PATHOGENICITY STUDY OF "WINTERFIELD 2512 G–61 STRAIN" OF INFECTIOUS BURSAL DISEASE VIRUS VACCINE (CEVAC® IBD L) IN COMMERCIAL CHICKENS

A THESIS

BY

MOHAMMAD ABU SAYED KHAN

REGISTRATION NO: 0905084
SESSION: 2009-2010
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MASTER OF SCIENCE (M.S.)

IN

PATHOLOGY

DEPARTMENT OF PATHOLOGY AND PARASITOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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DEDICATED TO
MY
BELOVED PARENTS
&
ENA

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The Author,
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ABSTRACT

A modified form of intermediate plus of infectious bursal disease virus vaccine (CEVAC® IBD L) prepared from the "Winterfield 2512 G-61 strain" of infectious bursal disease virus was tested for it's pathogenicity in commercial chickens. A total of 500 unvaccinated Cobb-500 commercial chicks, raised in relative isolation from day old were used. 21 chicks were collected from experimental farm at day D_{11}, D_{13}, D_{15}, D_{17}, D_{20} D_{23} and D_{26}, respectively. 3 chicks were collected randomly from experimental flock each respective day. Vaccine was administered at ocular route at D_{11} and D_{17} with drinking water. All the sampled birds were subjected to detailed necropsy. The visible gross morbid lesions, bursa-body weight ratios were recorded. The bursae were collected, preserved at 10% formalin, processed, sectioned and stained with Hematoxylin & Eosin for histopathology including determination of bursal lesions scores. Data were analyzed statistically. One typically affected flock was included in this study for the comparison.

The visible gross morbid lesion was not observed during necropsy and bursa-body weight ratios were 2.75±0.60, 2.71±0.39, 2.44±0.42, 3.39±0.13, 2.58±0.55, and 2.15±0.16, 2.41±0.28 at D_{11}, D_{13}, D_{15}, D_{17}, D_{20} D_{23}, and D_{26}, respectively. Histopathological lesions were characterized as normal to severe lymphoid depletion with varying degrees of follicular atrophy in the vaccinated flock of study work. The bursal lesions scored were 0.67±0.33, 0.67±0.33, 2.00±0.58, 0.67±0.33, 1.0±0.00, and 0.67±0.33, 0.33±0.33 at D_{11}, D_{13}, D_{15}, D_{17}, D_{20} D_{23}, and D_{26}, respectively. No outbreaks were noted in the vaccinated flock, but significant changes were found in the affected flock.

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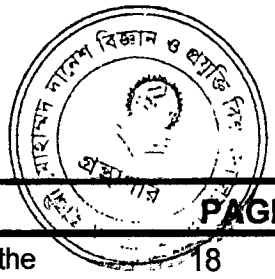

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LIST OF ABBREVIATIONS AND SYMBOLS

AbbreviatioSn and symbols

a	:	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
DPB	:	Day post boosting
DPV	:	Day post vaccination
D	:	Day
d.p.i	:	Days post inoculation or days post infection
ds		Double-stranded
DOC	:	Day Old Chick
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
nm	:	Nanometer
'C	:	Degree centigrade
ORF	:	Open reading frame
p.i		Post inoculation or post infection
PBS		Phosphate buffered solution
RNA		Ribonucleic acid

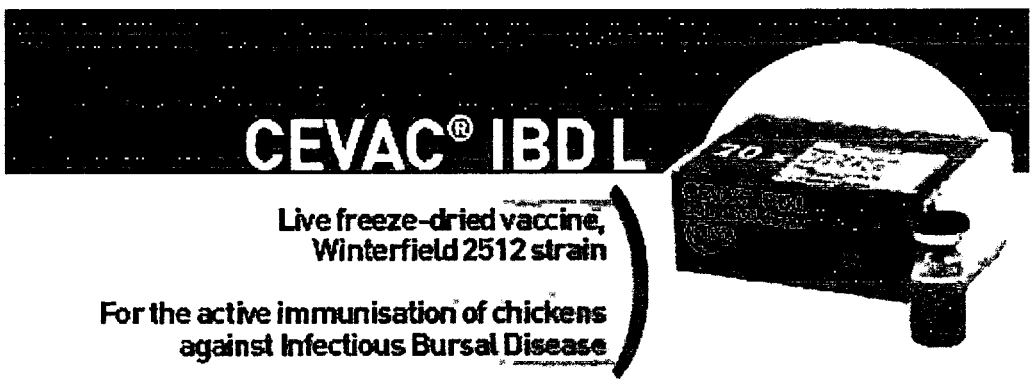
LIST OF ARBREVIATIONS AND SYMBOLS (CONTD.)

AbbreviatioSn and symbols

SIBW	:	Spleen1body 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
vvlBDV	:	very virulent infectious bursal disease virus
WIV	:	Weight/volume

CHAPTER I

INTRODUCTION



CHAPTER I

INTRODUCTION

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 (Cheville, 1967; Allan *et al.*, 1972; Hirai *et al.*, 1974; Fadley al., 1976; Rosenberger and Gelb, 1978; Saif, 1994; Lukert and Saif, 1997). Infectious bursal disease virus (IBDV), the oetiological agent of Gumboro disease, belonging to the genus Birnavirus (Murphy *et al.* 1995), sub-genus Avibirnavirus (Pringle, 1998), family Birnaviridae (Dobos *et al.*, 1979; Brown, 1986), has been widely studied mainly for two reasons:

Firstly, the highly contagious virus can cause severe economic losses in poultry industries due to high morbidity and mortality as a consequence 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: serotype1 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). Only serotype 1 viruses are virulent for chickens, replicating in and eventually destroying maturing B lymphocytes in the bursa of Fabricius (Cheville, 1967), inducing immunosuppression (Faragher *et al.*, 1972). 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 of poultry in 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).

IBDV is highly infectious and very resistant to inactivation. There is no alternative of vaccination in the prevention of IBD or Gumboro disease (Lukert and Saif, 1997), although the clinical outbreaks in vaccinated flocks are also reported (Chettle *et al.* 1989; van den Berg *et al.*, 1991; Eterradossi *et al.*, 1992; Muhammad *et al.*, 1996; Hafez *et al.*, 2002). In order to control IBD with live vaccine, it is critical to vaccinate commercial chickens that have maternal antibodies at optimum time. Live vaccines have the ability to overcome the maternal antibodies at certain level, vaccination during low marternal antibody titre shows better immune response than high maternal antibody titre (Giasuddin *et al.*, 2003). Neutralization of vaccine virus by the neutral antibodies is considered to be one 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). No vaccine based on vvIBDV is yet commercially available.

The immunogenecity of virus may differ between strain to strain (Rosales *et al.*, 1989a, b,b; Abdel-Alim and Saif, 2001). The intermediate vaccine strain produced moderate to severe bursal lesions reported by many researchers (Franciosini and Coletti, 2001). The better protection with more virulent strain of IBDV is due to more antigenic stimulation based on higher and longer replication in lymphoid tissues (Rautenschlein *et al.*, 2001).

The present study was carried out to investigate the pathogenic effect of Gumboro disease virus vaccine (Winterfield 2512 G-61 strain) in commercial chickens.

Objectives

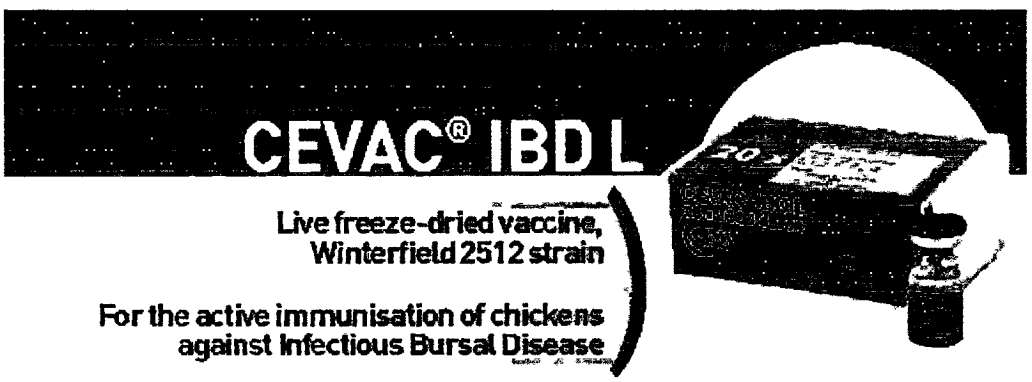
1. To study the gross morbid lesions including bursa-body weight ratios of the vaccinated flock
2. To study the sequential histopathological lesions of the bursa of Fabricius of vaccinated flock including bursal lesion scores
3. Plotted bursal lesions scores towards understanding the level of immunosuppression

Goal

- Evaluation of the vaccine prepared by live "Winterfield 2512 G-61 strain" of infectious bursal disease virus (IBDV), CEVAC® IBD L (CEVA) in the commercial chickens

CHAPTER II

REVIEW OF LITERATURE



CHAPTER II

REVIEW OF LITERATURE

Available literature for the determination of the pathogenic effect of Gumboro disease viral vaccine "Winterfield 2512 G-61 strain" in commercial chickens was reviewed in this part of the thesis after a brief overview on the history, epidemiology, oetiology, pathogenesis and pathology, clinical manifestations, immunosuppressive effects, adaptation of very virulent infectious bursal disease virus in CEF cell culture 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, southern 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). The term infectious bursal was proposed by Hitchner (1970). The etiological viral agent was isolated by Winterfield in 1962 (Lukert and Saif, 1997) who differentiated the disease from a previously established disease known as nephrotoxic viral infection of chickens. 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 (reviewed in 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 are of worldwide distributed, occurring in all major poultry producing areas (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, crows, 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 (Cosgrove, 1962; Winterfield and Hitchner, 1964; Hanson, 1967; Ley *et al.*, 1983). Sub-clinical infection has been reported to occur in chicks before three weeks of age (Allan *et al.*, 1972; Ley *et al.*, 1979; Lukert and Saif, 1997) 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; 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). Fishmeal in the feed contaminated with IBDV may act as a transmitter of the disease (Yongshan *et al.*, 1994), while lesser mealworm as well as mosqito may act as a reservoir of IBDV (Snedeker *et al.*, 1967; Howie and Thorson, 1981; McAllister *et al.*, 1995).

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.

2.2.6 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

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 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).

Mortality due to IBD on various farms ranged from 1 to 40% in broilers and from 2 to 40% in layers (Kurade *et al.* 2000) and from 1.5 to 30% in native and broiler flocks respectively (Saif *et al.*, 2000).

2.2.8 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 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 al.*, 1996
	Levels of MDA	Iordanides *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: serotype1 and serotype2 (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). Serotype1 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.

Serotype 2 strains cause neither mortality nor bursal lesions in SPF birds. Serotypel vaccine causes no mortality but possess residual pathogenicity with bursal lesions varying from mild to moderate or even severe. Virulent serotypel field strains induce both mortality and bursal lesions.

Several techniques, such as the virus neutralization test (VNT) (Jackwood and Jackwood, 1994), nucleotide sequencing (Kibenge *et al.*, 1990; Lana *et al.*, 1992; Lin *et al.*, 1993; Brown *et al.*, 1994; Brown and Skinner, 1996; Yamaguchi *et al.*, 1997), and reverse transcription /polymerase chain reaction-restriction fragment length polymorphism (RT/PCR-RFLP) (Giambrone *et al.*, 1994; Jackwood and Jackwood, 1994; Nakamura *et al.*, 1994; Jackwood and Sommer, 1999; Zierenberg, *et al.*, 2001), have been used to study the antigenic and genomic variation of the vvIBDVs. VNT (Skeeles *et al.*, 1979), AGPT (Cullen and Wyeth, 1975) and ELISA (Marquardt *et al.*, 1980) are the methods for IBDV antibodies detection.

2.3.3 Morphology of the virus

IBDV is a small, non-enveloped virus with icosahedral symmetry (Hirai and Shimakura, 1974). IBDV particles have a diameter of 55-60nm (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 molecular weight of the virus ranged from 2.2 to 2.5 X 106 daltons (Nick *et al.*, 1976; Müller *et al.*, 1979) with the buyoant density of 1.34 g/ml (Hirai and Shimakura, 1974; Nick *et al.*, 1976; Dobos *et al.*, 1979; Jackwood *et al.*, 1982).

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) arranged in the capsid, a single capsid shell composed of 32 capsomeres and a diameter of 60 to 70 nm (Hirai and Shimakura, 1974).

2.3.4 Physico-chemical properties

The virus is highly resistant to physical conditions and chemical agents. 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 IBDV can tolerate acidity as low as pH 2, but inhibited in pH12 (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 become 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).

2.3.5 Molecular biology of IBDV

The genome is composed of two double-stranded (ds) RNA segments designated A (larger segment, approximately 3400 base pairs) and B (smaller segment, approximately 2800 base pairs) (Dobos *et al.*, 1979: Muller

et al., 1979a). The major open reading frame (ORF) in the larger genome segment A encodes a polyprotein which is co-translationally and autocatalytically cleaved into the major structural protein, VP2 and VP3, and a viral protease VP4 (Muller and Betch, 1982; Hudson *et al.*, 1986; Azad *et al.*, 1987). A second ORF in segment A encodes a non-structural protein, VP5 (Mundt *et al.*, 1995). The smaller segment B encodes the multifunctional protein VP1, which has RNA- dependent RNA polymerase activity (Spies *et al.*,'1987) and capping enzyme activity (Spies and Muller, 1990).

The non-structural VP4 protein is mainly associated with type II tubules of 24 nm in diameter (Granzow *et al.*, 1997). VP2 and VP3 form the outer and inner layers, respectively (Bottcher *et al.*, 1997) and VP2 contains a major conformational neutralizing antigenic domain, stretching from amino acid 206 to 350 (Azad *et al*, 1987; Becht *et al*, 1988; Schnitzler *et al*, 1993). This region displays marked variations in the amino acid sequences among different strains of IBDV and therefore, designated as the variable domain (Bayliss *et al.*, 1990). Amino acid changes in this variable domain have found to be associated with antigenic drifts in IBDV (Heine *et al.*, 1991; Schnitzler *et al.*, 1993; Eterradossi *et al.*, 1998).

VP1 plays a central role in the transcription of viral RNA (Spies and Muller, 1990). VP2 is the major host protective immunogen (Azad *et al.*, 1987; van den Berg *et al.*, 1991; Fahey *et al.*, 1991; Snyder *et al.*, 1992; Vakharia *et al.*, 1993), displays the greatest amount of amino acid sequence variation between different strains (Bayliss *et al.*, 1990; Brown and Skinner; 1996;

Yamaguchi *et al*, 1997). The amino acid residues of VP2 are involved in the adaptation of IBDV to cell culture (Mundt, 1999; Lim *et al.*, 1999; Islam *et al.*, 2001b; van Loon *et al.*, 2001 and 2002). VP2 and VP3 are the major structural proteins that are processed by VP4, a virus encoded protease (Hudson *et al.*, 1986). VP5 plays an important role in the release of the virus particles from the infected cells (Lombardo *et al.*, 2000; Schrooder *et al.*, 2001). VP5 is not essential for the growth of virus in cell culture (Mundt *et al.*, 1997). It is a non- structural protein (Mundt *et al*, 1995). It is not essential for growth of virus in cell culture (Mundt *et al.* 1997). It plays a crucial role in viral pathogenesis by inducing apoptosis (Yao *et al.*, 1998).

Some membrane proteins have been identified as the possible receptor to IBDV in CEFs or in chicken lymphocytes (Nieper and Muller, 1996; Ogawa *et al.*, 1998; Setiyono *et al.*, 2001a and 2001 b), the actual nature of the receptor is still unknown.

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 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 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 bursal 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 IL-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 are increased.
- The follicular recovery becomes significantly faster

2.5.3 Role of chemokines in the pathogenesis

There are various chemical mediators such as IFN7 (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 IFNy (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 pagthogenesis

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 idenify 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, Islam *et al.*, 2008), oedematous (Chowdhury *et al.*, 1996; Czifra and Jonson, 1999, Islam *et al.*, 2008), haemorrhagic (van der Sluis, 1994; Chowdhury *et al.*, 1996; Haque *et al.*, 2001, Islam *et al.*, 2008) bursa , cheesy mass within the bursal lumen (Chowdhury *et al.*, 1996, Islam *et al.*, 2008) and finally, atrophy of the bursa (Jhala *et al,*. 1990; Chowdhury *et al.*, 1996, Islam *et al.*, 2008). 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).

2.6.2.3 Caecal tonsil

Haemorrhages(Chowdhury, *et al.*,1996) and partially damaged caecal tonsils are found in some cases (Islam *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 are 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, Islam *et al.*, 2008) and emaciation (Chowdhury *et al.*, 1996). Varyimg degrees of haemorrhages 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, Islam *et al.*, 2008), skeletal muscles are darkly discoloured (Nunoya *et al.*, 1992) and haemorrhages 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).

2.7 Histopathology

2.7.1 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, Islam *et al.*, 2008), interfollicular oedema (Czifra and Jonson, 1999; Hoque *et al.*, 2001; Franciosini and Coletti, 2001), necrosis (Islam *et al.*, 2008) 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, Islam *et al.*, 2008) with or without fibroplasia(Islam *et al.*, 2008) 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.,* 1989 a; 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.,* 1995 a; 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,* 1995 a; Rautenschlein *et al.*

2001;, Franciosini and Coletti, 2001) and increase of interstitial connective tissue (Franciosini and Coletti, 2001).

2.7.2 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).

2.7.3 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).

2.7.4 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.

2.7.5 Kidneys

Degeneration (Cosgrove, 1962; Chowdhury *et a1.*, 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 edematous space between many tubules and collecting ducts (Henry *et al.*, 1980) are found in the kidneys of IBDV infected birds.

2.7.6 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, haemorrhages and alveolar emphysema in the lungs (Islam *et al.*, 1997) are reported.

2.8 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.

2.9 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*, 1989 a, b, c; Mazariegos *et al.*, 1990; Tsukamoto *et al.*, 1995 b; 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). Thus, IBDV infection causes immunosuppression and the immunosuppression ultimately leads to increase the incidence of many diseases.

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
Mycoplasma	*Mycoplasma synoviae* infection or mycoplasmosis	Gimabrone *et al.*, 1977; Binta *et al.*, 1995
Other	Haemorrhagic aplastic anaemia	Rosenberger and Gelb, 1978

2.10 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 elsewhere (van den Berg *et al.*, 1991;

Eterradossi *et al.*, 1992; Muhammad *et al.*, 1996; Hafez *et al.*, 2002).Various vaccine against IBD are commercially available.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).

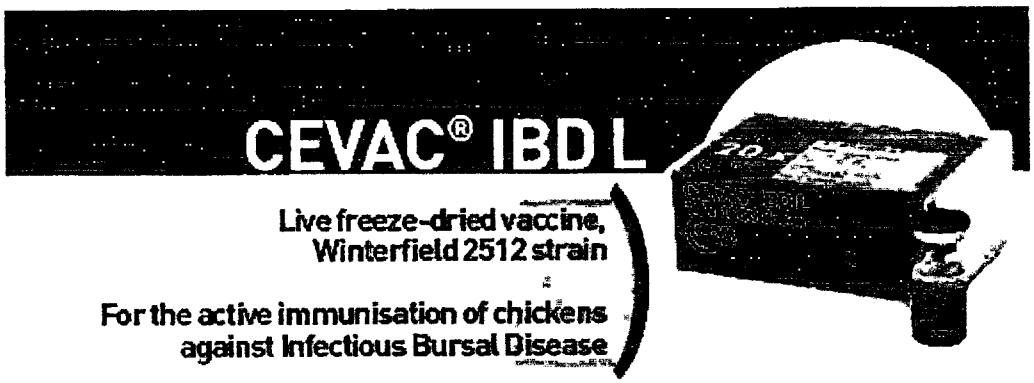
The invasive vaccine strains are able to break through higher maternal antibody levels (Kouwenhoven and van den Bos, 1994). Therefore, Vaccination during low maternally deriivied antibody titre shows better immune response than high maternal antibody titre (Giasuddin *et al.*, 2003) the chicks could 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 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 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 inactivated vaccine made from the vvIBDV provided full protection against challenge with classical virulent strain as indicated by the low bursa/body weight ratio (Abdel-Alim and Saif, 2001).Some vaccines were tested their protection level experimentally giving challenge with vvIBD and both significant and insignificant increase of antibody titre were reported (Islam *et al.*, 2005)

CHAPTER III

MATERIALS AND METHODS



CHAPTER III

MATERIALS AND METHODS

3.1 Experimental chickens

500 unvaccinated Cobb-500 Day Old Chicks (DOC) received from the "CP Bangladesh Ltd." by "Zahid Poultry Farm" were considered as the experimental chickens. Randomly selected three birds in each group were used.

3.2 Research area

Poultry farming and vaccination against IBDV was done in the above mentioned farm placed at Syedpur of Nilphamari district. The chickens were collected following experimental schedule and laboratory examination was done at the Department of Pathology and Parasitology of Hajee Mohammad Danesh Science and Technology University, Basherhat, Dinajpur.

3.3 Experimental period

The duration of the experiment was one year from June 2009 to May 2010.

3.4 Experimental design

Table 3: Experimental design of research work

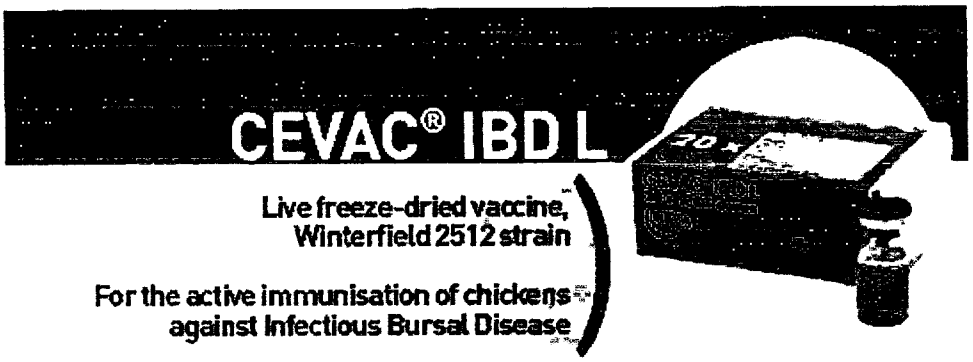
Primary vaccination with modified form of Intermediate plus of IBDV(CEVAC® IBD L)			
Sampling occasion at age of birds (Day)	Vaccination Status	No. of birds for Necropsy	Parameters studied
D_{11}	–	3	➢ Clinical signs and symptoms ➢ Gross morbid lesions ➢ Bursa– body weight ratios ➢ Histopathology ➢ Bursal lesion scores
D_{13}	2 Days Post Vaccination (DPV)	3	
D_{15}	4 DPV	3	
D_{17}	6 DPV	3	
D_{17} Boosting with modified form of Intermediate plus of IBDV (CEVAC® IBD L)			
D_{20}	3 Days Post Boosting (DPB)	3	➢ Clinical signs and symptoms ➢ Gross morbid lesions ➢ Bursa– body weight ratios ➢ Histopathology ➢ Bursal lesion scores
D_{23}	6 DPB	3	
D_{26}	9 DPB	3	
D_{23} (Affected flock)		3	

3.5 Management of chickens

The birds were maintained in relative isolation. The shed was made by rice straw and floor was constructed with brick. The shed was "Open Sided" and East-West in position. The room was thoroughly cleaned by sweeping and washing with tape water using hose pipe connected with a tape. The room was disinfected with a household phenolic disinfectant (phenyl) and fumigates the room. Optimum temperature in the brooder house maintained using electric bulbs in required number and at required distances. Rice husk was the littre material which was placed 2-3 inches in depth and it was replaced following wetting either by faeces or water or by both. For the first week brown paper was placed in the brooder which was replaced regularly. Feeding and watering was adlibitum for the first tow days birds were maintained on suji (a coarse flower of wheat), which was then replaced by commercial starter and grower feed accordingly. In addition electrolytes and vitamins were given in water time to time. Entry to the house was restricted. Wearing rubber boots and deeping boots in disinfectant foot bath were compulsory for the visitors during entry and exit. The measurement was taken so that the wild animals and birds could not enter into farm and spray the vehicles before entering into the farm.

3.6 Vaccines and vaccination

The vaccine used in this study was a commercial, manufactured modified live virus vaccine, obtained directly from the veterinary products seller and stored at 4°C until used. The vaccine was administered according to the manufacturer's (CEVA) recommendations. CEVAC® IBD L contains the "Winterfield 2512 G-61 strain" of Infectious bursal disease virus in live freeze dried form, which was used in this study.



COMPOSITION

CEVAC® IBD L contains the Winterfield 2512 G-61 strain of Infectious Bursal Disease virus in live, freeze dried form. The embryonated hen eggs used in the production of the vaccine are obtained from specific-pathogen-free (SPF) flocks.

INDICATIONS

For the active immunization of healthy chickens against the disease caused by classical and very virulent strains of Infectious Bursal Disease (Gumboro Disease).

CONTRA-INDICATIONS

CEVAC® IBD L should not be used for the immunization of flocks without maternally derived antibodies.

ADMINISTRATION AND DOSAGE

CEVAC® IBD L is administered through drinking water. Broilers require vaccination with CEVAC® IBD L from 10 to 18 days of age, depending on the level of maternally derived antibodies.
Pullets are usually vaccinated twice between the age of 16 and 26 days, allowing a six-day interval between administrations.
The exact date of vaccination can be determined by checking the level

of maternally derived antibodies by serological methods.

SIDE EFFECT

Usually none

STORAGE

- Store vaccine between +2°C and +8°C or 35°F and 45°F
- Protect from light

PRESENTATIONS

1,000 - 2,500 and 5,000 dose vials

20 x 1,000 dose vials / box

20 x 2,500 dose vials / box

20 x 5,000 dose vials / box

3.7 Sampling occasion

The birds were collected from the flock for laboratory examination as per as experimental design.

3.8 Necropsy

Necropsy of birds obtained from "Zahid Poultry Farm". The necropsies of the experimental birds were done following a standard procedure (Charlton, 2000).

3.9 Bursa-body weight (B/BW) ratio

Each bird was weighed before killing. The bursa of Fabricius was weighed and the average B/BW ratio was determined by the formula of Tanimura *et al.*, (1995) as following:

$$\text{B/BW ratio} = \frac{\text{Bursa weight in grams}}{\text{Body weight of individual bird in grams}} \times 1000$$

3.10 Histopathological study

During necropsy, bursa of Fabricius was collected, fixed in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed and stained as per standard method (Luna, 1968).

Materials required for histopathology

Equipment and appliances:

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

Processing of tissue for histopathology

Collection of tissue and Processing

During tissue collection the following point were taken into consideration-

The Bursa of Fabricius was collected in conditions as fresh as possible. The thickness of the tissues were as less as possible (5mm approximately).

The Bursa of Fabricius was collected from the experimental birds in the Histopathology Laboratory of Department of Pathology and Parasitology, HSTU, Dinajpur.

Fixation: 10% formalin was added in the plastic container (10 folds of the tissue size and weight) and fixed for 3-5 days.

Washing: The tissues were trimmed into a thin section and washed over night in running tap water to remove formalin.

Dehydration: The tissues were dehydrated by ascending ethanol series to prevent shrinkage of cells as per following schedule.

- 50% alcohol : one hour
- 70% alcohol : one hour
- 80% alcohol : one hour
- 95% alcohol : one hour
- *Absolute alcohol : three changes (one hour for each changes)*

Cleaning: the tissues were cleaned in chloroform for 3 hours to remove ethanol (1 and half hr in each, two changes).

Impregnation: Impregnation was done in melted paraffin (56- 60°C) for 3 hours.

Embedding: Paraffin blocks containing tissue pieces were made using templates and molten paraffin

Sectioning: 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.

- Then the sectioned tissues were dehydrated through descending grades of alcohol as per following schedule.
  - Absolute alcohol : three changes (three minutes for each)
  - 95% alcohol : two minutes
  - 80% alcohol : two minutes
  - 70% alcohol : two minutes
  - Dipping with distilled water for 10 minutes.
- The tissues were stained with Harris hematoxylin for 2-10 minutes.
- Washed in running tap water for 10-15 minutes.
- Then the tissues were dipped in ammonia water (few dips).
- Stained with eosin for one minute.
- Differentiated and dehydrated in ascending grade of alcohol.
  - 95% alcohol – three changes (2-4 dips for each.)
  - Absolute alcohol – three changes (2-3 minutes for each)
- Cleaned in xyline: three changes (five minutes each).
- Tissues were mounted with cover slip by using DPX
- The slides were dried at room temperature and examined under a low (10X) and high (40X, 100X) power objectives.

3.11 Scoring of bursal lesions

The slides were studied at 10X and 40X magnifications. The bursal lesions were scored on the basis of the following criteria (Raue *et al*, 2004):

Score 0: Apparently normal lymphoid follicles

Score 1: Mild lymphoid depletion

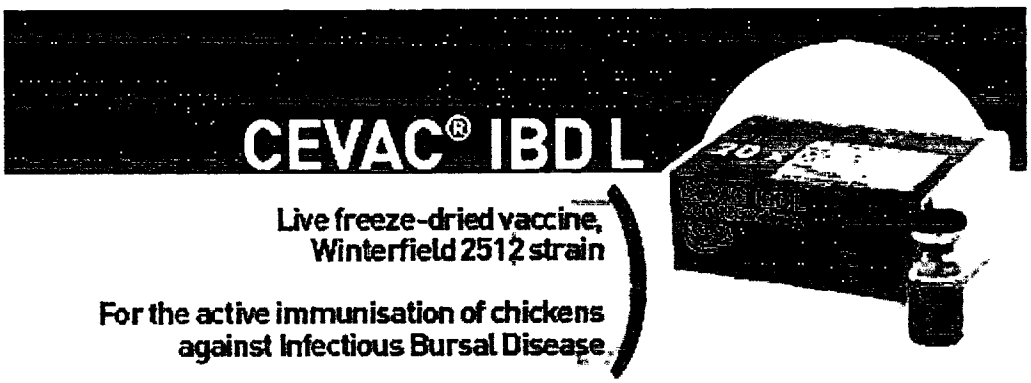
Score 2: Moderate lymphoid depletion

Score 3: Severe lymphoid depletion

Score 4: Atrophy of follicles with or without cystic spaces.

CHAPTER IV

Results



CHAPTER IV

RESULTS

4.1 Clinical manifestations of the vaccinated flock

Remarkable clinical signs in the birds of the vaccinated flock were not seen.

4.2 Necropsy / Gross morbid lesions

Necropsy of the birds was done thoroughly. The organs, such as bursa of Fabricius, spleen, caecal tonsil, thymus. Kidneys, liver, thigh and breast muscle, junction of proventriculus and gizzard were examined properly, but there was little pathological lesions. Only exception with liver, hematoma on the surface of the liver was seen in one case. In case of bursa of Fabricius, the size and weight of the bursae were variable according to the weight and age of the birds (Table: 3).

Typically affected flock was also included in this present study to compare the pathology. The birds brought to the laboratory of the Department of Pathology and Parasitology for the diagnosis and treatment of diseases was also included.

4.3 Bursa - body weight ratios

The Bursa-body weight (B/BW) ratios were determined at D_{11}, D_{13}, D_{15}, D_{17}, D_{20}, D_{23} and D_{26} including a affected flock and results were presented in Table- 4.

Table 4: Bursa-body weight ratios of experimental birds

Sampling occasion	Birds No.	Live body weight (gm.)	Bursa weight (gm.)	B-B ratio	Average
D_{11}	1	227.7	0.8	3.51	2.75
	2	255.5	0.4	1.57	
	3	125.8	0.4	3.18	
D_{13}	1	290.6	1.0	3.44	2.71
	2	308.7	0.8	2.59	
	3	190.7	0.4	2.09	
D_{15}	1	203	0.6	2.96	2.44
	2	435.2	1.2	2.76	
	3	373.3	0.6	1.61	
D_{17}	1	445.8	1.4	3.14	3.39
	2	569.3	2.0	3.51	
	3	255.1	0.9	3.53	
D_{20}	1	652.6	2.4	3.68	2.58
	2	414.3	0.8	1.93	
	3	329.6	0.7	2.12	
D_{23}	1	1169.8	2.2	1.88	2.15
	2	609	1.3	2.14	
	3	414	1.0	2.42	
D_{26}	1	507.7	1.0	1.97	2.41
	2	814.9	1.9	2.33	
	3	749.2	2.2	2.94	
D_{23} (Affected flock)	1	950	2.5	2.63	2.45
	2	900	2.1	2.33	
	3	875	2.1	2.4	

Table 5: Statistical analysis of live body weight

	Mean±SE							
	Live body weight (gm.)							
	D_{11}	D_{13}	D_{15}	D_{17}	D_{20}	D_{23}	D_{26}	D_{23} (Affected flock)
	203.00±39.43	263.33±36.69	337.17±69.42	423.40±91.39	465.50±96.69	730.93±226.54	690.60±93.40	908.33±22.05
P value	0.2131	0.0745	0.1562	0.2511	0.0341	0.0051	0.0441	0.0964
Level of significance	NS	NS	NS	NS	*	**	*	NS

NS = Not significant (p>0.05)

** Significant (P<0.01)

* Significant (P<0.05)

Table 6: Statistical analysis of bursa weight

	Mean±SE							
	Bursa weight (gm.)							
	D_{11}	D_{13}	D_{15}	D_{17}	D_{20}	D_{23}	D_{26}	D_{23} (Affected flock)
	0.53±0.13	0.73±0.18	0.80±0.20	1.43±0.32	1.30±0.55	1.50±0.36	1.70±0.36	2.23±0.13
P value	0.0038	0.0341	0.0756	0.0112	0.0002	0.0001	0.0029	0.0852
Level of significance	**	*	NS	**	**	**	**	NS

NS = Not significant (p>0.05)

** Significant (P<0.01)

* Significant (P<0.05)

Table 7: Statistical analysis of bursa-body weight ratios

	Mean±SE							
	B-B ratio							
	D-11	D-13	Day 15	Day 17	Day 20	Day 23	Day 26	Day 23 (Affected flock)
	2.75±0.60	2.71±0.39	2.44±0.42	3.39±0.13	2.58±0.55	2.15±0.16	2.41±0.28	2.45±0.09
P value	0.0044	0.0256	0.2144	0.0129	0.0051	0.0041	0.0036	0.1024
Level of significance	**	*	NS	**	**	**	**	NS

NS = Not significant (p>0.05)

** Significant (P<0.01)

*Significant (P<0.05)

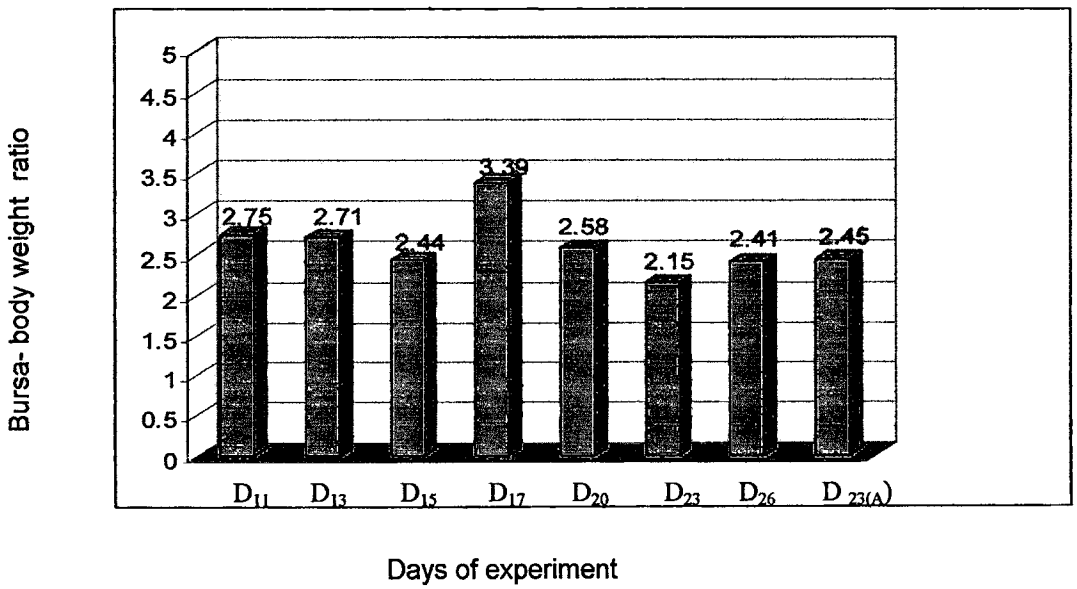


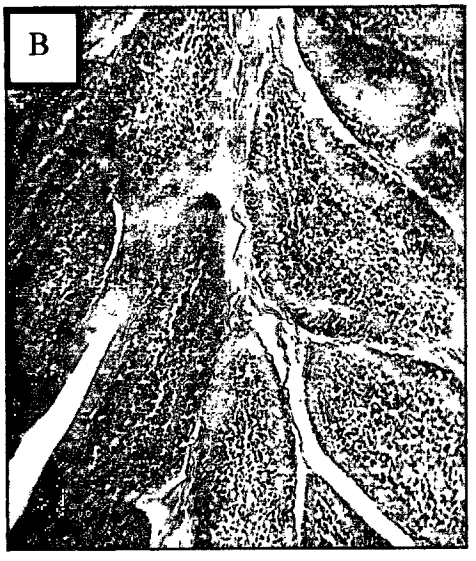
Figure 1: Graphical representation of bursa- body weight ratios

4.4 Histopathological lesions in bursa of vaccinated birds

- Most bursal follicles were apparently normal which were histologically characterized as unifomly cellular concentration in the follicles.
- Mild depletion of lymphoid cells was also found in some follicles in the same examined birds.
- Moderate depletion of lymphoid cells was found in few bursal follicles.
- Severe lymphoid depletion was also found in fewer follicles.
- Follicular atrophy without the development of follicular cysts was also observed, but this histopathological changes was marked in the flock showing typical outbreak of Gumboro disease.

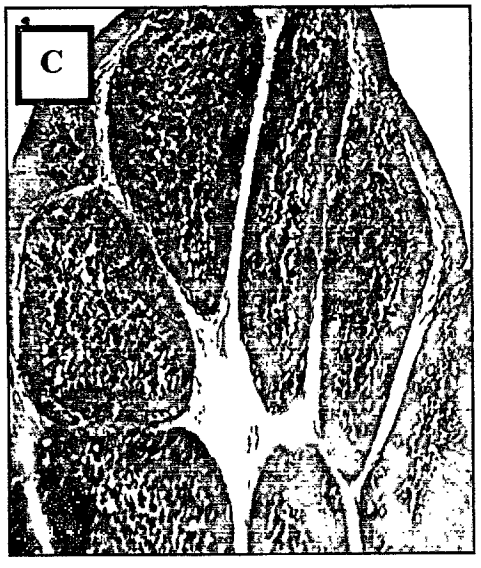
D_{11} (Unvaccinated group)

Apparently normal lymphoid follicles



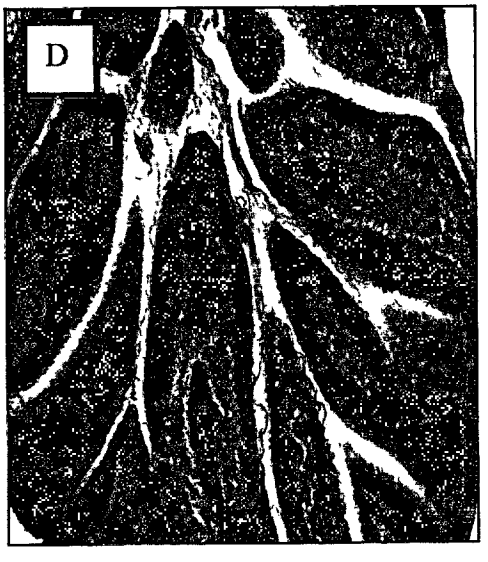
D_{13} (Primary vaccinated group)

Mild to moderate lymphoid depletion



D_{15} (Primary vaccinated group)

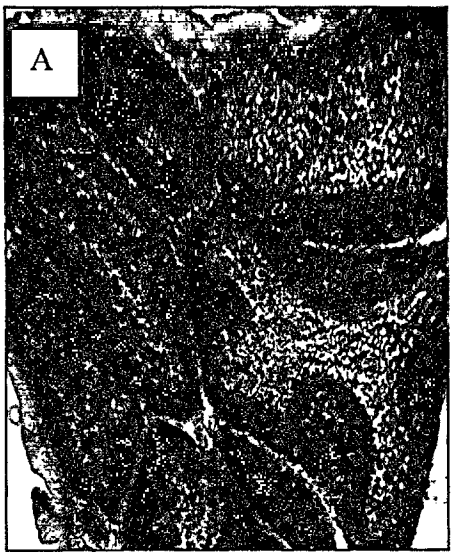
Mild to moderate lymphoid depletion



D_{17} (Primary vaccinated group)

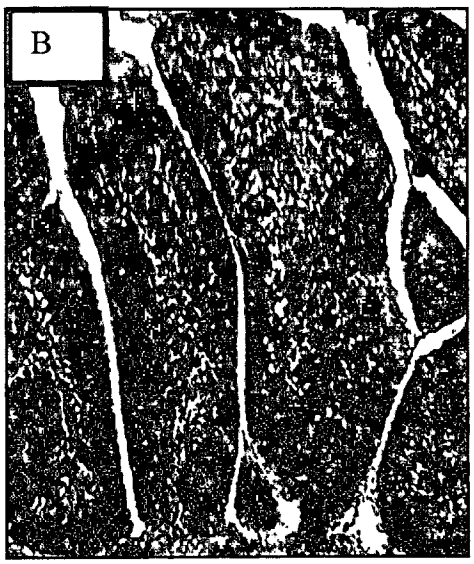
Mild lymphoid depletion

Fig-2: Bursal lesions at different age groups of experimental birds



D_{20} (After boosting)

Mild to moderate lymphoid depletion



D_{23} (After boosting)

Mild to moderate lymphoid depletion

D_{26} (After boosting)

Mild to moderate lymphoid depletion

Fig 3: Bursal lesions at different age groups after boosting of experimental birds

4.5 Bursal lesions score at different age groups of experimental birds

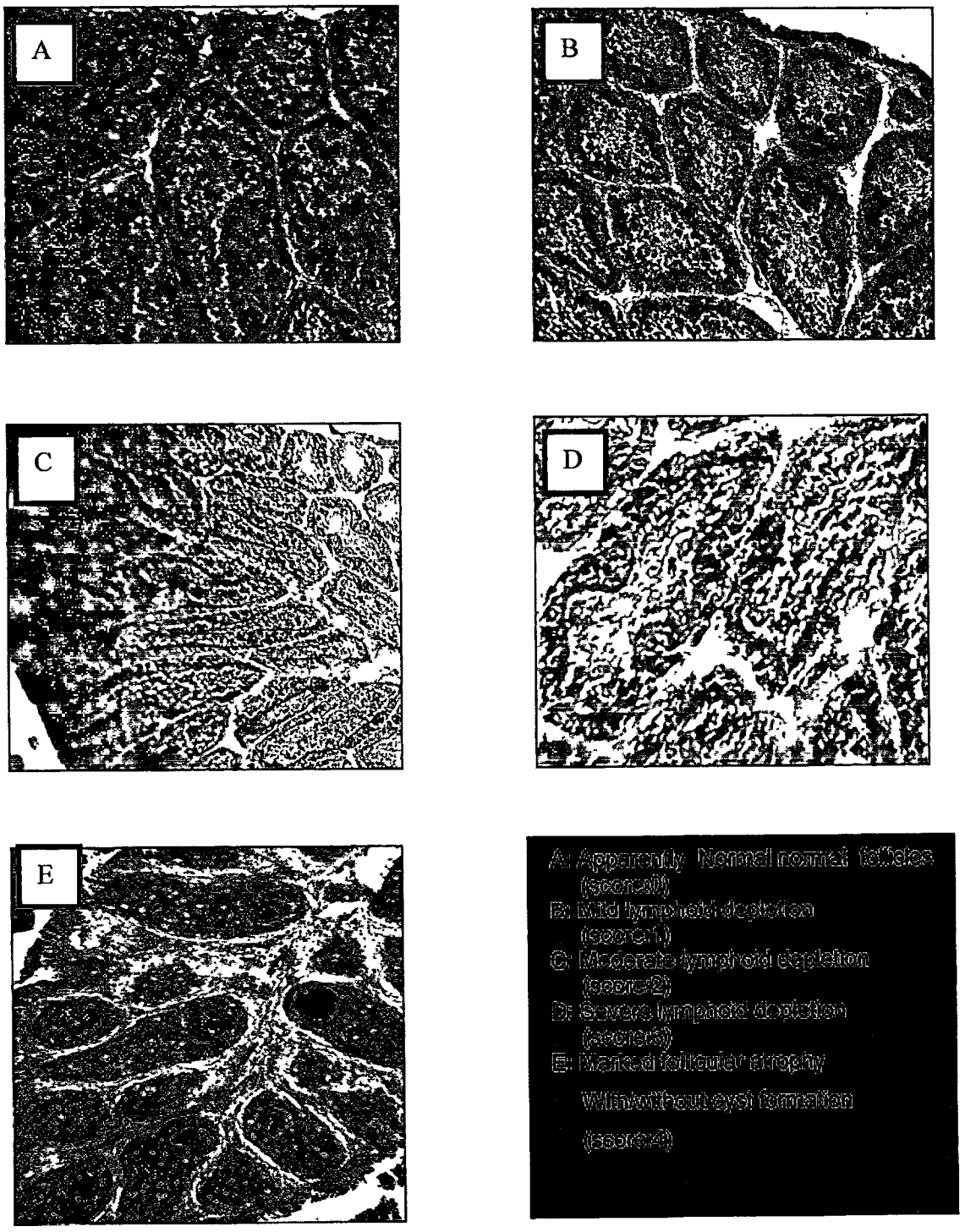


Fig 4: Criteria for scoring bursal lesions

Bursal lesions Scoring

The bursa lesions were calculated on the basis of present criteria (Fig-3).

Table 8: Bursa lesions Scoring

Sampling occasion	Histopathology	Bursal lesion score (Bird: 1, 2 , 3)	Average
Day-11	i. Apparently normal to Mild lymphoid depletion ii. A few atrophied follicle	0,1,1	0.67
Day-13	i. Mild to moderate lymphoid depletion ii. few atrophied follicle	1,2,1	1.33
Day-15	i. Mild to moderate and few severe lymphoid depletion ii. Atrophied follicle	1,2, 3	2.00
Day-17	i. Mild lymphoid depletion ii. Atrophied follicle	0,1,1	0.67
Day-20	i. Mild to moderate lymphoid depletion ii. Few atrophied follicle	2,1,1	1.33
Day-23	i. Mild to moderate lymphoid depletion ii. Few atrophied follicle	1,2,1	1.33
Day-26	i. Mild to moderate lymphoid depletion ii. Few atrophied follicle	1,2,1	1.33
Day-23 (Affected flock)	i. Severe lymphoid depletion ii. Follicular atrophy with formation of cystic spaces . iii. Intercellular edema with cellular infiltration. iv. Necrotic cellular debris. v. Loss of few follicle. vi. In folding of bursal epithelium	3, 3, 4	3.33

Table 9: Statistical analysis of Bursal lesion score

	Sampling occasion	**Bursal lesion score (Bird: 1, 2, 3)**	**Mean of the bursal lesion score**
	D_{11}	0,1,1	0.67±0.33
	D_{13}	1,2,1	0.67±0.33
	D_{15}	1,2, 3	2.00±0.58
	D_{17}	0,1,1	0.67±0.33
	D_{20}	2,1,1	1.00±0.00
	D_{23}	1,2,1	0.67±0.33
	D_{26}	1,2,1	0.33±0.33
	D_{23} (Affected flock)	3, 3, 4	3.33±0.33
P value			0.0003
Levels of Significance			**

** Significant (P<0.01)

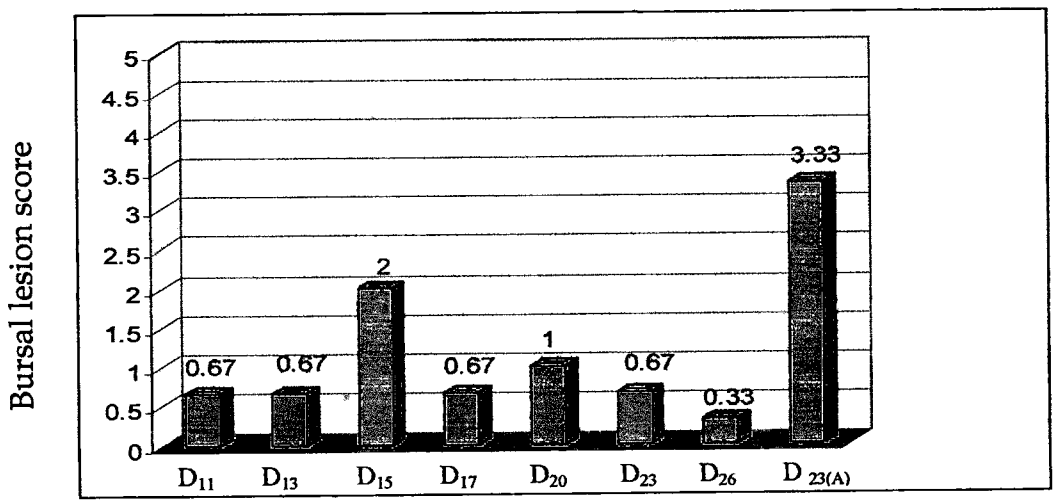
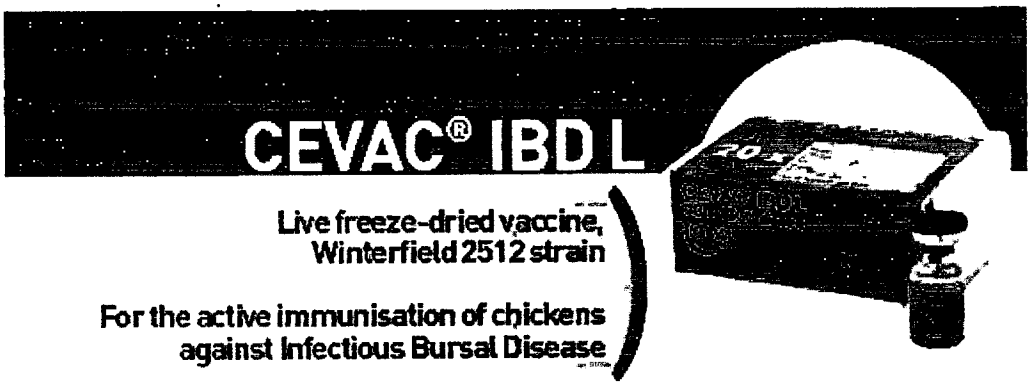


Figure 5: Graphical representation of bursal lesion score

CHAPTER V

DISCUSSION



CHAPTER V

DISCUSSION

Pathogenicity of the Gumboro vaccine prepared from "Winterfield 2512 G-61 strain" of infectious bursal disease virus was evaluated in commercial chickens (broiler) and showed relatively low pathogenicity in the broiler chickens under farm condition.

The present study was the reflection of the safety of infectious bursal disease vaccine. For this experimental study the following points were considered such as clinical signs, gross morbid lesions, bursa-body weight ratios, histopathological lesions of bursa and bursa lesions score.

The vaccination schedule was strictly followed as per manufacturer instruction. Vaccination schedule is the first and fundamental factor to achieve expected immunogenic protection of the vaccine (Lukert and Saif,1997). Faulty vaccination could play an important role to vaccine breaks and outbreaks of the disease. However, apparent clue related to vaccine break was not observed in this study.

Maternally derived antibody (MDA) is sustains in chickens for the first few days and this last for a variable times of age of chickens (Giasuddin *et al.*, 2003; Kouwenhoven and van den Bos,1994). This antibody is an important factor causing inactivation of the vaccine virus and results vaccination failure ((Lucio and Hitchner, 1979). However, experimental flock in this study was vaccinated at D_{11} and boosted at D_{17} without determining the MDA level and the sampling occasion was done following D_{11} and D_{17} (Table- 3).

Gumboro disease is a highly fatal disease where the morbidity rate was around 100% and mortality rate was variable and may reach up to 100 % (van den Berg *et al.*, 1991; Chowdary *et al.*,1996; Hoque *et al.*, 2001). However, there was no apparent morbidity recorded in the present study and mortality rate was also zero following vaccination. This finding is agreed with the previous study (Babiker *et al.*, 2004; Hasan *et al.*, 2004).
The clinical manifestations of the typically affected Gumboro disease is characterized as high fever, off feed, reluctant to move, depression , drowsiness, watery diarrhea and vent picking (Cosgrove, 1962; Islam *et al.*, 1977; Van den Berg, 2000) .

However, any of the signs stated above were recorded in the vaccinated and similar signs were also described previously (Hasan *et al.*, 2004). Vaccinated flocks also show different typical clinical signs which certainly determine the failure of vaccination (van den Berg *et al.*, 1991; Hafez *et al.*, 2002) developed either by one or one more factors of vaccine breaks (Rosenberger *et al.*, 1987; Islam and Saif, 1991; Eterradossi, 2001).

The routine necropsy was done following primary vaccination as well as boosting in the present study as per as experimental design (Table 3). There were no relevant gross morbid lesions recorded during the course of necropsy in the present experiment. But hemorrhage in the skeletal muscle, hemorrhage in the junction between proventriculus and gizzard, varying degrees of bursal lesions, enteritis, etc. were common gross morbid lesions observed both in the vaccinated flock (Cosgrove, 1962; Lukert and Hitchner., 1984; Hoque *et al.*, 2001; Islam *et al.*, 2008) and in the flock reared without Gumboro vaccination which indicate vaccination failure (Islam *et al.*, 2008).

Bursa-body weight ratios are the vital factor in determining the pathogenicity of the respective viruses and there is a proportional relationship between bursa-body weight ratio and the pathogenicity of the respective virus (Mazariegos *et al.*, 1990). However, the bursa-body weight ratios were 2.75±0.60, 2.71±0.39, 2.44±0.42, 3.39±0.13, 2.58±0.55, 2.15±0.16, 2.41±0.28 at D_{11}, D_{13}, D_{15}, D_{17}, D_{20}, D_{23} and D_{26} respectively which differed significantly ($p<0.01$) except D_{17}.

The bursa of typically affected flock histopathologically show mild to sever lymphoid depletion, follicular atrophy, cystic formation of follicles, bursal hemorrhage (Rudd *et al.*,2001; Hoque *at al.*, 2001; Islam *et al.*, 2008). The level of producing lesions in the bursa of Fabricius is also proportionally related with the degree of pathogenicity of the virus inoculated or infected naturally. In the present study the bursal lesions were histopathologically characterized as either normal follicles with or without mild to moderate lymphoid depletion without follicular atrophy or the development of cystic follicles. There was no indication of follicular regeneration in this study. However, the histopathological lesions observed in the present study did not mean the vaccine breaks because the lesions stated here might be developed by the vaccine virus and this agreed with many researchers (Rudd *et al.*, 2001; Alves *et al.*, 2007; Islam *et al.*, 2008) who characterized different bursal lesions produced by some vaccine strain.

Bursal lesions score was determined 0.67±0.33, 0.67±0.33, 2.00±0.58, 0.67±0.33, 1.00±0.00, 0.67±0.33, 0.33±0.33, at D_{11}, D_{13}, D_{15}, D_{17}, D_{20}, D_{23} and D_{26} respectively. The relatively low score was observed in all sampling occasion; these results are agreed with Raue *et al.*, 2004. Outbreaks in the

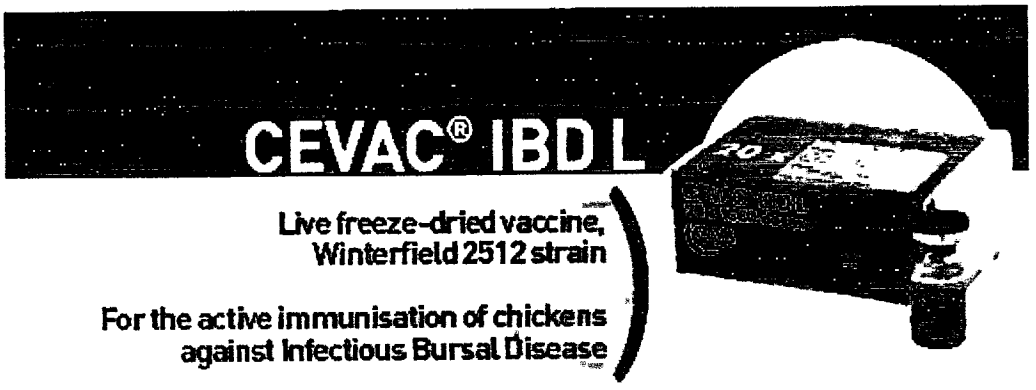
vaccinated flock is common in the experimental areas (Islam *et al.*, 2008) but it was inevident in the present study.

From the above facts and findings it was concluded that the virus used in the vaccine CEVAC® IBD L showed reduced pathogenicity and could be potential to prevent outbreaks in the flock which was characterized as –

1. Sound health without development of any clinical signs of vaccinated flock
2. Uniform bursa-body weight ratios
3. Uniform and reduced bursal lesions scores
4. Severe bursal lesions were unseen
5. No remarkable gross morbid lesion on necropsy
6. No outbreak in vaccinated flock

CHAPTER VI

SUMMARY AND CONCLUSION



CHAPTER VI

SUMMARY AND CONCLUSION

A commercially available CEVAC® IBD L vaccine contains the "Winterfield 2512 G-61 strain" of infectious bursal disease virus in live, freeze dried form was tested for its pathogenicity in commercial chickens. Samples were collected from unvaccinated group at the age of D_{11}, then from primary vaccinated groups at the age of D_{13}, D_{15}, D_{17} and at the age of D_{20}, D_{23} and D_{26} after boosting at D_{17}. After preservation and histological processing histopathological lesions were observed and bursal lesion scores were determined. Samples from affected flocks were collected, preserved and processed for the comparison of the study.

There was not any remarkable clinical signs in experimental flock. In the affected flock there were clinical signs including depression, ruffled feathers, inappetance, slightly whitish diarrhoea, emaciation, dehydration of IBD were observed. There was no mortality in experimental groups. Variation in bursa weight was according to age and individual bird. Significant changes in bursa-body weight ratios were observed in the birds of experimental flock except D_{17}.

Mean of bursal lesions scores were significant in the birds of experimental flock. All most normal to mild lymphoid depletion was seen in experimental flock except D_{15}, but in affected flock, severe lymphoid depletion, interfollicular oedema, active lymphoid necrosis and formation of cyst were seen in histopathological examination.

Considering above facts it may be concluded that the live freeze dried form of intermediate plus vaccine CEVAC® IBD L contains the Winterfield 2512 G-61 strain developed no remarkable clinical signs and necropsy changes, but induce mild histopathological changes that is not sufficient for disease production. The CEVAC® IBD L vaccine is safe in this respect.

From the research interest point of view following task may be scheduled for further study

- Evaluation of immunogenicity of CEVAC® IBD L against field challenge
- Serological evaluation of this vaccine in commercial chickens

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