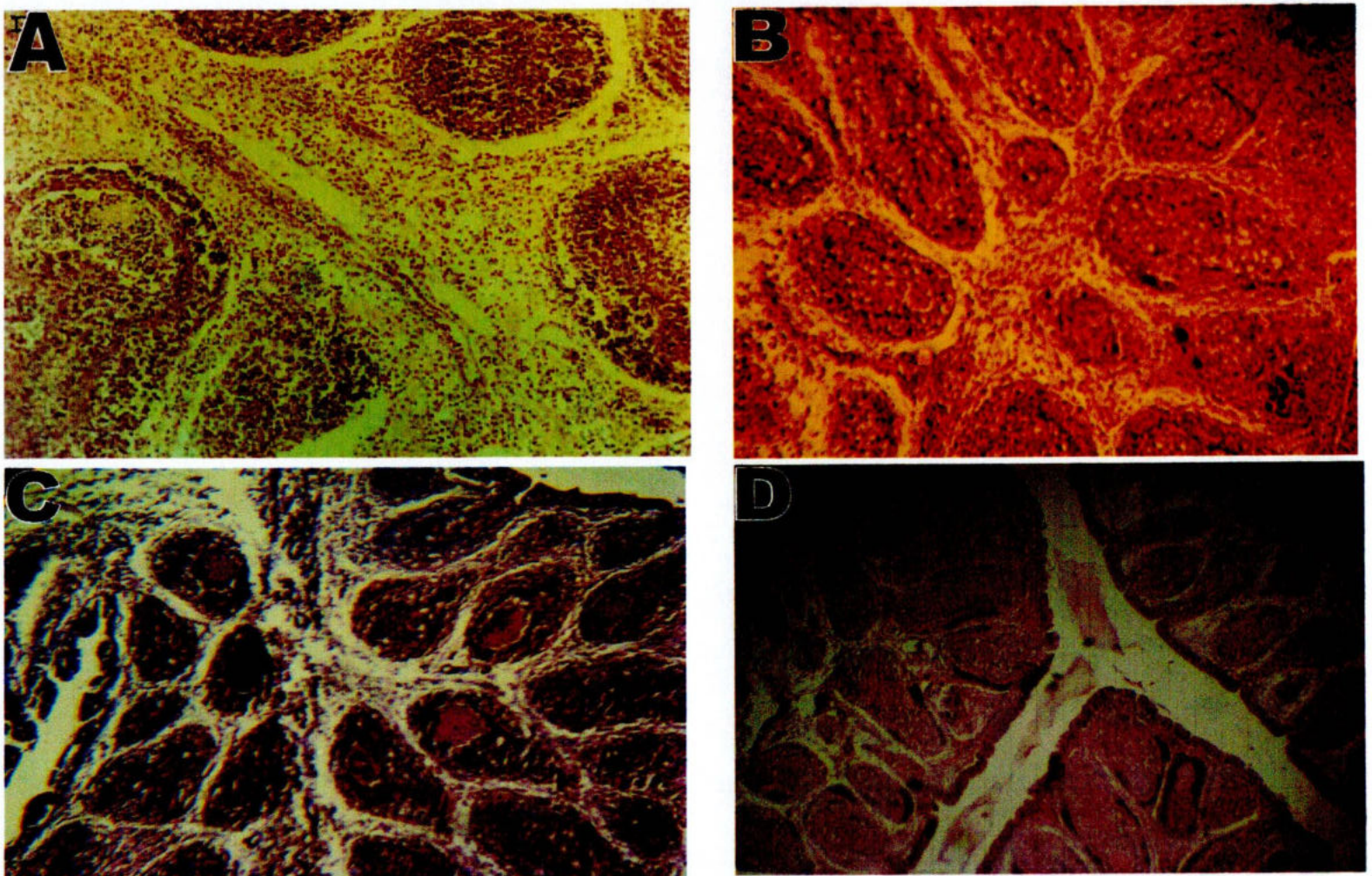


Figure 4: A: Densed interfollicular space, atrophy of the follicle, infiltration of reactive cells B: Atrophy of the follicle C: Cyst formation within the follicle D: Atrophy of the follicle

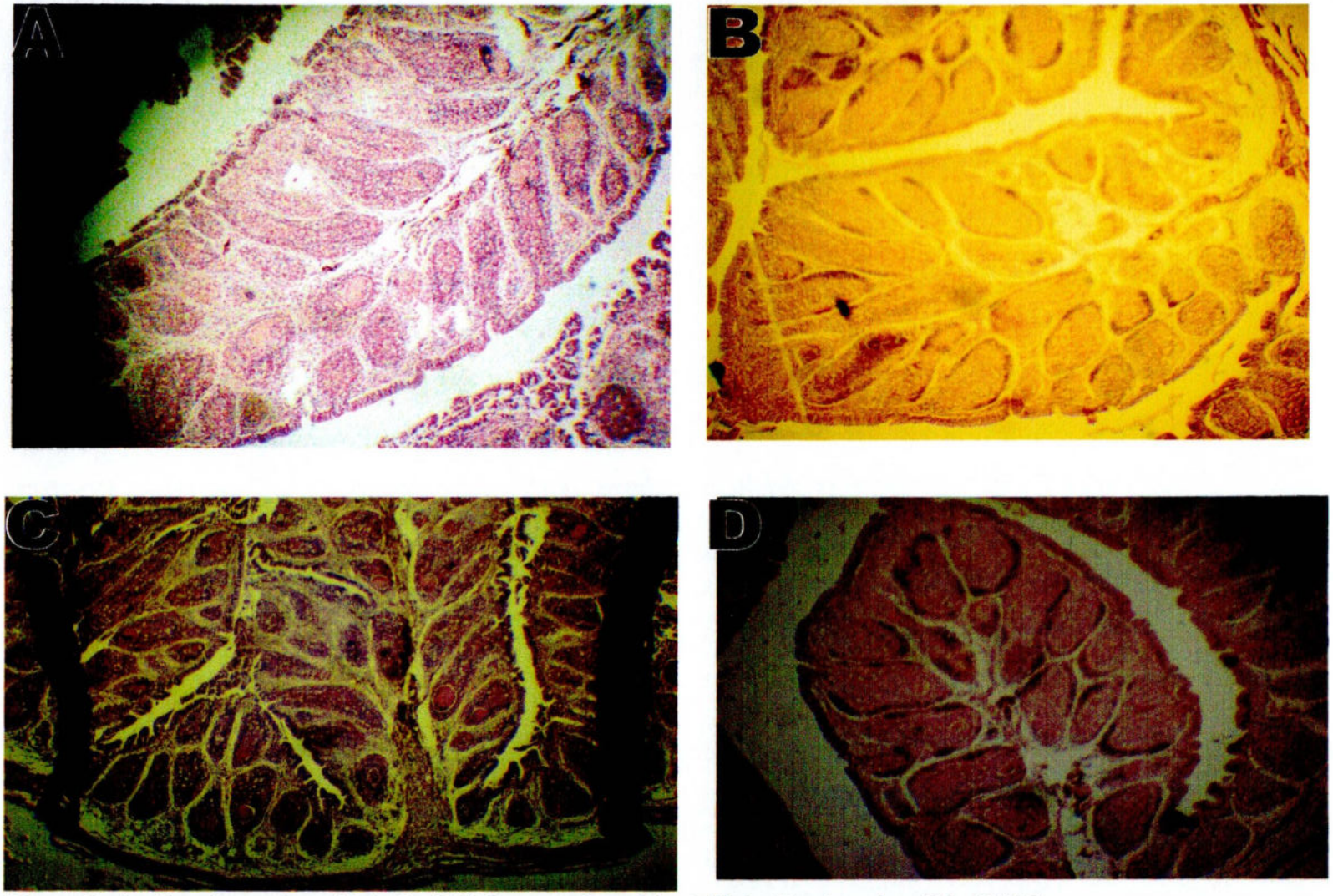


Figure 5: A: Cyst formation within the follicle , atrophy of the follicle B: Atrophy of the follicle C: Atrophy of the follicle, Cyst formation within the follicle D: Atrophy of the follicle

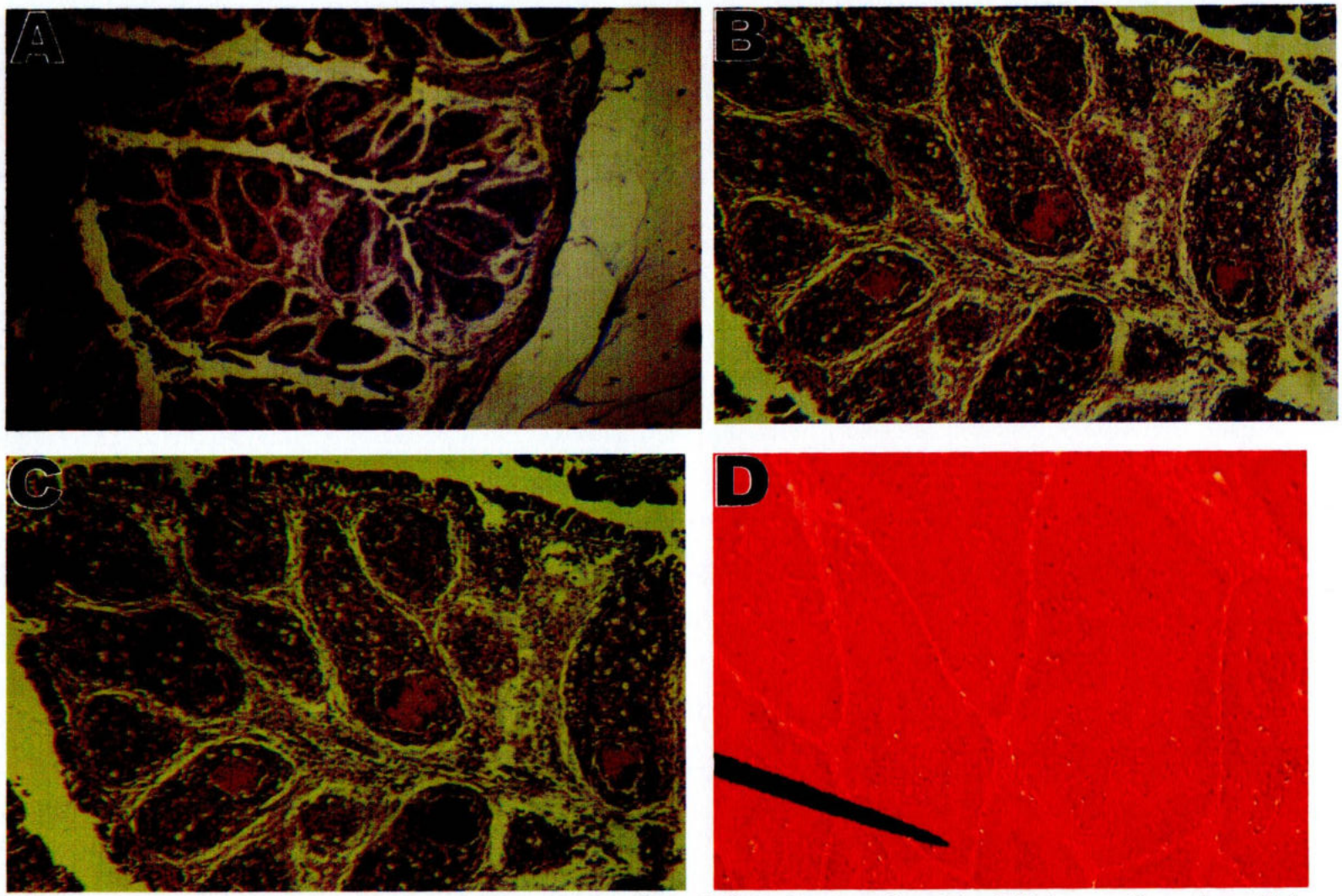
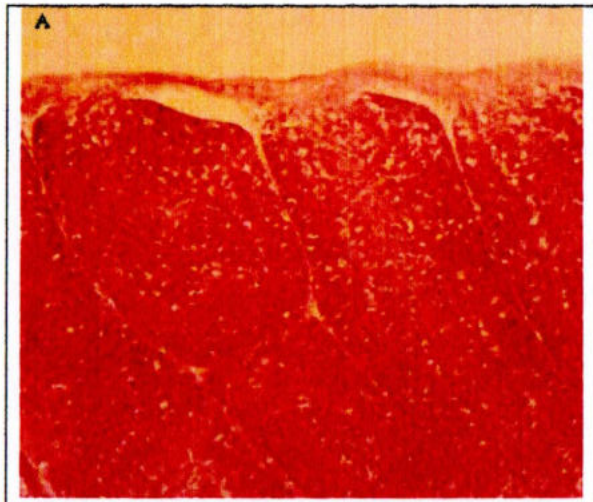
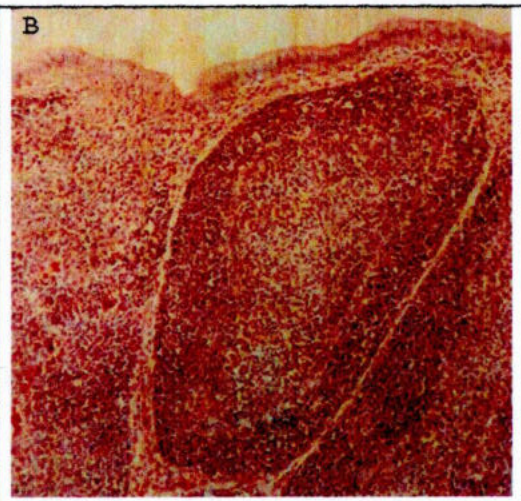
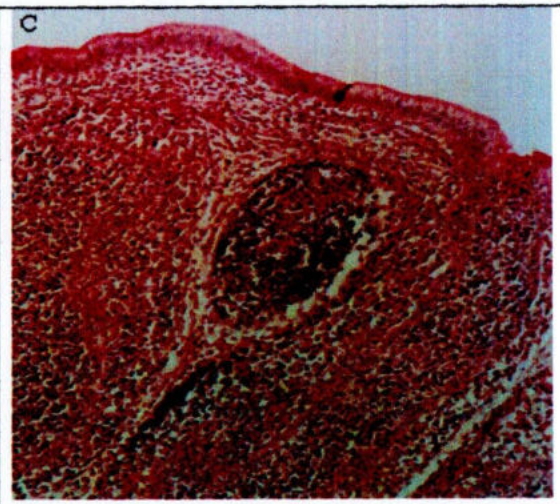
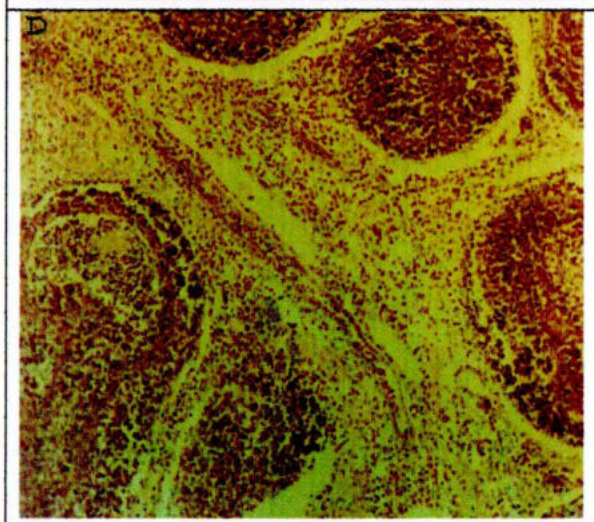


Figure 6: A: Cyst formation within the follicle , atrophy of the follicle B: Atrophy of the follicle, cyst formation within the follicle C: Cyst formation within the follicle, atrophy of the follicle D: Apparently normal follicle









BURSAL LESION SCORE

Score 0 : Apparently Normal follicle

Score 1: Mild lymphoid depletion

Score 2: Moderate lymphoid depletion

Score 3 : Severe lymphoid depletion

Score 4 : Marked follicular atrophy with/without cyst formation

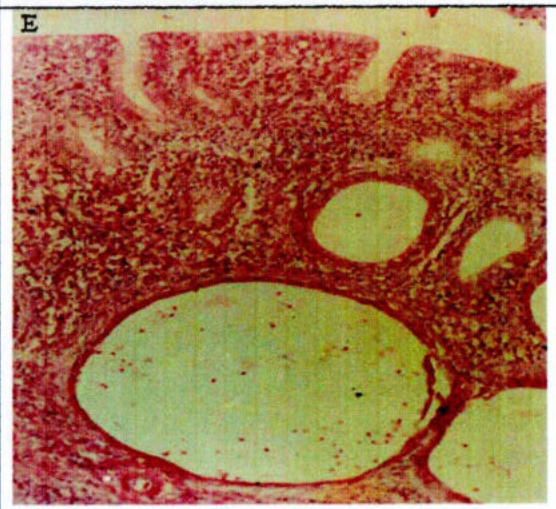


Figure 7 : Criteria of bursal lesion score: A) Apparently Normal follicle B) Mild lymphoid depletion C) Moderate lymphoid depletion D) Severe lymphoid depletion E) Marked follicular atrophy with/without cyst formation

CHAPTER V

DISCUSSION

The pathogenicity of IBDV virus related to outbreaks in the vaccinated flocks and the probable causes of vaccination failure, especially in connection with defective farm managements were investigated. A number of 20 such outbreaks were recorded, the pathogenicity of the virus was determined as highly virulent strain of IBDV and the probable causes of vaccination failure were suspected (Table 7).

Gumboro is the threat of poultry farming in Bangladesh and there is none alternative to prevent IBD without vaccination (Lukert and Saif, 1997). But Gumboro outbreaks in the vaccinated flocks were recorded elsewhere (Lukert and Saif, 1997; Hafez *et al.*, 2002). Various vaccines against IBD are commercially available. Some vaccines were tested their protection level experimentally giving challenge with vvIBDV and both significant and insignificant increase of antibody titre were reported (Islam *et al.*, 2005). Some commercially available vaccines became fail to give protection against IBD in a number of commercial poultry farms. Different factors related to Gumboro vaccine failure were suspected in the present study (Table 7). However, vaccination failure in connection to variation in the antigenicity among the IBD viruses (Rosenberger *et al.*, 1987), interference between the residual maternally derived antibodies and the vaccine virus (Eterradossi, 2001) and the appropriate time of vaccination were not determined in this study. It is critical to vaccinate commercial chickens that have maternally antibodies at the optimum time (Tsukamoto *et al.*, 1995). Vaccination during low maternally derived antibody titre shows better immune response than high maternal antibody titre (Giasuddin *et al.*, 2003). Again, the immunogenicity of the virus may differ between strain to strain (Rosales *et al.*, 1989a, b, c; Abdel-Alim and Saif, 2001) and the invasive vaccine strains are able to break through higher maternally derived antibody level (Kouwenhoven and van den Bos, 1994). The genetically engineered

tissue culture adapted vvIBDV was attempted to use as vaccine candidate, but the attempt was not yet successful for its reversion (Raue *et al.,* 2004).

The mortality rate determined in this study was upto 38% in the vaccinated flocks. Highest mortality recorded in flock 18.This picture of mortality principally found in the flocks infected by vvIBDV. IBDV strains isolated from the affected chickens induce severe clinical signs with high mortality in specific pathogen free (SPF) chickens (Nunoya *et al.,* 1992; Tsukamoto *et al.,* 1992). However, the mortality rate in the apparently normal flock in this study was o%. The clinical signs of the affected birds of the vaccinated flocks were variable (mild to the signs generally developed due to the infection with vvIBDV). The data clearly indicated the vaccination failure in the flocks. The scores of the different experimental groups clearly indicated the degree of severity of the disease which was closely associated with the pathogenicity of the IBD virus. The gross and histopathological lesions of the bursa of Fabricius were variable in different experimental flocks in the present study (Table 8).

Bursal lesion scores were surprisingly high in most cases except (Flock 1), where moderate bursal lesions were recorded. Depending on the residual virulence of the live attenuated viruses, some vaccine strains can cause bursal damage (Mazariegos *et al.,* 1990) and lead to immunosuppression in the vaccinated birds (Edward *et al.,* 1982; Reece *et al.,* 1982). Although highest bursal lesion scores with cyst formation (Tsukamoto *et al.,* 1995), lymphocytic depletion (Mazariegos *et al.,* 1990), acute necrosis (Rautenschlein *et al.,* 2001), follicular atrophy (Franciosini and Coletti, 2001), extensive bursal damage with follicular repopulation (Rautenschlein *et al.,* 2001) and increased interstitial connective tissue proliferation (Franciosini and Coletti, 2001) produced by intermediate vaccine strain of IBDV were reported. The high scores of bursal lesions especially found in the outbreaks with vvIBDV (Raue *et al.,* 2004).

The histopathological features and remarkably high score of bursal lesions in this study would evaluate the virus as undoubtedly highly pathogenic virus which could either be vvIBDV or vaccine virus. However, the pathogenicity of vaccine viruses were not yet determined in a separate experiment and further experiment to evaluate it can be conducted. Several suspected factors in connection to managemental errors in this study might be closely related to vaccination failure and outbreaks in the vaccinated flocks.

Vaccines were most repeatedly failed in those flocks where the batches of birds reared giving at least an interval to destroy the persistent IBDV and single dosing without boosting was followed. IBDV is highly infectious and very resistant to inactivation. The viruses could survive outside the host for at least for months (Allan *et al.*, 1982). Houses that contained infected birds are infective for innate birds after 54 and 122 days (Benton *et al.*, 1967). According to Godwin (2001), the factors causing vaccine breaks are either vaccine types, storage and handling; or condition of the birds including the level of maternally derived antibodies; or administration of vaccine. In this study, vaccination failure exclusively due to defective managements were thoroughly investigated and the suspected factors were listed (Table 5). However, the inactivation of vaccine virus may be due to careless transportation, preservation, preparation and administration of vaccines, and the vaccination and disinfection simultaneously by the farmers were noticed. All of these clues might be strongly associated with the vaccination failure, although the exact causes of vaccine breaks in connection with the antigenicity, immunogenicity and pathogenicity of vaccine viruses to protect the birds from this devastating malady are still obscure.

CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

Infectious Bursal Disease (IBD) is highly infectious disease of poultry. The pathogenicity of infectious bursal disease virus (IBDV) related to the outbreaks in the vaccinated flocks and the probable causes of vaccination failure were field based investigated during December, 2009 to May, 2010 at Dinajpur district of Bangladesh and the pathogenecity of the infectious bursal disease virus relation to the outbreaks in the vaccinated flocks were observed as highly pathogenic IBDV. The virus was pathologically determined as very virulent infectious bursal disease virus (vvIBDV) and the probable causes of vaccination failure were identified. Among the 20 Gumboro incidences in the vaccinated flocks were recorded during the course of observations. The number of the birds in the farms was variable ranging from 250-1250 and they were reared on litter. . One apparently normal flock was also included in this study for the comparison. The morbidity of the affected flocks was around 100%, and the mortality rate was variable ranging from 7-40%.

The clinical signs of the affected birds were more or less similar to the signs generally developed due to the infection with vvIBDV, and clinically characterized as anorexia, high fever, whitish diarrhoea, ruffled feathers, reluctant to move, trembling, huddling together, prostration and death. At necropsy, the birds were severely dehydrated and varying degrees of haemorrhages were found in thigh and breast muscles. The bursa of Fabricius was swollen, oedematous, haemorrhagic and atrophied containing cheesy exudates. Histopathologically, varying degrees of lymphoid depletion, necrosis, and reactive cells infiltration, cystic formation of the follicles were seen in the bursae.

The suspected causes of vaccination failure were also identified during the farm visit and from the farm records. The clinical and pathological findings with significantly high scores in the bursal lesions would suggest that outbreaks in the vaccinated flocks were undoubtedly vaccination failure, closely associated with different factors.

From the above facts and findings, it could be concluded that –

- ❖ Outbreaks of Gumboro disease in the vaccinated flocks is common
- ❖ Biosecurity is completely ignorged by the farmers
- ❖ Farmer's awareness to correctly apply of vaccine is questionable
- ❖ Vaccine breaks due to virus factor is prerequisite for the further investigation pathologically and molecularly

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